FGC extension note 08

The Reproductive Biology of Western Larch

February 2008

The Inland Empire Tree Improvement Cooperative

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Prepared for the Forest Genetics Council of British Columbia and the Inland Empire Tree Improvement Cooperative

Published February 2008

Library and Archives Canada Cataloguing in Publication Data

Owens, John N., 1936-
The reproductive biology of western larch
FGC extension note ; 08
"Author: John N. Owens."--P.
"Prepared for Forest Genetics Council of British Columbia and the Inland Empire Tree Improvement Cooperative."--P.
Includes bibliographical references: p.
ISBN 978-0-7726-5877-7

1. Western larch - Reproduction. 2. Western larch - British Columbia - Reproduction. I. Title. II. Inland Empire Tree Improvement Cooperative. III. Forest Genetics Council of British Columbia. IV. Title.
V. Series.

QK494.5.P66O93 2008 585'.2 C2007-960230-4

About the Forest Genetics Council of British Columbia

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

About the Inland Empire Tree Improvement Cooperative

Founded in 1968, the Inland Empire Tree Improvement Cooperative (IETIC) is a group of nineteen member organizations working together to apply classical plant breeding techniques to five native conifer species. Our mission is to identify and conserve genetically superior trees that can be used to produce seed for reforestation and ecosystem restoration. Our membership is a diverse mix of federal and state agencies, private companies, Indian tribes, universities, forest nurseries, private landowners and resource conservation and development councils. IETIC is based at the University of Idaho, College of Natural Resources, Moscow, Idaho, USA.

Acknowledgements

Preparation of this manuscript was funded by the Inland Empire Tree Improvement Cooperative (IETIC), and publishing and printing costs were paid through British Columbia Forest Investment Account allocations to the FGC. Most of the author's larch research that has formed the basis for the publication was funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada. The author thanks the several technicians who have assisted him in larch research over many years; Dave Kolotelo and Barry Jaquish of the B.C. Ministry of Forests and Range for use of some illustrations; staff at the Kalamalka Seed Orchard in Vernon, B.C.; and foresters in the Inland Empire for cone collections used for the cone and seed analyses. Thanks also to the following people who carefully reviewed the manuscript and offered many useful suggestions: Jeff DeBell, Jeff de Graan, Barry Jaquish, Mary Frances Mahalovich, Scott McLeod, Dan Miller, Larry Miller, Roger Painter, Dennis Parent, Marcus Warwell, Al Winter and Jack Woods. A special thanks to Marc Rust for his several careful reviews, helpful suggestions and for organizing and patiently guiding the project from beginning to end.

Foreword

Western larch (*Larix occidentalis* Nutt) is an important species with a natural range restricted to four western states (Washington, Oregon, Idaho, and Montana) and the interior of British Columbia. In its native range, it is a fast-growing tree that produces wood of exceptional quality and strength. This comprehensive and detailed publication is designed to assist foresters, seed orchard managers, and tree breeders interested in producing or enhancing larch seed crops.

The Inland Empire Tree Improvement Cooperative and the Forest Genetics Council of British Columbia are pleased to present this joint publication based on the extensive research of Dr. John N. Owens. While international boundaries often hinder scientific collaboration, the larch is not so easily confined but moves freely across the forty-ninth parallel. Thus, it is fitting that this joint publication should also span that boundary and encourage the exchange of information and ideas between tree improvement workers in the United States and Canada.

We thank Dr. Owens for producing such a complete and beautifully illustrated treatise on the reproductive biology of western larch and hope that readers on both sides of the border and beyond will find this work of interest and value.

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Figure 1. *Western larch cones.*

Introduction

Our knowledge of the reproductive biology of north temperate conifers has expanded rapidly in the last 40 years. During the first half of the 20th century, the reproductive biology of a few conifers, mostly within the family Pinaceae, and mostly European species, were studied. Very few species native to western North America were included in these early, classical studies. Most of this work was included in the early gymnosperm morphology texts of that time. In the mid-20th century, genetic tree improvement programs, breeding programs, and seed orchards in North America and Europe expanded rapidly. These programs required a better understanding of the life cycles and the structure, development, and physiology of conifer reproduction for commercially important conifers. Several species of larch were included in these programs, including western larch, *Larix occidentalis* Nutt (Fig. 2).

Figure 2. *Western larch (foreground) growing with ponderosa pine in Idaho.*

Since that time several studies, mostly in natural stands, have examined vegetative-bud development, cone initiation and cone induction, pollination, fertilization, cone and seed development, and factors affecting cone and seed production. Environmental and biotic factors affect all reproductive stages — some promote, but most reduce, cone and seed production. To understand the causes for good or poor cone and seed production, and perhaps enhance these, we must understand when and how each factor affects these processes.

Each species has a biological reproductive potential (RP) determined by the number of cones that can be produced and the number of viable seeds produced per cone. The RP may be very high for a species at a given site but it may be severely diminished during long reproductive cycles. The final reproductive success (RS) of a conifer, at a particular site or seed orchard, is often only a fraction of the RP present at the start of the reproductive cycle. By better understanding the causes for losses of cones and seeds, we may be able to more accurately predict the RS for a conifer in a natural stand or seed orchard. In seed orchards we may be able to enhance cone and seed production or prevent some of the losses. (See Appendix 6 for methods.)

The purpose of this manual is to describe the reproductive biology of western larch, identify each stage in the reproductive process, describe how each stage affects cone and seed production, and review the results of various studies and experiments designed to enhance cone and seed production or reduce seed losses. Many of the approaches described can only be applied effectively in seed orchards or seed production areas. However, the approaches described will help us understand what went wrong in natural stands, and this may aid in predicting cone and seed crops. Most of the detailed accounts are those drawn from work done on western larch, but some results are from other larch species where more or better experiments have been conducted.

Taxonomy and Distribution

The most recent and comprehensive report on conifer taxonomy places the genus *Larix* in the Pinaceae and lists 11 species and nine varieties of larch (Farjon 1998). Other authors consider that there are only 10 species: three endemic to North America and seven occurring in Europe and Asia (LePage and Basinger 1995). The genus is widely distributed across the boreal forest zone of Eurasia and North America. Most larch species occur in montane coniferous forests, often in pure stands at temperate latitudes, as in the Rocky Mountains of North America, the Himalayan Mountains of Eurasia, and the Sichuan Mountains of China. They also extend northward to the artic tree line in North America and Siberia. *Larix gmelinii,* generally a very stunted form, extends to 73°N and is apparently the northernmost conifer in Siberia, while *L. laricina* (tamarack) extends to about 65°N in Alaska and Canada.

Figure 3. *Western larch A. in the autumn as leaves begin to turn yellow, and B. a young tree in the winter after all leaves have fallen.*

All larch species are deciduous (Figs. 3A, B), meaning that they lose all of their leaves in the autumn each year. The needle-like leaves of larch are borne spirally on long-shoots for the first year and in clusters of variable numbers of leaves, depending on the species, on short-shoots on 2-year-old and older long-shoots (Fig. 1).

Three species are recognized in North America

Larix laricina (tamarack) is distributed almost continuously from the west coast of Alaska to the east coast of Canada and south into the Great Lakes Region and New England. *Larix lyallii* (subalpine larch) has a very limited distribution at higher elevations in southeast British Columbia; in southwest Alberta; and into the Inland Empire forest regions of Washington, Idaho, and Montana. Both of these species are generally small trees and are often stunted from the very harsh climates in which they grow. *Larix occidentalis* (western larch) is the largest of the North American larches and grows at moderate elevations in the Cascade Mountains of Washington and Oregon, in southeast British Columbia, southward into the Inland Empire forest regions of northeast Washington, Northern Idaho, and northwest Montana, and in southeast Washington and northeast Oregon (Fowells [compiler] 1965) (Fig. 4). One of the oldest trees recorded occurred near Seeley Lake, Montana and was about 915 years old. Some of the oldest western larch reached 2.3 m (93 in.) in diameter at breast height and over 45 m (150 ft.) in height. Western larch usually reaches its maximum reproductive potential when 30–50 m (100–150 ft.) tall and about 50 years old (Schmidt and Shearer 1995).

The genus *Larix* is ancient. The oldest fossils found so far have been on Ellesmere Island in northern Canada, and date back to the Eocene or Cretaceous, over 65 million years ago. Such fossils are clearly *Larix*, indicating a very long evolutionary history in the northern hemisphere (LePage and Basinger 1995). Although there is no clear fossil evidence to prove a relationship, *Larix* appears closely related to *Pseudotsuga* from Asia and western North America, and the lesser

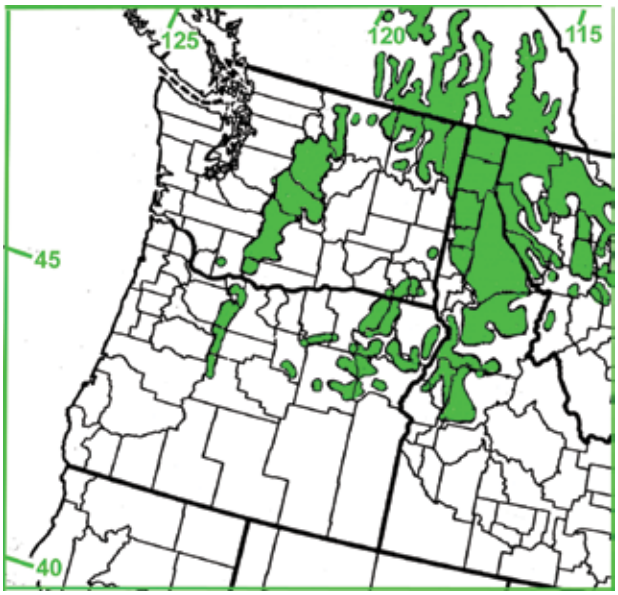


Figure 4. *Distribution of western larch (redrawn from Fowells [compiler] 1965).*

known Cathaya from China (Farjon 1998). There are striking similarities in reproductive biology, as well as morphological and anatomical features, between *Larix* and *Pseudotsuga*. Within the genus *Larix*, when all traits are considered, western larch and subalpine larch appear to be very closely related and fall into the same group as most Asian larches, whereas tamarack has greater affinity with the European larches (LePage and Basinger 1995).

Ecology and Economic Importance

Western larch is a pioneer species

Schmidt and Shearer (1995) have aptly stated, "Ecological requirements dictate that western larch play a pioneer role or it will not play at all." It becomes established before other more shade-tolerant woody species in areas where there has been fire, glaciation, volcanic activity, or other disturbances (Fig. 5A). These disturbances leave similar and very dramatic landscapes; however, the results of fire and volcanic activity appear very quickly while those of glaciation may take centuries. Fire, an integral part of larch ecology and regeneration, usually results in a mosaic of surviving and dead trees and other vegetation (Schmidt and Shearer 1995).

Glaciation has been a part of the Pacific Northwest region ecology for the last 16,000 years. About 12,000 years ago, the deglaciated areas became colonized by herb-dominated species and subalpine forests, but it is uncertain if larch was present at that time. The presence of summer rains would have prevented fires and would have favoured *Pseudotsuga*, but not larch. There is also some uncertainty about the presence of larch as opposed to *Pseudotsuga* at that time because recent fossil evidence is mostly based on fossil pollen deposits, and pollen grains from the two genera are not distinguishable.

At that time, larch may have been restricted to regions farther north in British Columbia. Only in the past few thousand years have the summer rains lessened, and fires become more frequent in the Pacific Northwest, allowing western larch to move south into the range it now occupies. The present distribution of western larch may thus represent remnants of earlier distributions. Currently, the reconstruction of the history of larch in the region is speculative (Whitlock 1995). Deep, well-drained soils are most suitable for growth of western larch, and these commonly form over glacial tills, alluvium, and volcanic ash. Western larch thrives in relatively cool temperatures, averaging 7°C (45°F) annually, but ranging from 41°C (106°F) to -37°C (-34°F). In its natural range, frosts can occur any month of the year, and the frost-free period usually averages 60–160 days. Rehfeldt (1995) suggests that cold hardiness may be a problem for western larch at low- to mid-elevations, and late spring frosts may be more damaging than early fall frosts. Most of the precipitation comes inland from the Pacific Ocean and averages about 760 mm (28 in.). Only about 20% of this falls during the most active growing season from May through August. Long hot and dry periods with low humidity usually occur in July and August. Snow usually covers the ground from November to April. In the southern part of its range, western larch is absent from the hot and dry south-facing slopes but it occurs abundantly on the adjacent northern slopes. In its northern range it can be found on all exposures (Schmidt and Shearer 1995).

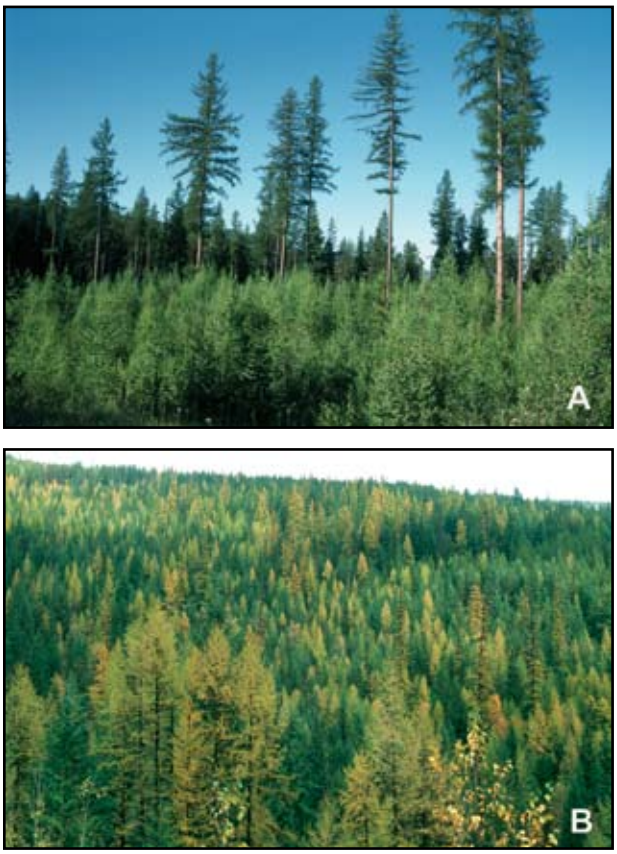


Figure 5. *A. Young western larch regenerating under older seed trees. B. Mixed stand of lodgepole pine and western larch (yellow) photographed in the autumn.*

Western larch is seral, rather than climax, in its habitat because of its intolerance to shade. Although it tolerates some shade for 1–2 years after germination, thereafter shade will severely restrict its development. Therefore, western larch is not climax in any forest habitat. Western larch commonly grows with other conifers in forests that generally have a rich herb and shrub understory (Fig. 5B). Up to 10 conifer species have been observed in these mixed stands, including Douglas-fir; lodgepole pine; Engelmann spruce; subalpine fir; and, on drier sites, ponderosa pine. These forests provide food and cover for a wide variety of animals including moose, elk, deer, bears, and numerous small rodents. The variety of tree species present also offers a wide range of habitats for birds (Fowells [compiler] 1965; Fiedler and Lloyd 1995; Schmidt and Shearer 1995).

Western larch is generally very tolerant to most insect pests and diseases that affect other conifers in the Northern Rocky Mountain region (Carlson et al. 1995). Usually, rather short-lived flare-ups occur for several insect pests including the western spruce budworm, larch casebearer, and larch sawfly but these usually result in little or no mortality (Schmidt and Shearer 1995). Most pathogens causing needle damage, or root and wood rots, do not cause significant damage. In the Inland Empire, *Meria* needle cast caused by *Meria laricinis* and the needle blight caused by *Hypodermilla concolor* can cause significant damage, particularly in repeated wet springs. Dwarf mistletoe (*Arceuthobium laricis*) is the most important disease organism of larch, often causing significant reductions in tree volume, growth, and vigor. This flowering plant parasite infects young shoots causing witches' brooms where ice and snow can accumulate and cause breakage of almost all branches on a tree. The parasite may infect over 40% of the larch in the region. This disease may be controlled in trees with light to moderate infections by commercial thinning and proper silvicultural practices (Carlson et al. 1995; Filip et al. 1995).

Very rapid height growth, equal to or better than its associated species, occurs in western larch until about 12 years of age. It then maintains a comparable or better growth rate than many associated species. Its growth rate is equal to, or better than, lodgepole pine to ages 50–100 years, but less than western white pine at age 100 years on productive sites (Fiedler and Lloyd 1995). Its large size and desirable wood properties make it a preferred species for lumber, utility poles, and firewood. It provides a wide variety of other primary products including plywood, pulp and paper, particleboard, fibreboard, house logs, and water-soluble gum (Keegan et al. 1995). Although it is usually considered to be a minor species in the Inland Empire and southeast British Columbia, in some areas like Montana it provides about 15% of the timber processed. The commercial value of western larch, combined with the ecological and aesthetic values of larch forests,

make western larch a truly valuable species that must be maintained and planted where needed. Plantings require effective seed production, and seed production is aided by an understanding of the reproductive biology of the species.

The Reproductive Cycle

The limited distribution of western larch to the mountainous interior regions of northwestern North America results in a reproductive cycle that varies little throughout its distribution. Temperature strongly affects the occurrence of reproductive events. Temperature variation from year to year or on different sites causes variation in phenology (the relationship of climate to periodic biological phenomena). The most easily observed stages in the reproductive cycle, such as pollination and cone and seed maturation, occur within a few weeks after dormancy in the spring or within a few weeks before winter dormancy in the fall, when weather is often more variable and extreme. Therefore, the rate of development at these times tends to be variable. Other stages, such as fertilization and embryo development, occur midway through the growing season and appear to be more uniform because they are less affected by weather. Even though the phenology may vary by a few weeks at the onset or end of the growth period, a single general description of the reproductive cycle is valid (Fig. 6).

The reproductive cycle of western larch is similar to most non-*Pinus* members of the Pinaceae in that it lasts about 15 months from cone-bud initiation and differentiation to cone and seed maturity and includes one winter dormant period (Owens 1995). Also, it is similar in that pollination, fertilization, and embryo and seed development occur during one growing season. Most species of pine have one extra year between pollination and fertilization.

The reproductive cycle of western larch (Owens and Molder 1979a, 1979b) is similar to that of other larches that have been studied (Schopf 1943) and very similar to that of Douglas-fir (Allen and Owens 1972). Pollen-cone and seed-cone buds differentiate from about mid-June through mid-July in the summer before pollination. Pollen-cone buds contain all of their microsporophylls and seed-cone buds contain all of their ovuliferous scales (scales) before winter dormancy begins.

Spring growth is commonly thought to begin when vegetative or cone buds burst in the spring, about April or May. However, considerable growth and development occur within the buds before bud burst (flushing). It is this growth that causes flushing. Vegetative and cone buds begin cell divisions and growth usually in March, with the first warm days of spring. This is followed by a few weeks of bud swelling as the buds enclosed by the bud scales enlarge.

Meiosis occurs in mid-March in the micro-sporangia of the pollen-cone buds, very soon after dormancy ends, and pollen quickly develops during the following month. Ovules resume development in early March, the pollination mechanism develops, and meiosis occurs within the ovules by mid- to late April. In southern areas, pollen-cone and seed-cone buds burst in late March or early April and pollination occurs over about two weeks. In northern Idaho and Montana, pollen shed frequently occurs in March or April. Pollen release and seed-cone receptivity are not always well synchronized. Pollen is taken into the ovules where it is held for several weeks while the ovules mature.

Ovule maturation, megagametophyte, and egg formation occur for about two months, from mid-April until mid-June. Early in June, pollen tubes form and penetrate the inner tissues of the ovules and fertilization occurs about mid-June. The fertilized eggs form proembryos within about one week, mid-embryos are recognizable in sliced seeds by mid-July, and embryos and seeds are mature by mid to late August (Fig. 6). Cones become mature, dry and shed their seeds from mid-August through the fall depending upon the weather. Dry, empty seed cones may remain on the trees for several years.

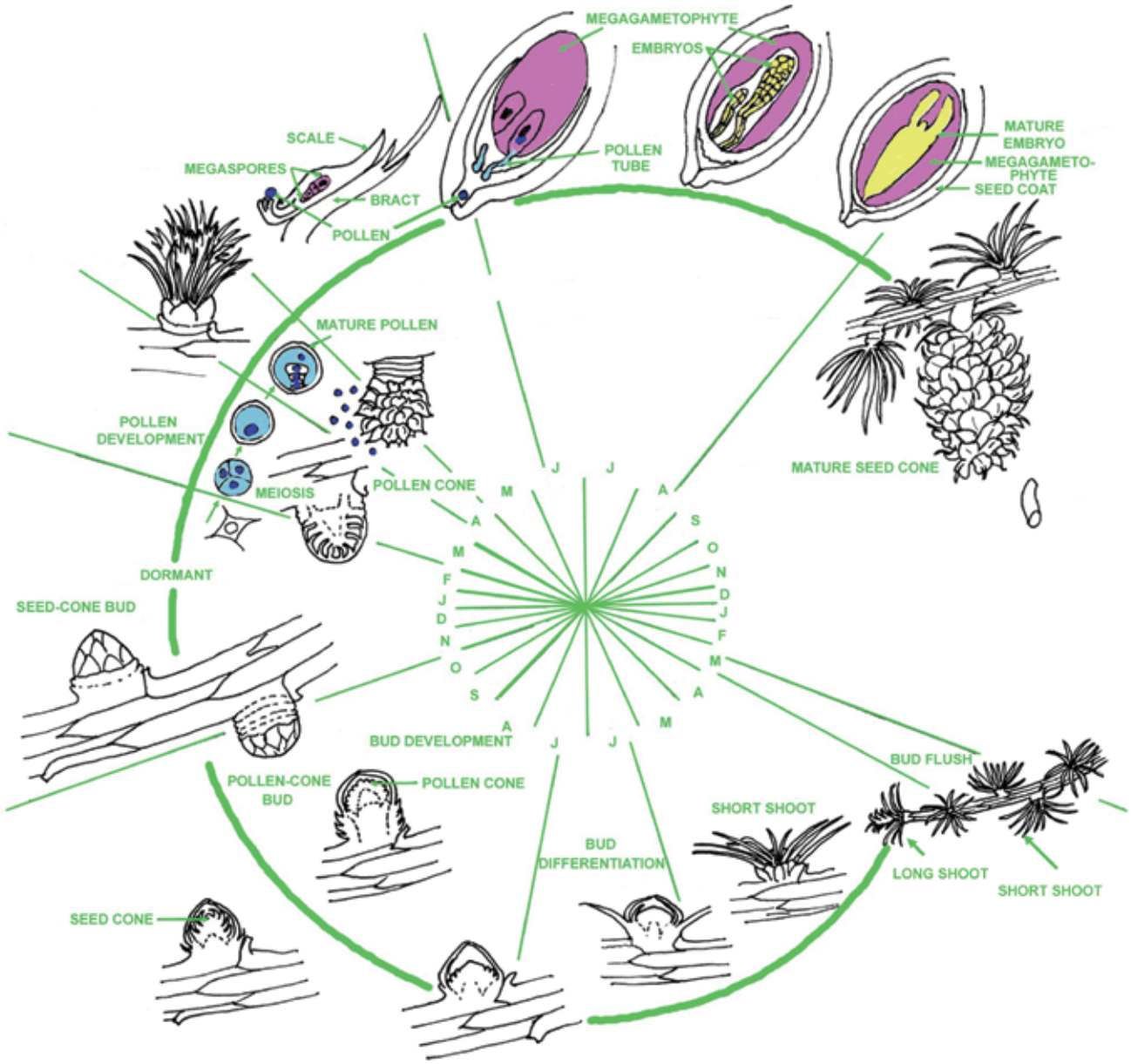


Figure 6. *The reproductive cycle of western larch (redrawn from Owens 1995).*

Vegetative-Bud and Shoot Development

All conifers shed some leaves or branches in the autumn. However, these leaves or branches are several years old, so most conifers remain evergreen. All larches are deciduous, and lose all of their leaves at one time in the autumn each year. This is similar to a few other conifers such as *Taxodium* (bald cypress) from the southeastern United States. Larches are also unusual in the types of vegetative buds produced and bud development. This trait is similar to *Cedrus*, common ornamental conifers in which all four species are native to mountainous areas in the Mediterranean region of southern Europe and the Himalayan Mountains. *Larix* and *Cedrus* produce long and short-shoots and although the same terminology is used for pine shoots, the structures and development are different from pines. Vegetative bud and shoot development are important in understanding reproduction because

they determine how, when, and where cone buds are initiated and develop.

Larch trees bear two types of vegetative shoots: long-shoots and short (dwarf) shoots. At the tip of each terminal or lateral long-shoot (branch) is a long-shoot terminal bud; subtending this are usually 1–3 long-shoot lateral buds. Axial (primary) leaves are spirally arranged along the long-shoot (Fig. 7).

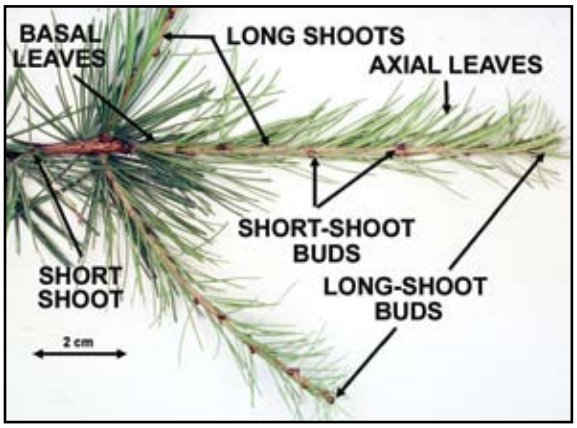
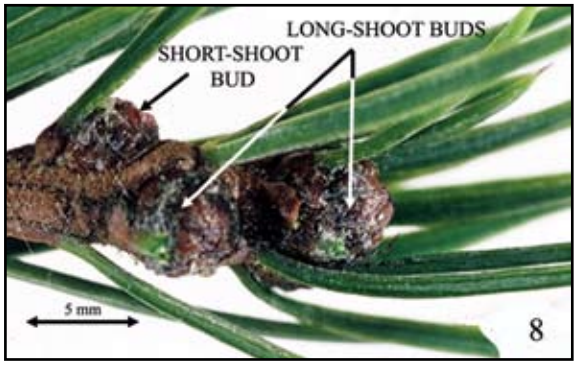
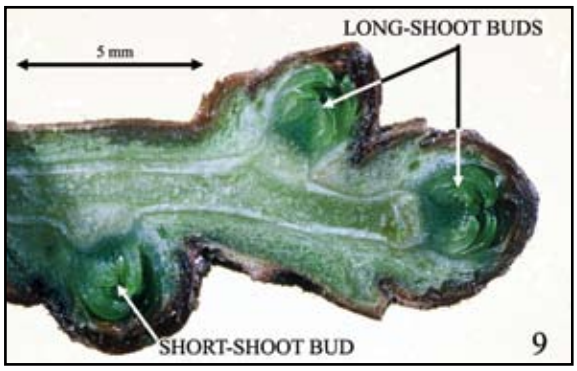


Figure 7. *Long and short-shoots and buds of western larch.*

A node is the site where a leaf attaches to the shoot and an internode is the space between adjacent leaves. Internodes are long throughout most of the long-shoot but short at the base forming a cluster of basal leaves. The leaf axil is just above, or distal to, where the leaf joins the stem. An axillary bud occurs in the axil of about every 5–7 primary leaves, but the number and location are not predictable and depend on the position of the shoot in the crown and positions of the leaves along the shoots (Fig. 7) (Remphrey and Powell 1984). All of the axillary buds on a long-shoot are initiated as the shoot is elongating in the spring. A small mound of meristematic cells (the apex or apical meristem) arises in the axil of some of the leaves. Each initiates bud scales that enclose and protect the bud. Once the long-shoot has completed growth, the axillary apices initiate leaf primordia. Then, late in the summer, the axillary buds become dormant (Fig. 8).

Two types of axillary buds develop (Figs. 8, 9). The distal few axillary buds on a shoot initiate





Figures 8 and 9. *Long-shoot and short-shoot buds, whole (Fig. 8) and sliced (Fig. 9) to show internal structures.*

bud scales followed by preformed leaf primordia before they become dormant. The terminal bud on the shoot does the same. These preformed leaf primordia represent about two-thirds of the total leaf primordia that will be initiated (Fig. 10). They are separated into large basal leaf primordia (Fig. 10) that develop into a cluster of basal leaves at the base of the long-shoot (Fig. 7) and small leaf primordia (Fig. 10) that develop into long axial leaves on the mature shoot (Fig. 7). The additional one-third of the leaf primordia are initiated after winter dormancy and they develop into neoformed (newly formed) axial leaves as the shoot elongates (Fig. 7). The presence of preformed and neoformed leaves on one year's growth of a shoot is rare in mature conifers but occurs in seedlings of some species. However, this is common in some mature hardwoods, such as poplars and birch. In birch, preformed and neoformed leaves develop differently and have different leaf morphologies on the same shoot. This is not true for larch.

In larch, the proximal axillary buds (below the distal long-shoot buds) (Fig. 7) initiate bud scales, then a tight spiral of about 40 preformed leaf primordia (Fig. 11). These short-shoot buds have a small apex buried in the centre of the long-leaf primordia (Fig. 12). These buds form the many short-shoots along the stem (Figs. 7, 9, 13, 14). Short-shoots do not initiate neoformed leaves after winter dormancy.

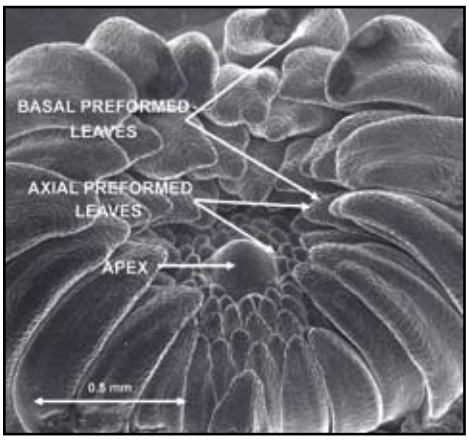
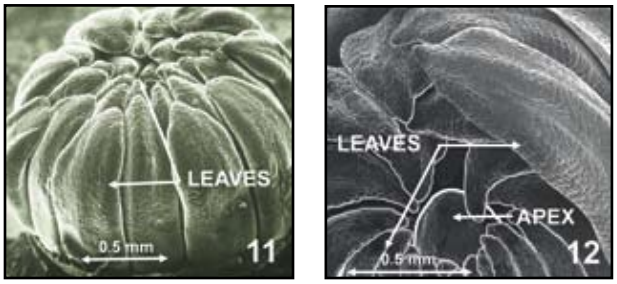
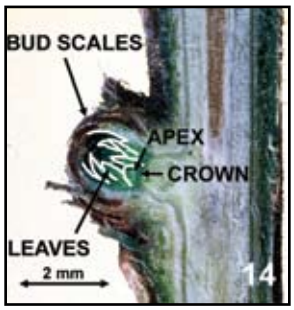


Figure 10. *Scanning electron micrograph of a dormant long-shoot bud after bud scales were removed.*



Figures 11 and 12. *Scanning electron micrograph of a dormant short-shoot bud after bud scales were removed (Fig. 11), and after some leaf primordia were removed to show the small apex at the base (Fig. 12).*



Figures 13 and 14. *Dormant whole short-shoot bud (Fig. 13) and a bud sliced down the centre (Fig. 14).*

In the next growing season, long- and short-shoot buds swell and flush. Long-shoots enlarge within the bud scales for about one month and then flush. This is followed by rapid shoot elongation for about one month. Elongation is usually completed early in June (Fig. 16). During shoot elongation, long-shoot apices initiate neoformed leaves and axillary buds. The distal long-shoot buds on the shoot initiate bud scales and preformed leaves, repeating the entire process as in the previous

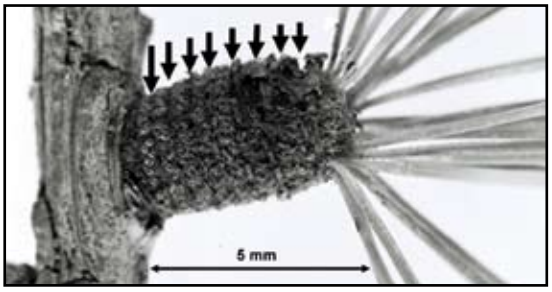


Figure 15. *A short-shoot that is eight years old, as indicated by the rings of bud-scale scars (arrows). It has few leaves and will probably die within 1–2 years.*

year (Fig. 16). After dormancy the short-shoots swell and burst and a cluster of leaf primordia emerge (Fig. 13). Short-shoots elongate only about 1 mm/yr. Each year they initiate bud scales and preformed leaves but no axillary buds. Short-shoots may repeat this process (Fig. 16) for up to 8–10 years (Fig. 15), each year producing fewer leaves than before, until they die (Owens and Molder 1979a).

Bud development in larch is very opportunistic, meaning that under environmental or physiological stresses and depending on the

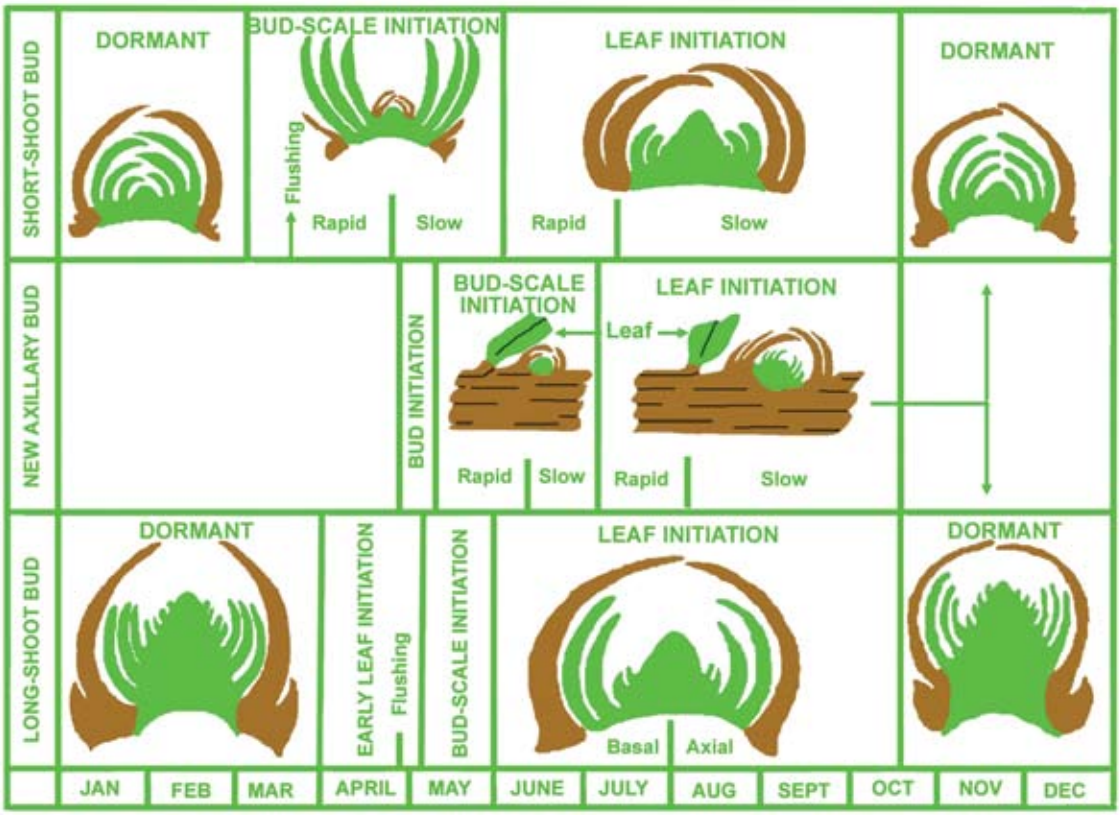


Figure 16. *The phenology of bud-scale and leaf initiation in newly initiated western larch axillary buds (centre), vegetative short-shoot buds (top), and vegetative long-shoot buds (bottom). The arrows (right-hand column) indicate that most newly initiated axillary buds differentiate into vegetative short-shoot buds and more-distal axillary buds differentiate into long-shoot buds. Bud scales are shown in brown and bud and leaf tissues in green.*

position of the buds on the shoots, long-shoot buds frequently change to become short-shoot buds and occasionally short-shoot buds change to become long-shoot buds or cone buds, or they may abort. There are no fixed rules and almost anything can happen. Thus, the branching pattern and the results from top pruning in larch are very unpredictable. The physiological controls for conversion from one bud type to another are not understood.

Cone Initiation and Pre-dormancy Development

Western larch in natural stands may start to form cones at 8–10 years of age, but frequent and abundant cone production usually does not begin before age 25. After trees are 40–50 years old they generally bear abundant cones for up to 400–500 years (Boe 1958).

Seed cones and pollen cones are often widely scattered throughout the crown. The patterns of pollen-cone and seed-cone distribution within the crown have not been carefully described for western larch but they have been for *L. laricina* (tamarack) in eastern Canada, as summarized by Powell (1995). The within-crown pattern of shoot development and cone position in tamarack, as the tree matures, is similar to that observed for western larch (Owens and Molder 1979a, 1979b). Lateral seed cones may occur on new long-shoots in young trees. This occurs because the newly initiated distal axillary buds may differentiate directly into seed-cone buds rather than long-shoot buds. Also, more proximal axillary buds may differentiate directly into seed-cone buds rather than spending the first year or more as short-shoots. In older trees, seed-cone buds form on short-shoots that are one or more years old and usually located distally on a branch. Pollen-cone

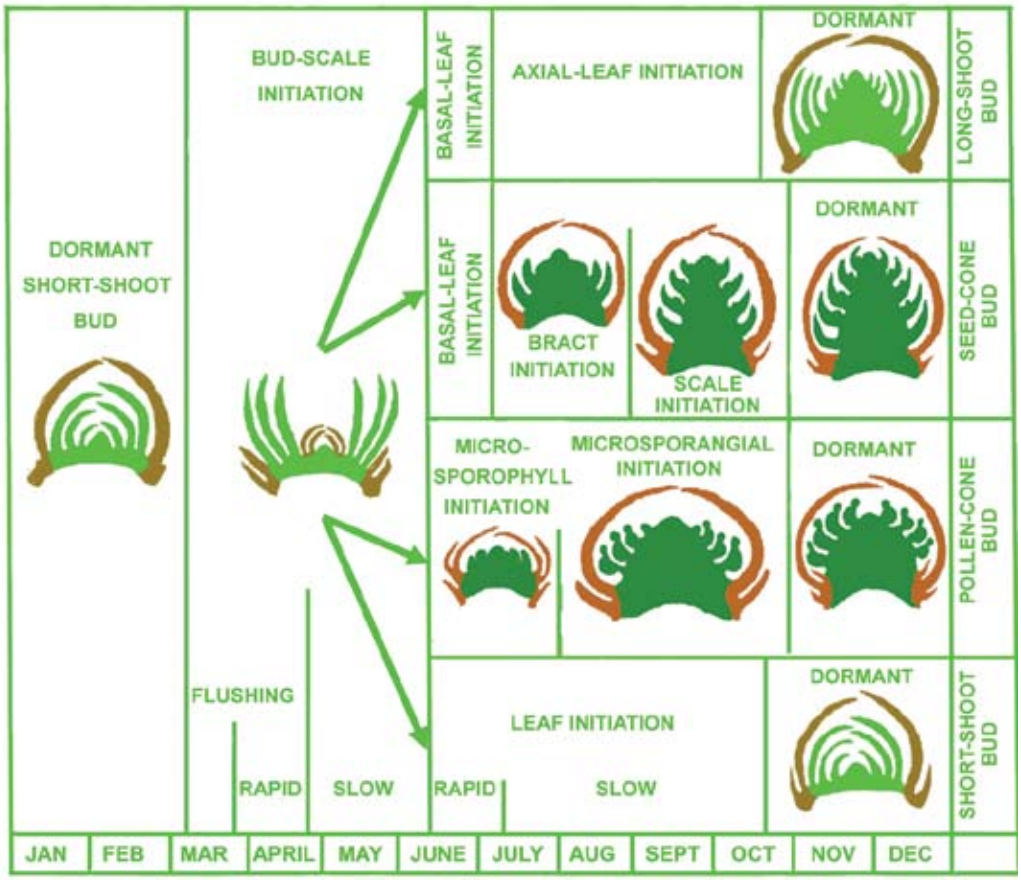


Figure 17. *Phenology of short-shoot bud development in the second or subsequent years. Most short-shoot buds remain as short-shoots for many years (lower row) while others may differentiate into pollen-cone buds, seed-cone buds, or occasionally long-shoot buds. Vegetative short- and long-shoot bud and leaf tissues are shown as light green and pollen-cone and seed-cone bud tissues are shown in dark green. Bud scales for vegetative buds are shown light brown and bud scales for pollen-cone and seed-cone buds are shown in dark brown.*

buds form on short-shoot buds that are one or usually several years old and located proximally on a branch. Pollen cones and seed cones may occur on the same branch and intermingle along the branch. Cones are generally more abundant on pendant branches than on horizontal or ascending branches. Seed cones are more abundant on more vigorous branches, whereas pollen cones tend to be more abundant on the less vigorous branches. In western larch, as in other larches, it is not possible to accurately predict the branch or position on a branch where a pollen cone or a seed cone will form.

The vast majority of seed-cone buds and all pollen-cone buds in reproductively mature western larch trees in natural stands are initiated from short-shoots. Figure 17 shows the development of short-shoot buds in western larch after the first year, as they develop again into vegetative short-shoot buds (VSS), pollen-cone buds, or seed-cone buds. In all three cases, the differentiation begins about mid-June, at the end of lateral long-shoot elongation (Fig. 18). Short-shoot buds end dormancy and begin growth in March (Fig. 17). Early development occurs entirely within the buds that, due to leaf elongation, swell then flush near the end of March. From March through early June, the apex initiates bud scales, first slowly then more rapidly. The outer brown bud scales cover and protect the apex before flushing occurs and the inner, membranous bud scales continue to be initiated. In about mid-June, bud-scale initiation stops. In buds that will remain as short-shoot buds for another year, the apex remains small

and initiates spirally arranged leaf primordia (Fig. 17). In buds that will become pollen-cone buds, the apex enlarges and becomes dome-shaped; then, for about two months, initiates spirally arranged microsporophyll primordia (Fig. 17). Microsporophylls are short leaf-like structures (Fig. 19), each of which initiates two microsporangia (pollen sacs) on the lower (abaxial) surface. In buds that will become seed-cone buds, the apex enlarges and for about one month initiates spirally arranged basal foliar organs (leaf primordia); then, for about four months, it initiates spirally arranged bract primordia (Figs. 17, 20). Most bracts initiate an ovuliferous scale (scale) on the upper (adaxial) surface from about mid-August until the end of October. Some basal bracts initiate no scales before dormancy. Most ovuliferous scales initiate two adaxial ovules and each ovule will form a central, enlarged megaspore mother cell. Short-shoot, pollen-cone, and seed-cone buds are well developed and become dormant at about the end of October (Fig. 17).

Occasionally, bisporangiate cone buds form in which microsporophylls are usually initiated in the base of the cone bud and bracts in the distal part of the cone bud. After dormancy these may form normal pollen and ovules, respectively, but when pollen is shed that portion of the cone may dry and abort. Occasionally, bisporangiate cones fully develop but the seed may not be viable (Tosh and Powell 1986).

Dormant short-shoot (Fig. 14), pollen-cone (Figs. 21, 22), and seed-cone (Figs. 23, 24) buds have a crown region at the base of the bud. This nodal diaphragm occurs in the buds of many species of Pinaceae (but not *Pinus*) and separates the new bud from the long-shoot. Its function is uncertain but it has been implicated in physiologically isolating the bud from the main shoot on which it is borne and in bud frost resistance in conifers (Krasowski and Owens 1989). In short-shoot buds, a new crown region develops each year and, like the external bud-scale scars (Fig. 15), tells the age of the short-shoot.

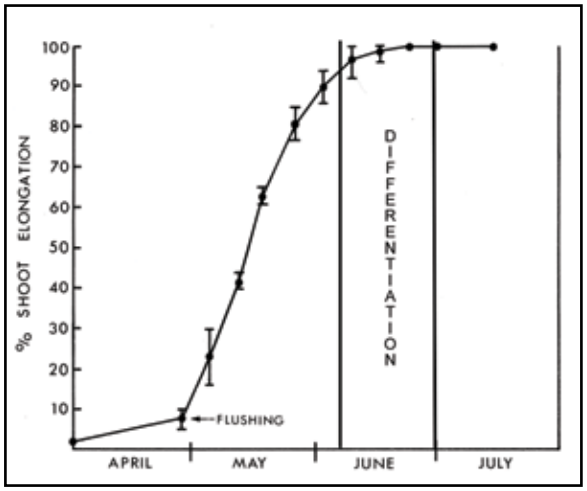
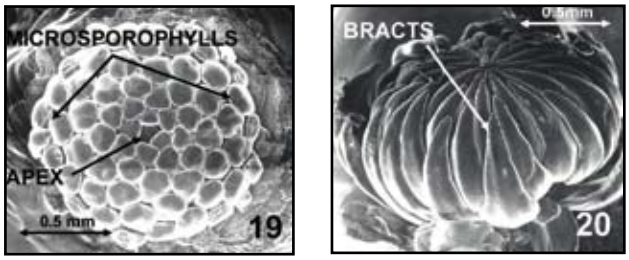
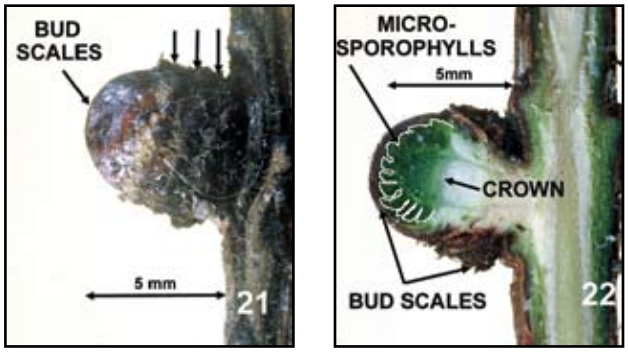


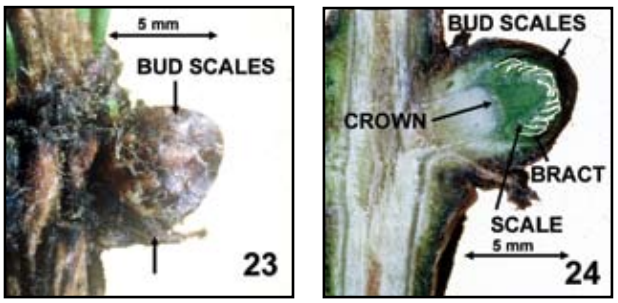
Figure 18. *The average lateral shoot elongation for four mature western larch trees shown as a percentage of final shoot length and the time of vegetative-bud flush and cone-bud differentiation.*



Figures 19 and 20. *Scanning electron micrographs of a dormant, dissected pollen cone (Fig. 19), and a seed cone (Fig. 20), showing microsporophylls and bracts.*



Figures 21 and 22. *Whole (Fig. 21) and sliced (Fig. 22) dormant pollen-cone buds on 3-year-old short-shoots (arrows show bud-scale scars).*



Figures 23 and 24. *Whole (Fig. 23) and sliced (Fig. 24) dormant seed-cone buds on 1-year-old short-shoots.*

Bud Identification and Forecast of Cone Crops

Identification of buds in mid- to late summer is possible if the position of the bud is carefully noted, buds are dissected or sliced down the centre, and the contents are observed using a hand-lens or dissecting microscope (Figs. 9, 13, 14, 21–24). As buds develop further, identification becomes easier. Numbers of cone buds should be estimated from samples of branches collected in the late summer, fall, or winter from upper and lower regions of the crown. Identification is more difficult in larch than in most other Pinaceae, such as Douglas-fir, because the distribution of cones in larch is less predictable. In most Pinaceae, cone buds are found only on the current year's growth — they were initiated following dormancy and differentiated on the elongating shoots. However, larch cone buds differentiate from short-shoot buds (Figs. 13, 14) that were initiated one or more years earlier. Rarely are they initiated on the current year's growth; rather, they occur on growth that is one to several years old. Pollen-cone buds (Figs. 21, 22) may be found on 1-year-old growth but are more common on shoots that are farther back and two to several years old. They often occur in large numbers all along the shoot or are scattered between short-shoot and seed-cone buds. They may occur on old shoots on which the terminal vegetative bud has died. Seed-cone buds (Figs. 23, 24) are most frequently found on 1-year-old growth. Both pollen-cone and seed-cone buds occur more frequently on pendant branches. These branches may be long, possess several years' growth, and hang down in the inner regions of the crown.

Long-shoot buds are usually not confused with cone buds since they occur as lateral buds only at the distal end of 1-year-old shoot (Figs. 8, 9), where seed-cone buds seldom occur and pollen-cone buds rarely occur. Short-shoot buds are usually easily distinguished from cone buds.

Short-shoot buds are only 2–3 mm in diameter, dark brown, and rather flat (Figs. 8, 13, 14). Upon dissection (Fig. 12) or slicing of the bud, the axis of the bud appears recessed below the bud scales (Fig. 14). The apex is tiny and buried in the centre at the base of the long, green leaf primordia (Figs. 12, 14).

Pollen-cone buds are broadly dome-shaped and about 5 mm in diameter. The bud scales are brown but fewer than in short-shoots or seed-cone buds, and they are easily dissected. In dissected or sliced buds, the pollen cone within is dark green (Fig. 22) and has the appearance of a flattened raspberry (Fig. 19). The many lobes are microsporangia on the microsporophylls (Fig. 19), giving it a rather shiny appearance. Some dormant pollen cones may be deep red-green.

Seed-cone buds, which are usually less numerous than either short-shoot or pollen-cone buds, are commonly scattered along the shoot among these other buds. They usually are found more distally than pollen-cone buds on the same year's shoot. They are about the same colour and diameter as pollen-cone buds, but are more pointed (Fig. 23) and have a thicker layer of bud scales. In dissected or sliced seed-cone buds, the cone is dark green, and has a long central axis and many long pointed bracts (Fig. 24) that completely cover the cone apex (Fig. 20).

Cone Induction

Terminology

Terminology regarding cone induction goes back about 50 years. The terms "cone enhancement" and "cone induction" are commonly used to describe the promotion of flowering in conifers

that have or have not had a previous history of cone production, respectively. Either term or simply "cone promotion" are commonly used in the literature without a clear distinction. The term "flowering" is usually restricted to flowering plants, including hardwoods; however, basic studies of the processes in flower initiation and cone initiation (Owens 1969) show that the processes are essentially the same at the early stages of flower and cone initiation. Anatomical differences in flowers and cones only occur at later stages of reproductive organ development. Thus, the terms "flowering" and "floral initiation" are commonly used in the conifer literature and designate a process and not a specific structure. However, use of the morphological term "flower" for cone is inaccurate because, although flowers and cones are functionally similar, they are structurally very different.

Cone production may be enhanced in natural stands or plantations by relatively inexpensive means, such as thinning and fertilizing. Cones may be induced in soil-based seed orchards or potted trees using exogenous application of the plant hormone (growth regulator) gibberellin $A_{4/7}$ ($GA_{4/7}$) usually with adjunct treatments such as girdling, root pruning, drought, fertilizer, or increased temperature (Owens and Blake 1985; Pharis et al. 1987). The earliest cone induction trials were done on larch in the 1960s, mostly using Japanese and European larches. Trials included thinning, branch and trunk girdling, root pruning, altering day length, tying and bending branches into downward positions, and applying treatments with a wide variety of plant growth regulators (see references in Owens and Blake 1985). During the last 30 years, experiments have focused on application of the plant growth regulator $GA_{4/7}$ in various concentrations and methods of application. Most of the techniques available have been tried on larch species in natural stands, seed orchards, or potted trees in Europe (Bonnet-Masembert 1982), Great Britain (Philipson et al. 1997), North America (Eysteinsson and Greenwood 1990; Ross 1991; Webber and Ross 1995), and Japan (Katsuta et al. 1981). Published results are available for a few trials using western larch (Ross 1991; Graham et al. 1995; Webber and Ross 1995).

As with other conifers, there have been many unpublished cone induction trials for western larch, usually in seed orchards or potted trees in British Columbia, Idaho, and Oregon. Results from this work show that cone induction is an important technique that can be used to stimulate cone production in young grafts to shorten the breeding cycle; in young seed orchards, to bring them into seed production earlier; in production seed orchards, to promote pollen and seed-cone production in clones with infrequent or low flowering levels; and in natural stands, to enhance wild seed production for reforestation (see Appendix 1).

About 30 years ago, research was active on developing practical techniques for cone induction for a wide variety of conifers, with some emphasis placed on determining the mechanism of cone induction based on environmental, physiological, developmental, and biochemical processes (Pharis et al. 1987). More recently, understanding the fundamental processes has received very little funding or emphasis; also less emphasis has been placed on improving the techniques for different species. As a result, we still do not understand the fundamental mechanism of flowering and cone promotion in conifers but field techniques have improved. Despite the lack of basic knowledge, cones have been induced in young field-grown larch in provenance trials, and in potted and seed orchard grafts of western larch. A brief summary of some of these experiments will give an idea of how such trials may be done.

Experiments with western larch

Experiments with western larch have been done using techniques similar to those in Bonnet-Masembert (1982). Ross (1991) did a cone induction experiment in a provenance trial near Enderby, in southeast British Columbia. He selected 120 non-forked trees, 4–15 cm in diameter at breast height (DBH), from six provenances. Trees were ranked according to

DBH and divided into groups of four trees each. One tree in each group was randomly assigned to each of four treatments: (1) no treatment (control); (2) girdling; (3) $GA_{4/7}$; and (4) girdling plus $GA_{4/7}$. As a result, the 30 trees per treatment had similar distributions of DBH plus a random representation of provenances.

Figure 25. *Thin, double-overlapping stem girdles shown at arrows.*

Double-overlapping stem girdles (Wheeler et al. 1985) were made with a pruning saw at the time of short-shoot flushing (Fig. 25). Some workers prefer using a fine metal hacksaw so that girdles heal more quickly. Each girdle should extend about 60% of the circumference of the tree, and be made about 1.5 m above the ground. The space between girdles should be about equal to stem DBH (Fig. 25). Holes 0.6 cm (¼ in.) in diameter and about 2.5 cm (1 in.) deep were bored at a downward angle (Fig. 26A). $GA_{4/7}$ was applied as two pulsed (repeated) stem injections using an adjustable pipette (Fig. 26B) (Philipson 1996; Philipson et al. 1997; Ross and Bower 1989). Ross (1991) made the first injection when long-shoots on 85% of the trees had flushed (mid-May). The second injection was made two weeks later. The $GA_{4/7}$ was dissolved in 95% ethanol at a concentration of 30 mg/L. The amount of $GA_{4/7}$ applied may be adjusted to tree size (DBH) by varying the number of holes evenly spaced around the stem. (See Appendix 1 for details.)

Figure 26. *A. Holes being drilled into the stem at a downward angle. B. Stem injections into bore holes using an adjustable pipette. C. Plugging the holes with grafting wax. D. Plugged hole.*

New holes, about 5 cm (2 in.) above the first and midway between the first holes, may be bored for the second injection since the old holes usually become plugged with resin. Ross plugged the holes with 0.6-cm (¼-in.) dowels to prevent loss of the solution after the injections. Some workers find it is easier to plug the holes with grafting wax (Fig. 26C, D).

Ross counted the number of seed-cones and pollen-cones at the time of flowering the following spring. As with most cone induction experiments, results from Ross' experiment were extremely variable, primarily because many trees did not flower. Consequently, non-parametric statistical tests had to be used to analyze the results (Conover 1980). In this experiment, none of the untreated control trees produced cones. In the trees that received only girdling, just seven trees produced seed cones and three produced pollen cones. In the trees that received only $GA_{4/7}$ injections or girdling and $GA_{4/7}$ injections, 50 and 40 trees produced seed cones and 23 and 20 trees produced pollen cones, respectively. These results indicate that $GA_{4/7}$ injections with or without girdling will induce both seed cones and pollen cones on young trees; however, there was no overall data on the number of cones induced per tree. This was only indicated in differences among provenances, which were extremely variable. When all provenances were combined, the average number of seed cones per tree was 9 (range = 3–14) and the average number of pollen cones per tree was 18 (range = 0–73). Provenance and locations where the provenances were being grown were very important factors in the experiment. Similarly, high variations can be expected among clones in seed orchards. These aspects should be considered in any study.

In a second, similar experiment done in the Invermere Forest District in southeast British Columbia, a stand of 14- to 20-year-old western larch was used (Webber and Ross 1995). A single stem injection of $GA_{4/7}$ was applied plus girdling at the time of long-shoot-bud flush. Of the 30 control trees, only one produced three seed cones and none produced pollen cones. Sixty-three percent of the treated trees produced seed cones, with up to 120 cones per tree, whereas only one tree produced pollen cones.

Experiments using Japanese and European larches

Experiments using Japanese and European larches were undertaken in France (Bonnet-Masembert 1982) to determine the best time for $GA_{4/7}$ treatments. May-June $GA_{4/7}$ applications maximized pollen-cone production and June-July applications maximized seed-cone production. Unfortunately, calendar date rather than shoot elongation determined the timing. The calendar date of flushing and subsequent shoot elongation can vary from year to year and on different sites; therefore elongation should be monitored and used as the more accurate prediction of time of cone initiation. Anatomical cone-bud differentiation in western larch in British Columbia occurs in mid-June, coinciding with the end of lateral shoot elongation (Owens and Molder 1979a). Because biochemical differentiation of conifer cone buds is thought to occur a few weeks before anatomical differentiation (Owens et al. 1986), the optimal time for treatments in western larch should be soon after flushing of long-shoot buds (Fig. 18).

Experiments using potted trees

Experiments using potted trees were done using grafted clones of European and Japanese larch (Philipson et al. 1997) in Great Britain and western larch in British Columbia (Webber and Ross 1995). In Great Britain, European and Japanese larch trees were grown for 2–3 years after grafting and were 1.5–3.0 m tall (2.0–2.5 m tall proved to be most suitable). They were originally in 28-L pots but some were transplanted into 45-L pots before treatments began. Cone production was enhanced simply by placing the potted grafts in a polyhouse for 3–4 months. $GA_{4/7}$ was also applied by stem injection but it did not increase pollen-cone or seed-cone induction. Application of drought stress usually increased seed-cone

induction. Potted grafts were moved into a heated polyhouse early in May after short-shoot buds had flushed and they were left there until the end of August. Wall and roof ventilators were kept closed early in the treatment but were opened on warm sunny days and during the latter part of the treatment. Temperatures were kept at 35–38°C. In September polyhouse doors and vents were left open but trees were left inside to protect them from frost during pollination the following spring. Results were variable and about 70% of the ramets in each clone produced some cones. From 20 to a few hundred seed cones were produced per tree. The numbers of pollen cones per tree were not counted. Larch pollen cones produced small amounts of pollen — usually less than 1 ml of pollen per 100 pollen cones. Seed production was also low, averaging only 10–15 seeds per seed cone.

Results were poor for similar polyhouse experiments using $GA_{4/7}$ as a foliar spray in British Columbia (Webber and Ross 1995). Scions from 40- to 60-year old trees were grafted onto seedling rootstocks and grown in bark-mulch media in 11-L pots in an open-sided polyhouse for three years. Crowns were pruned in the last two years, roots were pruned, and stock was repotted in 20-L pots just before vegetative bud swell in the third year. One to four ramets of each of 10 clones were randomly assigned to the controls and each of three treatments: (1) $GA_{4/7}$ applied at long-shoot bud flush and ambient temperatures; (2) $GA_{4/7}$ applied at long-shoot bud flush plus heat beginning at short-shoot bud flush; and (3) $GA_{4/7}$ and heat both applied at long-shoot bud flush. $GA_{4/7}$ was applied as a foliar spray at 200 mg/L in 5% Aromox C-12W ™ used as a surfactant. Spraying was repeated once per week for four weeks. Trees were rinsed three days after each spraying to prevent damage to foliage from the spray. Heat was applied in a closed polyhouse with temperatures at 25°C during the day (0800 to 2000) and 15°C at night. The numbers of seed cones and pollen cones were counted at pollination the next spring. All but Treatment 1 significantly *decreased* seed-cone and pollen-cone production when compared with the controls (no $GA_{4/7}$ and no heat), which had an average of 12 seed cones and 51 pollen cones produced per ramet. Clearly, there are serious problems with cone induction using potted western larch in polyhouses that we do not understand.

Results such as these indicate that western larch cone production may be increased in young trees using $GA_{4/7}$ as stem injections, but only in conjunction with adjunct treatments such as girdling, root pruning, and possibly nitrogen fertilizers in conventional soil-based seed orchards or other field sites. These are established techniques for the Pinaceae but the techniques tried for potted western larch have so far shown too many problems, including poor cone and filled seed production (Webber and Ross 1995), cone abortion, and low filled seed per cone, to warrant further investment at this time. Application of $GA_{4/7}$ as a foliar spray did not induce cones in potted western larch (Webber and Ross 1995) but did enhance cone production in soil-grown tamarack (Eysteinsson and Greenwood 1990). Foliar sprays of $GA_{4/7}$, generally, have not been as successful as stem injections for most of the Pinaceae on which they have been tried but foliar sprays have been quite successful for many of the Cupressaceae. Foliar uptake in the Pinaceae may be a problem.

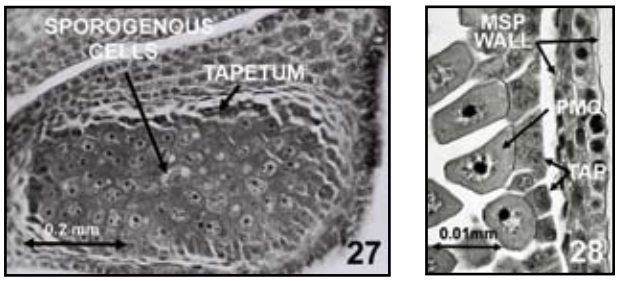
Pollen-cone and Pollen Development

Pollen-cone buds initiate microsporophylls from about mid-June through July and each microsporophyll initiates two abaxial microsporangia (pollen sacs) before winter dormancy in October (Fig. 17). A few sporogenous cells form within each microsporangium and these cells divide, forming a large compact mass of sporogenous cells (Fig. 27). Just before dormancy, the sporogenous cells divide and form about 100 large microsporocytes (pollen mother cells, PMC) (Figs. 28, 29B). The PMC begin the early stages of meiosis (a type of cell division in which the chromosome number is reduced by half), DNA is replicated, and chromosomes pair up and shorten

but the cells do not divide before winter dormancy. The PMC overwinter at the diffuse (diplotene) stage of meiosis (Owens and Molder 1979b). This differs from most conifers, in which meiosis does not begin until after winter dormancy.

The occurrence of the pre-dormancy stages of PMC meiosis was first discovered in *L. sibirica, L. decidua*, and *L. leptolepis* growing in Sweden (Ekberg et al. 1968; Eriksson 1968). It was suggested that the advantage of PMC overwintering at the diffuse stage was that they may be more frost resistant (down to -30°C) than pre-meiotic PMC and this may result in fewer meiotic defects (Ekberg and Eriksson 1967). Alternatively, it was suggested that the diffuse stage might result from the cold temperatures (Eriksson 1968) but this was found not to be true for western larch growing in the cold interior of British Columbia when compared with western larch growing in mild coastal regions (Owens and Molder 1979b). Trees at both locations had the diffuse stage. Another advantage of completing early stages of meiosis in the autumn is that it allows meiosis to be completed quickly, in a few days, in the spring following dormancy. This may be advantageous for trees growing where there is a short growing season, as is true for many larch species.

Outside the PMC is a single layer of tapetal cells (tapetum) and outside the tapetum is the microsporangial wall that is several cells thick. The tapetum is the tissue that breaks down releasing the thecal fluid, nutrients for pollen development, and materials for pollen wall synthesis during pollen development following dormancy. Spaces between PMC are filled with thecal fluid (Fig. 28).



Figures 27 and 28. *Sections of microsporangia showing the sporogenous cells before winter dormancy (Fig. 27) and the PMC in the diffuse stage during winter dormancy (Fig. 28). PMC, pollen mother cells; TAP, tapetum; MSP, microsporangial wall.*

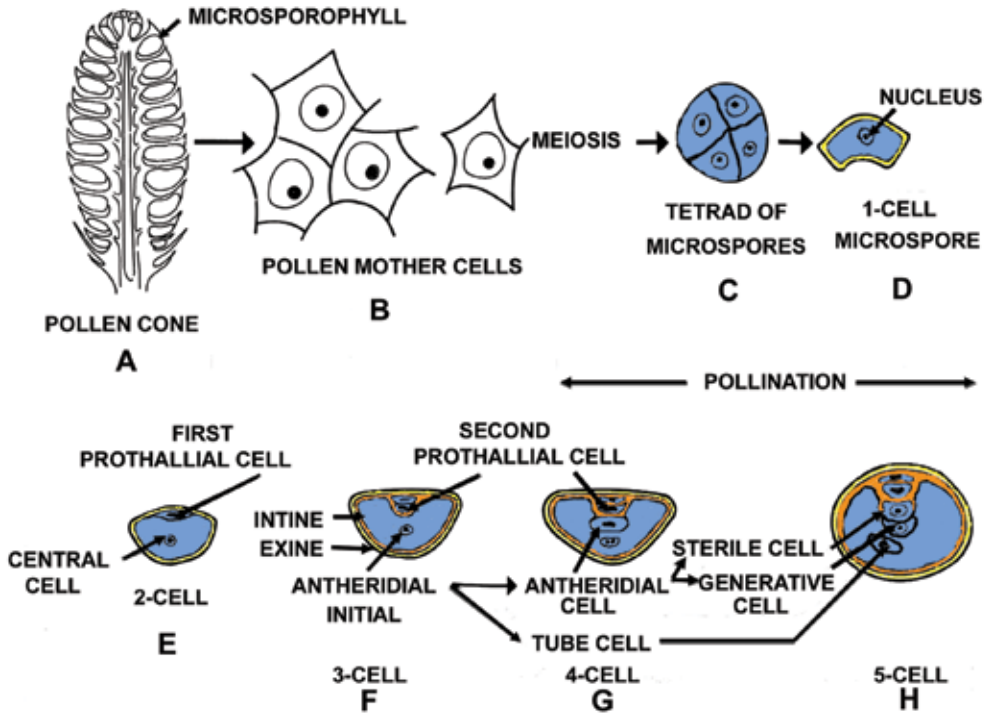
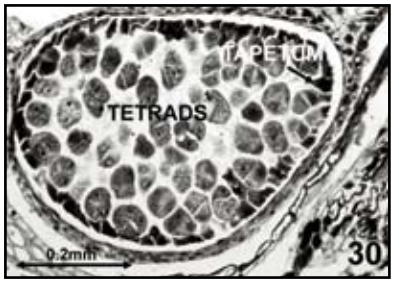


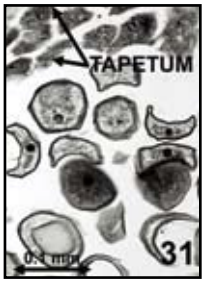
Figure 29. *Meiosis and pollen development in western larch. Blue indicates haploid (1N) cells.*

Pollen-cone bud development in western larch resumes in early March, 5–6 weeks before pollination (Fig. 29). Meiosis is completed within 1–2 weeks, during which each PMC replicates its DNA and chromosomes once and undergoes two nuclear divisions. This reduces the amount of DNA and the number of chromosomes by half, to the haploid number (1N = 12) from the original diploid number (2N = 24) present in the PMC. Four haploid microspores result from each PMC and remain together, as a tetrad of microspores, for a few days within the PMC wall (Figs. 29C, 30). The microspores quickly enlarge, burst out of the PMC wall, and float in the thecal fluid within the microsporangia. At this time they are lens-shaped and contain a single cell (Figs. 29D, 31). During meiosis the microsporangia and pollen cones swell, causing the pollen cones to burst out of their bud scales. The free 1-cell microspores enlarge and become round with one side indented; then the outer pollen wall (exine) thickens. Each microspore then divides unequally, forming a 2-cell pollen grain containing a small lens-shaped prothallial cell and a large central cell (Fig. 29E).

The central cell divides unequally to form an antheridial initial and a second, small lens-shaped prothallial cell that lies over the first prothallial cell. The inner cell wall (intine) forms around the prothallial cells and becomes a distinct layer inside the exine. This is the 3-cell pollen grain (Figs. 29F, 32A).

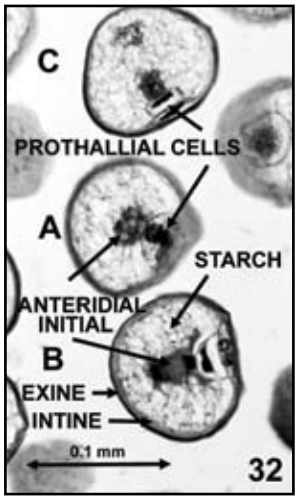
From this stage until pollen maturity, the terminology for pollen development may vary with the author and the species being discussed (Singh 1978). The terminology used here is correct for both conifers and flowering plants (Owens and Bruns 2000; Fernando et al. 2005). Prothallial cells in the Pinaceae have no known function and are absent in flowering plants and some conifer families, such as the Cupressaceae. In other families, such as the Auracariaceae, which contains the monkey-puzzle tree, numerous prothallial cells float freely in the pollen tube. These may help regulate the metabolism of the often multi-branched pollen tubes found in some species.

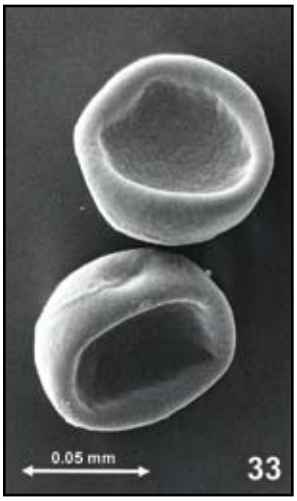




Figures 30 and 31. *Sections of microsporangia during pollen development in the spring. Fig. 30. Tetrads of microspores following meiosis. Fig. 31. Separate 1-cell microspores.*

The antheridial initial divides unequally (Fig. 32B) forming a small antheridial cell next to the second prothallial cell and a large tube cell. This forms the 4-cell pollen grain (Figs. 29G, 32C). The antheridial cell wall remains thin and the antheridial cell then divides equally forming a sterile cell (stalk cell) next to the second prothallial cell and the generative cell. The four cells form a stack. The tube nucleus may be located anywhere in the tube cell cytoplasm. The intine continues to thicken and forms a cup-shaped wall in which the sterile cell rests. This 5-cell pollen grain becomes filled with starch as it matures (Fig. 29H). All five cells within the pollen grain are haploid (1N), having 12 chromosomes each.





Figures 32 and 33. *Western larch pollen that has been sectioned (Fig. 32) at the A. 3-cell, B. dividing, and C. 4-cell stages and a scanning electron micrograph of mature pollen (Fig. 33).*

During pollen development, tapetal cells degenerate and release into the thecal fluid sporopollenin, proteins, and lipids that coat the outer exine wall giving it a finely granular texture and a very resistant coating. During the last week of pollen development, the thecal fluid dries and pollen dehydrates to about 10% water content. This causes the pollen to become more indented on one side. Mature western larch pollen is bowl-shaped due to the indentation, and averages 0.08 mm in diameter. Its surface is smooth except for faint ridges where the microspores fit together inside the PMC wall (Fig. 33). All *Larix* species have very similar pollen that varies only slightly in size (Owens and Molder 1979c; Owens and Simpson 1986).

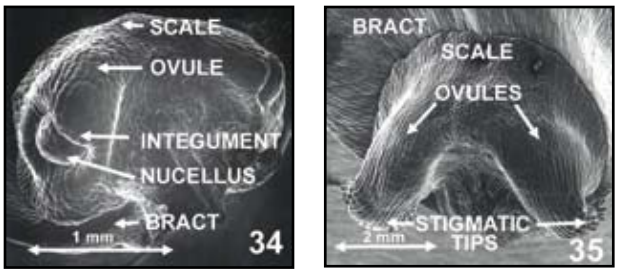
During pollen development the microsporangial wall forms a line of dehiscence on the lateral surfaces. As the pollen cones dry in the spring, the microsporangia split open along this line releasing the pollen. Each microsporangium contains about 500 pollen grains and each pollen cone produces about 60,000 pollen grains. Each gram of dry pollen contains about 3 million pollen grains.

It takes 5–6 weeks from the end of pollen-cone bud dormancy in early March until pollen is shed in April. High temperatures will hasten development and dry weather will hasten pollen shedding (dehiscence), whereas low temperatures will delay the end of dormancy and slow subsequent development. Wet weather will slow drying and delay dehiscence. Meiosis and pollen development can be monitored by microscopic observation of squashed and stained microsporangia. This requires a simple compound microscope, microscope slides, cover-slips, an appropriate stain, and a little practice (see Appendix 2).

Post-dormancy Seed-cone Development

When the dormant seed-cone buds resume development in March, cell divisions occur in the cone axis, bracts, and scales, causing the buds to swell. The cone apex initiates additional bracts at the tip of the cone for about one month, but these bracts usually initiate no scales and simply cover the cone apex. Occasionally, the cone apex initiates bud scales and a vegetative bud forms at the tip of the cone. This bud may abort or elongate and flush at the tip of the cone during the growing season (see Fig. 105), but the bud or the shoot formed dies when the cone matures late in the summer (Tosh and Powell 1986). These are called proliferated cones and occur more frequently in juvenile than in mature trees. They are commonly seen in seed orchards. The vegetative bud or shoot does not harm the cone or the seeds within the cone and the cones may be collected for seed extraction.

During dormancy the two ovule primordia, one attached to each lateral portion of the scale, are only small mounds of meristematic cells, each containing a large megaspore mother cell (MMC) surrounded by sporogenous cells. The sporogenous cells are enclosed by a nucellus; outside this is an integument. The tip of the integument forms a ring of meristematic cells that grows over the nucellus (Fig. 34). After dormancy this ring grows more quickly on the upper and lower margins forming a small abaxial (lower) lobe and a larger adaxial (upper) lobe. The lobes develop into the stigmatic tip of the ovule (Fig. 35). Cells on the adaxial lobe form many short hair cells while few hair cells form on the abaxial lobe. The stigmatic tips elongate beyond the margins of the scales and hang down in a spiral in space around the cone axis. Short hair cells also form on the cone axis. A slit-like opening remains between the two lobes and forms the micropyle, the opening through which the pollen must pass to enter the ovule (Fig. 36). Bracts elongate, each forming a trident (3-pronged) tip that extends far beyond the margin of the scales. Leaf-like primordia and sterile bracts (lacking axillary scales) form a cluster at the base of the cone (Fig. 43).



Figures 34 and 35. *Scanning electron micrographs showing the adaxial (upper) surface of the scales and ovules in erect cones. Fig. 34. The ovule tip forms as the integument elongates beyond the central nucellus. Fig. 35. Adaxial surfaces of the bract, scale, and two ovules, showing the stigmatic tips.*

Pollination

Pollination is the release of pollen from the pollen cones and transport of pollen by wind (anemophily) to the seed cones on the same tree or clone (self-pollination), or another tree or clone (cross-pollination). Pollination is completed once the pollen has entered the seed cone and the pollen or the pollen tubes have entered an ovule through the micropyle. Larch is monoecious, meaning that both pollen cones and seed cones are found on the same tree. The rate at which cones develop in the spring before pollination is primarily determined by temperature — with warmer temperatures, development is more rapid. However, pollen release (dehiscence) also depends on drying of the pollen cones. In wet weather, pollen release may be delayed by several days, and in dry weather hastened. This can lead to various kinds of dichogamy (Fig. 37), the temporal (events related to time) separation of male and female functions (Sedgley and Griffin 1989).

Dichogamy may take three forms: (1) seed-cone and pollen-cone buds may burst at the same time and seed cones are receptive at peak pollen release (homogamy); (2) pollen cones may release pollen before seed cones are receptive (protandry); and (3) seed cones may become receptive before pollen is shed (protogyny). All three situations can occur at one site in different years, and at different sites in the same year. Protandry is likely to occur in a warm, dry spring or site and protogyny in a cool wet spring or site. Because pollen cones

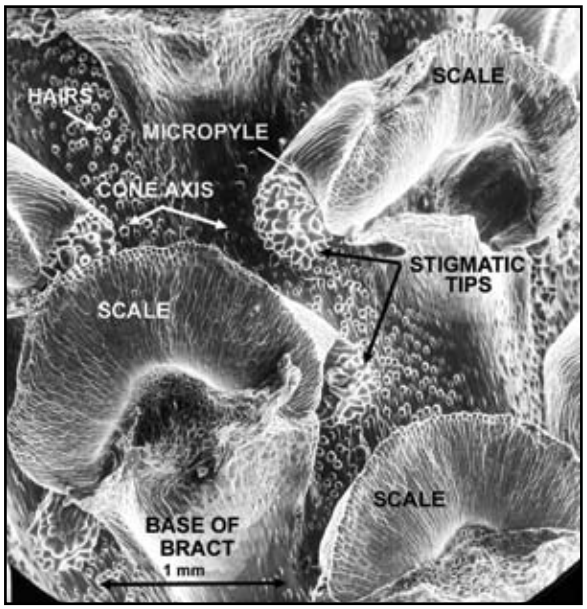


Figure 36. *Scanning electron micrograph showing a portion of the cone axis and several spirally arranged scales after bracts were removed. The abaxial surface of scales, the slit-like micropyle, and hairs on the stigmatic tips and cone axis are shown.*

and seed cones are in close proximity on many branches, some degree of protandry or protogyny is desirable because it reduces self-pollination. A light rain or overhead sprinkling within a seed orchard may delay pollen-cone drying and dehiscence by a day or more but may delay seed-cone receptivity very little.

It is useful to assign numerical stages of pollen-cone and seed-cone development before and during pollination to follow and predict the times of pollen release and receptivity. Predicting the time of pollen release is helpful in natural stands and seed orchards in which pollen collections will be made.

Predicting seed-cone receptivity is useful for making control crosses and both pollen-cone, and seed-cone predictions are needed to determine the need for supplemental mass pollinations (SMP) in seed orchards. In small seed orchards, all of the trees, and in large seed orchards a representative sample of trees that includes as many clones or families as possible, should be monitored every year to identify early- and late-flowering clones or families and those that produce varying amounts of pollen cones and seed cones. The sample size of

trees will vary with the staff available and size of the orchard. It takes less than five minutes to assess a tree and record the average stage of pollen cones and seed cones.

The assessment should be done regularly, at the same time every day. Northern or cool sites, where development is slow, may be assessed every second day.

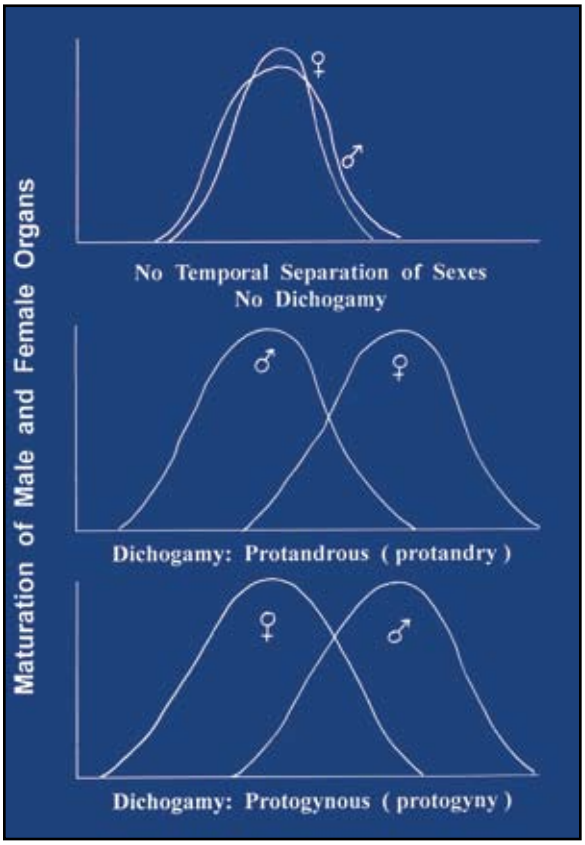


Figure 37. *Dichogamy, the temporal separation of sexes in terms of pollen release and female receptivity.*

Stages of pollen-cone development and pollen shedding

Stages of pollen-cone development and pollen shedding are recognizable. Stage 1 is when the pollen-cone buds swell and are starting to burst; meiosis is occurring (Fig. 38). At Stage 2, buds burst exposing the green microsporophylls but the yellow abaxial microsporangia are small and just becoming visible (Fig. 39). At Stages 3 and 4, the cone axis elongates opening the spaces between the microsporophylls and the yellow microsporangia quickly enlarge and become more easily visible (Fig. 40). At Stage 5, the pollen cones dry, and the microsporangia split open and shed the pollen (Fig. 41). By Stage 6, all pollen has been shed and the cones begin to wither on the shoot (Fig. 41). Dry pollen cones may remain on the shoot for the summer. Stages 3 and 4 are the best times for pollen-cone collection for pollen extraction. Pollen cones collected earlier may not be dry enough to open and shed pollen. Any pollen that is shed may have high moisture content. Pollen cones collected at late Stage 5 or after will have shed most of their pollen.

Development of larch pollen cones on a tree, and even on a branch, is variable. Therefore, there is always a mixture of several stages in a pollen-cone collection. This will reduce the amount of pollen extracted. In addition, the scattered distribution of pollen cones on the branch makes extraction of large quantities of larch pollen difficult and inefficient.

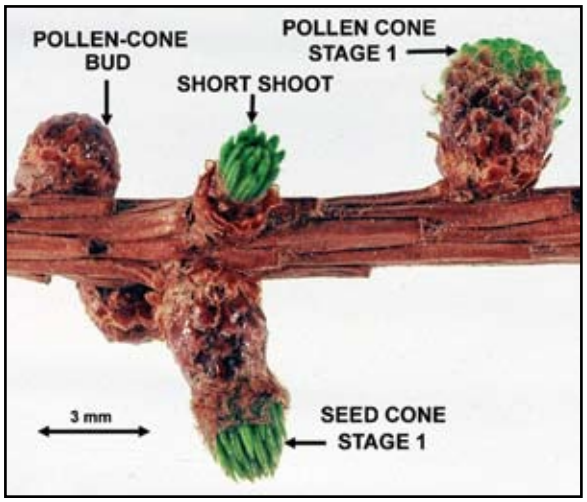


Figure 38. *A shoot bearing a pollen-cone bud that is swelling, indicating that meiosis is occurring. Also shown are a Stage 1 pollen cone and seed cone and a short-shoot, all of which are still partially enclosed within their bud scales.*

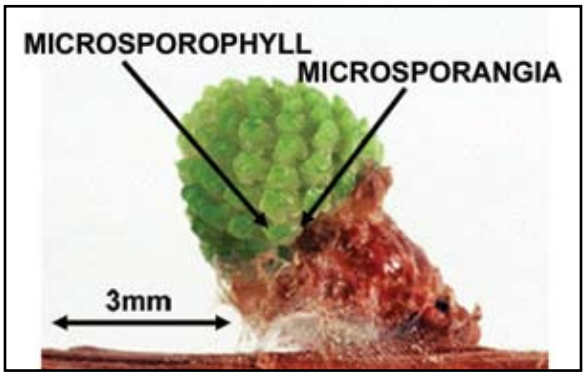


Figure 39. *A Stage 2 pollen cone that has burst through its bud scales and is elongating. Yellow microsporangia are slightly visible on the abaxial (lower) surface of the green microsporophylls. Pollen-cones collected at Stage 2 will yield only small amounts of pollen.*

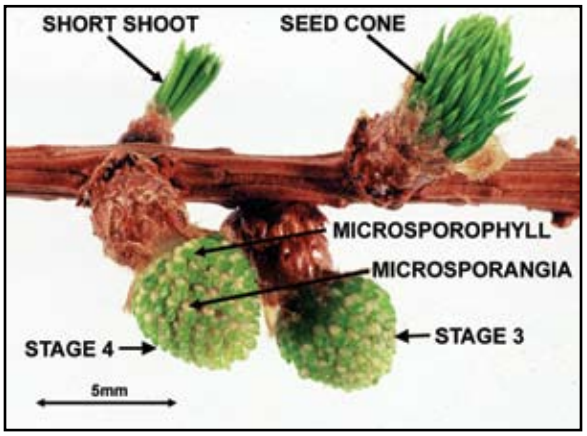


Figure 40. *Stages 3 and 4. Pollen cones have elongated, microsporangia are swelling and more obvious, but pollen has not started to be shed from either cone. Stage 4 cones will begin to shed pollen; this is the best stage for collection.*

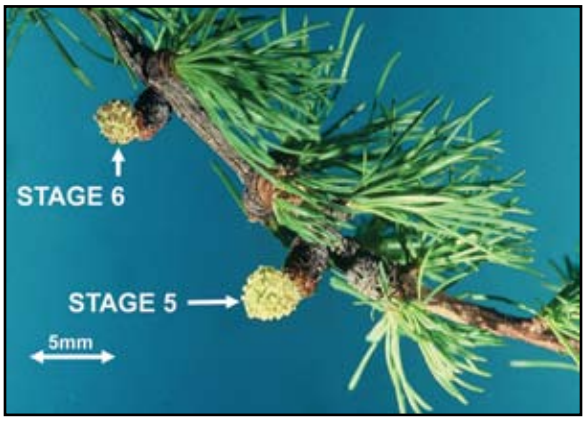


Figure 41. *Pollen cones at Stage 5, when pollen is being shed, and Stage 6 after most pollen has been shed. Few pollen grains will be obtained from cones collected at these stages.*

Different workers may assign slightly different stages to larch pollen cones, but as long as the staff that is monitoring pollen-cone development and harvesting cones for pollen extraction remain consistent from year to year, their numerical ranking will work.

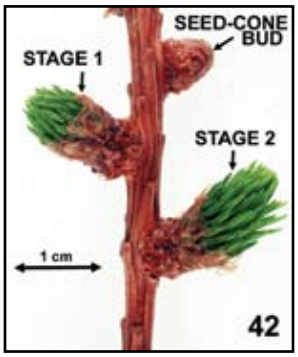
On a tree, pollen cones are generally not abundant, they may be at various stages of development and each pollen cone will shed all of its pollen in 1–2 days. Consequently, in western larch stands and seed orchards, there is usually not a large pollen cloud, as in some associated species such as Douglas-fir, and especially lodgepole pine. There are exceptions in some seed orchard trees in which abundant pollen-cones are produced. Larch species appear to have compensated for the limited amount of pollen usually available by having seed cones with a very efficient method of pollen collection.

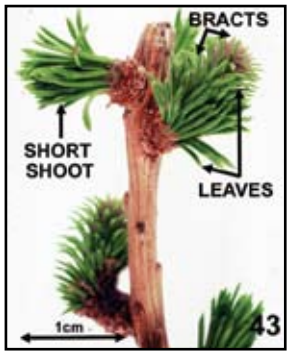
Stages of seed-cone development and receptivity

Stages of seed-cone development and receptivity used here have been changed from those used in the original research article (Owens et al. 1994), to be more consistent with the stages of pollen cones. Dormant seed-cone buds resume development early in March and become receptive about six weeks later. Seed-cone buds start to swell about two weeks after dormancy ends. Within another two weeks, seed cones burst through their bud scales and enter Stage 1 in which a tuft of bract tips extends beyond the bud scales that covers the base of the cone (Fig. 42). The cone elongates, more bracts appear, and some start to open (Fig. 42). Stage 1 and 2 seed cones at first may look similar to large flushing short-shoot buds (Fig. 38). A few pollen grains may enter the cones at Stages 1 and 2, but Stage 2 is considered the earliest stage of receptivity. The cone axis and bracts continue to elongate, the tips of the trident bracts become recognizable, and the cone begins to bend upward at Stage 3 (Fig. 43). Stage 3 seed cones are about 1 cm long and several long green leaves and sterile bracts at the base of the cone are distinguishable from the trident bracts that have green, red, or purple laminas (blades) (Fig. 43). The cones are

receptive at Stage 3 because the spaces between the bracts have become larger and bracts begin to reflex, which allows them to entrain more pollen.

By Stage 4, seed cones are 1–1.5 cm long and the axis is usually nearly vertical. The red or purple laminas of the bracts are more visible and there are wide spaces between the bracts. The tips of the trident bracts begin to curve downward and the leaves at the base of the cone bend outward (Fig. 44). Stage 4 is the most receptive stage. Stage 5 cones are 1.5–2 cm long and often red or purple because the bracts have elongated and the tips have bent downward exposing the coloured blades of the bracts (Fig. 45). Bract tips usually remain green. Small light green to red ovuliferous scales are visible between the bracts, but due to growth and thickening of the scales, the spaces between bracts and scales are reduced and pollen cannot enter the cone as easily. Stage 5 cones are still erect and may appear to be open but receptivity is coming to an end and SMP will be of little benefit.





Figures 42–43. *Stages of seed-cone development and receptivity. Fig. 42. Seed-cone bud and Stage 1 and 2 seed cones as they are starting to elongate. Stage 2 seed cones are slightly receptive. Fig. 43. Stage 3 seed cones are becoming erect and more receptive. The basal leaves can be distinguished from the more-distal purple bracts.*

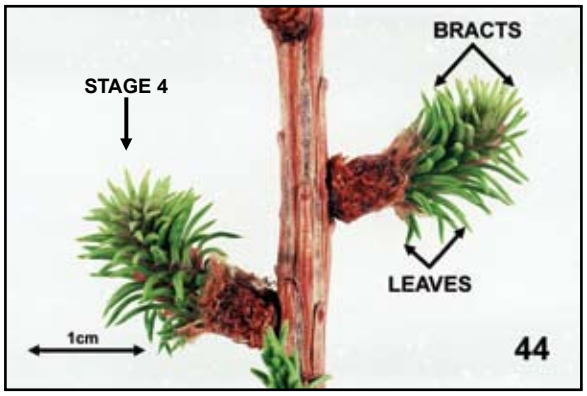
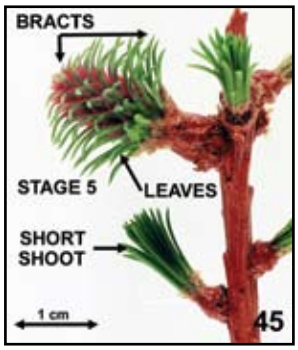
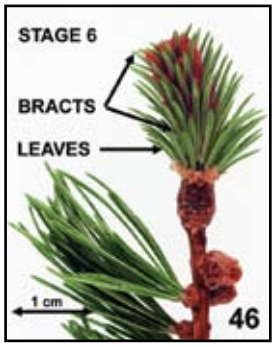


Figure 44. *Stages of seed-cone development and receptivity. Stage 4 seed cones have wide spaces between bracts and scales and are at the most receptive stage.*





Figures 45 and 46. *Stages of seed-cone development. Fig. 45. Stage 5 seed cone showing the red blades of the trident bracts but spaces between bracts and scales are small due to thickening of the scales making the cones less receptive. Fig. 46. Stage 6 seed cone showing prominent red bracts with green tips. Scales have thickened and spaces between bracts and scales are nearly sealed. Cones may still be slightly receptive at Stage 6.*

Stage 6 cones are about 2 cm long, bracts are no longer reflexed and have become nearly straight, but the coloured blades are still visible (Fig. 46). Spaces between bracts and scales are mostly sealed making Stage 6 cones only slightly receptive. After Stage 6, the cones thicken and the bract tips become broader but cones remain fairly erect on the shoots (Fig. 47). SMP at or after Stage 6 will be of little or no benefit.

Cone size may vary on a tree, and cone colours (from green to red) vary among trees and clones. Therefore, cone stages should be determined primarily by cone morphology (relative shapes and sizes of bracts and scales and spaces between), rather than cone size or colour (Owens et al. 1994).

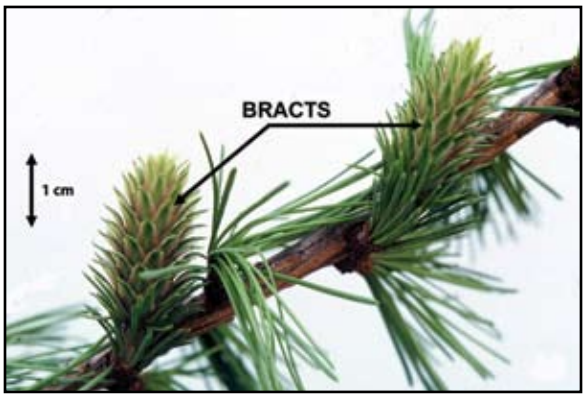


Figure 47. *Post-pollination seed cones are still erect and bracts are prominent. Leaves on short-shoots have fully elongated.*

Pollination Mechanisms

Five pollination mechanisms are recognized in living conifers based on differences in cone and ovule orientation at pollination, presence or absence of a pollination drop, pollen morphology, and the site of pollen germination (Owens 1993; Owens et al. 1998). Four of these are found within the Pinaceae and the other within the Cupressaceae and less well-known families (Fig. 48A-E). The mechanisms are: A. erect or variably oriented cones and ovules with a pollination drop and non-saccate (wingless) pollen, as in the cedars and junipers; B. erect cones with inverted ovules, a pollination drop and saccate (winged) pollen, as in spruces and pines; C. erect cones with inverted ovules, without a pollination drop (but artificial drops form from rain or dew) and saccate pollen, as in the true firs; D. erect cones with inverted ovules and no pollination drop — the non-saccate pollen is engulfed by the ovule tip, as in Douglas-fir and larch; and E. variably oriented cones and ovules with no pollination drop in which non-saccate pollen germinates outside the ovule and form long pollen tubes that grow into the ovule, as in western hemlock.

In western larch, and other larch species that have been studied, seed cones at pollination are usually erect or nearly erect, due to bending-up of the base of the cone axis, and ovules are inverted — causing the micropyle to face downward (Fig. 48D). The basal two-thirds of the trident bract has a broad green, red, or purple blade and the tip is green and needle-like. The cone scales are small and semi-circular with two ovules borne on the adaxial surface (Fig. 49). The ovule tip, where the micropyle is located, hangs below the lower edge of each scale. Cone scales and most of the ovule surface are smooth. The integument tip has two lobes, a short abaxial lobe with no hairs, and a larger adaxial lobe with many short stigmatic hairs (Fig. 50). The micropyle is a slit-like opening into the ovule, between the two lobes. The micropyle is too narrow to allow pollen to enter and no pollination drops are exuded from the micropyle to carry pollen into the ovule. The micropyle opens into a cylindrical micropylar canal formed by the tubular integument that encloses the nucellus. The micropylar canal extends from the micropyle to the surface of the nucellus, a distance of less than 0.5 mm (see Fig. 72).

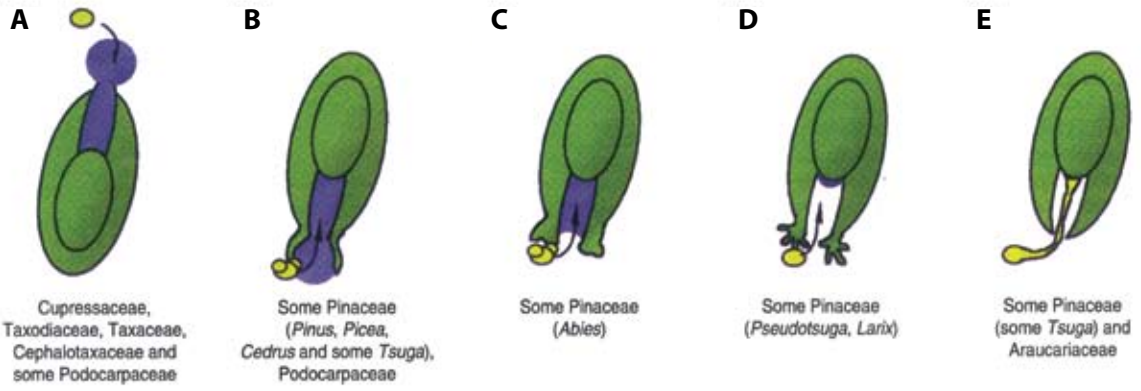
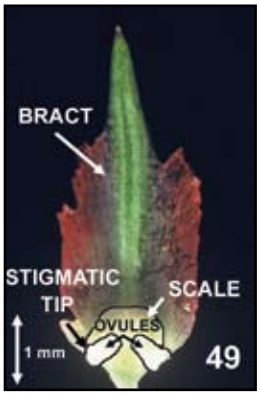
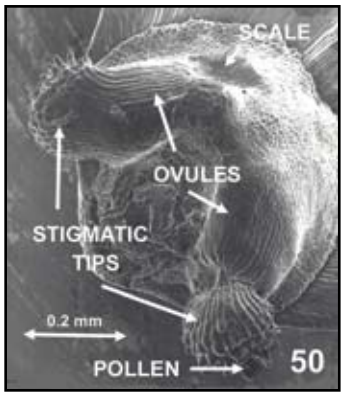


Figure 48A-E. *The five pollination mechanisms found in living conifers. Pollen and pollen tubes are shown in yellow, ovules in green, and the pollination drops in blue. Families and genera are listed in which these occur (redrawn from Owens et al. 1998).*

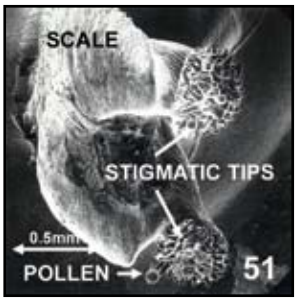


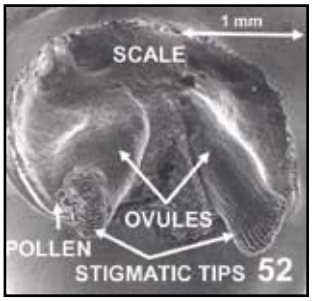


Figures 49 and 50. *Fig. 49. A bract-scale complex dissected from a western larch cone at pollination. Fig. 50. A scanning electron micrograph showing the adaxial surface of a scale at pollination, as shown in Fig. 49.*

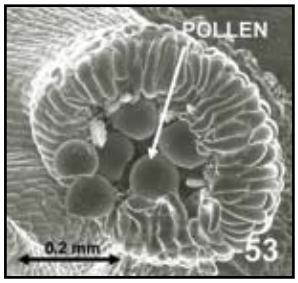
Bract surfaces are smooth and lack hairs; the bract is loosely attached to the scale. The bract and its adaxial scale, often called a bract-scale complex, are spirally arranged around the cone axis. As the cones elongate, bracts reflex and spaces between bracts and scales enlarge allowing pollen, carried by air currents, to pass among the bracts and scales. This slows the air movement and the air swirls around the bract-scale complexes allowing the pollen to settle-out on the bract, scale, or cone axis. Pollen grains landing on the smooth bract usually move down over the upper surface and come to rest at the base of the bract, just below a stigmatic tip of an ovule attached to the scale above. The neck of the ovule tip elongates, forcing the stigmatic tip close to the bract surface. Most of the pollen from the bract surface becomes entangled in the hairs on the adaxial lobe of the stigmatic tip (Figs. 50, 51). There are no visible secretions on the stigmatic hairs to which the pollen adheres.

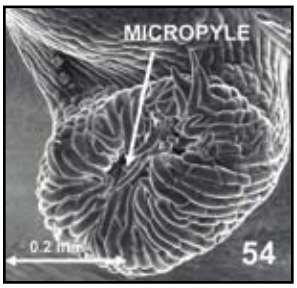
Pollen accumulates on the stigmatic tips for about one week. Up to 15 pollen grains may adhere to each stigmatic tip, but the average is usually 5–10 (Owens et al. 1994). About one week after receptivity begins for a cone, the outer cells of the stigmatic tip elongate and cells near the micropyle collapse. This causes the stigmatic hairs, with or without attached pollen, to be engulfed into the micropyle (Figs. 52, 53).





Figures 51 and 52. *Scanning electron micrographs of scales from specimens, as shown in Fig. 49. Fig. 51. The abaxial (outer or lower) surface of a scale showing the stigmatic tips with pollen attached to hairs. Fig. 52. The adaxial (inner or upper) surface of a scale showing the stigmatic tips as they start to engulf pollen.*





Figures 53 and 54. *Scanning electron micrographs of scales from specimens as shown in Fig. 49. Fig. 53. The stigmatic tip as it is engulfing pollen into the micropyle. Fig. 54. The stigmatic tip after it has engulfed pollen and the micropyle has become sealed.*

Once engulfment is completed, the micropyle becomes sealed and no more pollen can enter (Fig. 54). The engulfment process also occurs if no pollen is present on the stigmatic tip. Engulfment is neither selective nor is it triggered specifically by the presence of western larch pollen. Small insect eggs, spores, and microbes, as well as pollen from other species, may be engulfed.

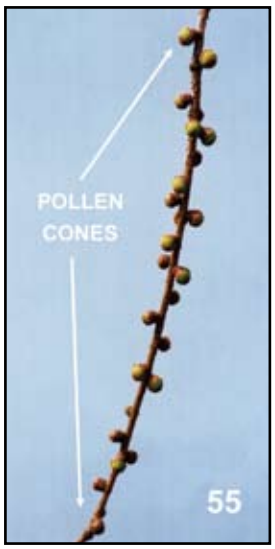
No pollination drop is exuded out of the micropyle of intact western larch cones during pollination. However, drops may be secreted from the ovules on scales that have been severed from the cone axis. These drops were called a "dissection fluid" in Douglas-fir (Takaso et al. 1996) but they have not been observed in intact larch or Douglas-fir cones and are not required for successful pollination in larch (Takaso and Owens 1997).

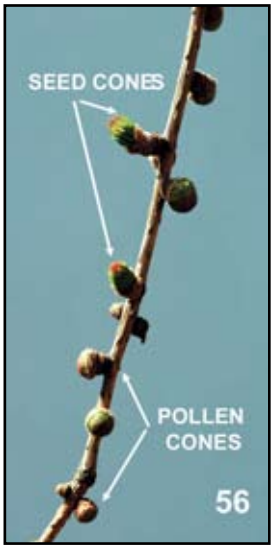
Pollen Management

Like many conifers, western larch in natural stands starts to produce cones at about eight years of age, and in clonal seed orchards cones may begin to be produced a few years earlier (see Fig. 103). In both cases, young larch trees usually begin to produce seed cones a few years before they produce pollen cones. This is to be expected in larch because pollen cones form from short-shoot buds that are usually several years old and commonly on older pendant branches (Fig. 55). Seed-cone buds form from short-shoot buds that are 1–2 years old, usually on younger, more vigorous shoots but they may also occur mixed with short-shoots and pollen cones on pendant branches (Fig. 56).

There has not been a detailed study to determine the age for the onset of flowering and pattern of pollen-cone and seed-cone production for western larch. However, detailed studies have been made for young *L. laricina* (tamarack) in eastern Canada (Powell 1995) and European and Japanese larches (Philipson et al. 1997) growing in Scotland. The pattern for western larch appears to be similar (Owens and Molder 1979b).

Pollen cones are produced less abundantly on larch than on most other Pinaceae and pollen cones are very scattered and mixed with other buds along branches in the lower crown (Figs. 55, 56). As a result, in seed orchards and in young stands, only small quantities of pollen are usually available for pollination. This means that pollen cones may have to be collected and pollen extracted, stored, tested, and then applied at the proper time for supplemental mass pollinations (SMP). Similar procedures for pollen collection must be used in breeding programs and the process is collectively called "pollen management." A comprehensive report on all aspects of pollen management has not been compiled for western larch, but has been for Douglas-fir (Webber and Painter 1996). Because Douglas-fir and larch are closely related and their pollen and ovule structure and development and pollination mechanisms are very similar, most of the recommendations for Douglas-fir pollen management may be applied to larch. The Douglas-fir manual by Webber and Painter (1996) should be consulted if detailed methods for western larch pollen management are being developed. Several aspects of pollen management that are applicable to western larch are summarized below.





Figures 55 and 56. *Pendant shoots in the mid-crown at pollination. Fig. 55. Pollen cones mixed with short-shoot buds that are flushing. Fig. 56. Seed cones mixed with pollen-cone and short-shoot buds.*

Monitoring pollen-cone and seed-cone phenology

Pollen management requires an understanding of pollen-cone and seed-cone phenology, including stages for pollen release and seed-cone receptivity. In seed orchards, pollen- and seed-cone phenology must be monitored in the same clones or families over several years. The numerical system for the stages of pollen cones and seed cones described earlier (see Figs. 38–46) works well, but other numerical systems may be applied if they are used consistently from year to year.

Phenology is very sensitive to temperature; temperature sum, in degree-days (DD), has been used for predicting time of pollen release and seed-cone receptivity in different years and at different sites (Sarvas 1962, 1968). Although the use of DD is reasonably precise, weather stations are required and expensive, and weather data must be collected daily and converted to DD.

Also, temperature sum is primarily a quantitative method for predicting pollen release and receptivity from year to year, and does not replace the need for monitoring trees, ramets, clones, or families within the orchard every year.

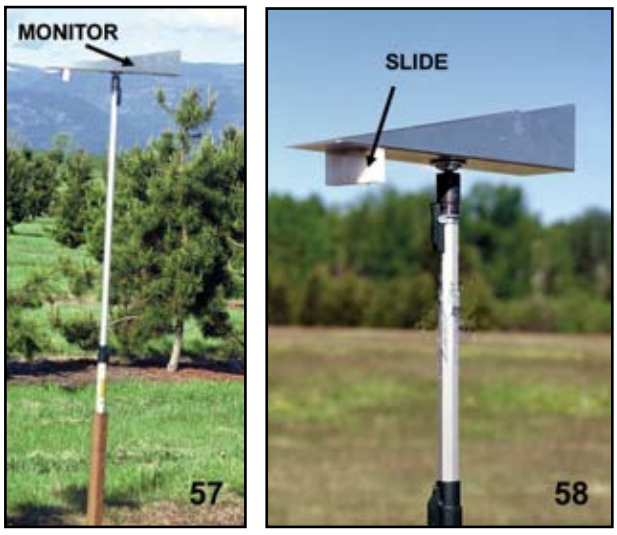
In a study of western white pine in coastal and interior British Columbia seed orchards over two years (1998 and 1999), the method of temperature sum using DD was compared with the daily monitoring of all 359 trees, representing 67 families at the coastal orchard, and a sample of 249 ramets, representing 50 clones at the interior orchard (Owens et al. 2001). Weather stations were not present at either seed orchard; consequently, government weather stations within a few miles had to be used and this gave only an approximation of the orchard temperatures.

Measuring and calculating temperature sums using a 0°C threshold was fairly precise. Results showed that pollen release and receptivity occurred at about 400 DD in both years at the interior seed orchard and at about 600 DD in both years at the coastal orchard. If the threshold temperature was increased to 5°C, the times increased to 900 DD and 1400 DD, respectively. Although choosing the threshold temperature is somewhat arbitrary, commonly 0–5°C, it is important to choose one that gives the least variation in DD (as in the white pine example above) from year to year. Essentially, threshold temperature is an estimate of the temperature at which development of the structure begins, but this is usually not known. The same threshold temperature must be used consistently from year to year, as must the stages of cone development. It may take several years to refine this method so that it becomes practical. However, once developed, the data needed to use this method would be easy to collect.

Unfortunately, many seed orchards do not have good weather recording equipment and distant weather stations may not produce good measures of weather within the orchard. The calculations of DD must be done every day or the information that is provided may be available too late to be useful for that year. In general, DD calculations take about as much time as monitoring a sample of 40 trees (1–2 h) in an orchard but DD will not identify early- and late-flowering trees, families, or clones, whereas monitoring does. Some monitoring will always be needed to check for insects, disease, or frost damage. Monitoring is no more expensive and just as fast and accurate as using DD. Also, orchard staff must learn the phenology of the species, and of the clones, and trees within an orchard, and monitoring is the best way to do this. Nevertheless, temperature sums may be useful in large orchards and orchards containing several species.

Monitoring pollen flight

Whether the orchard manager uses the method of monitoring trees, temperature sums, or both methods, pollen flight also must be measured using some type of pollen monitor. Pollen monitors are generally of two types. First, a simple and inexpensive (often home-made) weather vane can be mounted on a pole (Fig. 57). A microscope slide-coated with a petroleum jelly is mounted on the front facing the wind with a small roof to protect it from rain or water from sprinklers (Fig. 58).



Figures 57 and 58. *Fig. 57. A simple weather-vane type pollen monitor mounted on an extendable pole. Fig. 58. Close-up of monitor with a microscope slide coated with petroleum jelly mounted on the front facing the wind.*

The slides must be collected and replaced regularly, ideally every day. The second type are more expensive weather-vane monitors (these may not be commercially available) that contain a spring-wound or battery-powered drum that is covered with an acetate sheet coated with petroleum jelly. The drum is enclosed with a slit-like opening in front of the drum, facing the wind. The drum turns one revolution every week and allows hourly and daily counts of pollen. The acetate sheet is changed once a week (Webber and Painter 1996). Normally, daily pollen counts are all that are required but if pollen flight patterns during the day are required, a drum-type monitor is useful. Alternatively, microscope slides would have to be frequently changed. For either type of monitor, there should be several monitors per orchard. Four per orchard is adequate, one in each quadrant of the orchard, unless the orchard is very small (<0.4 ha). The monitors should be at mid-crown height and mounted on extendable poles (Fig. 57) to make changing slides easier.

Based on several years of seed orchard experience, we have found that the drum-type monitors are expensive and breakdown frequently. Compared with small microscope slides, pollen counts are difficult to make from the large acetate sheets coated in petroleum-jelly. Also, hourly counts are rarely required. The slide type of monitor requires that slides be replaced at the same time every day, except possibly for the earliest and latest days of pollen shedding when it may be done every second day because few pollen grains are being shed. However, calculations must be adjusted for two days rather than one day. When microscope slides are changed they should be placed in a covered microscope slide box to avoid dust and pollen contamination.

Petroleum jelly is spread on the acetate sheet with a paintbrush or on the microscope slides with a small paintbrush or your finger. The sheet or slide is then gently heated, causing the petroleum jelly to soften and slightly melt, forming a smooth thin layer on the surface. If this is not done, the coarse surface makes pollen counting difficult and pollen identification almost impossible. For microscope slides, a piece of graph paper (marked off in 1-mm^2 units) may be placed under the slide on the stage of a dissecting microscope and the number of pollen grains per square millimetre counted from a random sample of 10 mm^2. The average for the 10 mm^2 is then calculated. The relationship between pollen-cloud density, as measured using a drum-type pollen monitor, and filled seed per cone has not been reported for western larch but has for Douglas-fir which has the same type of pollination mechanism. In Douglas-fir at two orchards, maximum filled seed per cone was obtained if six or more pollen grains per square millimetre per day were recorded on the drum-type monitor (Webber and Painter 1996). One square millimetre would be about equal to the area of the stigmatic tip in western larch and Douglas-fir. Measures of pollen flight per day may be graphed with the phenology of seed-cone receptivity to determine the type of dichogamy that is occurring (Fig. 59).

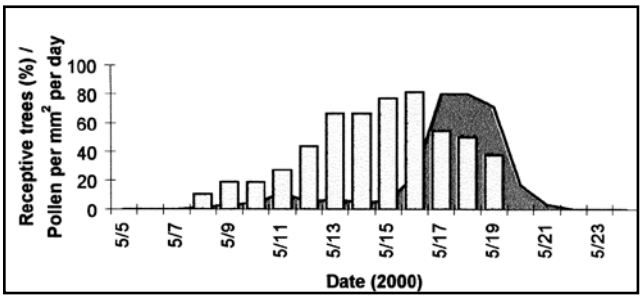


Figure 59. *Graph showing the phenology of seed-cone receptivity (vertical bars) and pollen flight (shaded area) for lodgepole pine at a seed orchard near Vernon, B.C.*

Monitoring pollination success (PS)

This directly measures the amount of pollen that has entered the seed cones and has been deposited on the stigmatic tips. PS can be determined at the end of the receptive period (Stage 6) but before engulfing has occurred, or at various stages of receptivity (Stages 3–5) from cones sampled at each stage. Sampled cones are placed separately in a vial with some damp tissue paper in the base of the vial to keep cones fresh. The cones may be taken to the laboratory and the basal one-third of

the cone sliced off and discarded. The bract-scale complexes can then be pulled off using forceps and placed scale-side up as shown in Figure 49. Ten bract-scale complexes should be sampled from the distal two-thirds of the cone and laid out on a microscope slide with the scale surface up. One intact ovule tip per scale should be observed using a dissecting microscope and the number of pollen grains per stigmatic tip counted (Fig. 60). Some ovule tips may break off when the bract-scale complex is removed from the cone. The average number of pollen grains for the 10 stigmatic tips is calculated as the PS. About five cones for each stage or each pollination experiment should be sampled and the number of pollen grains on each of 10 stigmatic tips per cone should be counted for a total of 50 stigmatic tips per stage or experiment. Results may be graphed to show PS for different stages of receptivity (Fig. 61) or for different types of pollination treatment (see Appendix 3).

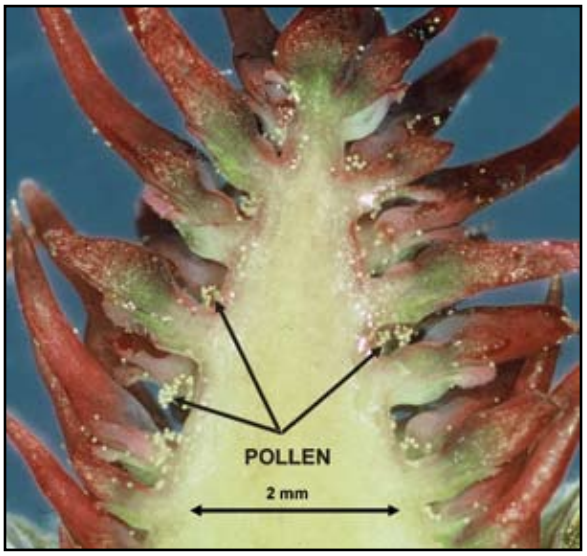


Figure 60. *Larch seed cone at pollination that has been sliced in half to count pollen on integument tips to determine the pollination success.*

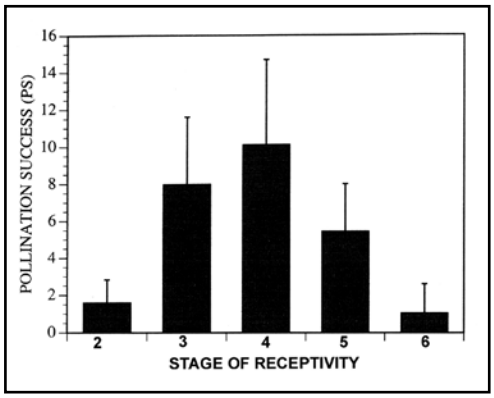


Figure 61. *Bar graph showing the pollination success (PS) as the average number of pollen grains per stigmatic tip in control pollinations of seed cones at different stages of receptivity in potted western larch.*

As a general rule, if there are less than about six pollen grains per square millimetre on the microscope slide, SMP should be applied, whereas 7–15 pollen grains are adequate and over 15 are abundant. Relatively few pollen grains per ovule are needed for good seed set in larch and Douglas-fir because their pollination mechanism is efficient for pollen collection and engulfing. It is easy to distinguish larch pollen (see Fig. 33) from that of commonly associated species having winged pollen, such as pines, true firs, and spruces but impossible to distinguish by size, shape, or colour from interior or coastal Douglas-fir pollen (Owens and Simpson 1986). When larch and Douglas-fir orchards are established on adjacent sites, larch appears to shed pollen much earlier than Douglas-fir (B. Jaquish, pers. comm.).

Pollen-cone collection and pollen extraction

Larch pollen cones should be collected at Stages 4 and 5 for pollen extraction. This is just before, or just as, pollen starts to be shed. These cones can then be dried and the shed pollen can be collected, cleaned, and stored. Pollen-cone collection for larch is more difficult than most other Pinaceae because there are often few pollen cones and they are small and widely scattered in the crown. An alternative that may work for larch is direct vacuum collection from the trees. Copes et al. (1991) described this equipment and showed that it could collect large quantities of Douglas-fir

pollen quickly from small seed orchard trees without affecting pollen quality. They suggested that vacuum-collected pollen to be used for SMP was more fertile than pollen extracted from picked cones because pollen being shed is more uniformly mature and of the desirable water content, thus requiring no further drying before storage or application. Vacuum collection could not be used for breeding purposes because pollen from other parents cannot be excluded. Pollen-cone buds, from which pollen is extracted and then used for breeding, should be rinsed to remove extraneous pollen before pollen extraction begins.

Various types of pollen-drying equipment have been developed and procedures for drying Pinaceae pollen have been generally described (Jett et al. 1993). Detailed descriptions are given for spruce (Webber 1991), Douglas-fir (Webber and Painter 1996), and western hemlock (Webber 2000), but not for western larch. Two types of drying apparatus are described in Appendix 4.

Douglas-fir and larch pollen are structurally the same and physiologically very similar; therefore, the procedures for Douglas-fir should work well for western larch. Western larch pollen-cone buds picked at Stage 4 or 5 may be stored for several days in paper bags at 4°C, if they cannot be dried immediately. However, immediate extraction is recommended for maximum pollen fertility and so cones do not become moldy. Douglas-fir pollen-cone buds dried at 28–30°C and 30–40% relative humidity shed pollen at 4–8% water content in approximately 48 h, but longer times may be required during wet weather (Webber and Painter 1996). Some large-scale drying methods described for pines, in which many pollen cones are easily collected (Eriksson 1993), may not work for larch.

Water content of pollen cones should be below 10% and in Douglas-fir this is indicated if the pollen-cone buds feel dry, crumble when touched, and shed pollen readily (Webber and Painter 1996). If further drying of extracted pollen is required, this may be simply done in dry interior regions by spreading the pollen in a thin layer on paper placed on a table in a dry room where there are no strong air currents and left for 24–48 h. This method could not be used for controlled breeding because samples could be contaminated with other pollen. Large-scale drying columns that use compressed air and desiccants for precisely controlled pollen drying can be built (Webber and Painter 1996).

Pollen storage

Extracted pollen is screened through 80–100 mesh screens to remove coarse debris (leaves and parts of pollen cones) and then sieved through fine 120 mesh screens (nylon stockings work well) to remove fine debris. Clean, dry pollen may be stored for a few weeks in tightly closed vials kept in a refrigerator at +4°C. For long-term storage of several months to one year or more, pollen of less than 10% water content can be stored in air-tight containers in conventional household freezers at about -25°C. Considerable research on Douglas-fir pollen has been done and the results should apply to western larch (Webber and Painter 1996).

Three factors are most important in maintaining high pollen fertility for conifers: water content; storage temperature; and to a lesser extent, storage atmosphere. Water content may be measured by the oven-dry technique, moisture probes, or one of several infra-red heating balances now on the market. The last two are rapid techniques but may require larger pollen samples (>500 mg of pollen) and equipment may be expensive. Results based on filled seed per cone from pollinations indicate that pollen that has a water content of 4% has higher fertility than that of pollen with 8% water content after three years of storage at -25°C in air-tight containers. Pollen stored in evacuated or nitrogen-containing containers had slightly higher fertility. Glass or plastic bottles, aluminum foil, or plastic pouches may be used as storage containers but the critical feature is that they must be air-tight, and ideally, vacuum sealed. Copes (1985, 1987) reported that dry pollen could be stored for long periods in liquid nitrogen. Because of the expense of the equipment and procedure, this technique

would not be practical for routine SMP but may be useful for breeding.

Pollen testing

Pollen that has been shed from mature, carefully handled pollen-cones usually contains over 95% live pollen. However, viability and vigor may be considerably reduced in pollen that has been improperly extracted or stored. Therefore, the viability of pollen should be tested before being used for SMP or breeding. The most convincing test is to use the pollen for pollination and evaluate the filled seeds per cone (seed set). However, this is usually not a practical method because of the time required and quicker methods have been developed for measuring or estimating pollen quality. One of the oldest methods is pollen germination, in which a small sample of pollen is placed in a sugar-water medium; after a few days, the percentage of pollen that has formed a pollen tube is determined. This method works well for pollen from pines, spruces, and true firs in which pollen tubes form immediately upon germination. A pollen tube is a good indicator of pollen viability and sometimes pollen vigor. Healthy pollen grains usually form pollen tubes quicker and the tubes grow faster than less healthy or less vigorous pollen. However, this test does not work well for larch (Ho and Rouse 1970) or Douglas-fir pollen because even viable pollen does not form a pollen tube immediately after the pollen germinates. It may be one or more weeks after germination before a pollen tube forms in the ovule (in vivo) (Owens et al. 1994; Takaso and Owens 1996) or in the germination medium (in vitro) (Fernando et al. 1997).

This does not mean that pollen "germination" cannot be used for larch pollen, but the method is only an estimate of pollen viability. Live dry pollen has an unevenly thickened exine and a thin dark intine. The large tube cell has a peripheral layer of lipid bodies and the rest of the cell is filled with large starch grains. Prothallial cells, the sterile cell, and a generative cell are visible (Fig. 62A). When dry, live pollen lands on the stigmatic tip it hydrates over about 24 h. If live, dry pollen is placed in water or a germination medium, such as Brewbaker's (Brewbaker and Kwack 1963) (see Appendix 5), it hydrates in about one hour. Hydration causes the pollen grain to swell and burst out of the exine. The exine may at first look like the shell of a broken egg but it is soon completely shed. The more flexible intine remains intact around the pollen grain (Fig. 62C). Pollen having this shape is considered to be alive (Ho and Rouse 1970). Dead pollen has no intact membrane and when placed in water or a germination medium it does not change in appearance. Water and solutes freely move in and out, so dead pollen does not swell and shed the exine, but it remains as a round to irregular pollen grain with dark shrunken contents (Fig. 62B). If pollen samples are observed after 24–48 h in a germination medium, percentage viable pollen may be estimated. Ideally, matching pollen samples should be applied to seed cones and the percentage of filled seeds determined at the end of the season. A correlation can then be made that may be used in subsequent years to estimate the quality of pollen. This approach may sound cumbersome, but once the initial correlations are determined, the germination test is quick, inexpensive, and accurate enough for most SMP and breeding needs.

It is of little value to leave larch pollen in germination media containing sugar for more than 48 h because fungi and bacteria grow too rapidly, and obscure or inhibit pollen germination and pollen-tube formation. The pollen cannot be surface-sterilized to kill the microbes without harming the pollen. However, if pollen cones are collected before pollen has been shed and the pollen cones are surface-sterilized using alcohol or bleach and rinsed in sterile water followed by drying in a sterile environment, small amounts of uncontaminated pollen may be extracted. Pollen extracted in this manner is free of contaminants and has been grown in a culture medium for six weeks or more. Under these conditions Douglas-fir (Fernando et al. 1998) and larch (Dumont-Beboux et al. 1998) pollen will germinate, elongate, and form a pollen tube. Unfortunately, this method

is not practical for routine pollen testing because of the large quantities of pollen needed, the numbers of pollen samples used in seed orchards, and the time required for the test, but it may be useful in breeding and has been applied in pollen physiology research.

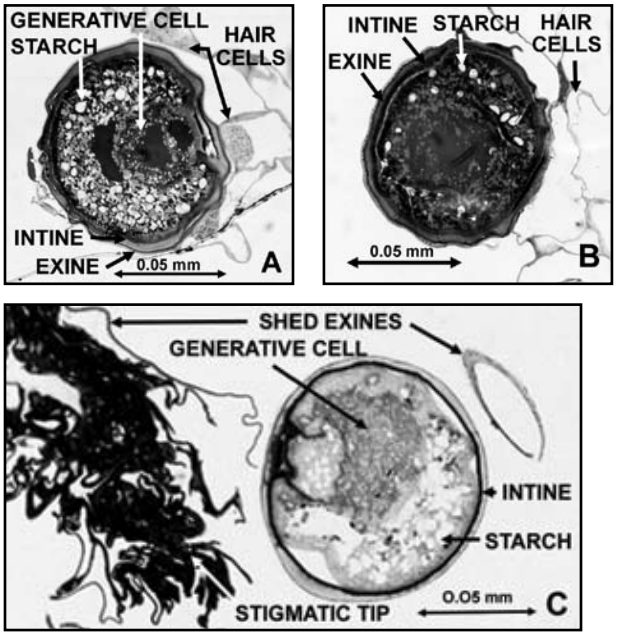


Figure 62. *A. Live dry pollen attached to the collapsed stigmatic hair cells as the pollen appears before it has absorbed water. B. Dead pollen as it appears after being placed in a germination medium for several hours or after being on the stigmatic tip for one or more days. C. Live, swollen pollen as it appears one hour after being placed in a germination medium or about 24 h after landing on the stigmatic tip. The cells within the exine and intine have become more visible. Observations of the germinating pollen may be made easier by using a simple stain such as aceto-carmine or cotton blue (see Appendix 2).*

Pollen germination tests have the advantage of requiring only inexpensive, easily acquired, and easy to use equipment (a few vials and a simple microscope). Moreover, only a few thousand pollen grains per test are needed, compared with other tests that require larger samples of pollen.

A second method, conductivity, is based on the principle that live pollen has a living cell membrane. When placed in a water solution, water is taken in through the membrane by osmosis causing the pollen to swell and burst the hard exine. The living cell membrane stretches but does not burst and does not release minerals from the live pollen. Dead pollen, on the other hand, has a dead membrane that releases the minerals from the pollen when the pollen is placed in solution. The more minerals that are released (leached) from the pollen, the higher the proportion of dead, non-viable pollen. The amount of minerals released is measured by the ability of the solution containing the leachate to conduct an electric current, as measured by a commercial conductivity meter. The greater the conductivity, the poorer the quality of pollen.

The third method, respiration or oxygen uptake, is based on the principle that live pollen is respiring and using oxygen. Therefore, samples of live pollen will use more oxygen in a given time than samples containing dead pollen. The pollen sample is put in an aqueous solution in a vial that is placed in a water bath at a constant temperature. A Clark-type polographic electrode attached to an oxygen monitor is placed in the vial and this measures the amount of oxygen in the solution over time. This information is printed on a chart recorder.

Both of these methods are accurate, reasonably quick, and based on sound physiological principles. Both methods are described in detail by Webber and Bonnet-Masembert (1989, 1993) and Webber and Painter (1996). Germination has the benefit of measuring the proportion of live and dead pollen grains (i.e., 75% live pollen), whereas the other two methods indicate the average physiological condition of the pollen sample (i.e., respiration is 75% of maximum), but this does not determine what proportion of the pollen is alive or dead. The method of choice must be determined by each agency based on the cost, the number of samples to be processed each year, the experience and availability of the technical staff, and the precision required by the agency for determining pollen quality. The more expensive methods may be available at nearby commercial, government, or academic laboratories for a reasonable cost.

Methods of pollen application and SMP

Tree breeders and geneticists each have their favourite methods of pollen application and this is usually a hand-held commercial or homemade

syringe applicator (Webber and Painter 1996) or commercial atomizer. The choice depends on the specific needs of the breeders and the species they deal with. Syringe applicators are inexpensive and relatively easy to assemble from simple laboratory supplies — rubber bulbs, glass tubing, syringes, and hypodermic needles. Since most breeders are using several pollen sources, many applicators are needed. Most can be easily cleaned, dried, and reused.

Seed orchard managers also use a range of mechanical means for SMP, depending on the orchard size, species, and amount of SMP needed. Over the years, most of the methods have been improvised using a range of often strange-looking equipment originally made for other purposes. Some large-scale methods have included large fans mounted on trucks or trailers or converted insect sprayers pulled by tractors to which pollen was added in rather unmeasured quantities. Pollen distribution was rather uneven but the purpose was mainly to increase the pollen cloud. These methods sometimes work but are wasteful of pollen and cannot be used on individual trees. If SMP is to be applied to individual small trees, hand-held spritzer-type dusters used for garden plants work well. Similar types of equipment may be fabricated from PVC pipe. For larger trees, hand-held sprayers, which hold vials (Fig. 63) or larger bottles of pollen, are commonly used for SMP. Sprayers fabricated from syringes and hand-held paint sprayers have been attached to canisters of compressed gas (inert nitrogen) by long hoses so that crowns of trees may be reached by ladder (Fig. 64) or the compressed gas may be carried in backpacks. Three types of compressed gas pollinators developed at the B.C. Ministry of Forests and Range, as well as a fourth type that was developed by the U.S. Department of Agriculture Forest Service, are described by Webber and Painter (1996). From the author's experience, the compressed gas method works very well for individual tree SMP using small bottles of pollen or vials of pollen for control crosses. Several spray units are required when doing control crosses with different pollen sources. An extra spray unit is often needed for SMP using small bottles of pollen, since the units may ice up with repeated usage.

Figure 63. *Hand-held compressed nitrogen pollinator fabricated from plastic and a spray paint air-gun. With modifications, vials or bottles of pollen may be attached.*

Alternative methods for SMP and their benefits

Other methods for SMP may be very simple or very elaborate and often expensive. French researchers found that SMP is essential for their hybrid larch orchards where phenology differs between European and Japanese larches. To increase the filled seeds per cone, they developed an electrostatic dusting technique. In a tractor-mounted device, pollen is charged with static electricity, then blown on a tree by an electrostatic gun. A high voltage is maintained between the pollen dispenser and the tree. They claim that 5–15 pollen grains are deposited per bract-scale complex. This increases filled seed per cone from 23 to 32% (Philippe and Baldet 1997).

Sometimes applying extra pollen may not be needed; simply stirring the existing pollen in the orchard is satisfactory. This may be done using blowers, such as trailer-type insecticide sprayers, in which the insecticide is not added. If the sprayer is pulled through the orchard, pollen is moved one or two rows in the orchard (Fig. 65).

Helicopters have been used for many years to move the pollen around within orchards. Although their rental cost initially appears expensive, helicopters create a very large, high cloud of existing pollen (Fig. 66) over the orchard in a very few minutes.

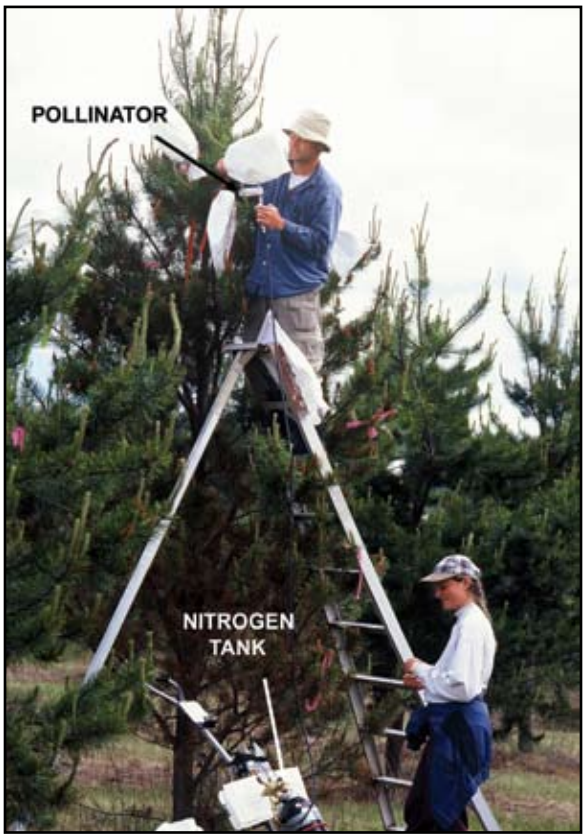


Figure 64. *Compressed nitrogen pollination equipment being used for pollination experiments in a lodgepole pine seed orchard.*

Figure 65. *Tractor-pulled insecticide sprayer, without insecticide, blowing pollen (white cloud) around within a lodgepole pine seed orchard.*

Figure 66. *A helicopter blowing pollen around a lodgepole pine seed orchard.*

Blowing pollen around in the orchard or SMP by various means may increase the pollen available to each cone, decrease self-pollination, or decrease the effects of pollen contamination from outside the orchard. Pollen may be carried long distances by wind and may contaminate the pollen produced within the orchard. SMP and blowing pollen are generally most effective at reducing contamination if they are done early in the receptive period. Unfortunately, orchard managers often wait to do SMP or blowing of pollen until the end of the pollination period, as one last measure to improve seed set. However, for most pollination mechanisms, including those for larch and Douglas-fir, the first pollen grains to enter the cones (Stages 2 or 3) are taken in preferentially over pollen arriving later. This is because early-arriving pollen grains usually occupy the sites on the stigmatic tip closest to the micropyle, and this pollen is more likely to be taken in than pollen landing farther from the micropyle (Owens and Simpson 1982; Webber and Yeh 1987). Also, early-arriving pollen has time to hydrate on the stigmatic tips and will germinate inside the ovule sooner than late-arriving pollen that has not had time to hydrate before being engulfed. In western larch, this allows pollen to shed the exine and move to the nucleus where a pollen tube forms before the late-arriving pollen germinates.

Overhead sprinkling in seed orchards

Overhead sprinkling in seed orchards is commonly used to delay pollen release or receptivity to lessen contamination from nearby trees. A variety of sprinkling or misting systems have been used, ranging from permanent underground systems, above-ground metal or PVC systems that may use sprinkling heads or misting heads mounted in the tops of crowns, and Bower guns. We have found that the standard sprinkling head is best and has less maintenance than misting heads that often become clogged and must be cleaned frequently. Also, the fine mist produced, although more localized, is easily blown by the wind resulting in only the parts of trees being sprayed.

The major problem with both sprinkling and misting systems is that they are commonly overused. It takes only about one hour of sprinkling in mid-morning to delay pollen shed for that day, and sprinkling during the receptive and pollen release period ultimately can only delay pollination by a few days. Excessive sprinkling (4 h every day) in a coastal Douglas-fir orchard caused very high early seed-cone abortion — in some trees all cones aborted. The cause was traced to the very high levels of fungal and bacterial growth in the young seed cones. Microbes are always present within the cones and show seasonal variation, with the highest amounts occurring during pollination. Excessive sprinkling significantly increased the microbial level of contamination and this, plus the excessive moisture, caused the cones to rot. Upon dissection of cones, browning (rotting) was observed to begin near the cone axis, where ovules and pollen were located. Within 1–2 weeks, rotting of the cones was externally visible and the entire cone turned brown and appeared the same as a frost-killed cone. Some of the bacteria within the cones were surface ice-nucleating bacteria, which, in agricultural crops, contain glycoproteins that cause water to nucleate and freeze at a slightly higher temperature than in the absence of the bacteria. This causes plants or parts of plants to be more susceptible to freezing (Colangeli et al. 1990). This should be less of a hazard in the dry interior than on coastal sites. However, to be safe, sprinkling should be minimized to obtain only the delay required in pollen release or receptivity for the seed orchard site.

Ovule and Megagametophyte Development

Ovules are initiated and ovule development begins in the late summer and fall before pollination. Dormant ovules each contain one large central megaspore mother cell (MMC) that is diploid (2N) and surrounded by sporogenous cells, all enclosed within the nucellus (Figs. 67A, B; 68). Seed-cone buds end dormancy in March after which bracts and scales grow rapidly (Fig. 67). The sporogenous cells divide, forming rings of cells around the MMC. The MMC enlarges, and about the time of pollination, undergoes meiosis forming four haploid (1N) megaspores (Fig. 67C). The innermost megaspore enlarges while the outer three degenerate leaving a single functional haploid megaspore (Fig. 67D). During the next two months, while the pollen is being engulfed, germinating, and forming a pollen tube, the haploid megaspore develops into a large multi-cellular megagametophyte that contains the eggs within the ovule (Figs. 67E-I).

Megagametophyte development is complex and only briefly described and illustrated here. The haploid functional megaspore divides by mitosis (division of the nucleus) but no cell walls form between nuclei for about three weeks. This creates a large sac that contains several hundred nuclei and is bounded by the megaspore cell wall. The centre of the sac is filled with fluid (Figs. 67E, F; 70). This unusual process allows for very rapid megagametophyte growth. Cell walls then quickly form between the nuclei and a parietal (outer) layer of cells is formed (Fig. 67F). These cells divide and the megagametophyte becomes filled with small prothallial cells (Fig. 67G).

Not all prothallial cells remain the same size. Several at the micropylar end enlarge, forming pyramid-shaped archegonial initials. The archegonial initials divide unequally, forming a

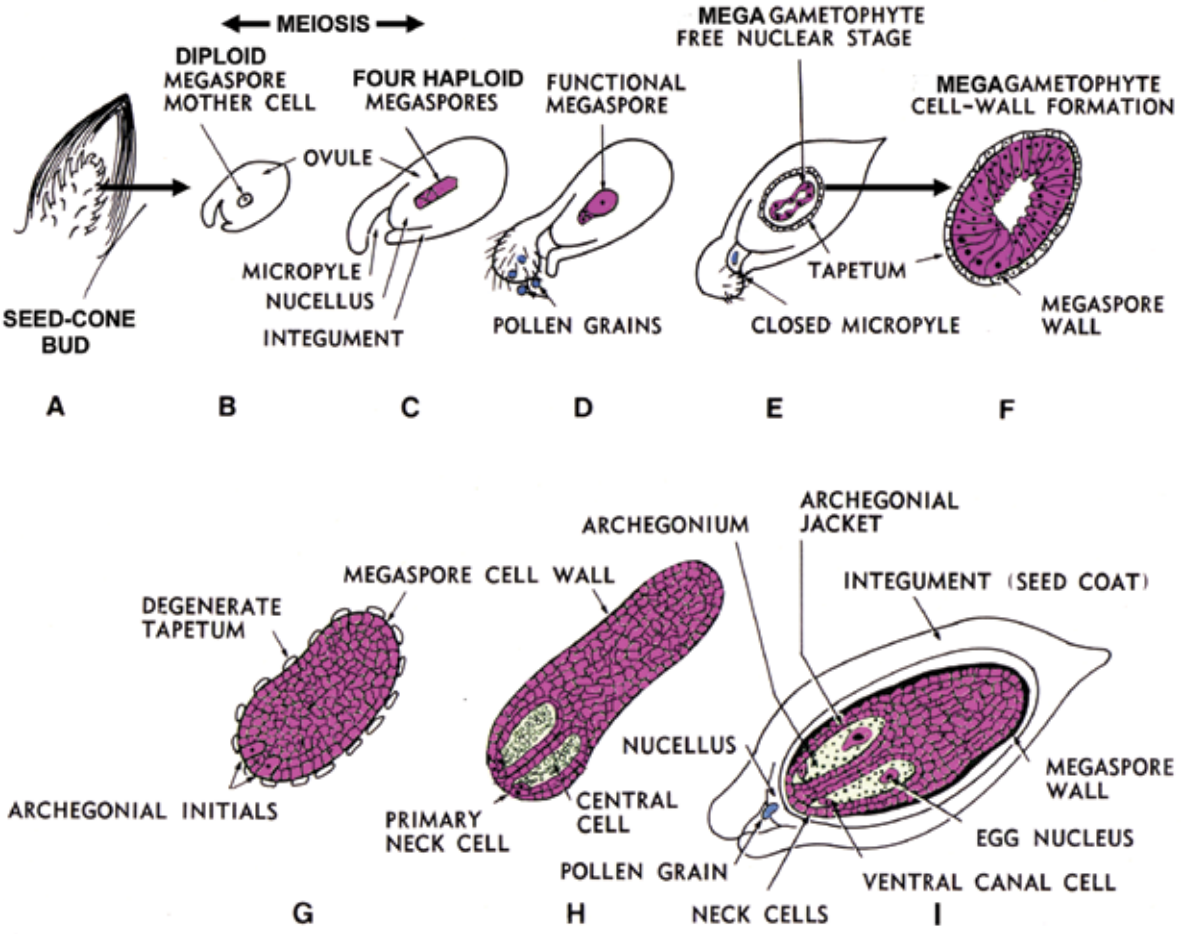
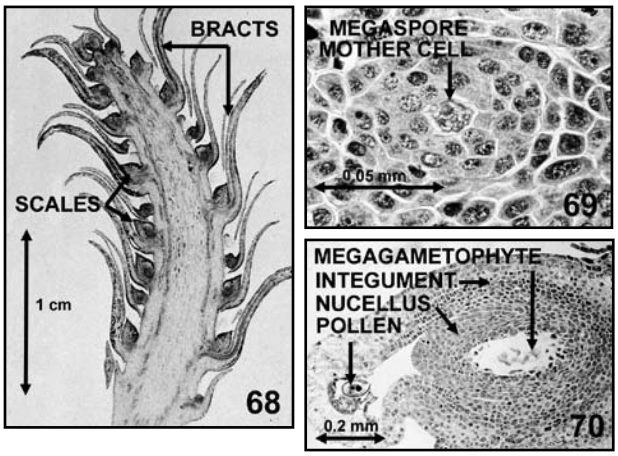


Figure 67A-I. *Ovule and megagametophyte development in western larch from the end of dormancy in March until the megagametophyte is mature and ready for fertilization in June. Haploid structures are shown in pink and pollen in blue. Figs. A-E and I show the entire ovule and Figs. F-H show only the megagametophyte without the surrounding ovule tissue.*

small primary neck cell and a large central cell (Figs. 67H, 71). The primary neck cell divides, forming a layer of neck cells; the central cell divides unequally, forming a small ventral canal cell and a large egg cell. A layer of small cells forms an archegonial jacket around each archegonium. An archegonium is the female structure and each contains one egg (Figs. 67I, 72). An average of four archegonia, thus four eggs, develop in each megagametophyte. There is rarely more than one megagametophyte per ovule.

Understanding megagametophyte development is important to our understanding of genetic differences in conifers. Within a megagametophyte the eggs are genetically identical because the megagametophyte develops from one haploid megaspore; however, the pollen grains entering the ovule are all genetically different. Archegonia keep the eggs separate within each ovule and allow each egg to be fertilized by a different pollen tube. The separation of the eggs allows for genetic selection before the seed develops. There may be competition between several genetically different pollen grains or pollen tubes before fertilization and competition between several genetically different embryos in one megagametophyte after fertilization.



Figures 68–70. *Light micrographs of sections of a seed cone at pollination (Fig. 68), an ovule at the megaspore mother cell stage (Fig. 69), and a free nuclear megagametophyte after pollen has been engulfed (Fig. 70).*

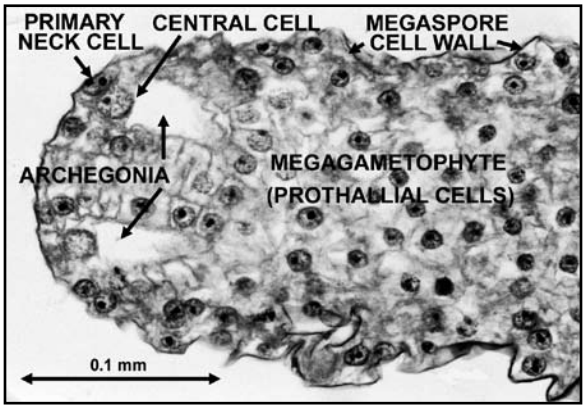


Figure 71. *Micrograph of a section of the micropylar half of a megagametophyte bounded by the megaspore cell wall and showing the prothallial cells and two archegonia, each containing a primary neck cell and a central cell.*

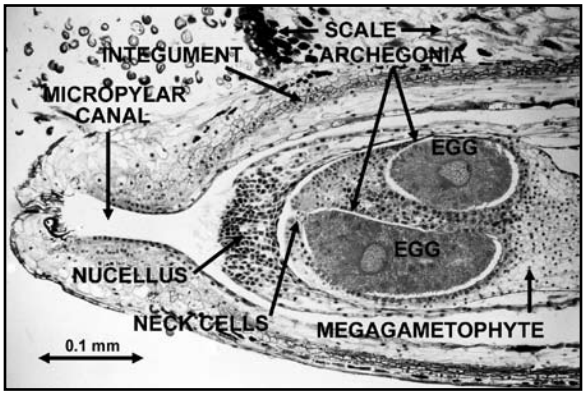


Figure 72. *Micrograph of a section of the micropylar half of an ovule, showing the sealed micropylar canal without pollen, the integument, and the nucellus containing a megagametophyte containing two archegonia each with one egg.*

Megagametophyte and ovule development have been more thoroughly described for western larch (Owens and Molder 1979c) than any other larch species. The development in western larch appears to be typical of *Larix*, and essentially the same as in other Pinaceae (Singh 1978). The fully developed ovule is ready for fertilization about mid-June. It is 4 mm long, about the same length as a mature seed (Figs. 67I, 72).

Fertilization

Fertilization in larch includes those events that occur from the time the pollen is engulfed into the ovule in March or April until the egg and sperm have fused in June. During this time the seed cones of western larch grow from about 1.5 to 3 cm in length and the base of the cone axis bends downward causing the cones to become pendant (compare Figs. 47 and 73). The seed cone at fertilization has green to purple scales and green-tipped bracts (Fig. 73). Cone colour at this stage varies among clones. In the pendant seed cones the ovules are inverted with the micropyle facing upward (Fig. 74).

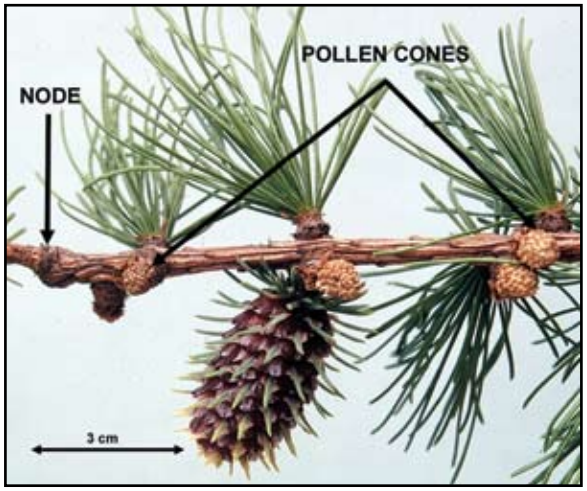


Figure 73. *Seed cone at fertilization. The node shows the end of one year of long-shoot growth.*

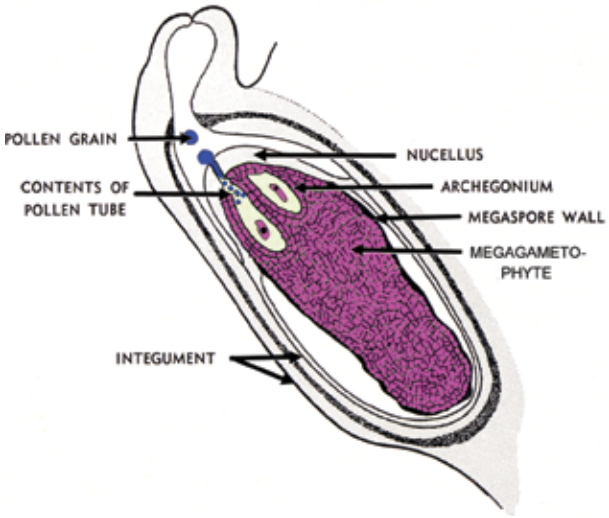


Figure 74. *Diagram of an ovule at the time of fertilization. The ovule is inverted with the micropyle facing upward. The haploid megagametophyte is shown in pink and the haploid pollen is blue.*

Early-arriving pollen landing on the stigmatic tip may remain there for several days (see Fig. 62A), hydrate, and swell but the exine usually is not shed. Regardless of the time pollen arrives on a stigmatic tip, all pollen on a stigmatic tip is engulfed at the same time.

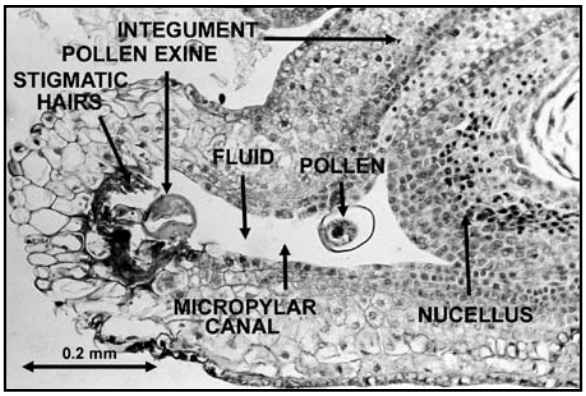


Figure 75. *Micrograph of a section of the ovule tip showing the micropylar canal with the pollen exine still attached to the engulfed stigmatic hairs and the pollen grain that was released from the exine is near the nucellus.*

Engulfment occurs at essentially the same time in a cone but may vary by a few days in different cones on a tree. Once engulfed into the micropylar canal, the hard exine is shed from hydrated, swollen live pollen but the exine remains around dead pollen (Fig. 62B).

Within the micropylar canal, pollen released from the exine remains enclosed in a thin and flexible wall, the intine (Fig. 62C), which consists mainly of cellulose. At this time, secretions produced from the nucellus tip and wall of the micropylar canal fill the micropylar canal (Fig. 75). Anatomical, electron microscope, and experimental studies using western larch and a hybrid of European and Japanese larches have shown the structure of the micropylar canal and nucellus — the tissues that produce the secretion (Owens et al. 1994; Takaso and Owens 1994, 1997). As the cones bend downward, the ovules become oriented upward (Figs. 73, 74).

Experiments were done to simulate the fluid-filled micropylar canal using pipettes lined with various membrane-like materials and filled with solutions similar to the ovule secretion fluid. Pollen was added to the fluid in the inverted pipettes and the movement of the pollen was observed (Takaso and Owens 1997). As the fluid in the pipettes decreased in volume by evaporation, similar to the fluid in the micropylar canal, the pollen slowly sank in the remaining fluid and settled near the bottom of the column of fluid. In the ovule, the pollen also sinks down in the micropylar fluid (Figs. 74, 75) and settles near the nucellar tip (Fig. 76).

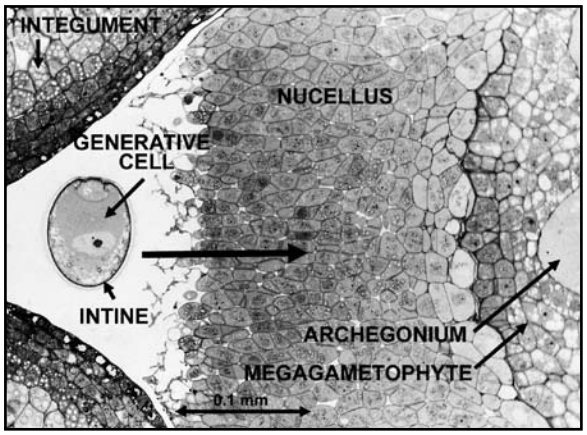


Figure 76. *Micrograph of a pollen grain in the micropylar canal, about six weeks after pollination. It has settled just above the nucellus on which the surface cells have broken down and secreted some of the fluid. The pollen tube will form and penetrate into the nucellus, as indicated by the large arrow.*

If the cone scale is severed from the cone axis or the ovule severed from the scale, the water tension within the vascular tissue of the cone is broken and a large drop of solution is exuded out of the micropyle (Fig. 77). This drop has been called dissection fluid in larch and Douglas-fir (Takaso et al. 1996). The fluid is not considered to be a pollination drop, as occurs in most other conifers, because its function is not to take pollen into the ovule but to stimulate pollen and pollen-tube growth. In larch, it also aids the movement of pollen down the micropylar canal. This process takes about six weeks, during which the cells within the pollen do not divide.

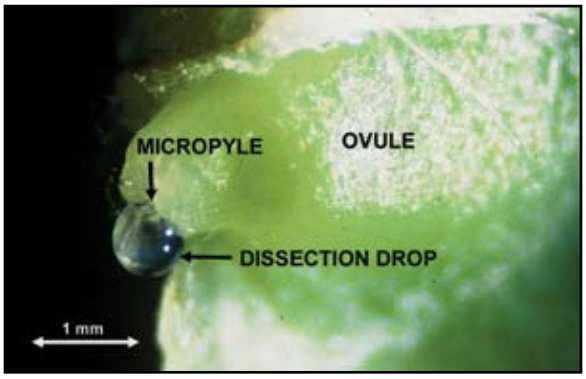


Figure 77. *Live ovule attached to a scale that has been severed from the cone, showing the dissection drop exuded from the micropyle.*

In western larch and Douglas-fir, a pollen tube does not form until the pollen is very close to (Fig. 75) or touching the nucellar tip. A small protuberance grows out from the pollen grain and forms the pollen tube. It penetrates the nucellus tip and grows between the cells of the nucellus. The tip of the pollen tube secretes enzymes that cause the nucellar cells it contacts to collapse and die (Fig. 78). Pollen tubes grow through the nucellus and reach an archegonium in about one week. Several pollen tubes may be growing in the nucellus but only one grows to each archegonium (Fig. 74). At fertilization, the ovule consists of an integument that has begun to differentiate into a 3-layered seed coat (Fig. 72). The integument encloses the nucellus that in turn encloses the megagametophyte. Consisting of about 2000 haploid cells, the megagametophyte is surrounded by a megaspore cell wall, often called the megaspore membrane (Fig. 74).

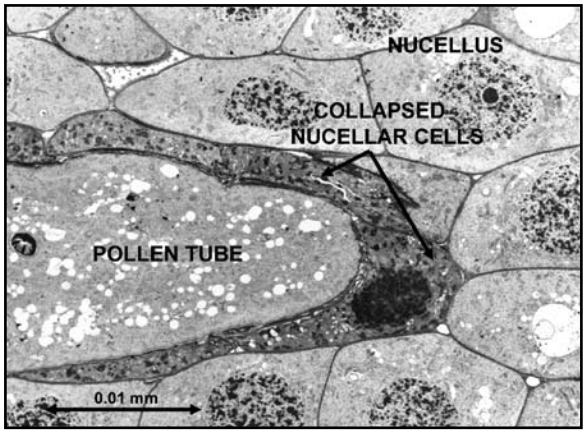
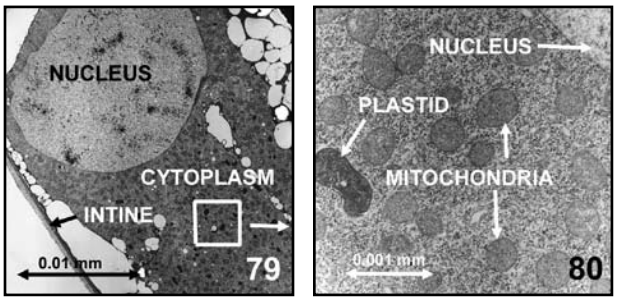


Figure 78. *Micrograph of a section through the nucellar tip showing a pollen tube growing between the collapsed cells.*

During pollen-tube growth, the small tube nucleus and large generative cell move from the pollen grain into the pollen tube (Fig. 79). They migrate along the pollen tube as it grows with the tube nucleus ahead of the generative cell. During this migration, the nucleus of the generative cell divides by mitosis forming two equal-size sperm that remain within the generative cell. In the older literature this structure was mistakenly called a 2-nucleate sperm. The generative cell is rich in cytoplasm containing many plastids and mitochondria (Fig. 80).

At fertilization, the pollen tube penetrates between the neck cells of the archegonia and bursts, releasing the generative cell and some pollen-tube cytoplasm into the egg. The generative cell also bursts, releasing the two sperm and generative-cell cytoplasm into the egg cytoplasm. This creates a receptive vacuole at the micropylar end of the archegonium (Figs. 81B, C). The 2-sperm move through the egg cytoplasm to the egg nucleus, but one sperm usually lags behind. When the leading sperm reaches the egg nucleus, it fuses with the egg nucleus (Fig. 81A) forming the zygote nucleus.



Figures 79 and 80. *Transmission electron micrographs of part of a pollen tube growing through the nucellus. Fig. 79 shows the large generative cell nucleus and cytoplasm just before the nucleus divides to form the two sperm; Fig. 80 shows the plastids and mitochondria in the generative-cell cytoplasm.*

The fusion of a haploid sperm, containing 12 chromosomes, with a haploid egg, also containing 12 chromosomes (syngamy), forms the fertilized egg or zygote containing 24 chromosomes, as 12 homologous pairs. This is the nuclear genome, contributed equally by both parents. The nuclear genome controls most of the development and physiology of the zygote as it develops into an embryo and then into a tree.

Cytoplasmic Inheritance

In addition to the nuclear genome that consists of the 24 chromosomes in each living cell, a second smaller genome is contained within the plastids and mitochondria. Plastids function in photosynthesis (chloroplasts) and storage while mitochondria function in energy release. Most of the genes controlling these functions reside within the nuclear genome but some reside within the organelles within the cytoplasm (the cytoplasmic genome). The cytoplasmic genome also helps regulate other traits within the tree, such as protein formation and disease resistance (Mogensen 1996). The cytoplasmic genome is not inherited equally from the sperm and the egg. This type of inheritance varies among some conifer families, but is the same within a family, such as the Pinaceae (Neale et al. 1986; Owens and Morris 1990, 1991; Bruns and Owens 2000). The molecular aspects of chloroplast inheritance, using DNA restriction fragment length polymorphisms (RFLPs), have been studied in European and Japanese larch and their hybrids (Szmidt et al. 1987) but not in western larch. Some aspects of the structural mechanism of cytoplasmic inheritance have been studied in western larch (Owens and Molder 1979c; Owens et al. 1994). From the various studies, it appears that cytoplasmic inheritance in larch is the same as in other members of the Pinaceae.

The generative cell of the pollen contains a large nucleus and darkly staining cytoplasm (Fig. 79) that contains many plastids and mitochondria (Fig. 80). The haploid generative nucleus divides equally by mitosis forming two genetically identical and equal-size sperm that remain enclosed within the generative-cell cytoplasm. The generative cell thus contains two separate genetic systems: the nuclear DNA, which is identical within the two sperm, and the cytoplasmic DNA contained within the plastids (cpDNA) and mitochondria (mtDNA).

During egg-cell maturation, nuclear and cytoplasmic DNAs are also kept separate. As the egg matures, the plastids that contain the cpDNA become transformed into structures called large inclusions or transformed plastids. The transformation involves each plastid engulfing large amounts of egg cytoplasm, causing the plastid to enlarge and become very complex. The transformed plastids migrate to the periphery of the egg. These two processes cause the plastids to be excluded from participating in fertilization. In contrast, the egg mitochondria, which contain the mtDNA, migrate to the centre of the egg, forming a zone around the egg nucleus. There they can be involved in fertilization.

When the pollen tube penetrates the neck cells and enters the egg cytoplasm, it bursts, releasing the generative cell and some pollen-tube cytoplasm. The generative cell also bursts, releasing into the egg cytoplasm its two sperm, plastids containing cpDNA, and mitochondria containing mtDNA. These paternal organelles move in small clusters with the sperm toward the egg nucleus. Most of the organelles remain near the leading sperm. When the leading sperm fuses with the egg nucleus

(syngamy), the paternal plastids and mitochondria remain for a short time as a cluster in the egg perinuclear zone. This new cytoplasm, made up of the egg perinuclear zone and the cluster of paternal organelles, is called the neocytoplasm (Fig. 81A). The paternal and maternal organelles do not intermingle until the neocytoplasm (containing four free nuclei of the proembryo) migrates from the centre of the egg to the far end of the egg opposite the micropyle (chalazal end) (Fig. 81B). The second, trailing sperm and its accompanying organelles, degenerate near the micropylar end of the egg (Fig. 81A-C). When cell walls start to form in the proembryo, each cell contains one diploid nucleus and cytoplasm derived from the neocytoplasm that contains both maternal and paternal organelles — cpDNA and mtDNA.

We do not know if these organelles are equally distributed in all proembryo cells. Molecular studies in the Pinaceae indicate that about 90% of the mitochondria are contributed from the egg and 10% are contributed from the paternal parent (Wagner et al. 1987). The large difference can be attributed to the larger amount of maternal cytoplasm making up the neocytoplasm and that most of the paternal cytoplasm is excluded from the neocytoplasm. Inheritance of plastids, thus cpDNA, may vary in different conifers. With rare exceptions in the Pinaceae, all of the plastids and cpDNA are derived from the paternal parent via the generative cell cytoplasm (Owens and Morris 1990, 1991; Bruns and Owens 2000; White 1990). However, some maternal inheritance of cpDNA has been reported for Japanese and European larch and six of their hybrids (Szmidt et al. 1987). To date, there are no reports of mitochondrial inheritance in *Larix*.

The two genetic systems, nuclear and cytoplasmic, are inherited differently. The nuclear genome comes equally from the sperm and the egg — 12 chromosomes from each — and is inherited in a Mendelian fashion. These chromosomes control most of the functions and development of the new embryo and tree. The cytoplasmic genome consists of the DNA contained in mitochondria and plastids. In the Pinaceae, most mitochondria come from the egg (maternal) and some from the generative cell in the pollen tube (paternal). Usually, all of the plastids are from the generative cell in the pollen tube, since all maternal plastids were transformed and excluded from the zygote. In the Cupressaceae, all mtDNA and cpDNA come from the male parent, whereas in most flowering plants, including the hardwoods, both are inherited from the female parent (Mogensen 1996).

At present, RFLP studies have a practical application in paternity determinations, showing interrelationships between conifer genera and species, and for classifying seedlots in regions of introgression (Szmidt et al. 1988). Other important functions and practical uses may be discovered in the future.

The Embryo

The embryo refers to the early developmental stages of plants. Embryogeny or embryogenesis refers to the development of embryos, and embryology is the study of embryo development. Embryogeny has been described for about 40 of the 80 conifer genera and has been summarized by Singh (1978). *Larix* embryogeny was first described by Schopf (1943) using European larch, tamarack, and some hybrid larches. A study of embryogeny was done for western larch (Owens and Molder 1979c).

For convenience, the following discussion is divided into proembryo, early embryo, mid-embryo, and embryo maturation stages. The distinction between the first two is clear, but the distinction between later stages is more subjective.

The proembryo

The *proembryo* develops within the archegonium and involves both free-nuclear and cellular stages. Immediately following fertilization the zygote nucleus divides by mitosis, forming two free nuclei suspended in the neocytoplasm in the centre of the archegonium (Fig. 81A). Each nucleus divides again and the resulting four free nuclei within

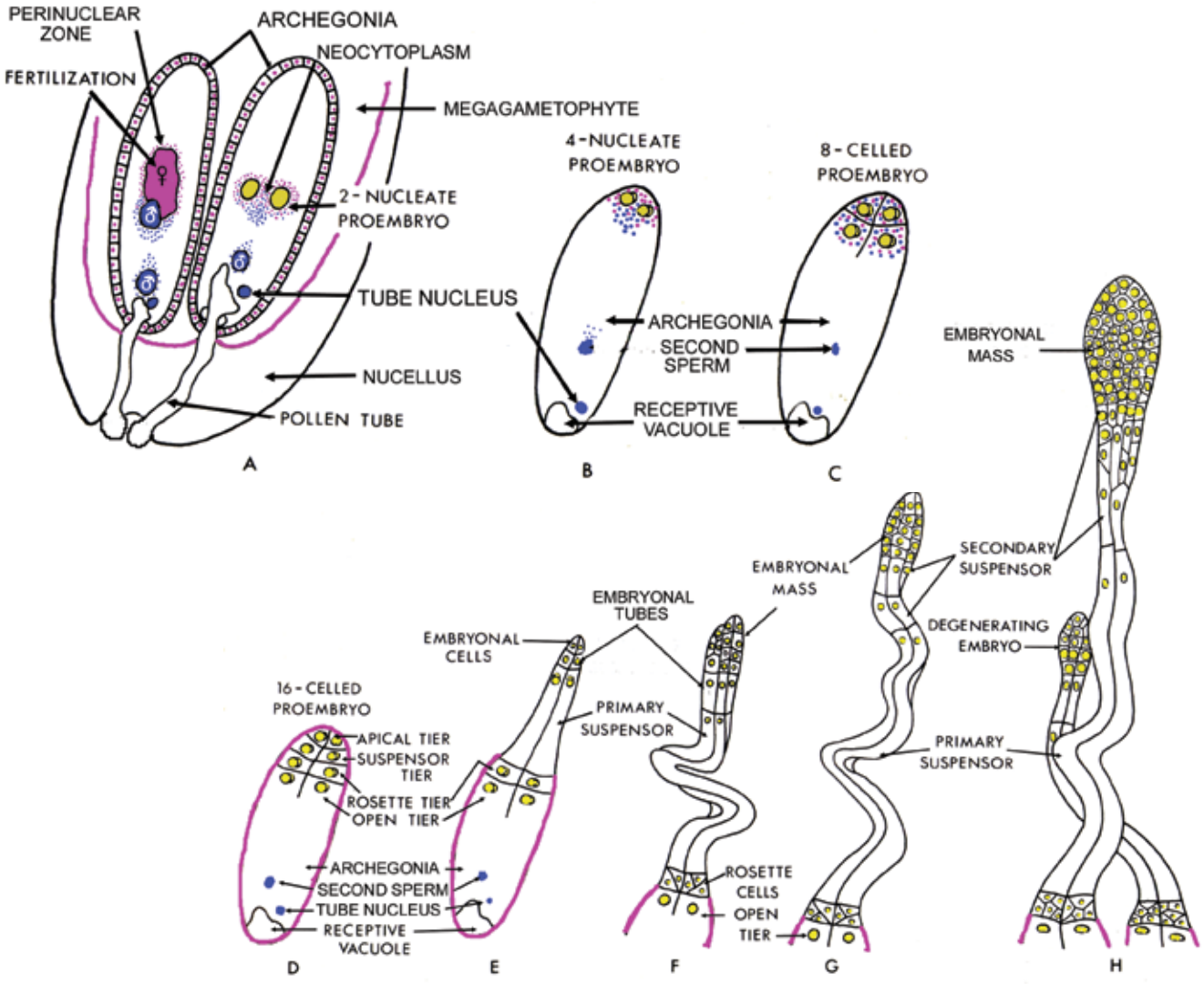


Figure 81. *Diagram of an ovule and two egg cells at fertilization (A), and during proembryo (B-D) and early embryo (E-H) development. A. Fusion of sperm and egg and distribution of paternal (blue) and maternal (pink) organelles in the egg are shown on the left and intermingling of organelles in the 2-nucleate proembryo are shown on the right. B. A 4-nucleate proembryo with paternal and maternal organelles starting to intermingle. C. Two-tier, 8-cell proembryo with all organelles intermingled. D. Four-tier, 16-cell proembryo. E. Early embryo. Primary suspensors are elongating, forcing the embryonal cells and secondary suspensor cells out of the archegonium and into the megagametophyte, ending the proembryo stage. F. Early embryo showing coiled primary suspensor cells and the formation of secondary suspensor cells. G. Early embryo stage showing the embryonal mass. H. The end of the early embryo stage showing one degenerating embryo and one developing embryo from two fertilized eggs (archegonial polyembryony).*

the neocytoplasm migrate to the chalazal end of the archegonium (Fig. 81B). The free nuclear stage takes only a few days. The four nuclei divide forming eight nuclei, then cell walls form between these nuclei producing an 8-cell (2-tier) proembryo (Fig. 81C). The four cells in each tier divide again and cell walls form producing a 16-cell, 4-tier proembryo (Fig. 81D). The four tiers making up the proembryo are (1) the apical tier, at the tip, that forms the embryo proper; (2) the suspensor tier below elongates forcing the apical tier out of the archegonium; (3) the dysfunctional suspensor (rosette) tier, as the name indicates, has no known function; and (4) the open tier at the base that remains open to the egg cytoplasm within the archegonium. The open tier is thought to facilitate the movement of materials from the degenerating egg cytoplasm to the distal tiers. The 16-cell proembryo stage is reached about one week after fertilization. During the proembryo stage,

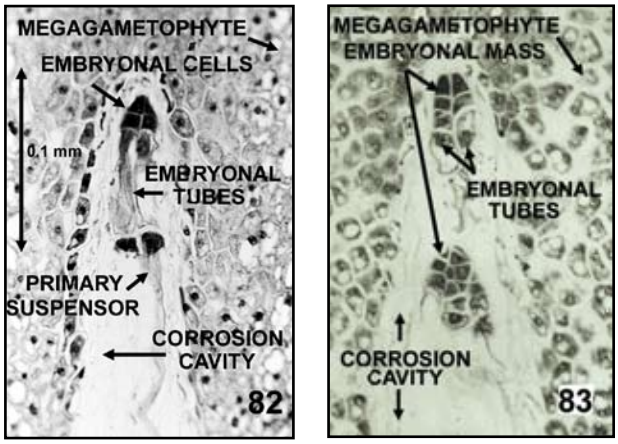
the remains of the egg cytoplasm and the second sperm and tube nucleus degenerate (Fig. 81A-D) and the archegonia start to collapse.

The early embryo

The *early embryo* stage starts when the primary suspensor cells elongate and push the apical tier cells out through the archegonial jacket and into the megagametophyte tissues (Fig. 81E). The four apical cells divide, each forming a file of embryonal tubes (Figs. 82, 83). The embryonal tube cells elongate, forming the secondary suspensor below the apical cells (Fig. 81G). Secondary suspensor cells elongate pushing the apical cells farther into the megagametophyte (Fig. 81H, 84). By the time the apical cells have been pushed about midway into the megagametophyte (Figs. 81F, 84), they divide and form many embryonal cells that are collectively called the embryonal mass (Figs. 81G, 83). During this development, the primary and secondary suspensors elongate and become coiled (Figs. 81F, G), as they push the embryonal mass deeper into the megagametophyte. Apical, embryonal, and embryonal mass cells secrete enzymes that cause the megagametophyte cells to break down, creating a corrosion cavity into which the early embryo grows (Figs. 82, 83).

Polyembryony

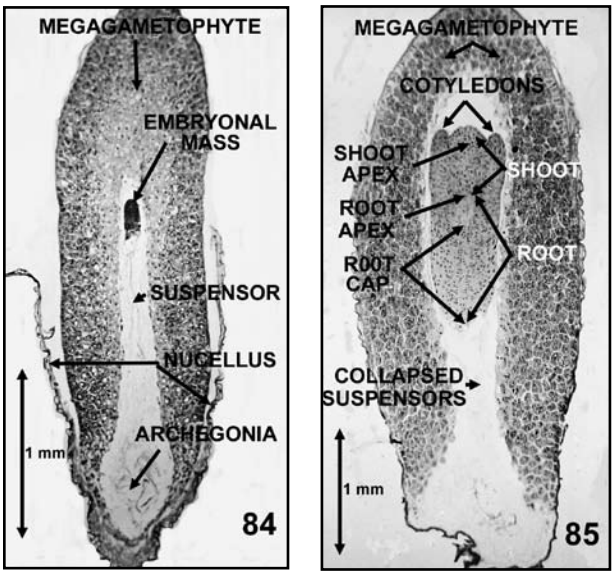
Polyembryony is when more than one egg in an ovule is fertilized and the zygotes formed may each develop into a proembryo. This is called *simple* or *archegonial* polyembryony. The number of proembryos that form is determined by the number of archegonia and the number of pollen grains that germinate and form a pollen tube that reaches an archegonium. Simple polyembryony is common when there is abundant pollen but rare if pollen is scarce (Owens and Molder 1979c). The presence of several pollen tubes and archegonia allows for pre-fertilization competition and selection among competing pollen grains. The existence of multiple fertilizations allows for post-fertilization competition and selection among competing embryos. Each embryo from simple polyembryony is genetically different from the others in the ovule. The greater the number of viable pollen grains and fertile archegonia, the greater would be the benefit from the selection process. Western larch usually has four archegonia, but ovules with three or five archegonia have been observed (Owens and Molder 1979c) all of which can be fertilized if there are enough fertile pollen grains.



Figures 82 and 83. *Micrographs showing early embryos in the corrosion cavities as the apical cells divide to form embryonal tubes (Fig. 82) and the embryonal mass (Fig. 83).*

Some conifers, such as pines, may have similar numbers of archegonia, but have an additional post-fertilization process called *cleavage* polyembryony. During cleavage polyembryony, the four files of cells in each early embryo (Fig. 81E) separate and each file may develop into a separate embryo, resulting in four early embryos per fertilized egg. All four embryos are genetically the same. This may result in many early embryos in each ovule (4 times the number of archegonia) but normally only one survives. An advantage in having cleavage polyembryony in addition to simple polyembryony may be that having four times more early embryos present, even if some are genetically identical, makes it more likely that one will survive. Cleavage polyembryony has not been observed in *Larix*. However, in *Larix* (Schopf 1943) and Douglas-fir (Allen and Owens 1972), although the files of cells in the early embryo do not cleave, not all files of cells develop equally and contribute equally to the embryo (Fig. 81F). This was called delayed, or incipient, cleavage

polyembryony by Schopf (1943), who considered it to be a derived character in conifer embryogeny. It does not appear to affect the final development of the embryo, and appears to be a relic of evolution found in some genera in the Pinaceae that lack cleavage polyembryony (e.g., *Larix, Pseudotsuga, Picea*).



Figures 84 and 85. *Micrographs of sections of ovules in mid- to late July showing developing mid-embryo stages in the corrosion cavity in the centre of the megagametophyte. Polarity and meristems are established in the embryonal mass (Fig. 84). Cotyledons, shoot, and root form as the suspensor collapses (Fig. 85).*

The mid-embryo

The *mid-embryo* stage starts by mid-July, about one month after fertilization. In this first month of embryo development, the most important developmental stages occur that determine the ultimate structure and development of the embryo. Cell divisions in the embryonal mass produce a few hundred cells (Figs. 81H, 84) and the directions in which cells divide become less random. More new cell walls form perpendicular to the long axis of the developing embryo, and distal and proximal meristems form. This establishes polarity within the embryo and separates it into two meristematic regions that develop into the distal shoot and proximal root of the embryo (Figs. 84, 85). The distal meristem forms the stele promeristem, delimited at the distal end by a dome-shaped surface that will form the cotyledons. Below this, cells will form the embryonic shoot axis. The proximal meristem forms the root meristem, which in turn forms the embryonic root and root cap; below this, a long rib meristem joins to the suspensor system.

Embryo maturation

Embryo maturation stages occur in August and involve mostly enlargement and maturation of embryo organs and tissues. The embryonic stem elongates, forming the hypocotyl-shoot axis that contains the provascular tissue and embryonic cortex. They are still referred to as embryonic because they are rapidly forming new cells and growing, but complex tissues such as xylem do not yet differentiate. At the top of the stem, 6–7 cotyledon primordia form (Fig. 85) and elongate, leaving a central shoot apex. Unlike true leaves that form in the seedling, cotyledons are not initiated from an apical meristem (apex) of the embryo. Also, the embryo shoot apex does not contribute to the development of the embryonic shoot; it only becomes active during germination of the seeds. The cotyledons each have a provascular strand that is continuous with the provascular tissues in the hypocotyl-shoot axis. Long secretory cells form outside the provascular tissues (Schopf 1943). The root apex forms the embryonic root or radicle. The embryonic root has a long root cap that consists of a central column of meristematic cells enclosed in a cylinder of pericolumn cells. The root cap is continuous with the collapsed suspensor below. The small embryo elongates rapidly toward the distal and proximal ends of the megagametophyte and fills the corrosion cavity (Fig. 86). Towards the end of late embryo development, the embryo and seed tissues dehydrate and a wider corrosion cavity forms between the embryo and megagametophyte (Fig. 87).

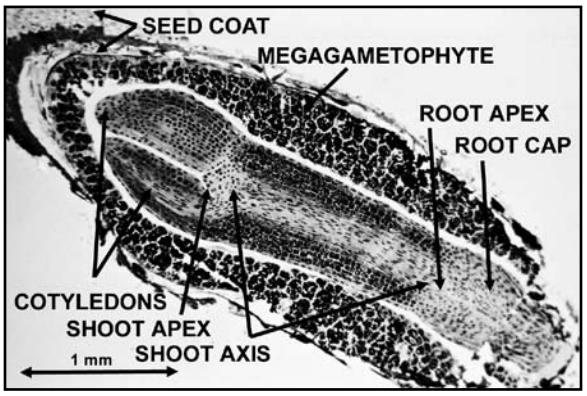


Figure 86. *Section of a nearly mature seed collected early in September, before it has dehydrated, showing the megagametophyte containing the embryo and the tissues within the embryo.*

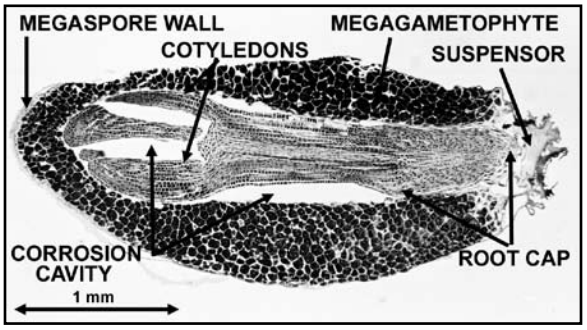
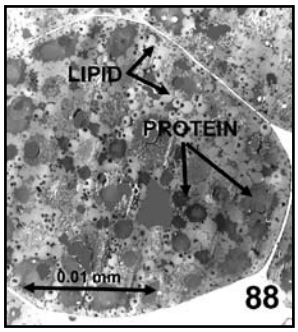


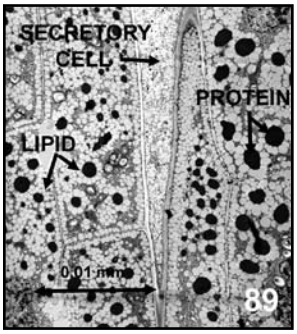
Figure 87. *Section of a mature dehydrated seed collected late in September showing the megagametophyte that contains the dehydrated and shrunken embryo in the corrosion cavity.*

Storage products

Storage products are abundant in the megagametophyte, the primary food storage tissue supplying the developing embryo, and the maturing and germinating seeds. Storage materials are formed by the conversion of soluble substances that are present in the megagametophyte at fertilization and other substances that may be transferred there after fertilization. However, there is no vascular connection between the ovule and the cone scale, and a seed coat is developing, so late transfer would be minimal. At fertilization, megagametophyte cells are small with thin walls. They have several large vacuoles, plastids containing starch grains, and a few small protein bodies. During seed and embryo development, most starch in the megagametophyte is broken down into soluble carbohydrates, proteins are synthesized and stored in large protein bodies, and many small lipid bodies form around the protein bodies (Fig. 88).

Cells in the early embryo are meristematic with large nuclei, many small vacuoles, a few starch grains, and a few small protein bodies that form within some of the vacuoles. During mid- and late-embryo development and seed maturation, protein bodies become very large and many small lipid bodies form that fill most of the embryo cells (Fig. 89). The root cap contains more starch grains than other tissues. Secretory cells, the shoot apex, and procambial cells have fewer and smaller protein and lipid bodies than other tissues.





Figures 88 and 89. *Electron micrographs of mature megagametophyte cells (Fig. 88) and mature embryo cells (Fig. 89) showing the large black-stained protein bodies and the small grey-stained lipid bodies.*

During embryo development, nutrients are transferred from the megagametophyte to the embryo. Storage products may be identified using cytochemical techniques, as has been done throughout embryo and seed development in Douglas-fir (Owens et al. 1993) but only partially for western larch. However, the presence and changes in amounts of storage products appear similar in these species and in most Pinaceae. In Pinaceae seeds that have been studied, lipids make up about 60% of the dry weight of the megagametophyte and 45% of the embryo. Proteins make up about 16% of the dry weight of the megagametophyte and 11% of the embryo. Soluble sugars make up only 2–3% of the dry weight of the megagametophytes and embryos.

The amount of starch is small and was not determined. The most important storage materials in seeds of the Pinaceae are lipids (Owens et al. 1993).

The Seed

The seed is a mature ovule and consists of an outer seed coat and a thin nucellus surrounding the megagametophyte that contains the embryo. The megagametophyte is enclosed by a thin megaspore cell wall (membrane) (Fig. 90), the same cell wall that enclosed the small functional megaspore at pollination. During ovule development, the megagametophyte enlarges several thousand times and the megaspore wall enlarges proportionately. If viewed with an electron microscope, it appears differentiated into several layers, similar to the pollen wall. In dissected seeds and using light microscopy, it appears as a thin grey membrane. In some pines it is thick and tough, may prevent entry of water, and affect seed hydration and germination. However, it remains relatively thin in western larch and does not have a major function in seed dormancy or germination. The nucellus becomes stretched and thin around most of the megagametophyte, except at the micropylar end where it remains several cells thick (Owens and Molder 1979c).

The seed coat (testa) develops from the integument, which thickens during early ovule development and begins to differentiate into three layers before fertilization. At fertilization the inner layer (endotesta) consists of several layers of

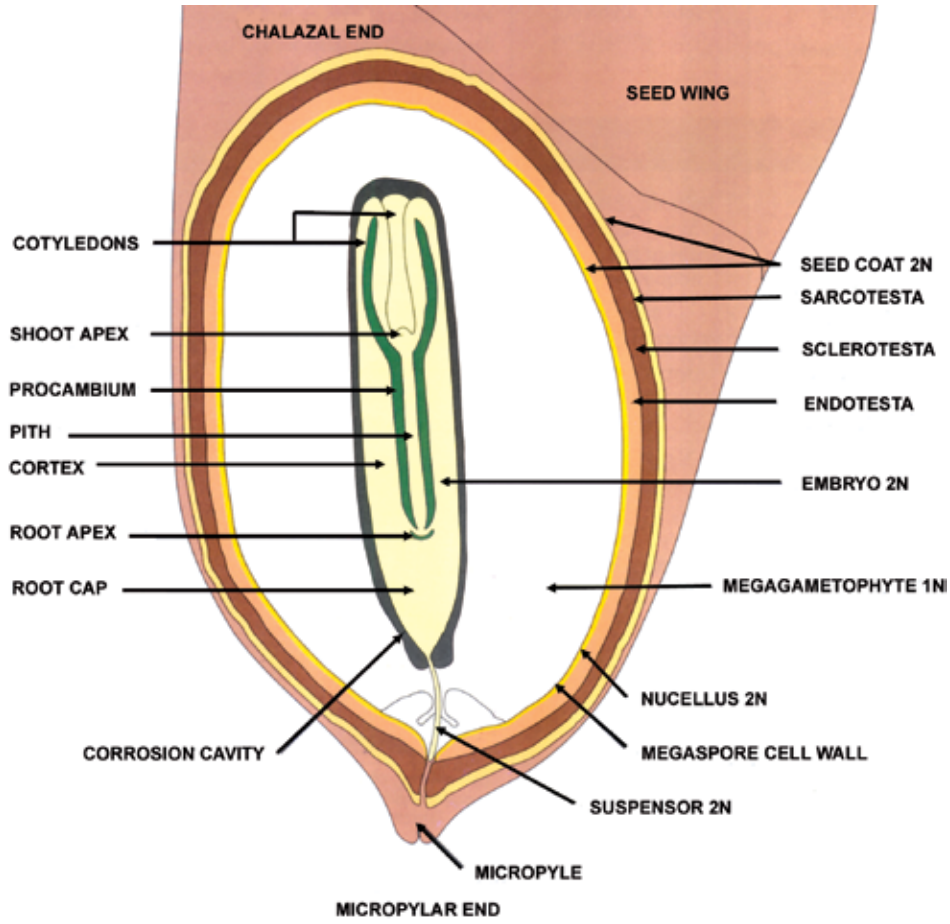
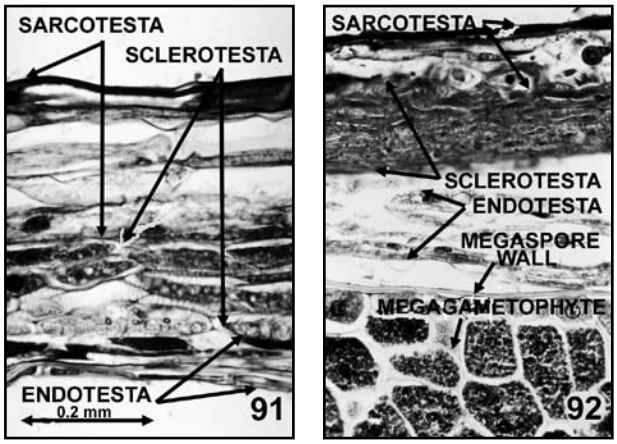


Figure 90. *Diagram of a mature seed showing the layers of the seed coat, nucellus, megaspore wall, megagametophyte, and embryo contained within the corrosion cavity. The seed coat, nucellus, and embryo are diploid (2N) and the megagametophyte is haploid (1N) (from Kolotelo 1997).*

parenchyma cells and these cells become stretched and flattened as the ovule grows (see Figs. 72, 91, 92). In the mature seed it is a thin membranous layer of elongated, flattened parenchyma cells that contain few phenolic compounds or other stored products (Fig. 92). It does not appear to restrict water movement into the seed or affect germination. The middle layer of the integument in the young ovule consists of 4–5 layers of parenchyma cells. They contain darkly staining phenolic compounds and during seed development they elongate slightly. At fertilization the cell walls begin to thicken and become filled with many microscopic pores (pits) (Fig. 91). In the mature seed this middle layer (sclerotesta) consists of a layer of very hard sclerenchyma cells (stone cells) that give the seed coat its strength and hardness (Fig. 92). They swell and soften when the seed is hydrated but may serve as a physical barrier to germination in some conifer seeds. The outer layer of the young ovule consists of layers of parenchyma cells (see Fig. 72). The cells remain thin walled, and elongate and become stretched as the ovule grows. At fertilization the outer layer has started to form a waxy cuticle and store phenolic compounds. This gives the mature seeds a dark brown colour. In the mature seeds, usually only the outer cell layer remains intact while the inner layers stretch and collapse (Fig. 92) (Owens and Molder 1979c). This layer restricts water movement and if damaged during seed processing, seed storage life and germination may be reduced.

The seed wing in larch, as in other members of the Pinaceae, develops from the ovuliferous scale and not the integument of the ovule, although it joins firmly with the ovule (Figs. 95, 96). The seed wing begins to develop from the surface of the scale soon after pollination. Cells beneath the ovule and the epidermis of the scale divide forming three layers of meristematic cells.

These layers raise the epidermis forming the outline of the wing. As the cone and seeds mature, the cell walls in the middle of these three layers of cells break down, causing the upper layer to separate from the scale and form the seed wing that is attached to the seed. The same layer of cells extends beneath the seed, and also breaks down, causing the seed to separate from the scale. This process is similar to the abscission layer at the base of a leaf that causes the leaf to abscise and fall (Owens and Molder 1979c).



Figures 91 and 92. *Micrographs of sections of seed coats at fertilization (Fig. 91) and in the mature seeds (Fig. 92) showing the development and structure of the outer (sarcotesta), middle (sclerotesta), and inner (endotesta). The megagametophyte and megaspore wall are shown in the mature seed.*

Insect damage may stimulate resin formation in the abscission layer causing seeds to stick within the cones. Similarly, if cones are not kept dry after being picked, mold may grow in this abscission layer, causing wings and seeds to stick to the scales. The function of the seed wing is to slow the rate of fall of the seeds from the cones, allowing seed to be carried farther by the wind.

Figure 93. *Mature western larch cones in August.*

Cone Maturation and Collection, and Seed Release and Extraction

Cones mature about mid-August (Fig. 93), dry, turn brown, open, and shed the seeds over the next month. Extensive studies have been made of Douglas-fir seed cone development, drying, and opening (Owens and Smith 1965); the process appears to be the same in western larch (Owens and Molder 1979c). In pendant seed cones, tissues at the base of each scale, on the upper (abaxial) surface, differentiate into long woody sclerenchyma cells with thick lignified cell walls. Cells on the lower (adaxial) surface of each scale, next to the seeds, remain as parenchyma cells with thin, non-lignified cell walls. Scales are spoon-shaped, and a band of vascular bundles occurs between these two tissues and is continuous with the vascular tissues in the cone axis. Thus, each scale is attached to the cone axis in a hinge-like fashion with the woody sclerenchyma cells on the upper surface. As the cone dries, the woody cells on the upper surface of the scales shrink more than the non-woody cells on the lower surface. The upper surface shortens, pulling the scale up and causing the cone to open. Since the tissues involved consist mostly of dead cells, cone opening is a drying process and not a growth process. If it rains, the woody tissues absorb water and swell causing the cones to partially close, only to open again, usually more widely, during the next dry period.

In Douglas-fir the first opening of cones begins when 19–34% of the wet weight is lost. Further opening occurs until about 51% of the wet weight is lost, at which time all cones are fully opened. Further drying does not cause greater cone opening; however, wetting of the open cones causes them to close and a second drying causes cones to open more fully than with the first drying (Owens and Smith 1965). This technique has been used in some commercial drying processes.

On a tree, most seeds are shed during the first cone opening so partially opened cones may have lost some of their seeds. Successive cone opening allows additional seeds to be shed over several months. However, seeds that are released during the first opening are usually of higher quality than seeds released in subsequent openings. Seeds that remain in the cones often have been damaged by insects or disease, resulting in resin secretions that cause seeds to stick within the cone. Seeds and seed wings abscise from the scales between fertilization and cone maturity, but are held tightly within the cone until it opens.

Figure 94. *Cones stored on racks in burlap bags until processed.*

Cones should be collected before they begin to open (usually about mid-August). They should be collected at the first signs of drying and turning brown, before they have lost 20% of their wet weight. Cones collected too early may not have the mechanical tissues fully developed and may case-harden and not open fully, or they may quickly get moldy causing seeds to stick to scales. Cones collected too late will have lost many seeds and the seeds that remain may have low viability. Until they are processed, cones may be stored in burlap bags that are not filled too full, labelled, and stored in a dry place with good air circulation (Fig. 94). Western larch cones may remain on the tree for one or more years after seeds were shed, but these usually have no viable seeds and are easily distinguished from mature cones by their darker colour and more weathered appearance.

The methods of commercial cone collection, post-collection cone handling and storage, and

seed extraction have not been prescribed for western larch. However, because of the similarity of cone and seed structure to that of Douglas-fir, the methods described for Douglas-fir (Kolotelo et al. 2001) are recommended (D. Kolotelo, pers. comm., June 2004).

Seed Condition

"Seed condition refers to the physical and physiological state of being and health of seeds" (Kolotelo et al. 2001). Differences can arise among and within species, provenances, clones, trees, and seedlots during seed development, extraction, handling, and storage. Therefore, seed condition is often measured at several times and for several traits during processing. We need to know if seeds are filled, empty, insect or disease damaged, and if they will germinate well. The specific techniques that can be used are described thoroughly in two B.C. Ministry of Forests and Range manuals (Kolotelo 1997; Kolotelo et al. 2001). These manuals should be consulted and only a brief discussion is given here. Seed condition can be effectively, quickly, and economically assessed by visual observations requiring no special tools, but this requires experience through repeated observations of seed morphology and anatomy.

Seed morphology

Seed morphology refers to the external appearance of the seed coat and seed wing (Kolotelo et al. 2001). The seed coat of western larch is hard and has no resin vesicles that can be damaged during processing. The lower surface of the seed and wing that is attached to the scale is slightly rough, brown, and not shiny. The lower surface of the seed is slightly mottled and well delimited from the wing. The upper exposed surface of the seed and wing is tan to brown, smooth, and shiny. The seed wing is about twice as long as the attached seed and is firmly attached to the seed coat, thus variable fragments of the wings remain attached to dewinged seeds (Fig. 95).

Seed anatomy

Seed anatomy refers to the internal structure of the seed (Kolotelo et al. 2001) and can be assessed by a simple cutting test in which the upper or lower half of the seed is sliced off using a sharp, single-edge razor blade (see Appendix 6). The following brief discussion is based on descriptions and illustrations of conifer seeds given by Kolotelo (1997) and Kolotelo et al. (2001). In sliced, healthy, dry seeds, the megagametophyte is white, opaque, and firm, and fills the seed (Fig. 96), whereas the embryo is light yellow and fits loosely in the corrosion cavity. In healthy, hydrated seeds the megagametophyte and yellow embryo are shiny and the embryo fits snugly into the corrosion cavity (Fig. 97).

Cutting tests using imbibed (hydrated) seeds are recommended. In healthy seeds, the embryo should fill about 90% the length of the megagametophyte. These would be considered mature seeds that will germinate. Seeds with embryos that fill about 75–90% have a high probability of germinating but germination rate, vigor, and storability may be reduced compared with mature seeds. Seeds with embryos filling only 60–75% of the megagametophyte have usually aborted late in embryo development (Fig. 98) and are not likely to germinate. Those below 60% will not germinate. Seeds in which the embryo appears normal but the megagametophyte has grey or yellow areas are considered to be slightly deteriorated and may germinate. Seeds in which the embryo is normal but the megagametophyte has deteriorated and become gel-like, grey or yellow, and transparent or translucent will probably not germinate. Seeds in which both the embryo and megagametophyte are discoloured or deteriorated will not germinate. Seeds in which the contents are rotten, mushy, shrunken, or shrivelled will not germinate.

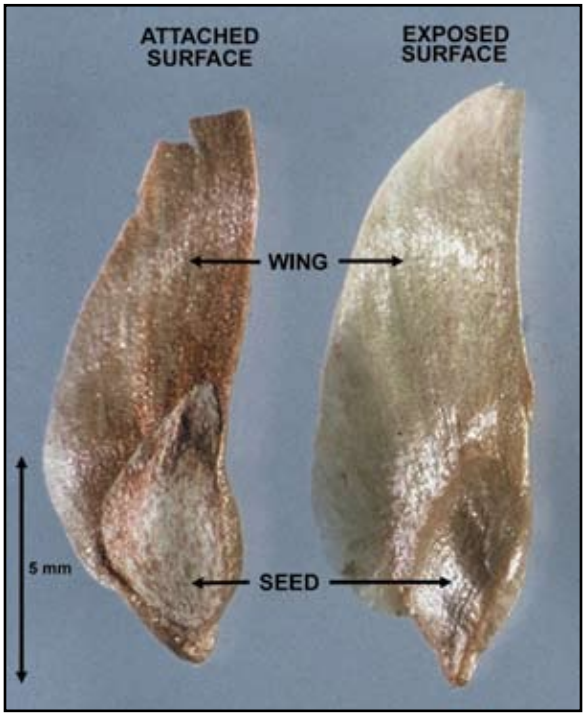


Figure 95. *Morphological differences between the lower (attached) and upper (exposed) surfaces of western larch seeds and wings.*

Figure 96. *Cutting test done with a razor blade showing a longitudinal section of a dry, filled, mature, healthy western larch seed (left) and a dry seed (right) in which the megagametophyte is shrunken, probably due to late abortion of the embryo.*

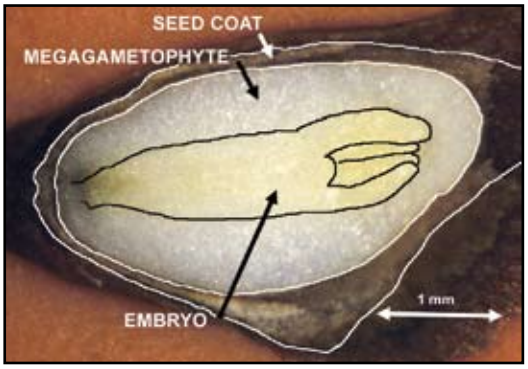


Figure 97. *Cutting test of an imbibed seed showing the shiny white megagametophyte and yellow embryo filling about 90% of the seed (from Kolotelo 1997).*

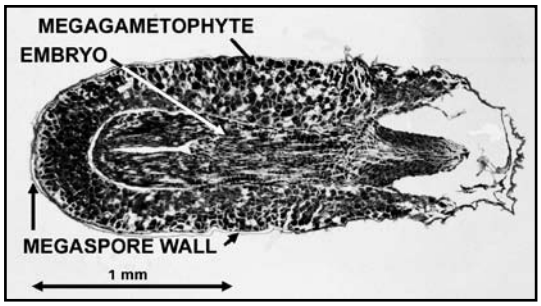


Figure 98. *Longitudinal section of a seed in which the embryo aborted during late development.*

Cutting tests can be used to determine if cones are ready to be harvested. If embryos are not fully developed, cones should not be harvested. If embryos appear to be fully developed but seeds appear wet and shiny when sliced (Fig. 97), the seeds may not yet be dehydrated enough for cones to be harvested. An easy test of dryness is to leave seeds exposed to air overnight: if the megagametophyte shrinks away from the seed coat (appearing similar to Fig. 96, right) the seeds have not dehydrated enough for the cones to be harvested (Eremko et al. 1989).

Use of radiographs is another way of visually assessing seed condition. Radiographs are normally performed on seeds having a storage moisture content of 5–10%, at which point anatomical details are clearly visible. The test is not valid if seeds are hydrated because anatomical details are obscured. This technique is effective for detecting damage to seed coats and insect-infested seeds (Kolotelo et al. 2001), but other subtle differences in moisture content and megagametophyte or embryo conditions often

give ambiguous results that are difficult to interpret. Also, radiographs require both very expensive equipment and supplies and about the same amount of time for preparation and interpretation as the cutting test.

Seed Dormancy, Storage, and Germination Test

Seed dormancy

Seed dormancy occurs in most temperate conifers. Dormancy is usually defined as the condition in a plant or plant structure when it does not grow even though placed under conditions favourable for growth. Seeds of most temperate conifers show different degrees of dormancy and low temperature is the most common dormancy-breaking treatment. Tropical tree seeds usually have no dormancy and seeds germinate soon after seeds fall and become hydrated. However, in most cases water content is high (>30%) and little hydration is needed. Because of this, seeds can only be stored for days or weeks. Some coastal conifer species, such as western hemlock, also show only slight seed dormancy. Western larch has seed dormancy that allows long-term storage of healthy dry seeds at low temperatures. Dormancy is broken in nature by the seeds becoming hydrated and receiving cold treatment over several months in the fall and winter on the forest floor.

Seed dormancy can be expressed as *physiological dormancy* and *physical dormancy* (Kolotelo 1997). Seeds of some conifers may have both. The most common type of dormancy in conifers is physical dormancy, also called seed-coat or external dormancy, which is caused by layers of the seed coat or megaspore wall restricting the entry of water and oxygen required for hydration and growth. Stratification helps to break down the seed coat, allowing entry of water and oxygen. Physiological dormancy is not well understood for conifer seeds. This is often called embryo or internal dormancy but no doubt results from an interaction between the embryo, megagametophyte, and environmental conditions. Some authors consider there to be several additional types of seed dormancy and that seeds of some species may possess several types. The literature on the subject is extensive and a recent review, such as that by Bonner (2004), is recommended if research or extensive germination trials are to be done. Most reforestation, seed, and seed orchard workers are primarily interested in the technical aspects of seed handling including collection, storage, and germination.

Seed storage

Seed storage is not difficult for healthy temperate conifer seeds that have dormancy. Healthy, dewinged, and cleaned western larch seeds may be stored for many years if dried to 5–10% water content and placed in air-tight containers (sealed plastic bags from which most air has been removed and placed in cardboard boxes) at -18°C. Under these conditions western larch seeds gained about 0.2% of water content per year. This is about twice the rate of most other conifer seeds, thus the water content of these seeds should be retested every 10 years (Kolotelo et al. 2001).

Seed germination testing

Seed germination testing is the most effective method for determining seed quality and estimating the number of seedlings that can be obtained from a given amount of seeds. Standard germination tests are based on four replicates of 100 seeds that have been pretreated using the best protocol for the species. The pretreatments for temperate conifers consist of soaking seeds in water in a vial or covered dish followed by cold stratification. For western larch, seeds are soaked for 24 h at room temperature in covered plastic germination dishes, about 15 cm^2, in which a layer of paper towel or lighter laboratory tissue paper is cut to fit the base of the container. About 50 ml of water is added to saturate the paper and excess water is drained off. Each replicate of 100 soaked seeds is blotted dry, spread evenly in the germination dish, and then placed in a refrigerator for stratification for 21 days at +2 to +5°C. After stratification, germination dishes are placed directly into a germination cabinet set at 20–30°C for 21 days. Counts of germinated seeds are

done three times per week (Monday, Wednesday, Friday). A seed is considered germinated when its radicle (embryonic or primary root) is four times the length of the seed. Germinants are counted, then removed from the germination box. Abnormal germinants are recorded but are considered to be non-germinants. Abnormalities may include aborted radicles, stunted hypocotyls, and seeds in which the hypocotyl has emerged before the radicle. Details of protocols and other useful information are given in Edwards (1986), Kolotelo (1997), and Kolotelo et al. (2001).

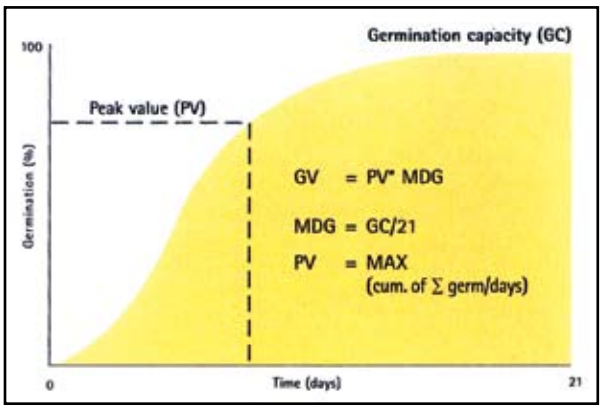


Figure 99. *Graphical representation of germination capacity (GC), peak value (PV), and germination value (GV) (from Kolotelo et al. 2001).*

The germination capacity (GC) is the percentage of seeds that have germinated over time to the end of the test period (21 days) (Fig. 99). The GC may be supplemented by germination value (GV), used to indicate seed vigor, but this is usually not necessary (Wang 1973). The GV is the product of the mean daily germination (MDG) and peak value (PV). MDG is the germination capacity divided by the number of days in the germination test. PV is the point at which the cumulative percent germination divided by the number of days is maximum. For examples of raw germination data and additional graphs of GC, GV, and PV, see Kolotelo (2001).

Cone and Seed Insect Pests and Diseases

Western larch is generally tolerant to most insects and diseases of vegetative structures (Carlson et al. 1995). This generalization also appears to apply to cones and seeds. However, since cones and seeds have not been extensively collected or studied and seed orchards are only now being established, there may be pests and diseases that we do not yet know about in larch. Also, seed orchards may create conditions more suitable for the introduction of cone insects and diseases. Most cone insects and diseases affect more than one conifer species and occur in specific geographic areas (Hedlin et al. 1980; Sutherland et al. 1987). Many seed orchards are located outside the species' natural range to prevent pollen contamination, but this may place a species in an area containing species with which they are not normally associated, thus bringing "new" pests and diseases. These factors, plus the more careful monitoring of larch cones, may result in western larch having more cone and seed insect and disease problems than we currently believe exist, or that have existed in small measure. Consequently, we should be aware of potential problems and closely monitor cone and seed problems in western larch seed orchards. Because of the similarities in cone and seed structure and life cycle between Douglas-fir and larch, problems in Douglas-fir seed orchards may become problems in western larch seed orchards, especially if both species are in the same orchard.

Insects

Information on cone and seed insects is contained in Hedlin et al. (1980) and summarized in Kolotelo et al. (2001) but there is little information specific to western larch. Insects that feed directly on cones and seeds are called conophytes. They may be subdivided into obligate conophytes, which must complete part of their lifecycle within cones or seeds, and hetereconophytes, which are facultative and do not require cones and seeds to feed on but do so when they are available (Turgeon and de Groot 1992).

Potential problem insects may be the Douglas-fir cone moth (*Barbara colfaxiana*), coneworms (*Dioryctria* spp.), the cone gall midge (*Contarinia oregonensis*), and seed wasps (*Megastigmus* spp.), all of which are obligate conophytes. The first two feed on cone tissues and the latter two feed on seeds. The larvae are found in the harvested cones and seeds. Damage from the first two insects is indicated by the caterpillar bore holes and frass on the surface of cones, and frass-filled tunnels within cones that have been sliced in half. Seed insects are detected in seeds using the cutting test or radiographs. It is difficult to identify species of larvae that overwinter in the mature cones and emerge as adults the following spring. Possible heteroconophytes, such as the spruce budworm, may feed on new foliage and young cones in the spring, causing cone damage or cone abortion.

A common problem in seed orchards is the western conifer seed bug (*Leptoglossus occidentalis*), which is an obligate conophyte that may feed on cones of several species. This large brown insect, 1–2 cm long (Fig. 100), has a long proboscis that it inserts into the cone scale and down to the developing seeds.

The proboscis penetrates the seed and the insect sucks out the soft seed contents, causing the megagametophyte to collapse. If feeding occurs before the seed coat is well differentiated, the seed may be indented. Many seeds within a cone can be destroyed by a single adult *Leptoglossus*. Damage is not detectable on the surface of the cone and the damaged seeds are difficult to distinguish from "empty" seeds due to problems arising at pollination.

In seed orchards, spraying insecticides can usually control most insect problems, and keeping the orchard grounds and orchard trees clean of old cones will prevent the emergence of adults of those insects that overwinter in the cones or seeds.

Diseases

Few cone and seed diseases have been reported for larch, and none specifically for western larch (Sutherland et al. 1987). Rusts are a common

Figure 100. *Two adult Leptoglossus (arrows) feeding on a Douglas-fir cone at about the time of fertilization.*

cone disease in spruce and several pines in natural stands and seed orchards. Rusts such as *Chrysomyxa* spp., which infect spruce cones, might also infect larch cones. Rusts have complex lifecycles that involve an alternate host on which other stages of the lifecycle occur. Non-rust fungi, such as *Caloscypha* spp. and *Sirococcus* spp., have simpler lifecycles, most of which occur in the duff on the forest floor. These diseases usually do not affect cones on the trees, but can damage seedlots and commonly spread the disease to the seedlings in the nursery or on the forest floor. Cones that contact the forest floor or are collected from squirrel caches may be infected with pathogens that spread quickly to the seeds and to other cones. If collected cones are stored under cool and moist conditions, the disease can spread quickly to the seeds within the cones and to seeds that have been extracted, ruining seedlots. Fungi may quickly destroy large numbers of seeds or lower the germination percentage. Sirococcus blight has been reported on cones and seeds of some species of larch and on Douglas-fir (Sutherland et al. 1987). Blights such as these can be prevented in seed orchards by removing all cones from the trees and the ground and by carefully handling cones and seeds.

Categories of Seeds and Their Developmental Causes

If seeds are examined using a cutting test or the less precise radiographic technique, other categories of seeds, in addition to full, empty, or insect- or disease-damaged, are evident. A full seed analysis can be done if cones are dried individually and all winged structures are shaken out or carefully pulled out of the cone with forceps. If these seeds are laid out in rows on masking tape attached to a small board, they can be sliced longitudinally with a razor blade, examined using a dissecting microscope (Figs. 96, 101) and placed into one of several categories. This technique was recently applied to western larch from five natural stands and from several ramets pollinated in various ways in a seed orchard in the Inland Empire and for 10 clones from a production seed orchard near Vernon, B.C. (Owens and Kittirat 2004). When this information is related to studies of pollination and seed development of western larch (Owens and Molder 1979a; Owens et al. 1994), many of the causes can be determined for the different categories of seed structures. Many causes originate at pollination but may not be expressed until later in the reproductive cycle. Seeds that appear normal (Fig. 95) are often found to be empty (Fig. 101), damaged, or aborted when sliced.

Generally, seeds may be categorized as filled (Figs. 96, 97), partially developed (Fig. 96), empty (Fig. 101), or rudimentary (Fig. 101). In addition, there are wings with no seed attached and seeds damaged or destroyed by insect and disease. Different categories of insect- and disease-damaged or destroyed seeds are illustrated and described by Kolotelo et al. (2001). Insect damage may result from eggs deposited within the cones at pollination. Some of these may be taken into the ovule with pollen, while others may hatch near the cone axis, inside the cone. The larvae then bore into the cone axis, scales, or seeds. Many insects lay their eggs on or near the ovules and these eggs may be engulfed with the pollen. Engulfed insect eggs hatch and the larvae mature as the megagametophyte and seed coat develop. The larva then feeds on the megagametophyte tissue. As a result, many insect-infested seeds appear normal. Spores of fungal pathogens carried by wind may be taken into the cone or ovule with the pollen while other spores infect the surface of cones (rusts) at other times in the reproductive cycle. Pathogens that enter and infect seeds during development may cause discolouration, rotting, or abortion of the developing embryo or megagametophyte. Large numbers of microorganisms were found in Douglas-fir cones at pollination (Colangeli et al. 1990).

It is common to find seed wings with no ovule or seed attached near the base and tip of the cone and scattered throughout the rest of the cone. These usually are not shed from the cone but can be removed with forceps. They form because the tiny ovule primordia present at pollination stimulated a seed wing to develop but the ovule stopped development very early and only a small wing remained. Or wings may be nearly fully formed and bear small, rudimentary, ovules (Fig. 101). These form because, although ovules were present at pollination, they aborted at about that time, and the wing continued to develop from the scale. Other small ovules abort soon after pollination and form slightly larger aborted seeds. These may or may not have been pollinated and the exact cause of the abortion is uncertain. Pollinated ovules may abort before fertilization because of low pollen vigor or early self-incompatibility.

Other seeds may abort soon after fertilization. In larch, the megagametophyte develops even if the ovule has not been pollinated with fertile pollen or has been self-pollinated. In both cases, the seed coat is well developed but the mature megagametophyte aborts soon after fertilization and degenerates into a collapsed brown sac. These are commonly called "empty seeds" because they are full-size and have well-developed seed coats and wings (Fig. 101). Unlike pines, a larch ovule does not have to be pollinated to develop a seed coat and a normal megagametophyte. Therefore, in larch it is impossible to distinguish empty seeds

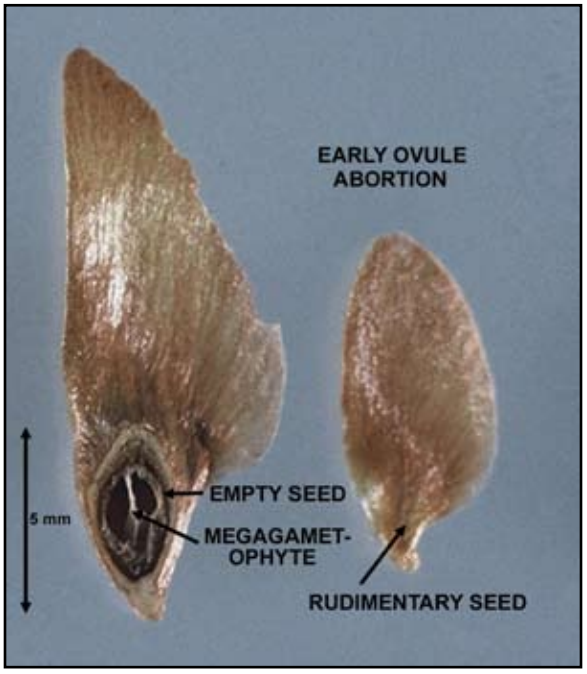


Figure 101. *On the right is a rudimentary seed that aborted early, at about or soon after pollination The seed coat is poorly developed and the seed contents have aborted. On the left is a seed that aborted at or soon after fertilization as a result of no pollination, being self-pollinated, or pollinated by pollen of low vigor. The seed is full-size with a well-developed seed coat but a brown, degenerated megagametophyte.*

that have resulted from unsuccessful pollination, pollen of low fertility, or self-pollination.

Self-pollination in conifers can cause poor pollen germination or slow pollen-tube growth resulting in no fertilization. This is referred to as early (pre-fertilization or pre-zygotic) incompatibility. Alternatively, self-pollination may result in pollen germination, pollen-tube formation, and fertilization, but abortion occurs at the proembryo or early embryo stage. In both cases, for many conifers including larch, the fully developed megagametophyte then aborts, leaving an empty seed that is not distinguishable from a seed that developed from an unpollinated ovule (Fig. 101). In other self-pollinated ovules of larch, the embryo may develop to the mid- to late stage, then aborts or embryo development is arrested. These seeds contain a shrunken but normal-coloured megagametophyte (Fig. 96) in which there is a small degenerated embryo or an embryo that fills only part of the megagametophyte (Fig. 98). This is sometimes referred to as self-inviability and results from post-zygotic events. Species vary in their ability to produce viable self-pollinated seeds. Pioneer species, such as larch, are generally self-compatible. A normal viable seed has a well-developed embryo that fills about 90% of the healthy megagametophyte and fits tightly within the seed coat. In hydrated mature seeds, the embryo fits tightly within the corrosion cavity (Figs. 86, 97). However, as the seed dehydrates, the embryo fits more loosely within the megagametophyte (Fig. 87).

Cone and Seed Production in Natural Stands

Western larch may start to form seed cones at 8–10 years of age, and the formation of pollen cones will begin a few years later, but abundant cone production usually does not begin before age 25. After 45–50 years of age, trees may bear abundant cones until they are 400–500 years old (Boe 1958). Western larch is considered to be a good seed producer in the Inland Empire. However, intervals between good cone crops are variable in different regions. Intervals of 10–14 years commonly occur in northern Idaho and eastern Washington. In Montana, intervals of 7–10 years are more common. Moderate cone crops may occur in the intervening years.

Good cone crops may occur in successive years if conditions are favourable. This occurs because the cones are usually rather scattered along long-shoots and form from short-shoots that are one or more years old. Therefore, the position of potential cone buds is far removed from rapidly growing long-shoot buds, which may compete for nutrients and growth regulators at the critical time of cone-bud differentiation, as occurs in many other Pinaceae (Owens and Blake 1985). It has been estimated that in about half of the years there will be enough seeds produced for the desired planting. Over 1.2 million seeds per hectare (500,000 per acre) have been measured in areas adjacent to timber edges of mature trees (Shearer 1959).

Some areas in northern Idaho and central Oregon and Washington have had several successive poor western larch crops resulting in regeneration failures. These have been attributed to late season frosts in the spring, which cause damage to pollen cones and seed cones at pollination. Cone production is strongly related to crown size of healthy mature trees because cones occur throughout the crown (Schmidt and Shearer 1995), especially in open stands. However, filled-seed production per cone is variable. Some reports indicate that larch is one of the best seed producers in some areas of the Mountain West (Schmidt and Shearer 1995). In contrast, results from a developmental study in British Columbia indicated that western larch produced few filled seeds per cone, primarily due to poor pollen quality (a high proportion of non-viable pollen) and inadequate pollen for pollination (Owens and Molder 1979c). An anatomical study of factors affecting seed yields in four *Larix* species concluded that, in general, *Larix* has a poor seed yield and poor seed quality. In that study, poor seed production was attributed to two factors: cone abortion at the pre-fertilization stages of cone development resulting in a loss of cones; and, embryo degeneration at the post fertilization stages of development resulting in a loss of filled seed (Shin and Karnosky 1995). Insect damage to cones may significantly reduce filled seed production in some natural stands (i.e., at higher elevations, in years when there are few cones). Years with good to heavy cone production will still have adequate numbers of seeds produced but insects may destroy the entire crop in poor cone years or on specific sites.

Seed production from mature western larch cones was studied for five or more trees from each of five natural stands in the Inland Empire (Owens and Kittirat 2004). Data were collected on cone size, cone weight, total cone scales, sterile cone scales (those lacking any winged structure), fertile cone scales (those having winged structures), seed potential (fertile scales ×2), filled seeds, empty seeds, early seed abortion, late seed abortion, insect or disease damaged seeds, and seed efficiency (SEF or the number of filled seeds/seed potential). Unfortunately, the number of trees per site and number of cones per tree varied and all cones from a tree were placed in one paper bag that was stapled shut. As a result, the cones shed their seeds into a common mix in the bag and numbers and types of seeds could not be traced to a particular cone. This resulted in variable sample sizes and the loss of information on among-cone variation within a tree. In the Inland Empire, the five sites were Plum Creek, IEPC, Goosmus, BCC, and an unnamed high-elevation site (Table 1). (See Appendix 6 for detailed methods.)

Cone length averaged 30 mm and cone weight, 2.8 g, but many of the seeds had been shed. Total scales averaged 62; sterile scales, 16; and fertile scales, 46. Seed potential averaged 90; filled seeds, 38; empty seeds, 45; early-aborted seeds, 3; late-aborted seeds, 6; insect- or disease-damaged seeds, 4 (1–10), and rudimentary seeds, 3. The seed efficiency (SEF) was 42%, meaning that this percentage of the fertile ovules were successfully pollinated, fertilized, and developed into viable seeds (Table 1, Fig. 102).

Although cone size varied considerably, this was usually tree-specific and often resulted from insect damage to cones during early development. In general, larger cones contained more filled seeds and had higher SEF, thus there is an advantage to collecting large cones. Many insect-damaged cones had few or no seeds left to analyze and data could not be collected. This result means that the importance of insects is greater than these data indicate and was very site and tree specific. Early- and late-seed abortion rates indicate that there were not high rates of self-pollination or that the species is self-compatible. About 50% of the potential seeds were empty, which could result from ovules not being pollinated (no pollen engulfed); early embryo abortion as a result of self-pollination; or damage from the western conifer seed bug, *Leptoglossus*. The last reason can only be determined by an anatomical study, which was not done. For this one year at these five sites, filled seed production per cone was high for

Site	No. trees	No. cones	Cone length cm	Cone weight gm	Total scales	Sterile scales	Fertile scales	Seed potential	Filled seeds	Empty seeds	Rudi-mentary	Early abortion	Late abortion	Insect/ disease	Total seeds	SEF %
PC	5	50	3.0	2.6	62	12	50	99	47	43	2.1	2.0	6	3.0	103	52
IEPC	5	50	3.4	3.1	73	9	64	127	64	61	8.8	9.0	11	10.0	164	51
Goos	5	25	2.8	2.0	60	25	35	69	25	37	0.4	0.4	5	1.4	69	32
BCC	9	47	2.7	3.2	56	14	42	76	31	39	1.7	2.0	3	1.0	78	42
HE	5	70	3.1	3.0	59	20	39	77	23	43	2.0	2.0	4	5.0	79	31
Total	29	242														
Avg			3.00	2.78	62.0	16.0	46.0	89.6	38.0	44.6	3.00	3.08	5.8	4.08	99.0	41.6
SD			0.27	0.49	6.5	6.4	11.5	23.7	17.3	9.5	3.31	3.38	3.1	3.66	38.7	10.0

Table 1. *Cone and seed analysis of 29 trees from five western larch sites in the Inland Empire in 2003.*

a member of the Pinaceae. Not surprisingly, the high-elevation site had the lowest filled seeds per cone and lowest SEF, but still produced an average of over 20 filled seeds per cone. Sites having the highest filled seeds per cone and SEF were IEPC and Plum Creek. Based on this information, recommendations for cone collection are (1) collect cones from trees that are fairly open-grown or at the edge of a stand; (2) collect cones from trees that have large crowns in which good cross-pollinations may occur; (3) collect large cones; (4) do not collect cones that show any external signs of insect damage; and (5) avoid collecting cones from wild stands that have a low component of western larch (B. Jaquish, pers. comm.).

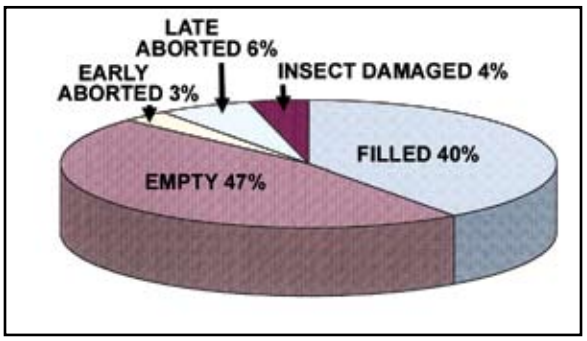


Figure 102. *Chart of western larch cone and seed analysis for five natural stands in the Inland Empire in 2003. See Table 1 for details of seed categories.*

Cone and Seed Production in Seed Orchards

Western larch genetics research and tree improvement programs began in the United States in the mid-1970s and in British Columbia in 1987. A western larch clonal seed orchard, which was established near Vernon, B.C., in 1987, began to produce some seed cones within about six years (Fig. 103). A few seed orchards have been established in the United States in the last 10 years (Jaquish et al. 1995). Some of these orchards are now coming into cone production (Fig. 104) but there have been no published data on production of cones and seeds per cone.

Figure 103. *Six-year-old clonal seed orchard near Vernon, B.C., which has just begun producing cones.*

Figure 104. *The same seed orchard as in Figure 103 at 13 years of age. It is producing abundant cones and about 55 filled seeds per cone.*

Several attempts at cone induction followed by small studies of cone and seed production in potted grafted clones in the United States and British Columbia have produced very mixed results (Webber and Ross 1995). Also, seeds per cone from potted, cone-induced western larch generally have been very low (10–20 seeds per cone), and the SEF with multiple control pollinations was about 28% (Owens et al. 1994).

In a well-managed seed orchard, where water and soil fertility may be optimized and insects and disease largely controlled, many cones may form on the young trees. Over-production of cones on a branch may occur and result in abortion of some cones soon after pollination, as cones are becoming pendant (Fig. 105). Also, in young and some older trees, the seed cones that differentiate on short-shoots may form a vegetative bud at the tip before they become dormant. These may remain as buds at the tip of the developing cone or they may burst and elongate following pollination (Fig. 105). These are proliferated cones and the vegetative buds or shoots at the tip of the cone do not make the cone less fertile than normal cones — the buds or shoots die as the cone matures and dries. Proliferated cones may be collected, since they produce viable seeds.

A study of western larch cone and seed production was conducted in 2003 in a clonal seed orchard near Vernon, B.C. This orchard was established in 1987 and is now coming into full production (Fig. 104). Five mature cones were sampled from each of 10 clones. In the field, each cone was placed in an inverted position in a stamp envelope; the envelope was sealed and labelled and the five envelopes were placed in a sealed and labelled paper bag. The cones dried and opened within the envelopes. The five cones from each clone were analyzed separately using the same procedures as described in the methods for trees in natural stands, except that for the clones, all seeds from a cone were analyzed (see Appendix 6).

Clone	Cone length cm	Cone weight gm	Total scales	Sterile scales	Fertile scales	Seed potential	Filled seeds	Empty seeds	Rudi-mentary	Early abortion	Late abortion	Insect/ disease	Total seeds	SEF %
A	4.2	4.5	73	8	64	127	60	65	0.0	0	2	0.0	127	47
B	3.6	3.8	79	6	74	148	50	96	0.4	0.2	2	0.2	147	34
C	4.0	4.2	74	5	69	138	74	58	0.2	0.2	6	0.0	133	54
D	3.9	4.2	69	10	56	112	63	39	0.2	0	10	0.4	113	56
E	3.4	3.1	68	4	61	121	43	77	0.4	0.4	1	0.0	121	30
F	3.4	3.0	72	8	115	129	90	29	0.0	1.8	8	0.2	129	70
G	3.0	2.3	59	10	53	106	51	46	1.2	0.4	8	0.0	107	48
H	3.6	3.3	73	2	71	141	34	104	1.8	0	3	0.0	143	24
I	2.7	2.2	64	10	53	106	16	88	0.0	1.2	1	0.0	106	15
J	3.5	3.2	62	9	52	103	62	31	0.0	0	10	0.2	103	60
Avg.	3.53	3.38	69.3	7.2	66.8	123.1	54.3	63.3	0.42	0.42	5.1	0.10	122.9	43.8
SD	0.45	0.79	6.1	2.8	18.7	16.1	20.7	27.2	0.61	0.61	3.7	0.14	15.6	17.4

Table 2. *Cone and seed analysis of five cones from each of ten western larch clones at Kalamalka Seed Orchard in 2003.*

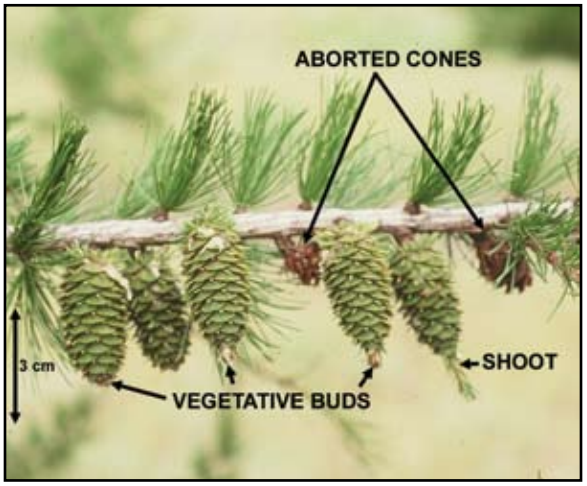


Figure 105. *Shoot on a young western larch seed orchard tree showing seed cones before fertilization, two seed cones that aborted before fertilization, and several seed cones that have proliferated and bear vegetative long-shoot buds or long-shoots at the tip of the cones.*

Cone length averaged 35 mm and cone weight, 3.4 g. Total scales averaged 69, sterile scales averaged 7, and fertile scales averaged 67. The seed potential averaged 123; filled seeds per cone, 54; empty seeds, 63; early aborted seeds, 0.4; late aborted seeds, 5; insect- and disease-damaged seeds, 0.1; and rudimentary seeds, 0.4. The SEF averaged 44% (Table 2, Fig. 106).

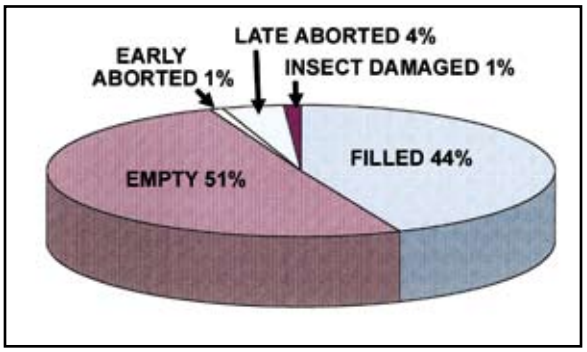


Figure 106. *Chart of western larch cone and seed analysis for five cones from each of ten clones at the Kalamalka Seed Orchard near Vernon, B.C., in 2003. See Table 2 for details of seed categories.*

With few exceptions, cone size and total scales varied clonally and there was little insect damage or disease that could cause cones to remain small. As in natural stands, larger cones (>4 cm long) contained more filled seeds (commonly >70 per cone) and fewer empty seeds. Very low rates of early abortion (<1 per cone) and late abortion (5 per cone) indicated that the level of self-pollination was not high. However, the fairly high level of empty seeds could result from lack of pollination, pre-fertilization, early post-fertilization incompatibility, or feeding by *Leptoglossus*. *Leptoglossus* feeding usually results in somewhat flattened or indented seeds (Bates et al. 2000; Kolotelo et al. 2001), but such seeds were rare and included in the late abortion category.

Compared with the study of western larch cones from five natural stands, this study indicated that, in a well-managed seed orchard on a suitable site, 10- to 15-year-old western larch grafts produced large numbers of cones (C. Walsh, Kalamalka Seed Orchard, pers. comm.) and filled seeds. Cones averaged about 54 filled seeds per cone without supplemental pollinations (Table 2, Fig. 106), which was much higher than the 38 filled seed per cone produced on mature trees in natural stands.

Unfortunately, not all western larch seed orchards produce abundant cones and abundant high quality seeds. A potted orchard in western Oregon produced less than 10 filled seeds per cone (R. Sniezko, pers. comm.) but a detailed cone and seed analysis was not done. A soil-based clonal orchard in northern Idaho produced many seed cones and a detailed cone and seed analysis was done on 48 cones, 19 of which were enclosed in insect bags after open pollinations and 29 of which were open-pollinated but not enclosed in insect bags. The 48 cones contained no filled seeds, 4546 empty seeds, and 43 rudimentary seeds. The seed analysis (see Appendix 6 for methods) indicated that the empty seeds resulted from a lack of pollination or from self-pollination. Enclosing cones in insect bags did not affect filled-seed production indicating that the cause was not *Leptoglossus*, which was common in the orchard. Slides from pollen monitors showed very few pollen grains (see Figs. 57, 58). Dissections of cones at pollination to determine pollination success (see Appendix 3) indicated that there was very little larch pollen released in the orchard

at the time of seed-cone receptivity. Location is likely the main cause, since the orchard lies in a narrow river valley where humidity is high and rain is very common during seed-cone receptivity. Observations of pollen cones and seed cones at the time of seed-cone receptivity indicated that, although seed cones and pollen cones developed normally, many pollen cones did not dry and release their pollen; those that did released pollen after most seed cones were no longer receptive. This would result in low or no pollination success producing empty seeds. The earlier discussion (see Categories of Seeds and Their Developmental Causes) explained that ovules of larch that are not pollinated develop into full-size but empty seeds.

Seedling Development

Seed germination takes place in two stages. Stage 1 is the time from seed fall or sowing until radicle emergence or emergence from the growing medium. In natural stands this would involve an overwintering period for seeds on the forest floor. Metabolic activity begins when the seed coat imbibes water, which hydrates the embryo and megagametophyte. This hydration activates enzymes and stimulates the formation of new enzymes that cause the conversion (hydrolysis) of the storage products, mostly lipids and proteins, to less complex organic compounds. These compounds can be further hydrolyzed to release energy, provide building blocks for growth, and increase osmotic potential within the cells of the embryo. Hydration causes the megagametophyte and embryo to swell and the seed coat to split open. This splitting is followed by frequent cell divisions within the embryo, mostly in the meristems of the embryo. This is accompanied by cell elongation — first in the radicle, then in the cotyledons and the hypocotyl — resulting in the emergence of the radicle which ends Stage 1. Unfavourable temperature and moisture conditions may delay the emergence of the radicle by a few days.

In Stage 2, the elongation and bending downward of the radicle and elongation of the hypocotyl raise the seed coat that covers the cotyledons above the surface of the medium. This type of germination is called epigeal. As the cotyledons become green and elongate, the seed coat is pushed to the tips of the cotyledons and holds them together. The seed coat is soon shed from the tips of the cotyledons, which then open outward, revealing a small shoot tip at the base of the whorl of cotyledons. Respiration and photosynthesis rapidly increase during this process, cell divisions become more restricted to the root and shoot meristems, and vascular tissues begin to differentiate in the cotyledons, embryonic shoot, hypocotyl, and primary root. At the end of Stage 2 the seedling becomes an independent plant, no longer relying on the stored reserves of the megagametophyte or embryo.

Stages 1 and 2 appear essentially the same in all of the Pinaceae and occur over a few weeks. By the time the cotyledons open, the shoot tip has begun to initiate spirally arranged primary leaf primordia that cover and protect the apex. They elongate as the primary shoot elongates. This process will occur until the middle of the growing season, about late June when the day length starts to decrease, and primary shoot elongation slows and stops.

The shoot apex then initiates a spiral of thick brown bud scales that cover and protect the apex. Within this terminal long-shoot bud, the vegetative apex initiates many thin bud scales, followed by a spiral of preformed leaf primordia before the seedling becomes dormant in the fall. Short-shoot buds may form in the axils of some of the primary leaves. Just below the terminal bud, lateral-long-shoot buds may be initiated. These long- and short-shoot buds will flush in the following spring and continue the cycle of bud and shoot development as described for mature trees (see Vegetative-Bud and Shoot Development and Fig. 16). In seedlings, long- and short-shoot buds are less fixed in development than in mature trees and buds may switch frequently from short-shoot to long-shoot or visa versa.

Summary

Western larch (*Larix occidentalis*) is a pioneer species of the Intermountain West region of North America and one of about 11 *Larix* species worldwide. This deciduous conifer is distributed at moderate to high elevations in southeast British Columbia, the Inland Empire, and Cascade Mountains of Washington and Oregon. Western larch is not a climax species but a seral species commonly moving in after fire or other disturbances. This large pest- and disease-tolerant species has valuable wood properties.

Its reproductive cycle is similar to most other members of the Pinaceae, with the exception of *Pinus*, in that pollination, fertilization, and seed maturation occur in one growing season. In larch, cone buds are initiated from short-shoot buds in the summer before pollination. Pollination occurs in the early spring, fertilization occurs in June, and cones and seeds mature late in the summer. Vegetative shoot growth, in part, determines the position and abundance of cones. Vegetative buds and shoots may be long or short (dwarf). Pollen-cone buds form from short-shoots that are commonly two or more years old and seed-cone buds form from short-shoots that are one or more years old. Vegetative and cone buds can be identified in the fall before pollination and cone crops can be forecasted.

Western larch is a moderate cone producer and a good producer of seeds per cone. Cone production begins at 8–10 years of age, peaks at 40–50 years, and may continue until 400–500 years old. Good quality seeds may be obtained from trees at all of these ages.

Cone induction can be accomplished on young or older trees or ramets in seed orchards using cultural treatments such as girdling, root pruning, and drought. Some or all of these treatments may be applied with stem injections of the plant hormone gibberellin $A_{4/7}$ ($GA_{4/7}$). The effects are commonly synergistic. Treatments must be given during the natural time of cone initiation, which is about mid-May until mid-June in the year before pollination — during the period of early long-shoot elongation (see Fig. 18).

Pollen-cone and seed-cone buds are preformed before winter dormancy, which begins in October and ends in early March. Pollen forms in the spring and is shed about April. Numerical stages are suggested for pollen-cone and seed-cone development, from the end of dormancy until the end of pollination. Seed-cone buds initiate ovules before winter dormancy but the pollination mechanism does not develop until after dormancy. The pollination mechanism is an engulfing mechanism, as in Douglas-fir, and lacks a pollination drop. Optimal stages are suggested for pollen collection and pollination.

Methods for pollen collection, extraction, storage, testing and application, as well as pollination techniques and equipment, are described. Methods are described for measuring the abundance of wind-blown pollen in seed orchards, pollination success in seed cones, and supplemental and control pollinations. There are both benefits and problems associated with supplemental pollinations, blowing pollen, and overhead sprinkling within an orchard.

Seed cones develop quickly following pollination. Pollen grains are taken into the ovule in April and pollen tubes form and fertilization occurs in mid-June. Several genetically different pollen grains may be taken into an ovule and an average of four genetically identical eggs occur within each megagametophyte. Pre-fertilization competition occurs among the genetically different pollen grains and pollen tubes. More than one egg may be fertilized, so post-fertilization competition also may occur among genetically different embryos. There are differences between the nuclear and cytoplasmic genomes. There are also differences in methods of cytoplasmic inheritance among some conifer families, but the pattern appears to be the same within a family. Differences are determined by the structure of sperms and eggs, the process of fertilization, and early embryo development. In larch, as in other Pinaceae, plastids are paternally

inherited and mitochondria are bi-parentally inherited.

Embryo development begins in June and is completed in about two months. Its four phases — proembryo, early embryo, mid-embryo, and embryo maturation — are similar in all the Pinaceae. Simple polyembryony occurs but cleavage polyembryony does not. Seed storage products are mostly lipids, followed by proteins and starch. They accumulate in the embryo but more abundantly in the megagametophyte.

The seed coat develops from the integument and the seed wing from the scale. During maturation, the seeds and wings separate from the scale and the seeds dry to about 10% water content before being shed. Cone opening involves the drying and shrinkage of woody mechanical tissues in the scale. Cones start to turn brown in late August or early September. Cones should be collected before they start to open, which takes place before they have lost 20% of the wet weight. Cones that are collected too early or stored where they will not dry or open properly may become case-hardened and moldy, reducing seed yield and viability.

Seed condition may be determined by seed morphology and anatomy. The cutting test for determining seed condition is recommended and the categories of seeds and the causes for each category are discussed. Physiological but primarily physical seed dormancy occurs in larch and most temperate conifers. Seed dormancy is related to methods of seed storage, testing, and germination. Cone and seed insect pests and diseases are not a major problem in natural stands of western larch but relate to intensive seed orchard management. Standard methods are used for seed extraction, handling, storage, germination, and determination of seed condition.

Two studies have shown that filled seeds per cone were higher (54 seeds per cone and 44% seed efficiency) in a western larch clonal seed orchard in British Columbia than in five natural stands of western larch from the Inland Empire (38 seeds per cone and 42% seed efficiency). These numbers demonstrate that western larch is a good seed producer in natural stands and can be a very good seed producer in well-managed clonal seed orchards. Several seed categories are illustrated and described and some of the possible causes for each category are discussed.

Seed germination is similar to other Pinaceae but early seedling development is different in larch because of the presence of long and short-shoots.

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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

Anemophily: wind pollination

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

Archegonial polyembryony: simple polembryony, when more than one egg, each within a separate archegonium within a megagametophyte, may be fertilized and develop into an embryo

Archegonium: multi-cellular haploid female sex organ that produces the egg

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

Basal: toward the base of a structure

Bract: modified leaf in a seed cone

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

Cone induction: causing cone production in trees that are reproductively mature and have not previously borne cones

Conelet: the young seed cone at pollination; often incorrectly called a "flower"

Conophyte: an insect pest that feeds on cones or seeds

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

Cortex: the portion of the embryo hypocotyl outside the vascular tissue

Cotyledon: embryonic leaf formed on the embryo

Crown region: a nodal diaphragm that extends across the pith at the base of a bud separating tissues from one year's growth from the following year's growth

Cytochemical: microscopic chemical tests for compounds in cells or tissues

Deciduous: the shedding of a structure from a plant. In larch it refers to the shedding of all the leaves each autumn

Determination: the 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

Dichogamy: the temporal separation of sexes

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

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

Distal: toward the tip of a structure

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

Dwarf shoot: (short shoot) a vegetative shoot that only elongates a few millimetres per year and bears many leaves in larch

Embryo: young sporophyte plant contained within the seed that results from fertilization of the female gamete

Embryogeny: development of an embryo

Empty seed: seed in which the contents have almost completely degenerated

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

Exine: outer resistant layer of a pollen grain or megasporophyte

Female gamete: haploid (1N) egg

Female gametophyte: haploid (1N) multi-cellular phase in the life cycle that produces the egg and is contained within the ovule

Fertile: capable of fertilization

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

Gibberellins: a large group of naturally occurring plant growth substances (hormones) some of which are used to induce or enhance cone production

Heteroconophyte: insect pests that do not require cones or seeds to feed upon but do when they are available

Homogamy: pollen being shed when seed cones are receptive

Hormone: a naturally occurring plant growth regulator that occurs in plant tissues and affects growth and development

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

Incompatibility: biochemical, physiological, or developmental differences that prevent pollen germination or pollen-tube growth, fertilization, embryo, or seed development

Initiation: earliest stages of the origin and 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 grain that forms the pollen tube

Lamina: the broad blade portion of a leaf or bract.

Lipid bodies: small droplets of lipid (fat or oil) in the cell that serve as stored food

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

Long shoot: a lateral branch or terminal shoot that elongates and bears many leaves along the axis

Long-shoot bud: a bud that develops into a long shoot

Male gamete: haploid (1N) sperm

Megagametophyte: the haploid (1N) female gametophyte that develops within an ovule

Megaspore: a haploid (1N) cell in the young ovule that results from meiosis of the megaspore mother cell

Megaspore cell wall: the thick cell wall that forms around the megaspore and the megagametophyte

Megaspore membrane: see megaspore cell wall

Megaspore mother cell (MMC): a large diploid (2N) cell within the young ovule that undergoes meiosis to form the megaspores

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

Micropylar: the end of the seed nearest the cone axis where the micropyle is located

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

Microspore: haploid (1N) cell that results from meiosis and develops into a pollen grain

Microsporocytes: (microspore mother cells) diploid (2N) cells in the microsporangia that undergo meiosis; each forms four haploid (N) microspores

Microsporophyll: modified leaf that bears the microsporangia

Mitochondria: within the cell, small organelles that produce energy

Mitosis: the type of nuclear division that duplicates and separates chromosomes such that each of the two daughter nuclei carry a chromosome complement identical to that of the parent cell

Nucellus: the inner tissues of an ovule and seed that contain the megagametophyte

Organelles: several kinds of microscopic structures within living cells that have specific functions such as storage or synthesis of cellular materials

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

Ovuliferous scales: the scales in seed cones that bear the ovules

Pericolumn: a cylinder of meristematic cells of the embryonic root that make up part of the embryonic stele (vascular cylinder) of the root

Phenology: science that relates periodic biological phenomena to climate, especially seasonal changes

Physical dormancy: seed-coat or external dormancy caused by layers of the seed coat or megaspore wall restricting the entry of water and oxygen

Physiological dormancy: embryo or internal dormancy caused by physiological changes required in the embryo or megagametophyte before growth can begin

Pith: the central core of cells in the hypocotyl of the embryo and stems of a branch or tree

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

Plastid: a type of organelle within a living cell that synthesizes and stores cellular products or pigments

Pollen: haploid (1N) male gametophyte

Pollen cone: the strobilus or male cone that produces pollen

Pollen mother cells: (microsporocytes) diploid (2N) cells that undergo meiosis in the microsporangia and each forms four haploid (N) microspores

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

Pollination drop: the watery secretion produced by the ovule that is exuded from the micropyle where it may pick up pollen and assist in taking pollen into the ovule

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

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

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

Post-zygotic: the stages of development following fertilization

Pre-zygotic: the stages of development before fertilization

Primordium: the earliest development stages of a structure such as a leaf, bract, scale, or ovule

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

Protandry: pollen being shed before seed cones are receptive

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

Protogyny: seed cones being receptive before pollen is shed

Radicle: the embryonic root of the embryo

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

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

Root initials: the meristematic tip (apex) of the root

Sarcotesta: the outermost of the three layers of the seed coat

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

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

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

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

Seed condition: the physical or physiological condition of the seeds based on seed morphology and anatomy

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

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

Seed wing: the thin wing that develops from the ovuliferous scale and is attached to the seed aiding in seed dispersal

Seed yield: the amount of seed produced

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

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

Self-incompatibility: mechanisms that prevent pollen from fertilizing an egg having the same genetic makeup or prevent the embryo resulting from self-fertilization from fully developing

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

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

Short shoot: (dwarf shoot) a vegetative shoot that only elongates a few millimetres per year and bears many leaves

Short-shoot bud: a bud that contains a vegetative short shoot

Simple polyembryony: when more than one egg, each within a separate archegonium within a megagametophyte, is fertilized and each may develop into an embryo

Sperm: the male gamete

Sporogenous: cells that are able to produce spores by meiosis, such as megaspores or microspores

Sporopollenin: a complex and highly resistant substance that forms part of the outer wall (exine) of a pollen grain

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

Strobilus: the simple pollen cone or compound seed cone

Supplemental mass pollination (SMP): extra pollen applied to a tree or seed orchard during the pollination period

Syngamy: the fusion of the sperm and egg

Tapetum: the layer of cells lining the microsporangium and affecting pollen development

Testa: the seed coat that develops from the integument

Thecal fluid: the fluid released from the tapetum into the pollen chambers of microsporophylls in which the pollen grains develop.

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

Vacuole: the organelles within plant cells that store water, food, pigments, and secretory or waste materials

Viable: alive and capable of growth

Zygote: the fertilized egg

Sources of Illustrations

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New or redrawn figures: Figs. 4, 29, 37, 64, 78

B. Jaquish: Figs. 3A, 5A, 5B, 25, 100

Kolotelo 1997: Figs. 87, 94

Kolotelo et al. 2001: Figs. 91, 96

Owens and Molder 1979a: Figs. 10, 12, 15, 16, 17

Owens and Molder 1979b: Figs. 18, 20

Owens and Molder 1979c: Figs. 30, 31, 34, 35, 50, 53, 54, 65, 66, 68, 69, 72, 80, 82, 83

Owens et al. 1994: Figs. 59, 60A, 60B, 60C, 74, 84, 95

Owens 1995: Fig. 6

Owens et al. 1998: Fig. 48

Appendix 1. Cone Induction

Most of the details for cone induction have been described in the text, including examples of trials using European, Japanese, and western larch. Some of these references should be consulted before large-scale trials are attempted (Bonnet-Masembert 1982; Phillipson 1996; Phillipson et al. 1997; Ross 1991; Webber and Ross 1995). It is important to consider the time of the treatments and this should not be done by calendar date but by the time of long-shoot bud flush and the degree of shoot elongation (Figs. 17, 18).

Gibberellin $A_{4/7}$ may be ordered in powder form from scientific supply firms as may the solvents, wetting agents, syringes, etc. used for the stem injections.

Below is a table (modified from Ross 1991) that lists the recommended number of injection holes and total amount of $GA_{4/7}$ (mg)/tree that should be injected for western larch having various DBH.

Tree DBH (mm)	Injection holes per tree	$GA_{4/7}$(mg)
16–20	4	240
21–25	5	300
26–30	6	360
31–35	7	420
36–40	8	480

Methods that have been commonly used for larch are described in detail in the text and illustrations of girdling (Fig. 25) and GA injection (Figs. 26A-D) are shown.

Appendix 2. Monitoring Pollen Development

Monitoring the stages of meiosis and pollen development in conifers is relatively simple and requires only an inexpensive compound microscope and simple dissecting tools. The stages of development are illustrated in Figs. 29A-H. Pollen cones may be sampled from branches, moistened, placed in plastic bags, and taken in a cooler to the lab. There, each cone is placed on a clean microscope slide and, using a sharp scalpel (pointed No. 11 blade), 2–3 green microsporophylls bearing yellow microsporangia are removed from the cone (Figs. 39, 40). This may be more easily done by first slicing the cone in half. Three or four microsporangia are then squashed on the slide using the flat surface of a razor blade or scalpel blade. The milky thecal fluid that contains the pollen comes out. Microsporangial walls and microsporophylls can be removed from the slide with a needle leaving a small amount of microsporangial contents on the slide. A drop of aceto-carmine is placed over material and a coverslip placed on the drop. The slide is then gently heated over an alcohol burner (warmed but not boiled) for a few minutes. Boiling precipitates the stain and ruins the slide. The slide is then placed on a flat surface and a piece of folded paper towel placed over the cover slip. Using your thumb, press firmly down on the towel to squash the cells in the specimen. Let the specimen stand for a few minutes and gently heat again.

Observe the specimen using the compound microscope. Pollen mother cells are large with large nuclei and thin walls (Figs. 28, 29B). Tetrads following meiosis show four angular cells within a cell wall (Figs. 29C, 30). Separate microspores are crescent-shaped at first (Figs. 29D, 31) but become round but smaller than pollen mother cells and have a thickened wall. Cell divisions occur within the pollen 2–3 weeks after meiosis (Figs. 29E-H, 32).

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

Appendix 3. Determining Pollination Success (PS)

The amount of pollen available in an orchard is commonly monitored using pollen monitors (Webber 1991). These usually give a good indication of how well the cones are pollinated. However, for breeding techniques and supplemental pollinations, individual cones may need to be sampled to determine how much pollen is actually in the cone. Pollination success is a measure of the number of pollen grains on the ovules of a cone. The method is simple and quick. A sample of cones at pollination can be sliced longitudinally down the centre and the two halves observed using a dissecting microscope (see Fig. 60). An alternative that works well for larch and Douglas-fir is to slice off the bottom one-third of the conelet at pollination. Then, using forceps, carefully pull down on the edge of the ovuliferous scale until it pulls off the cone axis. Place the ovuliferous scale on a microscope slide with the ovules facing upward and observe the stigmatic tips of the ovules. You may find it easier to remove the bract and scale together as a bract–scale complex (Figs. 49, 50). On a sample of 10 stigmatic tips, count the number of pollen grains on each. The pollen counted is that on the stigmatic tip and not on other surfaces of the ovule or ovuliferous scale. As a general rule, if there is an average of five pollen per stigmatic tip or less, the cone has been inadequately pollinated and SMP is necessary, otherwise seed set may be low; 5–10 pollen per ovule is satisfactory but SMP might increase seed set. A count of over 10 pollen grains per ovule is high and SMP is not needed. With natural wind pollination most ovules usually receive some pollen. However, using SMP, pollen may be applied infrequently, too early or too late, from one direction, or for a short time, and many ovules may receive few or no pollen grains.

More important than Pollen On (PO) the ovule is Pollen In (PI) the ovule — the number of pollen grains actually taken into the micropyle. However, counts of PI require careful slicing of tiny ovule tips to see the pollen taken in and this is not a practical method for monitoring pollination in western larch. Counting Pollen On is easy and quick but it is difficult to make counts of Pollen In. It is usually adequate to count the proportion of pollinated ovules from sliced or dissected seed cones and not try to determine the number of pollen grain in each ovule.

Appendix 4. Pollen Extraction

The methods vary depending on whether only small amounts of pollen are needed from specific clones or individuals for breeding or experiment, or large amounts of pollen are needed for supplemental pollinations. Several articles and manuals have been written on the subject and the most relevant for larch is that by Webber and Painter (1996) which discusses all of the current methods used for Douglas-fir. Since Douglas-fir pollen grains and seed cones are similar to that of larch, extraction methods are also similar, except that the scattered distribution of larch pollen cones makes collection of large quantities of pollen cones at the appropriate stages very difficult. Copes (1991) has developed and described a vacuum pollen collection mechanism with which pollen is collected directly from the trees. This avoids the tedious collection of pollen cones in larch but is not suitable for breeding and many experimental purposes because the pollen obtained may be contaminated with pollen from many sources other than the tree from which the collections were made. If pure pollen from specific clones or individuals is needed, then small numbers of pollen cones may be collected at stages 3–5 and dried in a small pollen drier or on a table where temperature, humidity, and air currents can be controlled to some extent. Drying and shedding may take 2–3 days, then pollen should be coarsely screened to remove pollen cones and debris and then finely screened (nylon stockings work well) to remove the fine debris. Pollen water content should be reduced to below 10% and this may be accomplished in dry regions simply by leaving the pollen in a thin layer exposed to the air. Various types of drying columns have been developed using converted air conditioners and columns or compressed air and desiccants in columns to precisely control water content for large quantities of pollen (Webber and Painter 1996). The text contains some additional information, but the original references discussed there should be consulted before attempting to construct or use a large-scale pollen extractor.

Appendix 5. Pollen Germination Test

For breeding and SMP it is necessary to collect, store, and test pollen. Several techniques are described in detail by Webber (1991), Webber and Bonnet-Masembert (1989), and Webber and Painter 1996).

The text briefly describes three methods: the germination test, conductivity, and respiration or oxygen uptake. The last two require equipment not usually available at a seed orchard and the original reference should be consulted (Webber 1991) for details if these methods are going to be used. Pollen germination is quick and easy and requires some simple glassware and an inexpensive dissecting microscope. This method also requires very small pollen samples that may be critical for breeders. The drawback, as mentioned in detail in the text, is that larch pollen does not form a pollen tube immediately after germinating as does pine pollen. Therefore, germination must be based on more subtle swelling of pollen and appearance of dead as opposed to live pollen (see Figs. 62A-C).The method involves placing a small sample (a few hundred pollen grains) in a germination medium, then placing a drop of the pollen–medium solution on a microscope slide and observing the pollen using a microscope.

The basic medium is modified from Brewbaker's medium (Brewbaker and Kwack 1963). The Brewbaker stock solution consists of the following dissolved in 100 ml of distilled water.

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

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

Hydrogen peroxide	1 ml
Brewbaker's solution	30 ml
Sucrose	10–15 g

Dispense about 5 ml of this solution into a vial or small flask and add a few hundred pollen grains (the exact amount is not critical). Cover the flask with foil and incubate it 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 produce a lot of water mold that will inhibit pollen germination and obscure pollen structure. If incubation is for about 48 h or less the hydrogen peroxide may be omitted. Reducing the sugar to 10% or replacing it with 20% polyethylene glycol also may reduce fungal growth (Webber and Painter 1996). After 48 hours of incubation, shake each sample and then remove a few drops of the pollen-medium and place it on a microscope slide and cover it 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 has swollen, split, or shed the exine (Fig. 62C). Do four replications of each germination sample. Fresh, high quality pollen should have over 90% germination.

If you wish to observe the pollen more carefully, a drop of aceto-carmine or cotton blue stain may be added to the pollen after it has germinated. The stain will stain and kill the pollen.

Do not leave the pollen in the medium too long waiting for a pollen tube to form. This may take several weeks and the fungal growth will obscure and kill the pollen. To observe pollen tubes in larch, you must use the detailed methods described by Fernando et al. (1997).

Appendix 6. Determining Reproductive Potential (RP) and Reproductive Success (RS)

Reproductive potential (RP) is a function of the initial number of cones per tree at pollination and the potential number of seeds per cone (SP). The Reproductive Success (RS) is a function of the final number of cones per tree and the filled seeds (FS) per cone.

To determine how well an orchard, clone, family or a tree within the orchard is performing, it is necessary to make some detailed counts of cones at different stages of development and an analysis of seeds within the cones that mature. This may be done using a sample of flagged branches on selected trees. The number of branches and cones should be fairly large (~50 cones per 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 or desirable to make cone counts of entire trees because the numbers of cones may be huge and impossible to accurately count. Sample branches should be flagged and be representative of the crown or the particular portion of the crown that is of interest (lower, higher, etc.). Branches should be labelled with metal tags because flagging tape may be lost. To obtain the most information, cones should be counted at three stages of development: 1. At pollination, to determine the initial number of cones; 2. About two weeks after pollination, to determine the number of cones lost (aborted), due to factors such as frost during or soon after pollination; 3. At cone maturity but 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 equal 1 or usually much less than 1.

At cone maturity, a sample of 5–10 cones from each tree, crown region, and treatment should be collected before the cones start to open on the tree. Each cone should be placed in a separate envelope (small coin or stamp envelopes) or bag that can be sealed so that, if the cone opens, the seeds are not lost or mixed with those of other cones. The cones should then be kept separate and dried in an oven if needed until the cones open. Most cones open without oven heating. To determine SP, the total cone scales are counted. To determine the total fertile scales (those bearing fertile ovules) the number of sterile scales at the base and tip of the cone are subtracted from the total scales. Sterile scales are those that bear no fertile ovules and at cone maturity may bear tiny wings but no seed attached to the wing. Fertile scales times two gives seed potential.

All seeds (Figs. 95, 96, 101), wings with very tiny undeveloped ovules (Fig. 101), and wings with no ovule attached should be shaken from the cone; any of the above that stick within the cone should be pulled out with forceps. These can be quickly separated into wings only, rudimentary seeds (wings with a tiny ovule at the tip) and normal-sized seeds. Rudimentary and normal size seeds 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 seeds should be stuck to masking tape or double-sided tape attached to a small board. The seeds then may be easily sliced longitudinally to reveal the contents of the seeds (Figs. 96, 97, 101). Slicing is best done with a sharp single-edge razor blade or a double-edge razor blade that for safely should be broken in half before removing the paper. Filled seeds will have a cream-coloured megagametophyte and white, yellow, or green embryo. The embryo should be about 90% of the length of the megagametophyte (Fig. 96, 97). Empty seed will contain a dried, brown, and empty megagametophyte (Fig. 101). Empty seeds result from abortion of the megagametophyte

about the time of fertilization and are commonly caused in larch by the ovule not being pollinated or by self-pollination or otherwise incompatible pollination. A few seeds may be round to flat with varying degrees of megagametophyte development and these may have aborted during late-embryo development (Fig. 96) or result from feeding by the insect, *Leptoglossus* (Fig. 100). Other seeds will have the contents destroyed by insects; 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) usually result from ovules that were developed enough to be pollinated and aborted at or soon after pollination (Fig. 101).

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 (RS) is the product of the cone:conelet ratio times the filled seed: fertile ovule ratio. RS = C/Cl × FS:FO.

In a western larch seed orchard in the dry interior region of British Columbia, the cones averaged 35 mm (26–44 mm) in length and 3.4 g (2–5 g). Total scales 69 (50–82), sterile scales averaged 7 (0–24), and fertile scales 67 (52–115). The seed potential averaged 123 (103–148), filled seeds per cone averaged 55 (4–108), empty seeds averaged 63 (17–157), early aborted seeds averaged 0.4 (0–1.8), late aborted seeds averaged 5 (1–10), insect- and disease-damaged seeds averaged 0.1 (0–0.4), and rudimentary seeds averaged 0.4 (0–8). The SEF averaged 44% (4–74%) (see Table 2 and Fig. 106). Results for five natural stands in the Inland Empire were similar (see Table 1, Fig. 102).

Although the above results for filled seeds per cone and SEF may sound low, it is actually moderate to high for a conifer and high for seed plants in general. The initial numbers of cones was not counted so RP and RS could not be calculated. In most hardwood forest trees RS is usually below 5% and commonly below 1%. This results from the extremely high abortion of fruits (analogous to cones) giving very low flower to fruit ratios. Any cultural treatments that can increase cone retention and seed efficiency, such as supplemental pollinations, reduction of selfing, or protection from frost or insect damage, can significantly increase filled seed production and RS in an orchard. Unfortunately, little can be done in natural stands.

THE REPRODUCTIVE CYCLE OF WESTERN LARCH