epigeal germination

# Anatomy & Morphology of Conifer Tree Seed

### procambium

**David Kolotelo** September 1997

Forest Nursery Technical Series 1.1

## megagametophyte



BRITISH COLUMBIA Ministry of Forests Nursery and Seed Operations Branch

parenchyma

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# Preface to Anatomy & Morphology: Beyond Height, Diameter, and Germination

In the 70 years that Canadian nurseries have grown conifer seedlings for reforestation programs, improvements in seedling quality have come about through improvements to seed germination (seed collection, processing, handling, storage, stratification) and nursery environmental controls that have hastened and increased height and diameter growth. Although improved germination and increased seedling size have been important crop changes, the grower should be aware of other characteristics that can affect seedling quality. It is possible to obtain seedlings that have taken the same time to grow, have achieved the same height and the same diameter, yet have a different morphology and anatomy and, as a consequence, may perform differently in the field. The objective of this series is to assist growers in developing an appreciation for the details of seed and seedling form and structure, leading to a better understanding and communication of what nursery cultural practices can and cannot do.

Plant morphology and anatomy are concerned with the form and structure of plants. By contrast, plant physiology is concerned with the functioning of plants. Plant morphology describes the exterior features of the plant or its parts—it describes characteristics that can be seen, without dissection, by the unaided eye. Plant anatomy describes the internal structure of the plant and its parts describing the organs, tissues, and cell types as seen by dissection and often requiring magnification. Morphological and anatomical features are the end products of the interaction of the processes of plant development with the environment. Although an understanding of the development of form, structure, and function is necessary for the cultivation of seedlings, a detailed integration of these features is beyond the scope of this series.

This series includes volumes on seed, buds, stems, foliage, and roots. Each volume provides an account and a description of these features and their relevance to conifer seedling growers. As a whole, the series seeks to bring together information and illustrations on conifer seed and seedling anatomy and morphology. Its content, organization, terminology used, illustrations, and type of presentation have been carefully selected with the conifer seedling grower in mind. It is not intended as a comprehensive review for the scientific community. The series contains many illustrations that are intended to reinforce and clarify the text. While many of the illustrations in the series have been obtained using techniques and equipment not available to growers, the structures illustrated can be readily identified using simpler techniques and less specialized equipment.

The descriptive content of the series is based on examination and experience with seed and seedlings. Many references on conifer seed and seedling anatomy and morphology of the last century have been reviewed and cited to provide the reader with reference to more comprehensive treatments of individual topics. Conflicting use of terminology has prompted us to choose the most informative, or in some cases, simplified terms to more effectively communicate the subject matter. We have included a glossary of terms presented in bold text, and provided synonyms to assist the reader when consulting other literature. We hope that this series can provide growers with the vocabulary necessary to communicate their successes and better describe their problems.

Throughout our consultation on conifer seed and seedlings, a common question that has been asked is, "What am I looking at?" This series attempts to answer that question and to encourage growers to look more closely at their seedlings. By referring to the illustrations, with seed and seedlings in hand, we hope to acquaint the grower with the smaller details that can make a significant difference to understanding crop culture. Although we have attempted to answer the "So what?" question so often posed by growers, it is, in the end, only the user of these series who can establish the authority of "So what?" for themselves.

## Acknowledgements

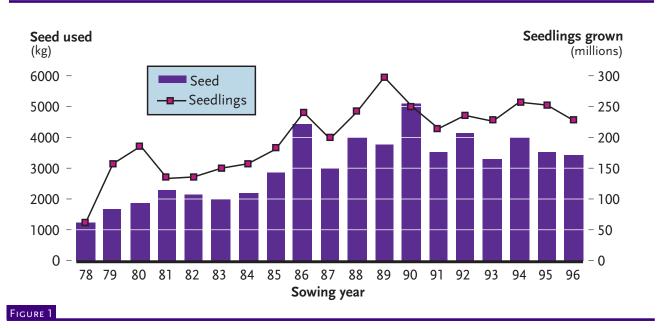
I would like firstly to thank the individuals that initiated this anatomy and morphology project for their comments, ideas and support: Rob Scagel, Pacific Phytometric Consultants; Eric van Steenis, B.C. MOF Nursery Extension Services and Dr. Joanne MacDonald, Canadian Forestry Service, Newfoundland. The following people have reviewed earlier drafts of this volume and have helped improve its quality and usefulness: Dr. John Owens, University of Victoria, Jol Hodgson, Pelton Reforestation; Wayne Gates, Pacific Regeneration Technologies; Don Summers, B.C. MOF Nursery Extension Services and Rob Bowden-Green and Heather Rooke of the Tree Seed Centre. The technical expertise and assistance of Gordon Draibye and Tim Mock of TM Communications, in photo imaging, design, editing, and layout, and of Rob Struthers for illustration, is greatly appreciated.

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# Introduction

Seed is essential in the regeneration of forests. It is the primary method used by conifers to reproduce, maintain genetic variability, and become established on appropriate sites. Currently in B.C., between 3000 and 4000 kilograms of seed are used annually to produce over 200 million seedlings (FIGURE 1). An average of 15 kilograms of seed is required to produce one million seedlings. This quantity varies greatly by **species**<sup>1</sup> from 5 kg for western redcedar<sup>2</sup> to 220 kg for Amabilis fir.<sup>3</sup>



Kilograms of tree seed used and number of seedlings grown in British Columbia 1978–1996.

This volume focuses on the **anatomy** and **morphology** of healthy, viable, mature, cleaned conifer tree seed. Seed characteristics will be explored from the dry seed in storage, through imbibition and stratification to radicle emergence and seed coat shedding. For simplicity, the term 'dry' is used throughout this volume to refer to seed at the preferred moisture content for longterm storage (4.9-9.9%) and 'imbibed' used to refer to seed that have fully saturated internal components. Examples of immature, damaged, and diseased seed are also presented to allow readers to identify and quantify through cutting tests some of the problems that can be found with tree seed. Emphasis is placed on those characteristics having operational significance.

<sup>&</sup>lt;sup>1</sup> Words in bold are defined in Appendix 2.

<sup>&</sup>lt;sup>2</sup> Scientific names are listed in Appendix I; common names are used throughout the text.

<sup>&</sup>lt;sup>3</sup> Based on PSB 415B styroblocks.

The objectives of this volume are to:

- consolidate available information and provide a highly pictorial reference to familiarize the reader with conifer tree seed anatomy and morphology
- encourage the use of cutting tests to evaluate seed quality.

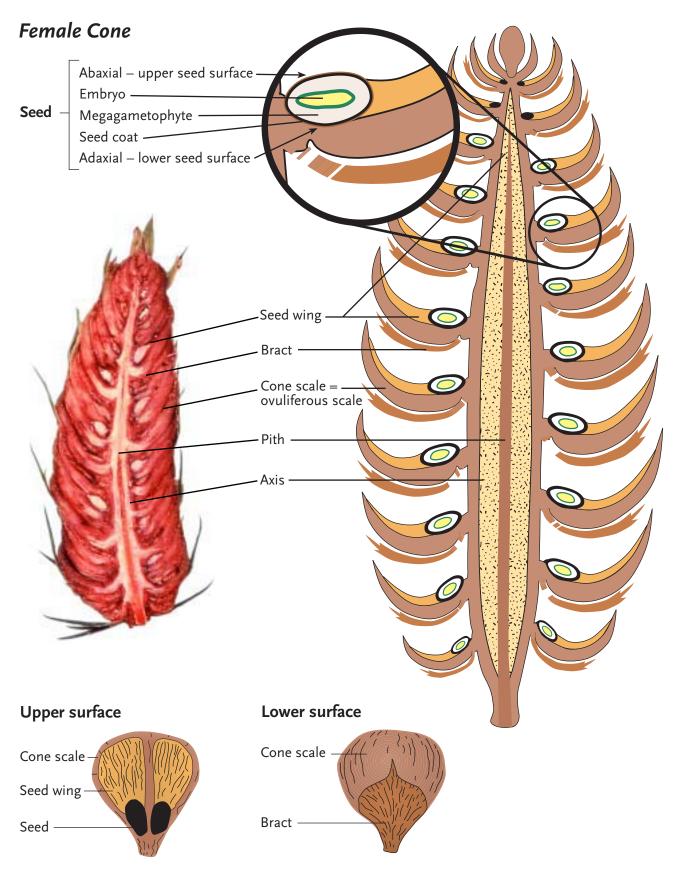
### Seed from Cones

In conifers, male cones produce pollen and female cones produce **ovules**. Seed are produced through the fertilization of an ovule by a pollen grain. In most conifers, both types of cones occur on the same tree. The female cones, which contain the seed, have ovuliferous scales and bracts that usually are arranged spirally around a central axis (FIGURE 2). In most species, two ovules are present on each ovuliferous scale close to the cone axis and each can produce a viable seed. The scales at the base and tip of the cone may not produce viable seed and the proportion of scales which can produce viable seed varies by species.

The size and shape of the cone and ovuliferous scale will influence seed morphology, particularly size and shape. When a seed is removed from an ovuliferous scale one can see the depression in which the seed sat and the impression of the seed wing. Reference is often made to the upper (abaxial) and lower (adaxial) surfaces of tree seed. The lower surface of the seed is in contact with the ovuliferous scale below it while the upper surface is free from attachment to an ovuliferous scale. In most seed, a raised junction or lip can be seen where the upper and lower surfaces of seed meet. It is along this junction that the seed coat will split, initially at the **micropyle**, to allow the radicle to emerge. The seed wing structure develops from the ovuliferous scale for species in the **Pinaceae** family. For yellow-cedar and redcedar (in the **Cupressaceae** family) the seed wing is derived from the outer portion of the **integument**, which also produces the seed coat, and is a more integral part of the seed. Complete dewinging should not be performed on western redcedar or yellow-cedar as extensive damage to the seed coat could result.

### **Anatomical Details**

A summary of the character, function, and occurrence of the common cell and tissue types is included as a reference to terminology used in the text (TABLE 1, page 4). The two most common cell types in conifer seed are parenchyma cells and sclerenchyma cells. Parenchyma cells are generally unspecialized, thin-walled and often contain chloroplasts. Sclerenchyma cells are thick-walled cells that provide support. The major tissue types are the vascular tissues: xylem, which transports water and nutrients, and **phloem**, which transports photosynthate from areas of production (leaves) to areas that utilize it (apices and growing areas). The **epidermis** or outer surface layer is generally important in regulating water and gas exchange. Other tissues such as **pith** and **cortex** are present, but these are mainly composed of parenchyma cells. Additional anatomical details on these cell and tissue types can be found in classic plant anatomy texts such as Esau[15] and Fahn[16].



#### Figure 2

A longitudinal section of a typical cone found in the Pinaceae family with details of the upper and lower surfaces of an ovuliferous scale.

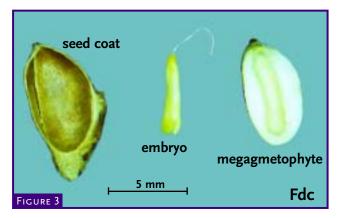
### TABLE 1 Characteristics, function, and occurrence of cell and tissue types found in the seed

	ction, and occurrence of cell and t		
Cell type	Characteristics	Function	Occurrence
Parenchyma 'unspecialized cells'	<ul> <li>cells alive at maturity</li> <li>variable shapes</li> <li>thin cell walls</li> <li>capable of division and expansion</li> </ul>	<ul> <li>photosynthesis</li> <li>storage</li> <li>wound healing</li> </ul>	<ul> <li>cortex</li> <li>pith</li> <li>xylem and phloem</li> <li>megagametophyte</li> <li>seed coat</li> </ul>
Sclerenchyma 'support cells'	<ul> <li>thick, lignified cell walls</li> <li>dead at maturity</li> <li>elastic properties</li> </ul>	<ul> <li>support by providing hardness and rigidity</li> </ul>	<ul> <li>seed coat (middle layer)</li> <li>xylem and phloem</li> </ul>
Tissue type	Characteristics	Function	Occurrence
Xylem 'water and nutrient transport'	<ul> <li>complex tissue of many cell types</li> <li>lignified, thick- walled tracheids dead at maturity</li> <li>also living, thin- walled parenchyma</li> <li>also thick-walled sclerenchyma, dead at maturity</li> </ul>	<ul> <li>tracheids function in conduction of water and nutrients and also support</li> <li>parenchyma function in storage</li> <li>some parenchyma function in conduction</li> </ul>	• in shoots, roots, and leaves
Phloem 'sugar or photosynthate transport'	<ul> <li>complex tissue with several cell types</li> <li>living thin-walled sieve cells predominate</li> </ul>	<ul> <li>sieve cells transport photosynthate</li> <li>parenchyma functions in storage and lateral conduction</li> <li>sclerenchyma provide support</li> </ul>	• in shoots, roots, and leaves
Epidermis 'surface layer or skin'	<ul> <li>various cell types may be present</li> <li>usually one layer of tightly packed cells</li> <li>can be modified into guard cells</li> <li>replaced by other protective tissue – periderm</li> </ul>	<ul> <li>prevents water loss and microbial infection</li> <li>mechanical support</li> <li>guard cells function in gas exchange</li> </ul>	<ul> <li>outermost layer covering shoots and leaves</li> <li>not found in roots of conifers</li> </ul>

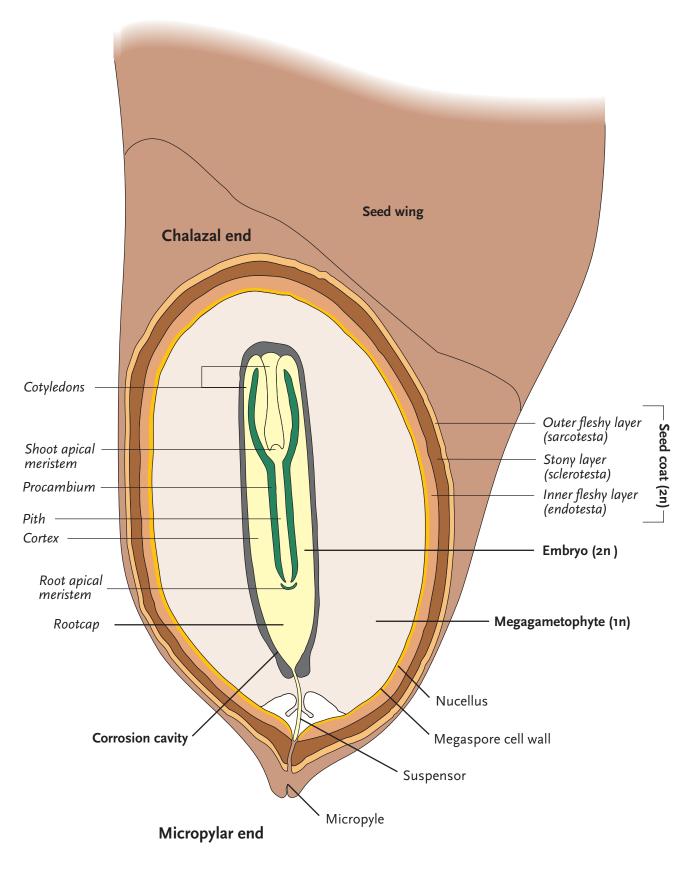
# Tree Seed Components

A conifer seed has three main components: the seed coat, embryo, and megagametophyte. These components are shown dissected from a longitudinal section of a Douglas-fir seed in FIGURE 3. The embryo or **zygote** is the result of the fertilization of the egg within an ovule by the sperm within a pollen grain. It has all the necessary structures and information to produce a tree. The **megaga-metophyte** serves as the food reserve for the embryo until it is able to **photosynthesize**. The seed coat protects the inner structures from damage, but can also restrict oxygen uptake, gas exchange, water uptake, or radicle emergence due to its anatomical structure.

An illustration of a typical conifer seed with the seed wing attached is presented in FIGURE 4 (page 6). The three seed coat layers are exaggerated in size to allow them to be differentiated. Throughout the text and in performing cutting tests this figure will be a useful reference in identifying the anatomical features of conifer tree seed. The thinner, pointed micropylar end of the seed is associated with the site of radicle emergence while the wider, more rounded **chalazal** end is associated with the cotyledons.



The seed coat, embryo, and megagametophyte dissected from a coastal Douglas-fir seed.



#### Figure 4

The anatomical details of a generalized conifer seed in longitudinal section. Chromosome complements for tree seed components indicated by 2n=diploid and 1n=haploid. A removable copy of this figure is located at the end of this volume.

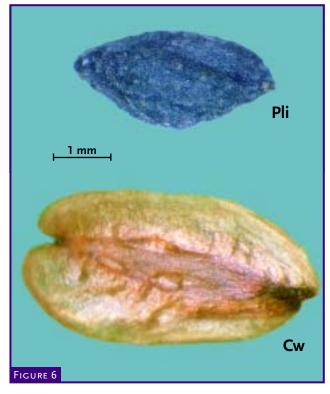
### Seed Coat

The seed coat is the readily observable feature of the seed and protects the inner tissues from insects, fungi, adverse conditions and mechanical damage. It is derived entirely from the mother tree, is **diploid** and appears similar for seed collected from a single tree. When seed from many trees are combined into a seedlot the seed coat appears quite variable (FIGURE 5). Seed coats vary in terms of colour, shape, texture, and the presence of resin vesicles. These attributes, along with seed size, form the basis for species identification using seed. The variability in seed coat morphology between species is illustrated with lodgepole pine and western redcedar in FIGURE 6. In lodgepole pine, the seed is quite dark, almost black, with characteristic ridges. No resin vesicles are present and the entire seed wing is absent. In contrast, western redcedar has a very light-coloured seed coat, resin vesicles, and a persistent seed wing.

Seed coat colour is related to the presence and distribution of **tannins** within the cells of the seed coat. The tannins accumulate during seed development giving rise to the change from light to dark seed coats. Not all seed coats are dark and within a seedlot one may see light-coloured seed, dark seed, and intermediate or mottled seed as shown for ponderosa pine (FIGURE 5). Variability in seed coat morphology can also be caused by abrasion of the



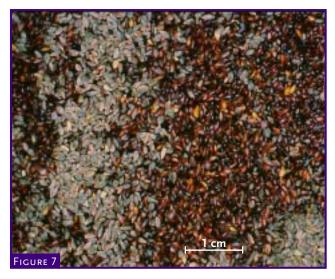
Variability in seed coat morphology from a single seedlot of ponderosa pine.



The seed coat morphology of lodgepole pine and western redcedar.

outer tannin containing parenchyma cells, exposing the lighter coloured middle seed coat layer. Even in the very dark seed coat of lodgepole pine (FIGURE 6), you can see some light-coloured cells that do not contain tannins. Seed coat colour has been implicated in differences in germination[46] and in susceptibility to damping-off fungi[35], but the results are not conclusive.

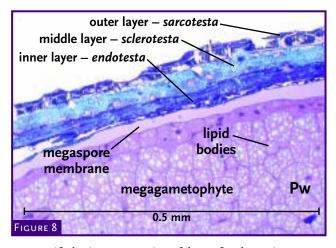
The colour or appearance of the seed coat is also influenced by the presence or absence of surface moisture. In FIGURE 7 (page 8), imbibed seed of interior spruce illustrate the difference between surface dry and surface moist seed. The darkcoloured seed have excess moisture on the seed coat while the lighter coloured seed have been surface dried to remove this moisture. The removal of excess surface moisture is important to enable the seed to be efficiently sown by mechanical sowing machines. Seed with excess moisture tend to clump together causing problems in sowing. To achieve uniform drying and to avoid removing moisture from within the seed, it is important to incorporate some movement or rotation of the



A spruce sowing request showing imbibed seed with excess surface moisture (darker) and seed that has reached a surface dry condition (lighter).

seed during drying. This is especially important in seed having a depth greater than a few cm to avoid drying only those seed in contact with the ambient air.

The seed coat of conifers is generally regarded as having three distinct layers: outer layer (sarcotesta), middle layer (sclerotesta), and inner layer (endotesta) (FIGURE 4, page 6, and FIGURE 8). The layers differentiate from the light-coloured integument or ovule wall at the time of pollination and may provide protection for the developing embryo. At seed maturity, the outer layer consists of unspecialized, tannin-filled parenchyma cells; the middle layer is generally thickest and consists mainly of specialized support cells with characteristic holes (simple **pits**) that allow for the passage of water



A magnified microtome section of the surface layers in western white pine.

between cells (FIGURE 8); and the inner layer consists of unspecialized cells without tannin deposits[39]. In FIGURE 8 note how the outer layer is thin, irregular, and appears broken in areas. The outer and middle layers are generally thicker at the tips of the seed, the micropylar and chalazal ends. The middle layer is often referred to as the 'stony layer' because of the predominance of thickwalled sclerenchyma cells that may slow imbibition or radicle emergence.

Most conifers do not possess vascularized seed coats, although many gymnosperms and angiosperms do. In coastal Douglas-fir, lack of a vascularized seed coat can be explained by the separation of the ovule from the ovuliferous scale at the time of fertilization. The precursors for storage products and development are already present within the ovule at fertilization, but their makeup will change during seed development. Initially the substances are primarily soluble, mobile substances that are converted into the less soluble starches, lipids, and proteins[32]. It is possible that diffusion of moisture will occur from cones to seed during development. The living cone still plays an important part in seed development by providing the proper microclimate and protection for seed maturation. Consequently, it should not be assumed that cones can be collected prematurely because a direct contact does not exist between the cones and seed.

It is suggested that growth inhibitors are present in the seed coats of conifers. One study with *Pinus pinea* L. concluded that inhibitors are present and that they are water-soluble. One inhibitor may be **abscisic acid** (ABA)[24]. It is probable that other genera contain similar inhibitors, but little research has been conducted in this area. Repeated washing of the seed removes the inhibitor and relieves dormancy[8]. This is a good reason to exchange water or perform running water soaks during seed imbibition. Another reason for exchanging water is to reduce the amount of fungal inoculum present on the seed coat.

True firs (Abies spp.), hemlocks, and western redcedar have resin vesicles in their seed coats. These vesicles are surrounded by **epithelial cells** 

that produce and secrete the resin into the vesicle. Resin vesicles form early in seed development in the middle or outer layer of the seed coat and are usually more abundant on the lower surface of the seed (formerly in contact with the ovuliferous scale). Resin vesicles in western redcedar are narrow, linear, and only slightly raised while those of the hemlocks are elliptical and prominently raised above the seed coat. In FIGURE 9 several seed of western hemlock are shown illustrating the prominent resin vesicles that originate from the middle seed coat layer. All but the top seed have had the outer seed coat layer removed. Its resin vesicles are hidden from view although their outlines are apparent. This seed morphology, with all three seed coat layers present, is the preferred product after seed processing to reduce resin vesicle damage.

The role of resin vesicles is not clear although they have been implicated in: i) preventing germination in the fall; ii) protecting the embryo from excessive drying; and iii) playing a role in seed coat dormancy. A reduction of germination through damage to these structures has been reported[17,21]. Seed with resin vesicles must be handled with extreme care to avoid a reduction in quality.

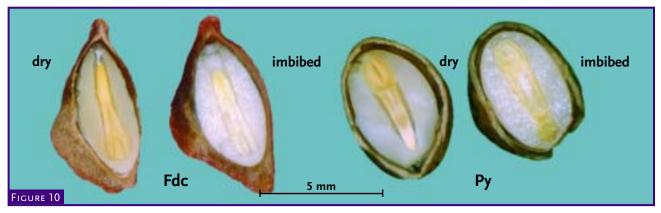


Seed of western hemlock displaying prominent resin vesicles. All seed, except the upper right, have had the outer seed coat layer removed.

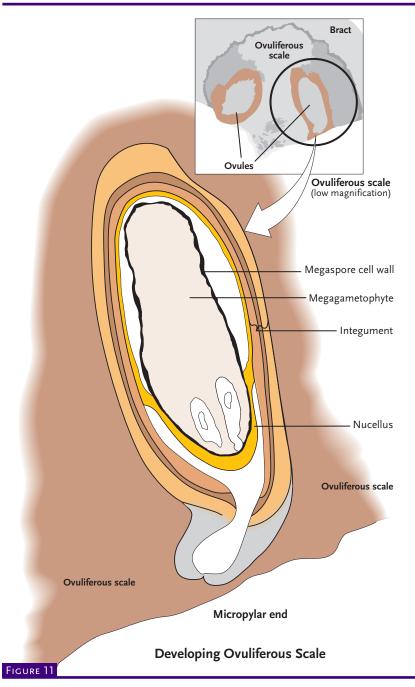
### Megagametophyte

The megagametophyte surrounds the embryo, providing protection and nourishment for initial growth. It originates from the mother tree and is haploid. In the developing seed, cells in the central portion of the megagametophyte, containing primarily starch[32], disintegrate to form the corrosion cavity into which the embryo develops. The megagametophyte cells are large, thin-walled, and spherical in shape (FIGURE 8). In Douglas-fir, the dry weight composition of the megagametophyte is 60% lipids, 16% proteins, and 2% sugars[32]. Lipids are an efficient means of energy storage due to the larger number of carbon-hydrogen bonds that release a greater amount of energy when oxidized than other organic compounds[42]. Although proteins are less abundant they are important as a source of amino acids (nitrogen-rich molecules) during germination. The lipids and proteins are found within specialized bodies (lipid and protein bodies) that are uniformly distributed throughout the megagametophyte[32]. Protein bodies also contain spherical structures, globoids, which store minerals for germination[34]. The lipid bodies can be seen as the white droplets in the cells of the megagametophyte in FIGURE 8.

The colour, condition, and texture of the megagametophyte varies with moisture content. In the 'dry' state (< 10% moisture content) the megagametophyte appears creamy-yellowish in colour and gaps usually exist between the megagametophyte, seed coat, and embryo. The structures appear shrunken. This is due to the natural dehydration that occurs during seed maturation. In the imbibed seed (≈30-40% moisture content) the megagametophyte is white and appears to occupy the entire space between the embryo and seed coat. The megagametophyte appears homogeneous and is often described as being shiny or crystalline due to the reflection of moisture in imbibed seed. It is the imbibed megagametophyte that deserves the common analogy of the 'firm meat of the coconut.'



Longitudinal sections of dry and imbibed seed of Douglas-fir and ponderosa pine.

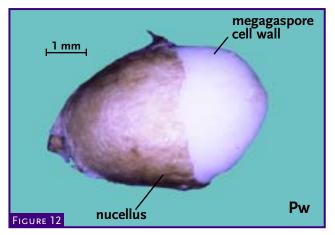


Longitudinal sections of a developing ovule at time of pollination.

In FIGURE 10 dry and imbibed seed of Douglas-fir and ponderosa pine are compared to illustrate the differences in morphology due solely to moisture content. Note the spaces around the megagametophyte and darker coloration of structures in the 'dry' seed. In the imbibed seed, the megagametophyte is white and appears shiny due to the reflection of moisture. In addition to causing changes in morphology, the addition of water also initiates many biochemical processes in the seed, the initial stages of seed germination.

Note the thicker seed coat in ponderosa pine and the incomplete dewinging of coastal Douglas-fir.

Although not strictly part of the megagametophyte, two other structures are evident; the **nucellus** and **megaspore cell wall**. The nucellus is the inner tissue of the ovule in which the megagametophyte develops. The nucellus becomes compressed during seed development (compare FIGURE 4, page 6, and FIGURE 11) and can be found as a papery covering outside the megaspore cell wall (FIGURE 12)

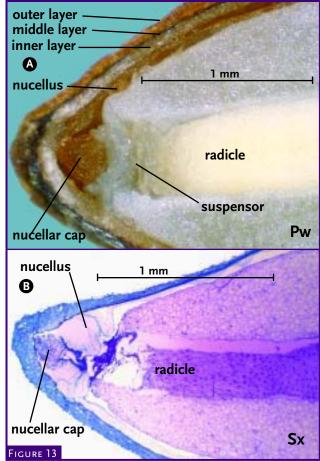


A western white pine seed with the seed coat removed revealing the nucellus and megagametophyte.

or reduced to a mass of tissue near the micropylar end of the seed, termed the nucellar cap (FIGURE 13).

The megaspore cell wall surrounds the megagametophyte. It is a lipid-rich, multilayered tissue that is more difficult to view with the unaided eye. This tissue is analogous to the pollen wall in the male gametophyte. In studies on Scots pine and Norway spruce, the megaspore cell walls were clearly shown to restrict water uptake[46,47].

The megaspore cell wall and nucellus are often compressed and difficult to distinguish in mature seed but distinct in the ovule at the time of pollination (FIGURE 11). Under high magnification the nucellar cap or 'plug' found in many species is clearly visible at the micropylar end of the seed (FIGURE 13). Allen and Owens[1] describe this plug as an infolding of the megaspore cell wall and hardening of the nucellus at the micropylar end forming a distinct brown tip where the micropyle and nucellus meet. This plug can be an impediment to radicle emergence and can sometimes act as a collar around the emerging germinant, restricting growth. Although these tissues do not usually play an active role in germination, they are readily observable and should not be mistaken for deteriorated seed contents or abnormal growth.



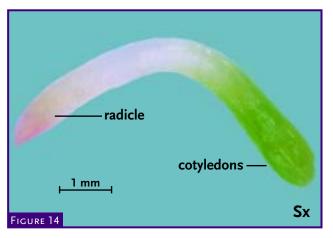
The anatomical detail of the micropylar end of (A) an imbibed western white pine seed from a longitudinal razor-cut section and (B) a 'dry' interior spruce seed from a longitudinal microtome section.

### Embryo

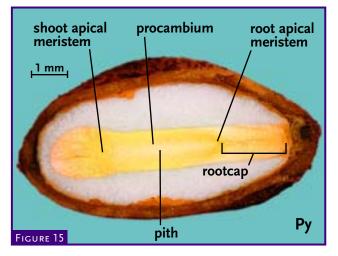
After the fertilization of an ovule, the embryo develops to maturity by a complex series of stages known as **embryogeny**. All of the rudimentary structures and information necessary to produce a mature tree are contained in the embryo. The embryo is the only seed component containing genetic information from both parent trees. The mature embryo is comprised of cotyledons, a shoot apical meristem, **hypocotyl**, radicle, root apical meristem, and rootcap (FIGURE 4, page 6). In FIGURE 14 a dissected, germinating embryo, illustrates the green cotyledons that will function in photosynthesis. The red on the radicle is the expression of **anthocyanins**.

In a cut seed of yellow pine (FIGURE 15) the **procambium** pith, shoot and root apical meristems, and the rootcap can be seen. The rootcap accounts for approximately one-third of the embryo in this seed. Remnants of the nucellus are obvious at the periphery of the megagametophyte where it has been torn during dissection. This photo also shows the embryo as being bright yellow. Although this yellow colour is not typical, when compared to other illustrated cut seed, it shows the variability of embryo colour.

The finger-like projections at the chalazal end of the embryo are the cotyledons, or 'seed leaves.' Conifers have a variable number of cotyledons ranging from two (western redcedar) to up to 12 or more (ponderosa pine). Cotyledons are arranged in an inverted 'umbrella-like' arrangement above the shoot apical meristem within the seed. The general cross-sectional shape of a cotyledon is triangular, but the specific shape depends on the number of cotyledons present as all cotyledons, regardless of number, will share the same 360° circle. If you slice through the cotyledons to obtain a cross-section, you can see a circle that is divided equally into the individual cotyledons (FIGURE 16).



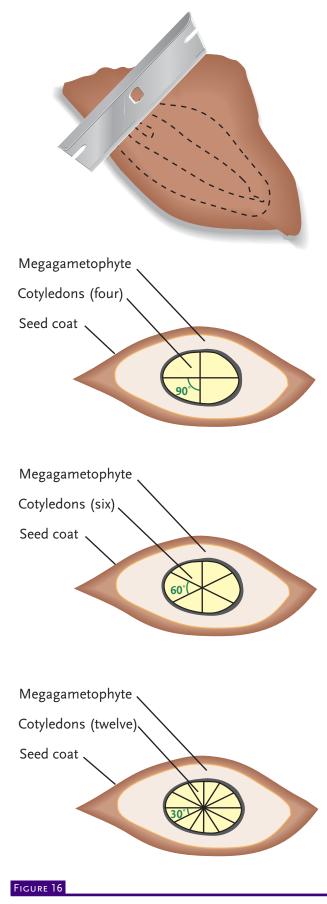
A dissected embryo from a germinating interior spruce seed.



A longitudinal section of a ponderosa pine seed.

If only two cotyledons are present they will be shaped as half-moons (180°), if four are present, they will appears as quarters of a pie (90°), and if 12 are present, they will each have a 30° angle as their upper surface. The cotyledons consist of an epidermis, cortex, and provascular tissues.

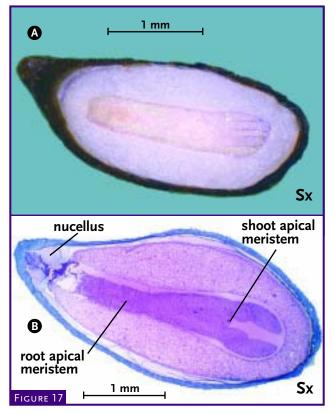
At the base of the cotyledons is the shoot apical meristem. This is a small, highly organized dome of inactive cells which are activated upon germination and will initiate all subsequent aboveground structures. Below the apical meristem is the embryonic stem or hypocotyl. With a good dissection, distinct tissues can be seen within the



A cross sectional; illustration of the origin of cotyledon shape.

hypocotyl (FIGURE 4, page 6). Good dissections are a function of technique, but also species. Hypocotyl details are readily observed in ponderosa pine or Douglas-fir, but very difficult to see in hemlock or larch. The innermost tissue of the hypocotyl is the pith which is mainly composed of loosely arranged parenchyma cells. The procambium surrounding the pith is a meristematic region that contains the first elements of the xylem and phloem[44]. Details of the type of vascular elements present in the dormant embryo are extensively reviewed by Berlyn[7]. Each cotyledon contains a provascular strand to allow for transport of water and sugar. These strands join to form the procambium slightly below the shoot apical meristem.

Outside the procambium of the hypocotyl is the cortex, which is composed of parenchyma cells that characteristically have fluid filled membranes (vacuoles). The epidermis is the outermost layer of cells in the hypocotyl and cotyledons that provides protection and reduces water loss. Compared to the cortex, the epidermal cells are quite compact without intercellular spaces. There is no obvious transition zone between the shoot and root in the embryo. Although the radicle does not contain pith it may exist in the primary root for up to 120 days[7]. The vascular structures of the root are similar to the hypocotyl. The root apical meristem is recognized as a spherical group of cells found about  $\frac{1}{2}$  to  $\frac{2}{3}$  of the way down the embryo toward the root tip. The remainder of the embryo consists of a large rootcap that protects the root apical meristem and aids penetration into the soil. The rootcap consists of parenchyma cells with abundant accumulated starch. The root does not have an epidermis[9]. The thread-like structure at the base of the embryo is the suspensor (FIGURES 3, 4 pages 5, 6), which acts like an umbilical cord to the very young embryo. The suspensor also aids in pushing the early embryo into the corrosion cavity through cell elongation.



Longitudinal section of an interior spruce seed from (A) an imbibed razor-cut section and (B) a 'dry' microtome section.

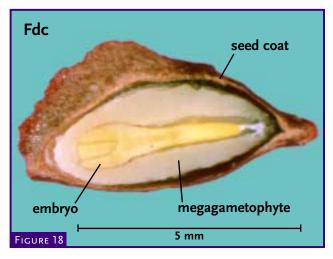
In FIGURE 17, a comparison of a razor-cut longitudinal section and a microtome-cut longitudinal section of a spruce seed is presented. FIGURE 17A is a fully imbibed seed with the embryo, megagametophyte, and seed coat clearly visible. The seed is not cut exactly in half in this example and the procambium and rootcap are not visible. A proper cut would dissect through the micropylar end of the seed coat and give a better view of the embryo. If a cut is not perfect it may still yield enough information to predict the probable fate of the seed. FIGURE 17B shows a dry seed in which one can clearly see the shrinkage around the embryo and megagametophyte. The number of cotyledons visible is reduced to just two with the thin microtome section passing through one cell layer. The shoot apical meristem lies between these two cotyledons, and the root apical meristem can be identified by the clustering of more densely staining cells. Other seed structures like the megaspore cell wall, nucellar cap, and compressed suspensor can be seen in this photograph.

# **Cutting Tests**

Cutting tests are 'seed anatomy tests' used to estimate germination and characterize a seedlot through visual inspection. Seed are dissected and classified into categories displaying similar characteristics. Cutting tests are essential as a decision tool in seed processing and upgrading, but are useful in any situation where an estimate of germination is desirable. Cutting tests can also reveal the presence and extent of problems (i.e., % and degree of immature or deteriorated seed) within a seedlot.

It is not always possible, based on seed anatomy, to predict whether a seed will germinate, but for most seedlots, cutting tests provide a good approximation of the proportion of viable seed. Although cutting tests can be performed on dry seed, the imbibed seed usually provides a better illustration of seed condition and a better prediction of germination. The moisture status, presence of deterioration, insects and fungi can all be viewed within the tree seed – take a look.

Cutting tests are most commonly performed on 50 seeds, but this can vary depending on seed condition and precision desired. Seed are generally cut **longitudinally** (lengthwise) and the best view of internal structures is obtained if one dissects through the thinnest dimension of the seed (FIGURE 18). Various means can be used to hold the seed in this unstable position: forceps, tape, fingers [be careful] or even a small template for the larger seed. It is safest to cut the seed so that its narrowest edge is placed on a firm surface



A longitudinal section of a 'dry' Douglas-fir seed.

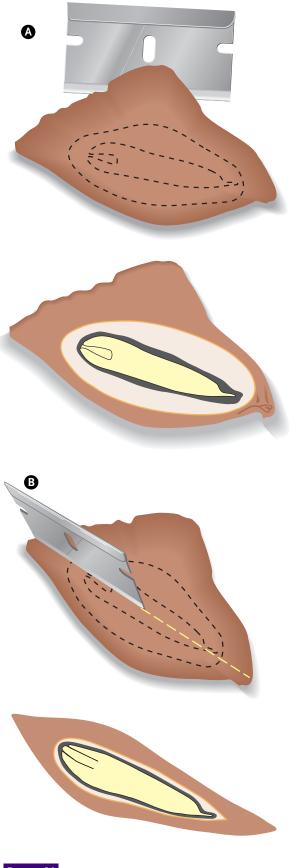


Figure 19

Methods and results of performing cutting tests (A) through the thinnest axis or (B) with the seed placed on a flat surface.

and a clean, sharp razor blade or scalpel pushed downwards through the middle of the seed with constant pressure (FIGURE 19A). With care, holding seed between your fingers and slicing can be a quick and effective method. Practise with larger seed first.

Seed can also be cut with the widest dimension resting on a firm surface making the seed much more stable, but resulting in a less illustrative view of the internal components, especially the megagametophyte (FIGURE 19B). It is more difficult to consistently cut the embryo in half with this method. Some experimentation may be required for each species. An aid in classification and counting is to place seed on masking tape and then cut and separate the seed halves allowing for examination of both halves. This eliminates seed moving during the assessment and allows for a direct comparison between adjacent seed. Employ the most comfortable method for you that produces longitudinal sections enabling you to assess seed quality.

### **Seed Problems**

This section illustrates some problems causing seed mortality (inability of a seed to germinate under any conditions) or reduced seed quality that can be observed in cutting tests. These illustrations will assist in the classification of seed required in cutting tests. Some seed morphologies are easily interpreted as being incapable of producing a germinant, but it is not always possible to determine if a seed will or will not germinate based solely on its anatomy and morphology. The best one can do is point out the important characteristics to look for in a cutting test.

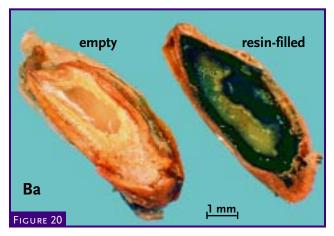
The two most easily recognized reasons for failure of seed germination are: lack of an embryo (resulting in an empty seed) and the complete deterioration of all seed contents (generally called dead-filled seed). These two morphologies are illustrated with Amabilis fir in FIGURE 20. Empty seed are a common feature in most conifers as the seed coat and megagametophyte can still develop without fertilization. The megagametophyte of the unfertilized seed usually degenerates leaving only the megaspore cell wall. This results in an empty seed that appears flat from the outside. In most species, empty seed are easy to remove during seed processing due to their lower **specific gravity** and are therefore not common in processed seedlots. If one is performing cutting tests on unprocessed seed include an empty seed category in your cutting tests.

The megagametophyte will not develop in species of *Pinus* if the ovule is unpollinated. This is the reason that the crush test is used by some to evaluate lodgepole pine seed. In the crush test, you simply apply pressure to the seed and if the exudate appears white, indicating the presence of the megagametophyte, you can assume an embryo is present. This may be suitable to some situations, but it gives no indication of embryo development or health. The longitudinal seed cut is recommended.

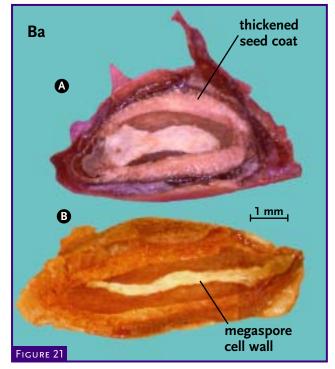
It has been observed that empty seed of *Abies* spp. often appear to have thicker seed coats than seed that develop with a fertilized embryo. One often knows that contents are absent in *Abies* spp. by the difficulty in cutting the seed. In FIGURE 21A, a thickened seed coat of a subalpine fir seed with a deteriorating megagametophyte is shown. With continued deterioration all that will remain within the seed coat is the megaspore cell wall (FIGURE 21B).

Empty seed can also be the result of insect damage as shown in FIGURE 22 with the complete destruction of seed contents by the *Megastigmus* seed chalcid in coastal Douglas-fir. There are no morphological clues to the presence of this pest in a seedlot, but they can be detected by x-ray radiographs. More detailed coverage of insect problems of cones and seed can be found in the suggested readings.

Fully deteriorated or dead-filled seed often contain a brown tar-like material, but no embryo or megagametophyte can be recognized. These seed have specific gravities similar to viable, filled seed and can be difficult to remove during the processing of some species. FIGURE 23 illustrates one hypothetical pathway for this type of deterioration. In FIGURE 23A, the embryo and



Comparative morphology between an empty and resin-filled Amabilis fir seed.



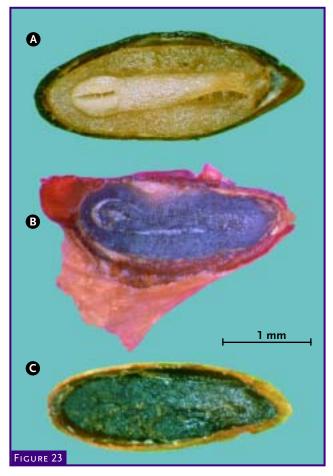
Empty seed of Amabilis fir displaying (A) a thickened seed coat and deteriorating megagametophyte and (B) completely deteriorated megagametophyte.



Destruction of a Douglas-fir seed by a Megastigmus larva.

megagametophyte appear translucent, have an uncharacteristic colour and a rubbery texture. In FIGURE 23B, the seed is undergoing a transition to a solid resinous consistency, but the outline of the embryo can still be recognized. Fungal mycelia are present between the megagametophyte and the embryo. It is not common to see seed at this intermediate stage of deterioration. In FIGURE 23C, the tissue is completely transformed into what is referred to as resin-filled, dead-filled, or 'woody' seed. This problem is quite common in *Abies* spp. but has also been observed in spruce, hemlock, western redcedar and yellow-cedar.

In FIGURE 24, an interior spruce seed displays deteriorated contents that are interspersed by fungal hyphae. This type of marbled seed is not common in seed cuts and is probably another intermediate stage in deterioration similar to FIGURE 23B. Many fungi can be found on the seed



A possible deterioration pathway for resin-filled seed from (A) gummy tissues with structures distinct, to (B) solidified contents with structures barely visible, and (C) solidified contents with no structures evident.



Fungi inside a deteriorated seed of interior spruce.

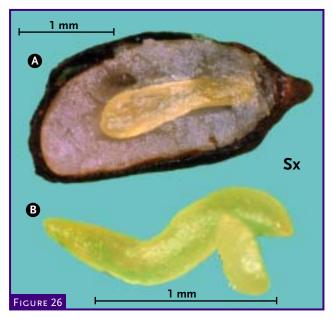
coats of conifers and are usually a combination of pathogenic and non-pathogenic fungi. Three seedborne fungi have been identified as problematic in B.C. conifer nurseries: *Fusarium* sp., *Caloscypha fulgens*, and *Sirococcus strobili*. This manual will not go into the biology of these species, but growers should be aware that fungal assays are being performed for these pathogens on seedlots in storage. For further details on disease problems consult the 'Suggested Readings' section.

A common type of deterioration is the discoloration of tissues or apparent 'bruising.' In FIGURE 25, a healthy seed is compared to seed with a completely deteriorated and partially deteriorated megagametophyte. The reserves of the partially discoloured megagametophyte have been depleted and it is uncertain whether enough remains to supply the energy for germination to occur. The cause of the discoloration is not known and it is probable that several factors may produce similar results. Seed deterioration can be minimized by storing seed at low temperatures and low moisture contents. Seed quality at time of initial storage is another factor influencing seed longevity. Gentle handling of seed during seed processing, seed preparation, and sowing is important to maintain the seedlots potential.

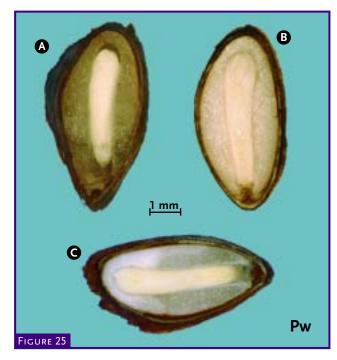
Another sign of deterioration that has been observed in some seedlots, particularly spruce, is the presence of a translucent embryo as shown in FIGURE 26. This morphology is abnormal and it was anticipated that these seeds would not germinate. Comparison between proportions of these seed in a seedlot and final germination clearly indicated, however, that at least some seed with this morphology do germinate. It was found that this translucency was a transient character in many seed and upon imbibition and stratification many seed returned to a morphology more representative of spruce. Cause of this morphology is unclear, but heat damage is a possibility. It is exceptions like this that make it difficult to prescribe strict guidelines for determination of germinable seed; there will always be exceptions.

Seed reach their maximum viability and peak maturity at the time of natural seed shed. Variation in seed maturity has been noted between stands, between trees within a stand, and between cones on the same tree[57]. Seed maturity is usually based on the presence of a fully developed embryo. In B.C. the primary, but not sole, criterion used is that the length of the embryo should be at least 90% of the corrosion cavity. Immature seed also possess a megagametophyte that is soft, milky, but generally not firm as those found in mature seed. By cutting seed longitudinally and examining the embryo length you can determine when seed maturity is achieved. Seed not achieving this degree of maturation may still germinate, but seedling vigour and storability will probably be reduced[13].

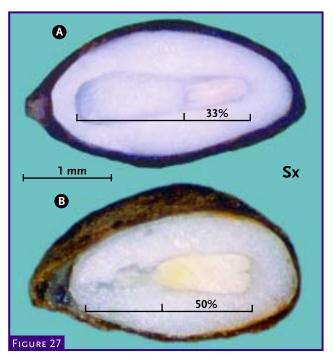
In FIGURE 26A, the embryo has elongated to about 33% of the corrosion cavity, the cotyledons are not very apparent, making it unlikely that this seed will germinate. In FIGURE 26B, the embryo is about 50% of the corrosion cavity, the cotyledons are distinct, but not fully elongated. This seed may germinate, but it will likely be slow to develop and will probably not produce a sellable seedling.



Interior spruce with a translucent embryo (A) within the seed and (B) excised from the seed.



Western white pine seed showing (A) healthy seed, (B) total deterioration of the megagametophyte tissue, and (C) incomplete or ongoing deterioration of the megagametophyte.



Immaturity of spruce seed displayed by (A) 30% embryo development and (B) 50% embryo development.

### **Seed Classification**

The first step in classifying a seed sample is to examine the external morphology. Are cracked or decorticated (seed coat removed) seed present? Does the seed show high variability or uniformity in size or shape? Is resin vesicle damage evident as a dark grey colour, sticky feel, and distinct aroma? Are fungi present on the seed coat? Many external characteristics are useful in alerting one to problems inherent in a seedlot (i.e., decorticated or cracked seeds sink quickly when placed in water allowing for relatively easy upgrading).

The act of cutting the seed also provides information related to seed quality. A viable seed can be cut fairly easily with a crisp, clean cut. Ease of cutting varies by species with yellow pine and Amabilis fir being the most difficult to cut due to their relatively thick seed coats. Seed that cut very easily are probably empty or immature. The 'feel' to cutting seeds will come with experience (repetition).

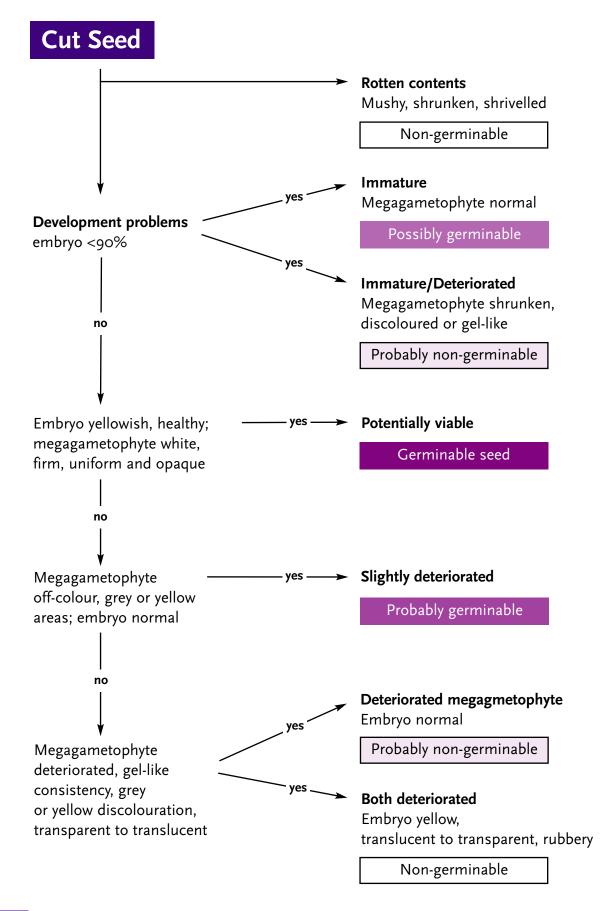
Once seeds are cut they need to be classified to provide an assessment of seedlot quality. Seed should be categorized as soon as possible after cutting as exposure to room conditions will result in dehydration, discolouration and changes in morphology. The heat from microscope lights can also change seed morphology and exposure should be kept to a minimum.

Many methods of classifying cut seed can be constructed. Depending on species and seed quality, the categories can vary greatly and one classification will not be suitable to all situations. Some characteristics to look for are the colour, **opacity**, consistency, texture, and degree of development of the seed components. Cutting tests are subjective and although most people will obtain similar results for good quality seedlots, interpretations may vary greatly for 'problem' seedlots (i.e., seedlots with a large proportion of seed marginal in appearance).

One of the simplest and most common classifications is to divide the cut seed into three categories : i) viable seed, ii) damaged and discoloured seed, and iii) immature seed. Start with this simple classification and add more categories if required. A more detailed classification may be required because the damaged and discoloured category needs to subdivided. One can subdivide based on the component (embryo, megagametophyte, or both) and/or the observed morphology (i.e., yellow, translucent contents, discoloration of the megagametophyte).

A flowchart illustrating one classification key that has been used at the Tree Seed Centre is illustrated in FIGURE 28 with a corresponding cutting test sheet in FIGURE 29 (page 22). The chart initially looks at the two most easily identified problems: rotten and immature seed. The rotten seed are definitely non-viable, but immature seed may still germinate and are differentiated based on whether the megagametophyte appears healthy. The potentially viable seed are the 'good' seed, but the slightly deteriorated seed also germinate and the best estimate of germination capacity is usually obtained by the sum of the proportions of seed in these two categories. The last two categories contain seed with a deteriorated megagametophyte with or without a deteriorated embryo. The cutting test sheet shows additional categories that are further subdivisions of those illustrated in the flowchart.

This volume provides a baseline for what mature, healthy, viable seed should look like in longitudinal section for many conifer species. It also illustrates some of the observable problems found in seed that can affect germination. Some of these problems (empty seed, totally deteriorated contents) enable you to determine that a seed will not germinate, but other problems (immaturity, bruising, fungal infection) vary in degrees and it is more difficult to predict whether germination will occur. One factor common to most species is that slight discoloration (greying) of the megagametophyte does not usually preclude germination. Providing an optimal germination environment is important. Proper record-keeping of cutting tests and comparison with the germination obtained is critical. This will help you predict the fate of the marginal seed grown in your nursery.



Seedlot/ # of Good Seedlot/ # of Seedlot/ #											
# of seeds	0				EMBRYO	0					
# of seeds		Good	Normal	Normal	Deteriorated translucent rubbery	Development problems	Development problems	Rotten C	Other En	Empty C	Comments
seeds				Σ	MEGAGAMETOPHYTE	РНҮТЕ					
	0 0	Good/Thin seed coat	Slight discolouration	Grey/Yellow deteriorated	Grey/Yellow deteriorated	Normal	Shrunken/ discoloured	Rotten	Other		

FIGURE 29

A cutting test sheet for classifying seed and recording comments.

# Seed Dormancy

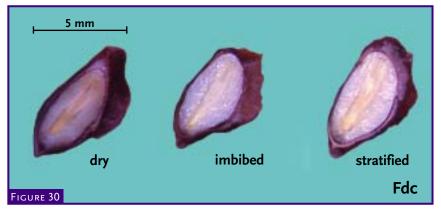
Seed dormancy is defined as the condition when mature, viable, imbibed, and healthy seed fail to germinate given suitable conditions. Dormancy is considered a mechanism that eliminates the risk of germination in the autumn after seed have been released from their cones. Seed dormancy is overcome through the technique of imbibition stratification (moist-chilling), which exposes the seed to cool  $(2-5^{\circ}C)$  temperatures for a specific duration following the imbibition of the seed. Stratification terminates dormancy and enables the seed to achieve maximum germination in minimum time. Most seed are given at least a 24-hour soak, or imbibition period, to prepare them for stratification. The imbibition and stratification times used operationally for B.C. conifers are included in Appendix 3.

Seed dormancy can be of two types: physiological and physical. These two categories are not mutually exclusive and are often found together. In physical seed dormancy, the seed possesses anatomical features that either restrict the entry of substances such as water and oxygen or restrain the emergence of the radicle. The physical restraint of the seed coat accounts for the majority of the dormancy exhibited by some pines from the south-eastern United States[3]. The relative ease of germinating longleaf versus loblolly pine can be explained by the lack of a dense, stony layer that is characteristic of longleaf pine[5]. The seed coat thickness of ponderosa pine has also been shown to be a constraint to germination[4]. Compare the force required to cut a ponderosa pine seed versus a Douglas-fir seed and you will appreciate how much energy may be required to split the seed coat.

Physiological dormancy, also called embryo dormancy, is not well understood although it is widespread among conifers. One notable exception is western redcedar which does not have embryo dormancy. The cool moist conditions encountered by seed during the winter, or through stratification, cause biochemical changes within the seed that overcome the impediments to germination. Internal seed morphology changes substantially following imbibition, but subsequent stratification does not show any morphological changes as changes at this stage are biochemical in nature (FIGURE 30).

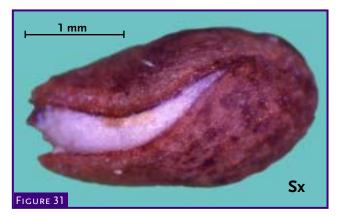
Embryo dormancy is generally considered a balance of germination-inhibiting and -promoting hormones[20], although changes in tissue sensitivity to these hormones may be more important[48]. One of the initial changes caused by stratification is the removal of a block preventing lipid breakdown[36]. Western white pine is an example of a species displaying both physical and physiological dormancy. Physical dormancy is due to the megaspore cell wall restricting water uptake and physiological dormancy is substantiated by germination improvements following extended periods of stratification[22]. Although dormancy is mainly a species attribute, variability between seedlots is present and some seedlots may require special pretreatments. This may be the result of improper collection timing or it may occur in seedlots collected from the extremes of a species range.

For effective removal of physiological dormancy an optimal moisture content of between 30 and 35% exists[13]. For nursery operations, this moisture content is very practical as it coincides with the point at which the seed exhibits no excess moisture on the seed coat after internal components have imbibed moisture. This is essential for efficient sowing as the seed flows freely and can pass smoothly through mechanical sowing machines. The surface dry status of seed is easily observed for most species and is usually indicated by a lighter coloration of the seed coat (FIGURE 7, page 8).

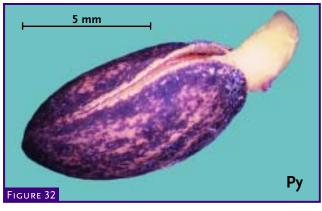


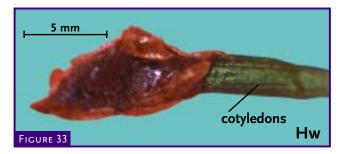
A comparison of dry, imbibed and stratified seed of Douglas-fir in longitudinal section.

# **Seed Germination**



An interior spruce seed splitting at the micropylar end, the junction of the upper and lower seed surfaces.





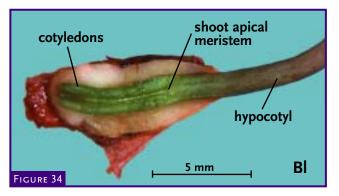
A germinating seed of ponderosa pine showing radicle emergence.

A germinating seed of western hemlock with cotyledons beginning to emerge.

Seed germination is recognized by the emergence of the radicle from the seed. The cotyledons may or may not emerge during germination. When the cotyledons emerge, germination is termed **epigeal** and this is characteristic of the conifers. When the cotyledons do not emerge, germination is termed **hypogeal** (e.g., oak). The germination process is actually initiated upon imbibition of the seed components. Imbibition is accompanied by an immediate release of gases and the initiation of respiration, enzyme activity, protein synthesis, and organelle activity within the embryo[8,10].

Germination is an energy requiring process and the megagametophyte supplies the energy and nutrients for embryo growth and emergence. Lipids, proteins, and reserve phosphorous compounds are used for the synthesis of carbohydrates, and various structural and soluble compounds in the germinant[10]. The breakdown of protein bodies precedes the breakdown of lipid bodies[38], releasing free amino acids exported immediately to the embryonic axis[23].

The initial sign of germination is radicle emergence, which results from both cell elongation and cell division at the root apical meristem[8]. Water is required for cell expansion and development and free water should be available during the rapid growth phase that occurs during germination. The pressure exerted by the radicle causes a splitting of the seed coat at the micropylar end along the junction that joins the two seed surfaces (FIGURE 31). The radicle will emerge through this opening causing further splitting of the seed coat (FIGURE 32). Radicle elongation will continue, followed by growth in the cotyledons and hypocotyl. This, in turn, will force the cotyledons to begin to emerge (FIGURE 33). A longitudinal section through a subalpine fir germinant illustrates the green coloration of photosynthetic parts and a prominent shoot apical meristem. The megagametophyte has become discoloured and softened by the translocation of its reserves to the growing embryo (FIGURE 34).



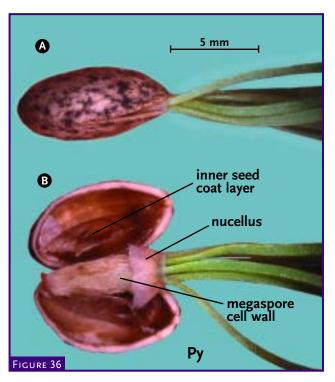
Longitudinal section of a germinating subalpine fir seed illustrating the deteriorating megagametophyte.

As growth of the cotyledons continues, the seed coat will be elevated and will eventually be shed (FIGURE 35). Prior to seed coat shedding seed morphology does not appear different from the exterior, but many anatomical changes have occurred within the seed (FIGURE 36). In the dissected seed of ponderosa pine the megagametophyte has almost completely been utilized and the main remaining feature is the megaspore cell wall. The nucellus is obvious and extends over about one quarter of the megagametophyte. On the interior of the seed coat one can see how the inner layer has torn away from the much thicker stony layer. The hypocotyl and radicle are not visible (FIGURE 36).

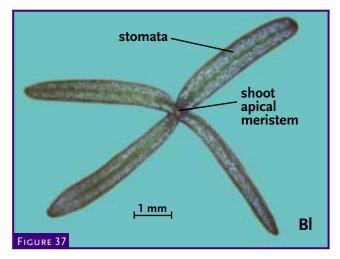
The fully expanded cotyledons maximize sunlight reception and photosynthesis to provide energy to the germinant for growth after the reserves of the megagametophyte are utilized. The hypocotyl is also photosynthetic[37]. Maturation of both cotyledons and hypocotyl tissues involves differentiation of stomata and parenchyma cells with abundant chloroplasts[37]. Stomata contain guard cells in the epidermis that act to regulate the exchange of gases and water vapour between the plant and the external atmosphere. The location, number and time of initiation of stomatal lines varies by species. FIGURE 37 presents four cotyledons of subalpine fir that have been dissected from a germinant. The white flecks are the stomata that are filled with wax and for this species they are arranged in lines on the upper surface. The apical meristem is apparent in the centre of the cotyledons and will produce all subsequent stem growth above the cotyledons (epicotyl) through cell division and expansion.



Germinants of Douglas-fir in the nursery prior to seed coat shedding.



Cotyledon emergence of ponderosa seed (A) from the exterior and (B) from the interior displaying the structures remaining at this stage.



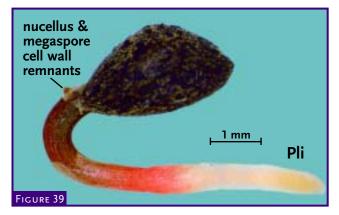
Four cotyledons with stomatal lines surrounding the shoot apical meristem in subalpine fir.

The typical reddish radicle tips found in western hemlock are displayed at various stages of germination in FIGURE 38. The green tissue evident in the larger germinants is due to the development of chloroplasts in the hypocotyl. In lodgepole pine seed anthocyanins are present in the hypocotyl, but the radicle tip is white (FIGURE 39). Note the remnants of the nucellus and megaspore cell wall above the point of radicle emergence. The radicle of interior spruce initially does not appear pigmented, but as the germinant advances the hypocotyl will turn green (FIGURE 40). The intensity and colour of the hypocotyl varies by species, but can be affected by pH[16]. Abnormal hypocotyl colouring may be a clue to check the pH of your growing media.

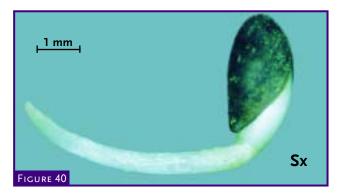
FIGURE 41 details a ponderosa pine germinant. The central pith remains unpigmented while the development of chloroplasts within the cortex produce the green colour in the hypocotyl. The cortex tissue that is still contained within the seed does not contain differentiated chloroplasts. The darker area between the pith and cortex is the procambium. This area will be differentiating rapidly to keep up with the increasing demands for water and photosynthate transport. The outer epidermal layer has accumulated pigments where it resides outside the seed.



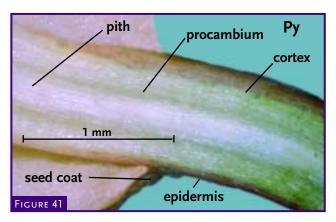
Germinating seed of western hemlock displaying the characteristic red-tipped radicles.



A germinating seed of lodgepole pine.



A germinating seed of interior spruce.



A magnified view of the tissues present in the hypocotyl during germination of a ponderosa pine seed.



Germination of pelleted seed of western redcedar.

FIGURE 42 shows how pelleted western redcedar radicles must penetrate both the seed coat and then the pellet before they can become established. This will slow germination, but most nurseries consider pelletting a necessity for seeding western redcedar because of the light weight, winged, and irregularly shaped seed. The species is not imbibed or stratified before pelletting. In FIGURE 43, two germinants of western redcedar are shown, one with a pellet present and one with it already shed or dissolved. Notice the amount of epicotyl growth above the cotyledons.

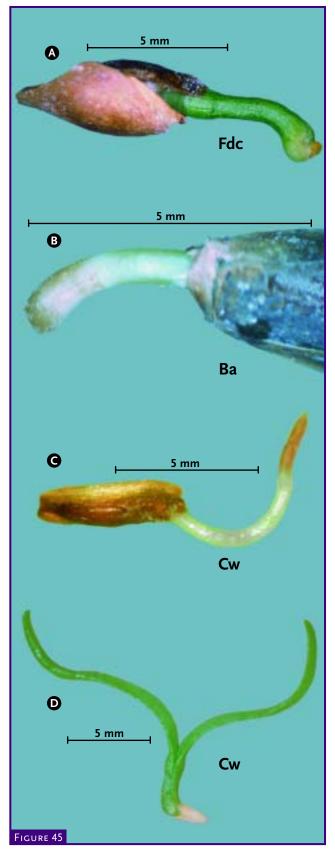
Abnormal germinants generally constitute a small percentage (less than 1%) of a seedlot. Abnormal germinants are defined in the ISTA International Rules for Seed Testing[24] and are not included in the germination percent recorded. The most common abnormal type is the reversed embryo or 'breached' germinant as displayed in FIGURE 44. In this situation the cotyledons emerge first before the radicle. It is unlikely that this germinant would survive due to the difficulty in establishing a root system. A description of germination variables is included in Appendix 4.



Germinants of western redcedar in the nursery prior to pellet breakdown.



A reversed or 'breached' embryo emerging with the cotyledons before the radical from a lodgepole pine seed.





Advanced development of two embryos in an interior lodgepole pine seed.

Another common abnormality is the stunting or retarding of tissues, generally the hypocotyl or radicle. This may take several forms and FIGURE 45 displays the variety of stunted radicles found in several species. The possible reasons for stunting are physical impacts destroying the root apical meristem, a deleterious mutation, or pathogen infection.

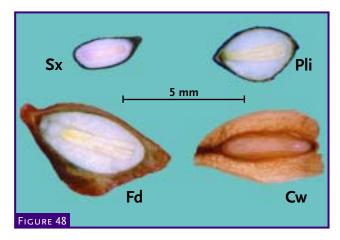
A rare situation is the development to maturity of two or more embryos in a seed (FIGURE 46). This can occur through the process of polyembryony, which is common in conifers. Usually one embryo becomes dominant and the other degenerates at an early stage. Various types of polyembryony occur and a detailed anatomical analysis of polyembryony can be found in Berlyn[8].

Examples of stunted radicles in (A) Douglas-fir, (B) Amabilis fir, and (C), (D) western redcedar.

# **Genera Profiles**



A comparison of seed coat morphology between interior lodgepole pine and interior spruce.



A comparison of longitudinal sections of interior spruce, interior lodgepole pine, Douglas-fir, and western redcedar.

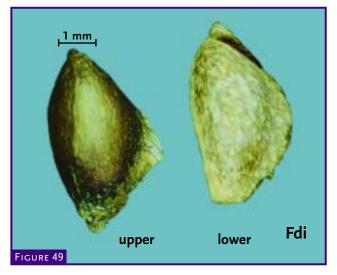
The genera profiles section provides illustrations and descriptions of the individual species within each genus commonly grown in western Canada for reforestation. The genera in the Pinaceae family are covered first followed by the Cupressaceae.

The importance of interior spruce and lodgepole pine to reforestation in B.C. cannot be overemphasized: they consistently account for 75% of the reforestation in the province. Due to their similar small size and lack of wing remnants, these species are sometimes confused, but upon closer examination differences are obvious (FIGURE 47). Lodgepole pine seed are generally larger, heavier, quite dark (almost black), while spruce seed are smaller, brownish, and more variable in colour, usually with a mottled surface. At the extremes of both species there is an overlap in seed size, weight, and colour.

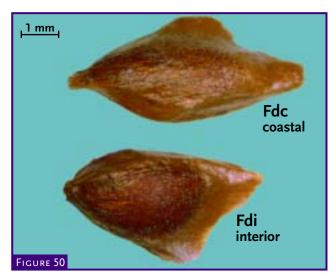
In FIGURE 48 a comparison of longitudinal seed sections of the four major reforestation species are presented. Combined, these account for about 87% of the seedlings grown in B.C. This diagram illustrates some of the differences in seed length, width, and embryo size between these species. A striking feature is the reduced size of the megagametophyte and presence of persistent wings in western redcedar. The increased ease of handling the larger Douglas-fir seed is often offset by the presence of wing remnants, which can reduce purity and decrease sowing precision.

Each species has unique attributes (TABLE 2). The seed size attributes can be useful in screening or sizing seed to maximize the efficiency of sowing with some seeders. TABLE 2Seed and embryo attributes for B.C. reforestation species. Measured variables are based on 10 seed<br/>from 5 seedlots and are presented with means and (ranges) in millimetres.

Common		Seed per	Seed	Seed	Embryo	Cotyledon number	Resin vesicle
name	Code	gram	length	width	length	number	number
Amabilis fir	Ва	29	10.4 (8–12)	4.4 (3–5)	6.7 (4–8)	4.6 (4–6)	6.2 (4–10)
grand fir	Bg	46	9.6 (7–13)	5.5 (4–9)	6.5 (5–8)	5.2 (4–6)	8.4 (6–13)
subalpine fir	BI	83	6.4 (5–8)	3.4 (3–4)	4.8 (4–6)	4.2 (3–5)	5.9 (3–9)
coastal Douglas-fir	Fdc	91	6.6 (5–8)	3.4 (3–4)	3.6 (3–5)	6.7 (5–8)	n.a.
interior Douglas-fir	Fdi	103	5.2 (4–7)	3.1 (2–4)	3.6 (3–4)	6.8 (6–8)	n.a.
mountain hemlock	Hm	461	3.6 (2–5)	1.8 (1–2)	2.2 (1–3)	3.3 (3-4)	2.8 (1–5)
western hemlock	Hw	494	3.1 (2–4)	1.9 (1–2)	2.3 (2–3)	3.0 (2–4)	3.8 (1–7)
western larch	Lw	283	4.4 (3–6)	2.2 (2–3)	2.1 (2–3)	5.7 (4–8)	n.a.
coastal lodgepole pine	Plc	375	3.2 (2–5)	1.8 (1–2)	2.5 (2–3)	3.5 (3–5)	n.a.
interior lodgepole pine	Pli	346	3.4 (2–5)	1.9 (1–2)	2.5 (1–3)	3.6 (3–5)	n.a.
western white pine	Pw	52	6.8 (5–8)	3.9 (3–5)	4.4 (3–6)	8.1 (6–10)	n.a.
ponderosa pine	Ру	20	8.9 (7–12)	5.7 (4–8)	7.8 (6–10)	8.9 (6–12)	n.a.
Sitka spruce	SS	412	3.1 (2–4)	1.9 (1–2)	2.0 (1–3)	5.4 (4–6)	n.a.
interior spruce	Sx	439	2.8 (2–4)	1.7 (1–2)	2.0 (1–2)	5.6 (4–7)	n.a.
hybrid Sitka spruce	SxS	458	2.8 (2–4)	1.6 (1–2)	2.0 (1–3)	5.5 (4–7)	n.a.
western redcedar	Cw	788	5.2 (4–7)	2.8 (2–4)	2.9 (2–4)	2	7.7 (4–12)
yellow-cedar	Су	225	4.4 (3–6)	4.7 (3–6)	2.7 (2–3)	2	n.a.



A comparison of the morphological features between the upper and lower surface of a Douglas-fir seed.



A comparison of the external seed morphology of coastal and interior Douglas-fir.



A comparison of a winged and dewinged Douglas-fir seed.

### Douglas-fir Pseudotsuga genus Pinaceae family

The seed of Douglas-fir is relatively easy to identify by its size and distinct upper and lower surfaces. The upper surface is rounded and dark

ADDITIONAL DOUGLAS-FIR PHOTOS					
FIGURE#	PAGE <sup>#</sup>				
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brown while the lower surface is light coloured, mottled, and much flatter (FIGURE 49). In studies of the effect of seed orientation on germination, it was found that seed with their lower (lighter coloured, mottled) surface facing upwards germinated 1.7 days earlier under laboratory conditions. The practical application of these results is difficult to imagine, but suggest that characteristics such as seed orientation can affect germination. It was also observed that coastal Douglas-fir seed from environments with the shortest growing seasons germinate quickest[53].

In western Canada, Douglas-fir is subdivided into a coastal **variety** (var. *menziesii*) and an interior variety (var. *glauca*). The varieties can be distinguished by shape and presence of markings on the seed coat. The coastal variety generally has longer and narrower seed with a pointed tip that is pinched in at the micropylar end. It also has a more pronounced ridge on the darker side that is wrinkled near the micropylar end. The interior variety is broad and more circular in shape. Its seed coat is often brighter and commonly the dark side is marked by stripes that may continue into the seed wing[2] (FIGURE 50).

Removal of the seed wing is important to improve seeding efficiency and is performed on all species in the Pinaceae family. In Douglas-fir, the seed wing and seed are integrally connected and dewinging is accomplished by breaking the wing as close as possible to the seed coat. A winged and dewinged seed of interior Douglas-fir illustrate the persistent remnants of the wing attached to the seed coat (Figure 51). Incomplete dewinging can result in decreased **purity** over time due to the continual breakage of wing remnants. This wing material may be sown instead of a seed resulting in an empty cavity. The seed wing of



A longitudinal section of an imbibed Douglas-fir seed.

Douglas-fir is composed of two layers of sclerenchyma cells, both of which develop from the ovuliferous scale and not the ovule. The outer layer is continuous with the outer layer of the seed coat[39]. This seed wing origin and structure is considered typical of the Pinaceae although the integrity of the seed to wing attachment varies by species.

In FIGURE 52 an imbibed longitudinal section of a Douglas-fir seed is presented. The embryo is characteristically yellow in dry seed and gradually grows paler following imbibition (FIGURE 10, page 10, FIGURE 30, page 24). The dry megagametophyte is a grey-cream colour that changes to white following imbibition (FIGURES 10, 30). This colour change following imbibition is characteristic of most conifer seed. In coastal Douglas-fir the megagametophyte accounts for 65% of the dry weight of the seed followed by the seed coat (28%) and embryo (7%).

Seed of the coastal variety are generally larger, but embryo lengths of both varieties were found to be equivalent (TABLE 2, page 31). The more rapid germination found in interior Douglas-fir may be due to its proportionately larger embryo. A suspensor is usually obvious at the micropylar end of the seed.

The anatomy of Douglas-fir seed has been intensively studied in Canada. The classic work of Allen and Owens[1] in 1972 has opened the door to studies on many other conifers and today we are gaining an understanding at greater levels of detail about the conifer seed[27,38,56,57]. Many of these details are beyond the scope of this text.

### Spruce Picea genus Pinaceae family

The species in the spruce genus are considered together, as their seed are quite similar in anatomy and morphology (FIGURE 53) (TABLE 2), although their ecological niches vary considerably. The species represented

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31	25
40	27
47	30
48	30

in B.C. include Sitka spruce on the coast and interior spruce (a species complex including white spruce at lower elevations; Engelmann spruce at higher elevations; and hybrids between the two species at intermediate elevations). Along the north coast, Sitka spruce also hybridizes with interior spruce and these seedlots are designated SxS. In the boreal part of the province black spruce is also present, although currently not planted to any extent in B.C. Black spruce can be identified by its small, dark seed (FIGURE 54).

The morphology of a spruce seed coat appears mottled (FIGURE 55), although it can be predominantly dark or light. Notable differences between upper and lower seed surfaces are not as apparent



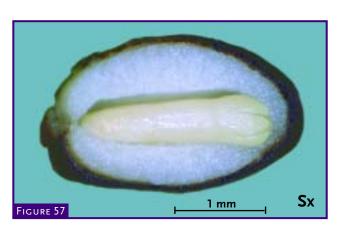
A comparison of seed morphology between interior spruce, Sitka spruce, and hybrid spruce.



A comparison in the seed morphology between interior spruce and black spruce.



A magnified view of the surface morphology of an interior spruce seed.



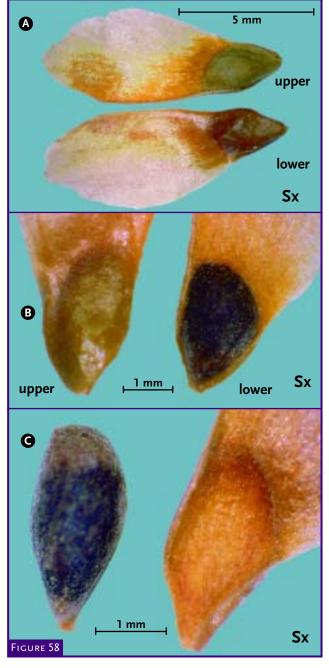
A longitudinal section of an interior spruce seed with the embryo unsliced.

as other genera. FIGURE 56 illustrates seed of interior spruce from two different trees. Both were fully mature and germinable, but very little pigmentation is present in the light-coloured seed. In Finland, Norway spruce seed are consistently darker from the north, but no such findings have been reported for Canada. In FIGURE 57, a typical imbibed seed of interior spruce in longitudinal section is displayed. The cream-coloured embryo has not been sectioned to present its three-dimensional morphology with three distinct cotyledons.

A comparison of the upper and lower surfaces of winged spruce seed is presented in FIGURE 58A. The seed wing, derived from the ovuliferous scale, is continuous with the upper surface of the seed. The lower surface of the seed is exposed due to a separation layer which forms beneath the seed before fertilization (FIGURE 58B)[34]. The lower seed surface sits imbedded in the surrounding ovuliferous scale. With the addition of water the seed wing expands and is easily removed from the seed (FIGURE 58c) resulting in very clean dewinging. Compare the complete wing removal of spruce with the incomplete breaking that must occur with Douglas-fir (FIGURE 51, page 32).



Comparison of interior spruce seed from two mother trees.



A comparison of (A) the upper and lower surfaces of winged interior spruce seed, (B) a magnified view of wing attachment, and (C) a seed removed from the adjacent wing attachment.

# Pine

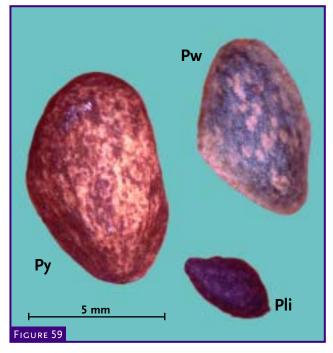
# *Pinus* genus Pinaceae family

This section will concentrate on the three pine species used for reforestation in the province: lodgepole pine, ponderosa pine, and western white pine (FIGURE 59). The pines we will discuss all have seed wings, but pine seed larger than 90 mg are generally bird dispersed and wingless[7]. In ponderosa pine and white pine the

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	Ру	5	7					
	<sup>*</sup>	10	10					
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r		32	25					
		36	26					
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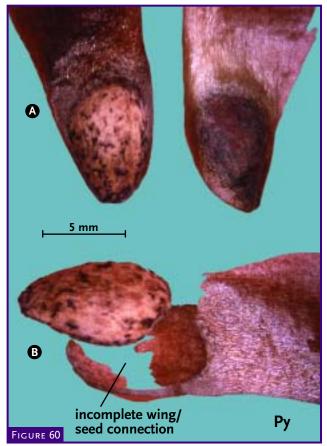
attachment of the wing to the seed is usually similar to spruce (FIGURE 60). The connection of the wing to the seed coat is relatively weak and in this example incomplete, allowing for its easy separation. In lodgepole pine and sometimes ponderosa pine the attachment is pincer-like where the cells from the wing do not cover the upper surface or lower surface of the seed, but clasp the seed at the junction of upper and lower seed surfaces (FIGURE 61). In either case the addition of moisture ensures efficient dewinging.

Lodgepole pine is subdivided into a coastal variety (var. *contorta*) and an interior variety (var. *latifolia*). Seed differences are not great, although the interior variety has slightly larger seed and

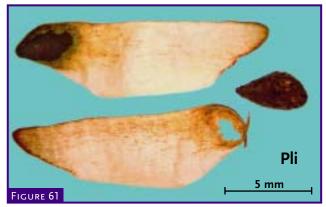


A seed morphology comparison between lodgepole, ponderosa, and western white pine.

about one hundred times more are planted in B.C. Both varieties germinate very quickly. A longitudinal section of a lodgepole pine seed shows numerous cotyledons and a well-defined cylinder of the procambium and pith (FIGURE 62). The embryo is cream coloured in this seed, but characteristically lodgepole pine has a distinct white embryo. The megagametophyte accounts for a large proportion



Method of wing attachment in ponderosa pine illustrated by (A) the upper and lower surfaces of a winged seed, and (B) the morphology of structures following dewinging.

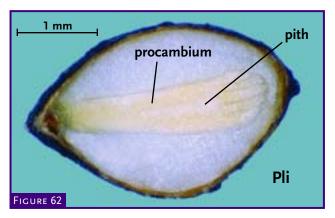


The morphology of the seed wing attachment in lodgepole pine.

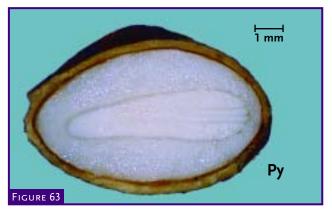
of the seed. In estimates of the energy invested in seed, the megagametophyte and embryo account for 77.6%, the seed coat 13.7%, and the seed wing 8.8%[51].

Ponderosa pine have the heaviest seed with thick seed coats that may act to constrain the megagametophyte and embryo (FIGURE 63). The seed coat weight is lightest in the interior of the range and heaviest with increasing elevation. This indicates seed coat dormancy is greater at higher elevations in the interior portion of the range[4]. The greatest number of cotyledons are found in ponderosa pine (ranging from 6 to 12) giving the species a large base for photosynthesis before leaves are initiated. Other literature on the southern pines would probably be applicable to ponderosa pine as their general anatomy and morphology are similar.

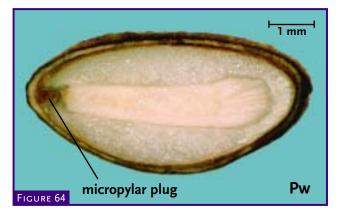
Western white pine is well known for its low and erratic germination. The species requires a long stratification period (deeply dormant) and has shown variability in the degree of physical and physiological dormancy between seedlots. A longitudinal section of a western white pine seed is shown in FIGURE 64. The embryo occupies a large portion of the seed and a prominent micropylar plug is commonly seen. The seed coat layers can be clearly seen at the chalazal (cotyledon) end of the seed (see also FIGURE 8, page 8).



A longitudinal section of an imbibed interior lodgepole pine seed.



A longitudinal section of an imbibed ponderosa pine seed.



A longitudinal section of an imbibed western white pine seed.

# True firs Abies genus Pinaceae family

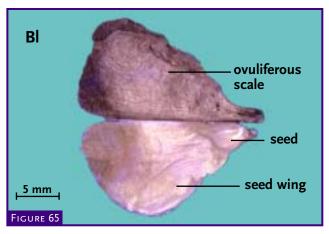
The true firs are represented in B.C. by Amabilis fir, subalpine fir, and

DITIONAL TRUE FIRS FIGURE# PAGE Ba 20 17 17 21 45 29 Rİ 34 26 37 27

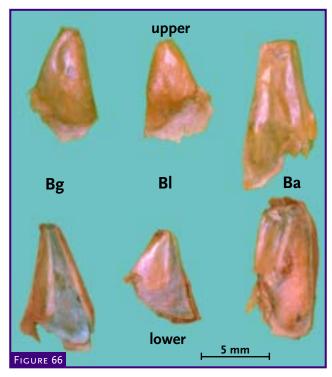
grand fir. All of the *Abies* spp. have cone scales that abscise from the cone axis. Therefore, kilning is not required for seed extraction. An example of an abscised cone scale with one seed attached is displayed in FIGURE 65. Note the continuity of the seed wing and outer seed coat over the upper surface of the seed and the relatively large size of the seed wing compared to other conifers.

A comparison of the upper and lower seed surfaces of grand, subalpine, and Amabilis fir are presented in FIGURE 66. Species can usually be identified based on seed morphology without much difficulty. Amabilis fir seed is tan-coloured, quite large, and elliptical in shape; subalpine fir is relatively small, triangular, and can have a purple coloration (FIGURE 67); Grand fir is intermediate in size, triangular, and contains the most resin vesicles. The lower surface of the seed is characterized by a discontinuity in the outer seed coat layer (FIGURE 66) as a result of the separation of the seed from the ovuliferous scale. This discontinuity exposes some of the resin vesicles on the lower seed surface to potential damage.

All *Abies* spp. have resin vesicles that form within the integument during seed development. The resin vesicles, which appear outside the stony layer of the seed coat in Amabilis fir[33], subalpine fir[48], and grand fir[49], are more common on the

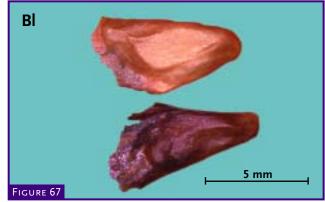


An ovuliferous scale of subalpine fir with one winged seed present.



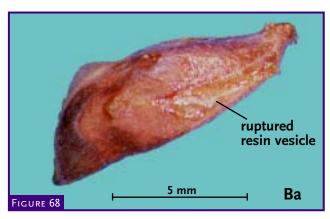
The upper and lower surfaces of the seed of grand fir, subalpine fir, and Amabilis fir.

lower surface of the seed and cause depressions in the megagametophyte. The stony layer is reduced in size under the resin vesicles. If processing is excessive, the entire outer seed coat layer and wing tissue can be removed exposing a larger number of resin vesicles to potential damage. An Amabilis fir seed without its outer seed coat layer illustrates a recently damaged resin vesicle (FIGURE 68). Seed with damaged resin vesicles can be identified by a sticky or pitchy feel, presence of resin, and a characteristic odour and dark grey colour. The outcome of resin vesicle damage is a reduction in germination[17,21].



Different morphologies of mature, viable seed of subalpine fir.

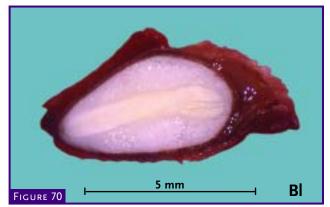
Seed of grand fir contain the greatest number of resin vesicles; five are obvious in FIGURE 69 causing depressions in the megagametophyte. Grand fir seed are also considered less dormant than the other species which is reflected in its shorter stratification regime (Appendix 4). The smaller seed of subalpine fir have the least number of resin vesicles in the true firs (average is 4) and only one is visible in FIGURE 70 at the chalazal end of the seed. A longitudinal section of an Amabilis fir seed is displayed in FIGURE 71. The characteristically yellow-cream coloured



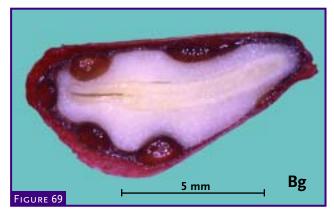
A damaged resin vesicle in an Amabilis fir seed.

embryo occupies nearly the entire length of the seed, cotyledons are large and well developed and the procambium is visible. Remnants of the seed wing can be seen. A short vascular strand was reported to be found in the seed coat, at the chalazal end, during seed development[41].

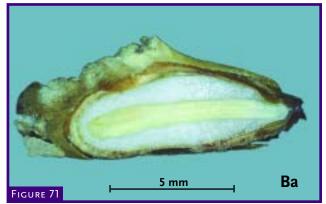
Details on cone and seed processing are beyond the scope of this volume, but an overview of changes in the morphology of material present in a subalpine fir seedlot during processing is presented to provide an overview (FIGURE 72).



Longitudinal section of an imbibed subalpine fir seed.



Longitudinal section of an imbibed grand fir seed.

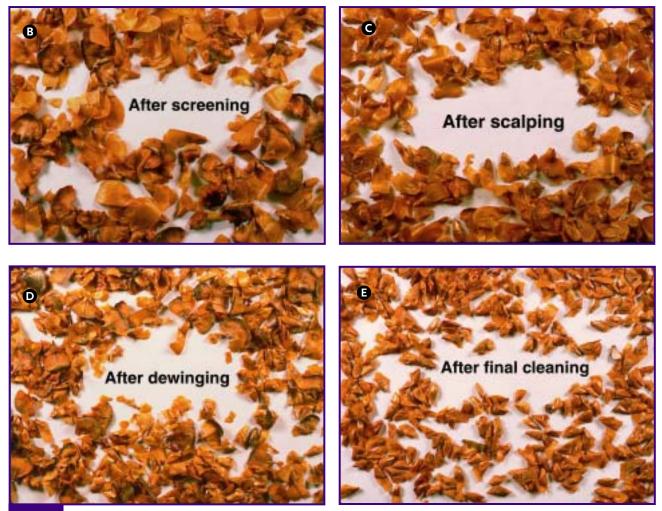


A longitudinal section of an imbibed Amabilis fir seed.

The cones are slowly dried until they are completely broken up (FIGURE 72A). A large amount of debris (cone axis, scales, and fine debris) are removed first by screening the material (FIGURE 72B). The



remaining material will be scalped (more refined screening) to further purify the seed and remove debris that may cause mechanical damage (FIGURE 72c). Dewinging seed using a rotary drum is the next processing step, and similar to Douglas-fir the wings are broken off because of the integral connection with the seed coat (FIGURE 72D). Dewinging is a critical point in processing as it is best to remove as much wing material as possible while not damaging the seed coat or resin vesicles through excessive tumbling. The final stage of cleaning is to run the seedlot over a fanning mill, gravity table, and/or pneumatic separator to remove any non-viable seed and remaining impurities (FIGURE 72E).



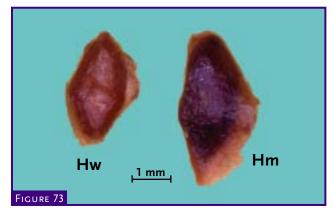
**FIGURE 72** Morphological differences in a subalpine fir seedlot during processing: (A) before processing, (B) after screening, (C) after scalping, (D) after dewinging, and (E) after final cleaning.

### Hemlock Tsuga genus Pinaceae family

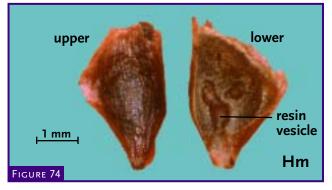


This genus is represented by western 38 27 and mountain hemlock in B.C. Mountain hemlock seed are considerably larger and darker in colour than western hemlock (FIGURE 73). The seed of western hemlock is very small and only western redcedar is lighter in weight. Both *Tsuga* spp. have resin vesicles which only occur on the lower surface of the seed (FIGURE 74). Although western hemlock seed are smaller, they contain more resin vesicles than mountain hemlock.

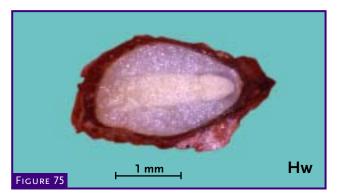
Like western redcedar, western hemlock is considered to have a low level of seed dormancy. Stratification of four weeks is used, but it is considered a treatment to increase the germination rate rather than the capacity[12]. The longitudinal section in FIGURE 75 reveals the main components. However, anatomical details are difficult to distinguish; this is typical of the hemlocks. The longitudinal section of mountain hemlock is from a dry seed (FIGURE 76). Cotyledons are prominent, but no shrinkage is present as is found with other species in the dehydrated state. The megaspore cell wall surrounds the megagametophyte in western hemlock, but in mountain hemlock only a few irregular cells occur and a continuous sheath is not formed[26].



The morphological differences between a western and mountain hemlock seed.



The upper and lower surfaces of mountain hemlock seed.



Longitudinal section of an imbibed western hemlock seed.



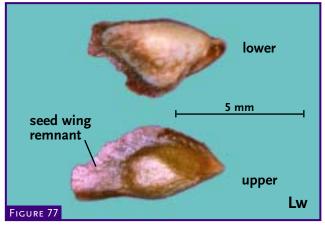
Longitudinal section of a dry mountain hemlock seed.

## Larch Larix genus Pinaceae family

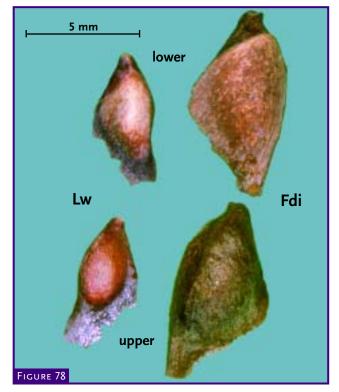
There are three larch species in western Canada: western larch, subalpine larch, and tamarack, but only western larch is currently used for reforestation in B.C. Western larch seed have a dark, raised upper surface and a mottled lower surface. A larger than average seed wing remnant is displayed in the lower seed (FIGURE 77). Dewinging is performed on dry seed, but it is a difficult species on which to achieve consistently thorough dewinging. Empty seed are common in western larch as the ovule and seed coat are well developed at fertilization relative to other conifers[35]. Western larch, although much smaller, is quite similar to Douglas-fir in terms of shape and differences between the upper and lower seed surfaces (FIGURE 78). The mottled appearance of the lower surface as well as the obvious tip of the seed at the micropyle are common to both species (FIGURE 78A). The upper surface shows similarity in form, although colour varies considerably and the western larch seed appears more rounded (FIGURE 78B).

These two species also show higher susceptibility to pre-emergence damping-off caused by *Fusarium* spp. in nurseries. Some of the anatomical characteristics that the species share may allow the fungi a relatively easy entry into the seed or provide the spores with suitable sites for attachment. Recent work has shown that treatment with hydrogen peroxide (3% for 4 hours) is an effective means of reducing *Fusarium* spp. without negatively affecting germination in these species[25].

FIGURE 79 displays a longitudinal section of an imbibed western larch seed. The prominent micropyle and wing remnant resemble Douglas-fir, but the seed is fatter with more megagametophyte tissue relative to its size. Within the embryo the cotyledons are visible and are beginning to turn yellow. Just prior to emergence, these will turn green. No anatomical details are observable within the embryo. Western larch and the hemlock species present the greatest challenge for viewing anatomical details of the embryo with cutting tests.



The morphological differences on the lower and upper surfaces of western larch seed.



The morphological differences and similarities between seed of western larch and Douglas-fir on (A) lower and (B) upper surfaces of the seed.



A longitudinal section of an imbibed western larch seed.

# Western redcedar Thuja genus Cupressaceae family

Western redcedar is the only species from this genus in western Canada.

As illustrated in FIGURE 80, the seed is quite different from other conifer seed: the wings are an integral part of the seed, resin vesicles are linear in appearance and reduced in size compared to Abies and Tsuga spp., and the seed is extremely light in weight. The seed coat is very thin and consists of three layers that are thickened at points of attachment to the persistent wings[37]. Western redcedar is generally considered to have nondormant seed. This can be problematic if proper care is not given during post collection handling as germination can occur while seed are still in the cones (FIGURE 81). This 'pre-germination' occurs because there are no internal restrictions to germination. If adequate moisture is present within the seed, the factor controlling germination is temperature build-up. In FIGURE 81B the radicle of the germinating seed is stunted, which may result from impact damage, dehydration, or fungal infection.

DDITIONAL

REDCEDAR PHOTOS

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FIGURE#

6

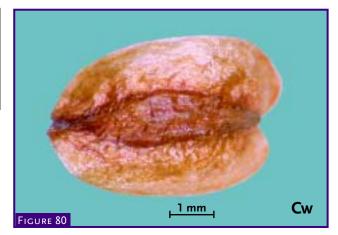
42

43

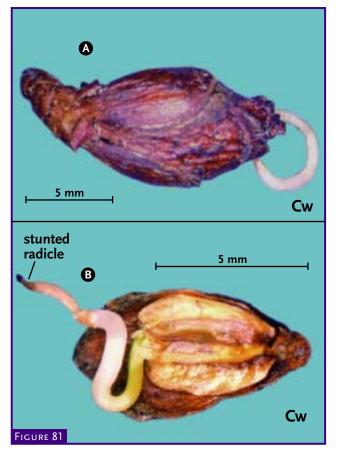
45

48

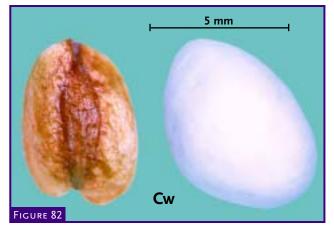
The very light weight and irregular shaped seed of western redcedar provide problems in mechanical sowing at the nursery. Seed are not easily transferred to containers using common seeding equipment. The current solution is to pellet seed of western redcedar (FIGURE 82). Dry seed are pelletized with a mixture of diatomaceous earth and various binders that are slowly built up over the seed with misting to produce an elliptical pellet. Pelleting does not involve imbibition and therefore does not affect seed physiology or storability. The lack of dormancy in western redcedar make it suitable for pelletting. It is probable that the seed coating protects the resin vesicles from damage as well as providing a regularly shaped propagule.



The morphology features of a western redcedar seed.



Premature germination in western redcedar displayed (A) in the cone and (B) with the cone scale dissected away.



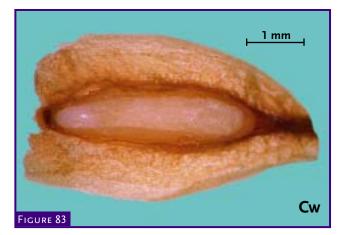
A comparison of a naked and pelleted seed of western redcedar.

The embryo of western redcedar occupies a larger proportion of the seed than other species (FIGURE 83). The megagametophyte is greatly reduced restricting the amount of storage reserves available and making its condition difficult to assess. Due to the limited megagametophyte and water holding reserves in western redcedar seed it is important that water is available to initiate and continue the germination process. In addition to thoroughly soaking blocks, misting will aid in pellet breakdown and avoid problems of pellets becoming 'cemented' to the germinant. A reduction in grit depth will also improve the chances of all viable seed germinating before their reserves are fully utilized.

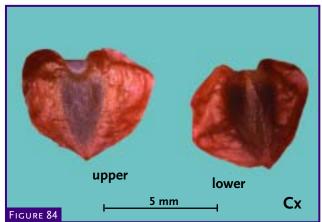
The germination environment is a crucial stage to manage in western red-cedar. Initial photosynthesis will also be less than other species due to the presence of only two cotyledons.

### Yellow-cedar Chaemacyparis genus Cupressaceae family

Only one species in this genus is found in Canada. It has a restricted range, but it is highly desirable for reforestation. Persistent seed wings, much thicker than western redcedar, are present giving the seed an oval appearance (FIGURE 84). No resin vesicles are present. The seed wings are covered in wax, which is also present as deposits in sclerenchyma cells and within the nucellar region. Compared to Pinaceae species, all of the seed coat layers in yellow-cedar appear compact with no cavities between them. The sclerenchyma cells of the stony layer continue to thicken until the seed matures, and this may also be implicated in physical dormancy[32]. The species is considered to have the deepest embryo dormancy of conifers in western Canada (requires longest and most complex pretreatment), and is suspected of having physical dormancy associated with the tissues surrounding the megagametophyte. Although western redcedar cones resemble those of the Pinaceae, yellow-cedar cones are spherical without a central stalk (FIGURE 85). All cone scales originate from a central spot limiting the number of potential seed from three to five per cone. Another complication with yellow-cedar is the variation found in the reproductive cycle enabling seed to mature in either one or two years following pollination depending on environmental conditions[15]. Mature and immature cones can be



A longitudinal section of an imbibed western redcedar seed.



The upper and lower surfaces of a yellow-cedar seed.



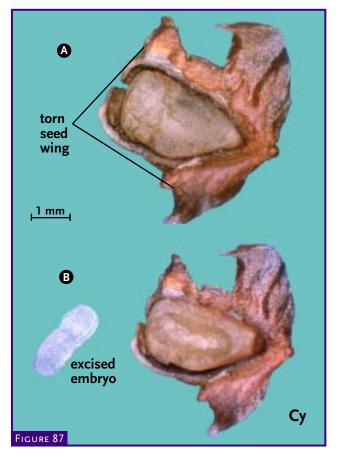
Mature and immature cones of yellow-cedar.

found on the same branches and both may be combined in a collection. The morphology of the mature and immature seed varies dramatically. Seed from immature cones are white, soft, and moist[27]. The seed can usually be easily separated during seed processing, but will decrease seed yield. Also, the extra moisture associated with these immature seed is undesirable.

A longitudinal section of a stratified yellowcedar seed is shown in FIGURE 86 with the laterally elongated wings that give the seed an oval appearance. The embryo does not occupy as great a volume of the seed as western redcedar, but more than the species in the Pinaceae. In dry seed the embryo is difficult to distinguish from the megagametophyte due to its clear coloration (FIGURE 87), and this is more problematic with immature seed.



A longitudinal section of an imbibed yellow-cedar seed.



A dry yellow-cedar seed in (A) longitudinal sections and (B) with the embryo excised.

# **Literature Cited**

- 1 Allen, G.S. 1960. A method of distinguishing coastal from interior Douglas fir seed. British Columbia Lumberman 44(8) 26–28.
- **2** Allen, G.S. and J.N. Owens 1972. The life history of Douglas-fir. Dep. Environ. Can., Can. For. Serv. Ottawa, ON. 139 p.
- **3** Barnett, J.P. 1976. Delayed germination of southern pine seed related to seed coat constraint. Can. J. For. Res. 6:504–510.
- 4 \_\_\_\_\_. 1991. Relating the seed coat of Pinus to speed of germination, geographic variation and seedling development. *In* Proc. 21st S. For. Tree Imp. Conf., June 17–20, Knoxville, TN. pp. 266–275.
- **5** \_\_\_\_\_. 1993. Handling longleaf pine seed for optimal nursery performance. South. J. Appl. For. 17:180–187.
- **6** Benkman, C.W. 1995. Wind dispersal capacity of pine seed and the evolution of different seed dispersal modes in Pines. Oikos 73:221–224.
- Berlyn, G.P. 1972. Seed germination and morphogenesis. *In* Seed Biology Vol. 1 Importance, Development and Germination. T.T. Kozlowski (editor).
   pp. 223–312. Academic Press, New York, NY. 416 p.
- **8** Bewley, J.D. and M. Black. 1994. Seed physiology of development and germination. 2nd Ed. Plenum Press. New York, NY. 445 p.
- **9** Bogar, G.C. and F.H. Smith. 1965. Anatomy of seedling roots of *Pseudotsuga menziesii*. Am. J. Bot. 52: 720–729.
- **10** Ching, T.M. 1966. Compositional changes of Douglas fir seed during germination. Plant Phys. 41:1313–1319.
- 11 Edwards, D.G.W. 1973. Effects of stratification on western hemlock germination. Can. J. For. Res. 3:522–527.
- **12** \_\_\_\_\_. 1980. Maturity and quality of tree seeds a state-of-the-art review. Seed Science and Technology 8:625–657.
- 13 \_\_\_\_\_. 1982. Collection, processing, testing and storage of true fir seeds a review. *In* Proc. Biol. and Mgmt. of True Fir in the Pac. Northwest Symp. Oliver, C.D. and R.M. Kenady (editors). USDA For. Serv., Univ. Washington., Coll. For. Resources, Seattle, WA, Inst. For. Resources Contrib. No. 45:113–137.
- 14 El-Kassaby, Y.J. Maze, D.A. MacLeod, and S. Banerjee. 1991.
   Reproductive-cycle plasticity in yellow-cedar (*Chamaecyparis nootkatensis*).
   Can. J. For. Res. 21:1360–1364.
- **15** Esau, K. 1977. Anatomy of seed plants. 2nd Ed. John Wiley & Sons Inc. New York, NY. 550 p.
- 16 Fahn, A. 1990. Plant anatomy. Fourth Ed. Pergamon Press. Oxford, UK. 588 p.

- Gunia, S. and M. Simak. 1970. Effect of damaging resin vesicles in the seed coat on the germination of silver fir (*Abies alba* Mill.) seeds. International Symp. Seed Phys. of Woody Plants. Inst. of Dendro. and Kornik Arboretum, Polish Academy Sci. Sept 3–8, 1968. pp. 79–83.
- **18** Hoff, R.J. 1987. Dormancy in *Pinus monticola* seed related to stratification time, seed coat and genetics. Can. J. For. Res. 17:294–298.
- 19 ISTA. 1993. Seed Sci. & Technol., 21, Supplement. International Rules for Seed Testing. 1993. 288 p.
- **20** Khan, A.A. 1975. Primary preventive and permissive roles of hormones in plant systems. Bot. Rev. 41:391–420.
- **21** Kitzmiller, J.H, J.M. Battigan, and J.A. Helms. 1973. Effect of resin vesicle damage on germination of stored *Abies concolour* seed. True fir Mgmt. Coop., Sch. For. and Cons., Berkeley, CA. Internal Rep. #1. 16 p.
- **22** Krasowski, M.J. and J.N. Owens. 1993. Ultrastructure and histochemical postfertilization megagametophyte and zygotic embryo development of white spruce (*Picea glauca*) emphasizing the deposition of seed storage proteins. Can. J. Bot. 71:98–112.
- **23** Lammer, D.L. and D.J. Gifford. 1989. Lodgepole pine seed germination. II The seed proteins and their mobilization in the megagametophyte and embryonic axis. Can. J. Bot. 67:2544–2551.
- **24** Martinez-Honduvilia, C.J. and A. Santos-Ruiz. 1978. Germination inhibitors in the pine seed coat. Planta 141:141–144.
- **25** Neumann, M. 1997. Sanitation methods for conifer seeds, soaking tanks and screens to control seedborne *Fusarium*. Contract report prepared for B.C. Min. For., Surrey, B.C. 49 p.
- **26** Owens, J.N. and M. Molder. 1975. Sexual reproduction of mountain hemlock (*Tsuga mertensiana*) Can. J. Bot. 53:1811–1826.
- **27** \_\_\_\_\_. 1975. Pollination, female gametophyte, and embryo and seed development in yellow cedar (*Chamaecyparis nootkatensis*). Can. J. Bot. 53: 186–199.
- 28 \_\_\_\_\_. 1977. Sexual reproduction of Abies amabilis. Can. J. Bot. 55:2653-2667.
- **29** \_\_\_\_\_. 1977. Sexual reproduction of white spruce (*Picea glauca*). Can J. Bot. 57:152–169.
- **30** \_\_\_\_\_. 1979. Sexual reproduction of *Larix occidentalis*. Can. J. Bot. 57:2673–2690.
- **31** \_\_\_\_\_. 1977. Sexual reproduction of western red cedar (*Thuja plicata*). Can. J. Bot. 58:1376–1393.
- **32** Owens, J.N., S.J. Morris, and S. Misra. 1993. The ultrastructural, histochemical development of the post-fertilization megagametophyte and the zygotic embryo of *Pseudotsuga menziesii*. Can. J. For. Res. 23: 816–827.

- **33** Owens, J.N. and F.H. Smith. 1965. Development of the seed cone of Douglas-fir following dormancy. Can. J. Bot. 43: 317–332.
- **34** Pittermann, J., M. West, and J.N.A. Lott. 1996. Characterization of globoids and iron-rich particles in cotyledons of *Pinus banksiana* seeds and seedlings. Can. J. For. Res. 26:1697–1702.
- **35** Rosochacka, J. and A.P. Grzywacz. 1980. The colour of *Pinus silvestris* L. seeds and their susceptibility to damping-off II. Colour of seed coats and their chemical composition. Europ. J. For. Path. 10:193–201.
- **36** Ross, S.D. 1969. Gross metabolic activity accompanying the after-ripening of dormant Douglas-fir seeds. Bot. Gaz. 130:271–275.
- **37** Sasaki, S. and T.T. Kozlowski. 1970. Effects of cotyledon and hypocotyl photosynthesis on growth of young pine seedlings. New Phytol. 69:493–500.
- **38** Simola, L.S. 1974. The ultrastructure of dry and germinating seeds of *Pinus sylvestris* L. Acta Botanica Fennica 103:1–31.
- **39** Singh, H. 1978. Embryology of gymnosperms. Borntaeger, Stuttgart, Germany.
- **40** Singh, H. and J.N. Owens. 1981. Sexual reproduction in subalpine fir (*Abies lasciocarpa*). Can. J. Bot. 59:2650–2666.
- **41** \_\_\_\_\_. 1982. Sexual reproduction in grand fir (*Abies grandis* Lindl.) Can J. Bot. 60:2197–2214.
- **42** Slack, C.R. and J.A. Browse. 1984. Synthesis of storage lipids in developing seeds. *In* Seed physiology. Vol. 1 Development. D.R. Murray (editor). Academic Press Inc., Orlando, FL. pp. 209–244.
- **43** Smith, C.C. 1970. The coevolution of pine squirrels (*Tamiasciurus*) and conifers. Ecol. Monogr. 40:349–371.
- **44** Spurr, A.R. 1950. Organization of the procambium and development of the secretory cells in the embryo of *Pinus strobus* L. Am. J. Bot. 37:185–197.
- **45** Sorenson, F.C. and R.K. Campbell. 1981. Germination rate of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seeds affected by their orientation. Ann. Bot. 47:467–471.
- **46** Tillman-Sutela, E. and A. Kappi. 1995. The morphological background to imbibition in seeds of *Pinus sylvestris* L. of different provenances. Trees 9:123–133.
- **47** Tillman-Sutela, E. and A. Kappi. 1995. The significance of structure for imbibition in seeds of the Norway spruce, *Picea abies* (L.) Karst. Trees 9:269–278.
- **48** Trewavas, A. 1981. How do plant growth substances work? Plant, Cell and Environment 4:203–228.
- **49** Zasada, J.C. 1988. Embryo growth in Alaskan white spruce seeds. Can J. For. Res. 18:64–67.

### Suggested Reading

#### A Guide to Collecting Cones of British Columbia Conifers.

R.D. Eremko, D.G.W. Edwards, and D. Wallinger. Can. For. Serv., B.C. Min. For. FRDA Rep. #55. Victoria, B.C. 1989.

#### A Guide to the Biology of Forest Tree Seeds

C.L. Leadem B.C. Min. For. Land Manage. Handb. 30. Victoria, B.C. 1996.

#### Cone and Seed Diseases of North American Conifers

Edited by: J.R. Sutherland, T. Miller, and R.S. Quinard. N. A. For. Comm. CFAN/COFAN/NAFC Publ. #1. 1987.

#### Cone and Seed Insects of North American Conifers

A.F. Hedlin, H.O. Yates III, D.C. Tovar, B.H. Ebel, T.W. Koerber, and E.P. Merkel. Canadian Forestry Service/US Forest Service/ Secretaría de Agricultura y Recursos Hidráulicos, México, 1980.

#### Diseases and Insects in British Columbia Forest Seedling Nurseries

J.R. Sutherland, G.M. Shrimpton, and R.N. Sturrock. Can. For. Serv., B.C. Min. For., FRDA Rep. #065. Victoria, B.C. 1989.

#### **Dormancy and Barriers to Germination**

Proc. 1991 Symposium of IUFRO Project Group P2.04-00 – Seed Problems. D.G.W. Edwards (compiler and editor). Forestry Canada, Pac. For. Cen., Victoria, B.C. 1993.

#### Forest Tree Seed Production

J.N. Owens and M.D. Blake. Canadian Forestry Service, Infor. Rep. PI-X-53. 1985.

#### High-quality Collection and Production of Conifer Seed

Proc. 1979 workshop, Edmonton, AB. R.F. Huber (compiler). Canadian Forestry Service, Infor. Rep. NOR-X-235. 1981.

#### Methods and Procedures for Testing Tree Seeds in Canada

D.G.W. Edwards. Canadian Forestry Service, Pac. For. Cen., For. Tech. Rep. #36. Victoria, B.C. 1987.

#### Seed Physiology: Development

Volume 1. D.R. Murray (editor). Academic Press. 1984.

#### Seed Physiology: Germination and Reserve Mobilization

Volume 2. D.R. Murray (editor). Academic Press. 1984.

#### Seeds of Woody Plants in the United States

C.S. Schropmeyer (editor). US Forest Service, Timber Management Branch US Dep. of Agric., Forest Service. Agriculture Handbook # 450. 1974.

### Textbook of Dendrology: Covering the Important Forest Trees of

the United States and Canada Sixth Edition. W.M. Harlow, E.S. Harrar, and F.M. White. McGraw-Hill Series in Forest Resources. 1978.

#### Tree and Shrub Seed Handbook

A.G. Gordon, P. Gosling, and B.S.P. Wang (editors). International Seed Testing Association; Zurich, Switzerland. 1991.

#### Tree Physiology: Biology and Control of Reproductive Processes in Forest Trees

Proc. 1993 Symposium of IUFRO J.E. Webber, J.N. Owens, and M.U. Stoehr (editors). Heron Publishing, Victoria, B.C. 1995.

# Appendix 1 – Names of B.C. Conifers

# Pinaceae family

Amabilis fir	Ва	Abies amabilis (Dougl.) Forbes
Grand fir	Bg	Abies grandis (Dougl.) Lindl.
Subalpine fir	Bl	Abies lasciocarpa (Hook) Nutt.
Noble fir	Bn	Abies procera Rehd.
Coastal Douglas-fir	Fdc	Pseudotsuga menziesii (Mirb.) Franco
Interior Douglas-fir	Fdi	Pseudotsuga menziesii var. glauca (Beissn.) Franco
Mountain hemlock	Hm	Tsuga mertensiana (Bong.) Carr.
Western hemlock	Hw	Tsuga heterophylla (Raf.) Sarg.
Western larch	Lw	Larix occidentalis Nutt.
Whitebark pine	Pa	Pinus albicaulis Engelm.
Limber pine	Pf	Pinus flexilis James
Coastal lodgepole pine	Plc	Pinus contorta Dougl.
Interior lodgepole pine	Pli	Pinus contorta var. latifolia Dougl. ex Loud.
Western white pine	Pw	Pinus monticola Dougl. ex D. Don
Ponderosa pine	Ру	Pinus ponderosa Laws
Black spruce	Sb	Picea mariana (Mill.) B.S.P.
Sitka spruce	SS	Picea sitchensis (Bong.) Carr.
Interior spruce	Sx	Picea glauca (Moench) Voss, Picea engelmannii Parry ex Engelm and hybrids
Sitka $\times$ interior spruce hybrid	SxS	Picea x lutzii Little
Cupressaceae family		
Yellow-cedar	Yc	Chamaecyparis nootkatensis (D. Don) Spach
Western redcedar	Cw	Thuja plicata Donn ex D. Don

# Appendix 2 – Glossary

Abaxial: Referring to the upper seed surface.

**Abscisic acid (ABA):** A naturally occurring plant growth substance that promotes leaf fall and dormancy in seeds and buds.

Adaxial: Referring to the lower seed surface.

**Anatomy:** The study of the structure of living organisms, especially of their internal parts by means of dissection and microscopic examination. (compare *Morphology*)

**Angiosperms:** The flowering plants, which are the plants with the most advanced structural organization in the plant kingdom. Monocots with one cotyledon, dicots with two cotyledons.

**Anthocyanin:** One of a group of flavanoid (naturally occurring phenolic compounds) pigments. Occur in the cell vacuoles\* of various plant parts.

**Cambium:** A plant tissue consisting of actively dividing cells that is responsible for increasing the girth of the plant (i.e., it causes secondary growth). The two most important cambia are the vascular cambium and the cork\* cambium. The vascular cambium occurs in the stem and root; it divides to produce secondary xylem\* and secondary phloem\*. In mature stems the vascular cambium is extended laterally to form a complete ring.

**Chalazal:** The part of a plant ovule\* where the nucellus\* and integuments\* merge. Associated with the cotyledon end of the seed.

**Chloroplasts:** Chlorophyll containing organelles of plant cells involved in photosynthesis.\* Generally lens shaped and bounded by a double membrane.

**Chromosomes:** A threadlike structure found in the nucleus of plant cells. They carry the genes (DNA\*) that determine an organisms individual characteristics. **Conifers:** Seed-bearing plants comprising the conifers, including the pines, firs, and spruces. Gametes are carried in male and female cones, fertilization usually being achieved by wind-borne pollen. The ovules\* and the seed into which they develop are borne unprotected (rather than enclosed in an ovary, as in the Angiosperms\*). Internal tissue and cell structure of these species is not as advanced as in the angiosperms. Typically evergreen trees inhabiting cool temperate regions and have leaves reduced to needles or scales. (see also *Gymnosperm*)

**Cortex:** The tissue exterior to the vascular system in plant stems and roots. It is composed of parenchyma\* and shows little structural differentiation.

**Corrosion cavity:** The cavity in the central portion of the megagametophyte that forms through cell breakdown. The embryo will grow into this cavity.

**Cupressaceae:** A family of gymnosperms\* characterized by persistent scale-like leaves and cones in which the bracts and scale is wholly fused. Genera present in B.C. include *Thuja*, *Chamaecyparis* and *Juniperus*.

**Cytoplasm:** The jelly-like matrix of a cell in which the organelles are suspended.

**Diploid:** (2n)Twice the haploid number of chromosomes\* characteristic of the species. The diploid number is designated as 2n. Two sets of chromosomes are present, one set being derived from the female parent and the other from the male.

**Embryo:** The structure in plants that develops from the zygote prior to germination.

**Embryogeny:** The process of embryo formation and associated changes occurring within an ovule.

Definitions have been obtained primarily from the Oxford Dictionary of Biology, but other references have been used, including Esau and Fahn, if no definition was found.

<sup>\*</sup> Indicates those words found elsewhere in the glossary.

**Endosperm:** A nutritive tissue, characteristic of flowering plants, that surrounds the developing embryo\* in a seed. Its cells are triploid and many plants, such as cereals and oil crops, are cultivated for the rich food reserves in the endosperm.

**Epicotyl:** The region of a seedling stem above the stalks of the seed leaves (cotyledons) of an embryo plant. It grows rapidly in seed showing hypogeal germination and lifts the stem above the soil surface. (compare *Hypocotyl*)

**Epidermis:** The outermost layer of cells covering a plant. It functions to protect the plant from injury and to reduce water loss. Some epidermal cells are modified to form guard cells or hairs of various types.

**Epigeal:** Describing seed germination in which the cotyledons emerge from the seed and function as leaves. Typical of the gymnosperms.

**Epithelial cells:** A compact layer of cells, often secretory, covering a free surface or lining a cavity or duct.

**Family:** A category used in the classification of organisms that consists of similar or closely related genera.\*

**Genus: (pl. Genera)** A category used in the classification of organisms that consists of similar or closely related species.

**Guard cell:** Specialized semicircular epidermal cells, whose movements (due to changes in water content) control the size of the opening of the stomate.

**Gymnosperm:** Any plant whose ovules,\* and the seed into which they develop, are borne unprotected, rather than enclosed in ovaries. (The term gymnosperm means naked seed.)

**Haploid:** (1n)Describing a nucleus, cell, or tissue with a single set of unpaired chromosomes.\* The haploid number is designated as n.

**Hypocotyl:** The region of the stem beneath the cotyledons and directly above the root of an embryo. It grows rapidly in seedlings showing epigeal germination and lifts the cotyledons above the soil surface. (compare *Epicotyl*)

**Hypogeal:** Describing seed germination in which the cotyledons remain contained within the seed coat.

**Imbibition:** The movement of water into substances that do not dissolve resulting in swelling. Full imbibition of seed is characterized by all seed contents becoming filled with water or 'imbibed'. It is critical in imbibition that water reaches the embryo.

**Integument:** The outer protective covering of a plant ovule.\* It is perforated by a small pore, the micropyle. Usually two integument(s) are present in angiosperms\* and one in gymnosperms.\* After fertilization the integuments form the seed coat.

**Lignin:** A complex organic polymer that is deposited within the plant cell walls during secondary thickening. Lignification makes the walls woody and rigid. (see *Sclerenchyma*)

**Lipid:** Any of a diverse group of chemical compounds, occurring in living organisms and having a variety of functions, that are insoluble in water, but soluble in organic solvents such as chloroform, benzene, etc. They have many carbonhydrogen bonds and release a larger amount of energy than other organic compounds.

**Longitudinal section:** Of or pertaining to length; placed or running lengthwise.

Megagametophyte: Female gametophyte.

**Megaspore cell wall:** The lipid-rich cell wall of the megagametophyte. Synonymous to the pollen wall in the male gametophyte.

**Micropyle:** A minute opening in the integument of an ovule through which the pollen tube normally passes to reach the embryo sac, usually closed at maturity to form a superficial scar and usually the point of radicle emergence. **Microtome:** A machine used to cut thin sections (3–5 mm thick) of plant or animal tissues for microscopic observation.

**Moisture content:** A derivation of the proportion of moisture within an object. For seed, moisture content is based on the proportion of moisture relative to the initial (fresh) weight and is usually presented as a percentage. The calculation is: moisture content = (fresh weight – ovendry weight)/ fresh weight and multiplied by 100. For other materials (i.e., wood) moisture content is calculated on a dry weight basis and it is this term which appears in the denominator.

**Morphology:** The study of the form and structure of organisms, especially their external form. (compare *Anatomy*)

**Nucellus:** The tissue that makes up the greater part of the ovule\* of seed plants. It contains the embryo\* sac and nutritive tissue. It is enclosed by the integuments\* except for a small gap, the micropyle.

**Opacity:** The inability of an object or substance to allow light to pass through. The term for the opposite condition is translucency.

**Ovules:** The part of the female reproductive organs of seed plants that consists of the nucellus,\* embryo\* sac, and integuments.\* The ovules of gymnosperms\* are situated on ovuliferous scales of the female cones while those of angiosperms\* are enclosed in the ovary. After fertilization, the ovule becomes the seed.

**Parenchyma:** Roughly spherical relatively undifferentiated cells, frequently with air spaces between them. The cortex and pith are composed of parenchyma cells.

**Phloem:** A tissue that conducts photosynthate\* in vascular plants from regions where they are produced (notably the leaves) to regions where they are needed (meristems). **Photosynthate:** The product of photosynthesis.\* It passes from the leaves or stem to areas of need through the phloem.

**Photosynthesis:** The chemical process by which green plants synthesize organic compounds. Chloroplasts absorb the energy of sunlight to initiate a complex set of reactions to produce sugars used in the production of plant material.

**Pinaceae:** A family of gymnosperms\* characterized by persistent or deciduous spirally arranged leaves, distinct bracts, and scales in a woody cone. Genera present in B.C. include *Abies, Larix, Picea, Pinus, Pseudotsuga,* and *Tsuga.* 

**Pit:** A depression or cavity in the secondary wall of a plant cell that facilitates the movement of substances between adjacent cells.

**Pith: (syn Medulla)** The cylinder of parenchyma\* tissue found in the centre of plant stems interior to the vascular system.

**Polyembryony:** The formation of more than one embryo in a plant seed.

**Procambium (provascular tissue):** A plant tissue formed by the apical meristems of shoots and roots. It consists of cells elongated parallel to the long axis of the plant. The procambium subsequently gives rise to the primary vascular tissue.

**Purity:** A seedlot chracteristic describing the weight of pure seed in relation to the weight of seed and debris. Purity standards are currently 97%. The purity is used to derive the seeds per gram variable from the average weight of 100 seeds.

**Sclerenchyma:** Plant cells whose cell walls have become impregnated with lignin.\* Due to the added strength that this confers, sclerenchyma plays an important role in support. The cell walls contain pits, enabling the exchange of substances between adjacent cells. Mature sclerenchyma cells are dead, since the lignin makes the cell wall impermeable to water and gases. (compare *Parenchyma*) **Seed coat:** The lignified \* or fibrous protective covering of a seed that develops from the integuments\* of the ovule\* after fertilization.

**Seedlot:** A quantity of cones or seeds having uniformity of species, source, quality, and year of collection. To register a seedlot the moisture content must be between 4.9 to 9.9 % and the purity above 97%.

**Species:** A category used in the classification of organisms that consists of a group of similar individuals that can breed among themselves and produce fertile offspring.

**Specific gravity:** The ratio of the density a substance to the density of water at 4°C. The density of water is 1.00 g/cm<sup>3</sup> and specific gravity is unitless.

**Stomata: (pl.; sing. stoma)** Pores that function in the exchange of gases between the plant and atmosphere. Large numbers are aggregated on the epidermis and usually more numerous on the lower surface. Each stoma is bordered by two guard cells.\*

**Stratification:** A technique used to overcome embryo dormancy in seed. Stratification is synonymous with moist-chilling. Seed are imbibed and then put into cool conditions (2–5°C) for the duration required to overcome embryo dormancy.

**Tannin:** One of a group of complex organic chemicals commonly found in leaves, unripe fruits, seed coats, and the bark of trees. Their function is uncertain though the unpleasant taste may discourage grazing animals. Some tannins have commercial uses, notably in the production of leather and ink. **Vacuoles:** A space within the cytoplasm\* of a living cell that is filled with air, water, or other liquid. In plants there is usually one vacuole bonded by a single-layered membrane.

**Variety:** Variety designation is used when organisms from different geographic areas have anatomical and morphological differences not considered large enough for designation as different species.

**Vascularized:** Possessing organized vascular tissues (see *Xylem*\* and *Phloem*\*).

**Xylem:** A tissue that transports water and dissolved mineral nutrients in vascular plants. In flowering plants it consists of hollow vessels that are formed from cells (vessel elements) joined end to end. The end walls of the vessel elements are perforated to allow the passage of water. In less advanced vascular plants, such as conifers\* and ferns, the constituent cells of the xylem are called tracheids. The walls of the xylem cells are thickened with lignin,\* the extent of this thickening being greatest in secondary xylem. Xylem contributes greatly to the mechanical strength of the plant: wood is mostly made up of secondary xylem. (see *Fibre*; compare *Phloem*)

**Zygote:** A fertilized female gamete. The product of fusion of the nucleus of an ovule with the nucleus of a pollen grain.

# Appendix 3 – Germination Test Codes

Code	Species	Soak (hours)	Surface dry	Strat.∘ days	Temp. (°C)	Strat. redry	Strat. days	Redry temp. (°C)	Test days	Germinator temp. (°C)*
G10	Fd, S, Lw	24	у	21	2	n	0	-	21	30 – 20
G20	Pl, Py	24	у	28	2	n	0	-	21	30 – 20
G31	Hw, Hm	24	у	28	2	n	0	_	28	20 – 20
G32	Bg	48	у	28	2	n	0	-	28	30 - 20
G44	Ba, Bl, Bn	48	n	56	2	n	0	-	28	25 – 15
G52	Yc	48	n	28	20	n	56	2	28	30 - 20
G55	Pw	336	n	98	2	n	0	_	28	30 – 20
G64	Ba, Bl, Bn	48	n	28	2	у	56	2	28	25 – 15
D1	Cw	0	_	_	_	n	_	_	21	30 – 20
W1	PI, S	24	-	0	-	n	-	-	21	30 – 20

♦ Strat. is the abbreviation for stratification.

\* 8 hrs at higher temperature, 16 hrs at lower temperature.

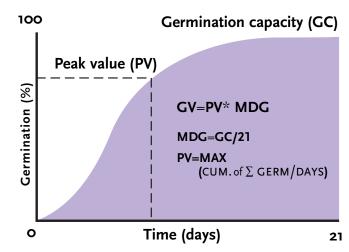
# Appendix 4 – Germination Variables

The germination capacity (**cc**) is the main criteria used to define seedlot quality. The **cc** is the percentage of seeds that have germinated during a germination test (21 or 28 days depending on species – see Appendix 3). Germination tests consist of four replicates of 100 seed samples. Germinants are counted and removed from dishes on Monday, Wednesday, and Friday once the radicle is four times the length of the seed coat. Seeds which germinate abnormally are not included in the **cc**. The **cc** is used in determining sowing and oversow factors and in calculating the grams of seed required to meet a request. It is a very useful variable, but it should be supplemented with a variable describing the germination rate (faster germination usually equates to a more uniform crop).

The Germination Value (**Gv**) has historically been used to define 'vigour' and is the other variable in addition to **GC** that is available on SPAR.<sup>1</sup> The **GV** is a product of two additional variables: mean daily germination (**MDG**) and peak value (**PV**). **GV=MDG** \* **PV**.

The **MDG** is simply the germination capacity divided by the number of days in test [**MDG=GC**/ #days in test]. For example, a Sx seedlot which is tested for 21 days, with a **GC** of 91% would have a **MDG** of **4.3**. **MDG** is a linear description of germination, but germination is not linear and this parameter alone is not very useful.

The **PV** is the point at which the cumulative germination percent divided by the number of days is maximum. The **PV** describes germination rate and is best understood with an example from a germination test sheet as illustrated below. The first step is to obtain the mean germination for each test date [i.e., for day 7: (52+57+55+60)/4 = 56]. For each test date we will then calculate the average cumulative germination [i.e., for day 7: 20.75+56=76.8]. The cumulative germination is then divided by the test days and the maximum value for the cumulative germination divided by test days is the peak value [i.e., for day 7: 76.8/7 =11.0]. In this example, the peak value is 11.0 and it occurs on day 7. The PV is more informative if presented as cumulative germination and days to arrive at this level [77%/7 days] rather than simply a single number [11]. The **cc** is equal to 91% and the **MDG** is equal to **4.3**. The **GV** is then the product of MDG\*PV = 11.0\*4.3 = 47.3 or 47 as GC ad GV are usually presented as whole numbers.



Test Day	3	5	7	10	12	14	17	19	21
Rep			Num	iber of Nor	MAL GERMIN	NANTS COUI	NTED		
1	0	20	52	10	8	0	0	0	0
2	0	21	57	10	4	2	0	0	0
3	0	23	55	8	5	1	0	0	0
4	0	19	60	7	3	1	2	0	0
Mean	0	20.8	56.0	8.8	4.0	1.0	0.5	0.0	0.0
Cumulative	0	20.8	76.8	85.5	89.5	90.5	91.0	91.0	91.0
Cum./Day	0	4.2	11.0	8.6	7.5	6.5	5.4	4.8	4.3

1 SPAR – Seed Planning and Registry System – a MoF mainframe system for registering new seedlots and for securing seed for reforestation.

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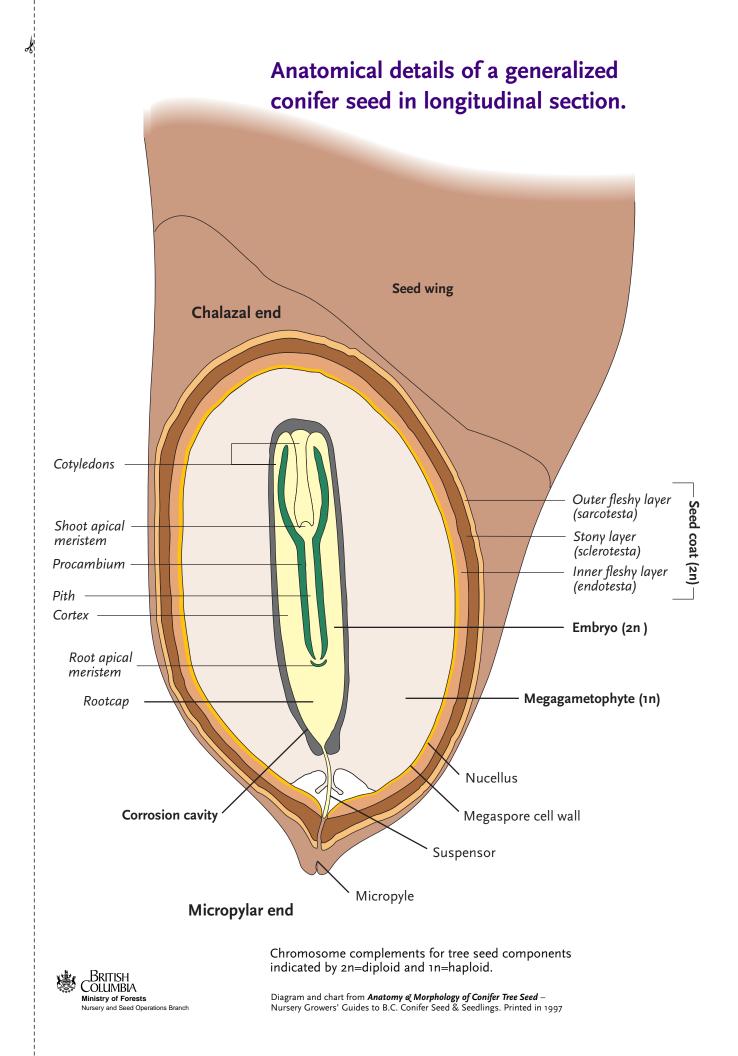
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		Viable		Question	Questionable Viability	ty	Immature	ture			Non-	Non-viable
						EMBRYO	Ő					
		Good	Good	Normal	Normal	Deteriorated translucent rubbery	Development problems	Development problems	Rotten	Other	Empty	Comments
Seedlot/	# of				Z	MEGAGAMETOPHYT	РНҮТЕ					
Sample	seeds	Good	Good/Thin seed coat	Slight discolouration	Grey/Yellow deteriorated	Grey/Yellow deteriorated	Normal	Shrunken/ discoloured	Rotten	Other		

20

A cutting test sheet for classifying seed and recording comments.