Seed Pretreatment

Seed pretreatment refers to a group of activities carried out to better prepare seeds for sowing and germination. The activities include dormancy-breaking treatments that mimic the recommended test procedure, pelleting of western redcedar seed, sanitation procedures used to disinfest seeds of fungi, and other methods used to improve germination characteristics (i.e., uniformity). Details on seed sanitation are covered in a separate chapter. The major difference between testing and operational pretreatment is the quantity of seeds being treated. A variety of methods involving regulation of moisture uptake and the germination process (priming) are also reviewed here, although they have not received widespread operational use with conifers.

The effectiveness of stratification in overcoming seed dormancy requires that certain conditions be met: an appropriate moisture level, temperature, duration, and access to oxygen

The process of **stratification** (imbibition followed by moist-chilling) is covered in detail due to its importance for overcoming dormancy and allowing germination to proceed. Stratification has mainly been described as a method to overcome embryo dormancy, but stratification may overcome coat-induced dormancy as well as offering other benefits. Many studies have indicated that stratification will improve the speed of germination and the uniformity of germination which are important considerations in producing a uniform seedling crop. The earlier in a crop cycle one introduces variability the more difficult it will be for the grower to correct this variability. Stratification also increases the 'vigour' of the seeds or their ability to germinate over a wide range of conditions (e.g., an expanded temperature range for maximum germination). The requirement for light in some species also seems to be overcome if conifer seeds are properly stratified. Recent evidence also indicates that natural repair mechanisms are activated during stratification (Wang and Berjak 2000). While stratification may be as close as one gets to a panacea in forestry, there are seedlots that perform better dry or with only a soak treatment. This is probably due to improper collection timing, mechanical damage to the seed coat, or fungal infection. Although seeds may be shipped dry to the nursery, it is recommended that they be soaked and surface dried prior to sowing when stratification is not recommended to facilitate rapid and uniform germination.

The effectiveness of stratification in overcoming seed dormancy requires that certain conditions be met: an appropriate moisture level, appropriate temperature, appropriate duration, and access to oxygen. If one of these elements is not adequately supplied, germination will be hampered through the incomplete removal of dormancy. Trial work on 10 seedlots of lodgepole pine and interior spruce stratified at moisture contents between 15 and 45% indicated that maximum germination occurred at 30% moisture content for both species (Hannam 1993). In white spruce, moisture contents below 20% were not adequate for stratification to be effective, while efficacy increased abruptly at 25% moisture content (Downie et al. 1998). The optimum stratification temperature falls between +2 and +5°C. Temperatures below freezing should be avoided as they are ineffective in breaking dormancy and may injure the imbibed seeds (Stokes 1965). Stratification at 5°C resulted in faster germination than at 2°C, for ponderosa pine and Douglas-fir, but if the higher end of this range is chosen one should frequently monitor the seeds as germination under stratification conditions is possible (Danielson and Tanaka 1978). No information is available on optimum oxygen levels, but the embryo and megagametophyte should have access to support respiration requirements of these tissues. Lack of oxygen will also promote the activity of anaerobic organisms that may contribute to seed deterioration, especially at elevated moisture contents and temperatures.

Scarification (abrasion of seed coat), although important for many plant species, is not considered essential for germination in most conifers. Species in which scarification may prove beneficial are whitebark pine and western white pine (Pitel and Wang 1990; Hoff 1987). On an operational scale the procedure is difficult to perform effectively and efficiently in a repeatable manner. Although not widely used, a modified barley pearling machine has been suggested (Larsen 1925) as a suitable piece of equipment.

Seed pretreatment begins with the withdrawal of seeds from long-term storage (-18°C) (**Figure 65**). For sowing requests the amount of seed required is calculated based on BC Ministry of Forests sowing guidelines (see "Seed Sowing" chapter), but one can override these based on discussions between the seed owner and nursery. There is a significant difference between sampling for seed testing and operational seed pretreatment withdrawals for sowing. In testing, the sample taken to estimate germination is random and representative of a seedlot, but in operational seed preparation, seeds are withdrawn on an individual container basis. Logistics make it impossible to provide random, representative sampling for more than 5000 requests,

Figure 65 a) Withdrawing seeds from long-term storage and b) weighing pelleted western redcedar seeds for a sowing request.

primarily received within the few months that sowing occurs in BC. This is one area where differences between germination test results and germination of sowing requests can arise. A summary of all areas in which differences in germination results could occur is presented in the nursery results chapter.

Seed Soaking

Seed soaking is the first step in seed pretreatment for all species, except western redcedar. This step is also referred to as hydration or imbibition. As seeds gain moisture they become more susceptible to damage from mechanical impact, freezing, or high temperatures. Imbibed seeds should be handled very carefully to avoid damaging them.

Soak duration for operational pretreatment is equivalent to those given for testing in **Table 8** (page 56), although actual procedures differ. Once seeds have been weighed and a label prepared, the seeds are placed into a nylon mesh net, which is tied and labelled. It is important not to tie the net too tightly around the seed mass as all seeds should have equal access to moisture for uniform uptake. The nets are placed into water baths of approximately 11°C that have a slow, but continuous flow (**Figure 66**). This 'running water soak' is intended to reduce the amount of seed-borne fungi on the seed coat through dilution and possibly aid in the removal of inhibitors in the seed coat (Martinez-Honduvilia and Santos-Ruiz 1978). For species with higher risks (i.e., coastal Douglas-fir or western larch) of significant pathogen levels, separate soaking compartments are used to avoid fungal cross contamination.

There is a significant difference between sampling for seed testing and with drawing seed for sowing requests

Running water soaks are applied to all sowing requests above 60 grams. After the soak the nets are withdrawn from the water bath and hung so that the excess moisture on and between the seeds drains away (**Figure 67**). For small sowing requests (<60 g), the seeds, without nylon mesh screens, are imbibed in labelled plastic bags filled with water. After these requests are soaked, run them over a screen to remove excess water and capture the seeds.

Seed soaking is the first step in seed pretreatment for all species, except western redcedar

The temperature of water used for seed hydration may affect the amount of moisture absorbed and possibly seed and seedling performance. It is generally accepted that at higher water temperatures imbibition will be faster, but Edwards (1971) found that if the imbibition period in Noble fir was extended past 14 days, a 5°C soak produced higher seed moisture content compared to a 25°C soak. Imbibition in

Figure 66 Placing sowing requests into a running water soak tank with a cover to ensure all seeds are submersed.

Figure 67 Draining seeds of extra moisture following the running water soak.

Pinus sylvestris was shown to vary by individual seeds and that a 10°C soak seemed to produce more uniform imbibition than at 5°C (Tillman-Sutela 1996). In Noble fir, a 48 hour soak at 4°C produced a reduction in germination capacity and this was attributed to the bulk pretreatment method, but no other soak temperatures were investigated (Jones et al. 1991). For loblolly pine, the imbibition temperature affected the moisture uptake rate, germination, and seedling development illustrating how a simple factor such as water temperature may have long ranging consequences in seedling production (Barnett and Vozzo 1985).

Surface Drying

Once draining is complete the seeds are surface dried with forced air and seed movement to facilitate uniform drying and then placed in cold stratification (Figure 68). Requests under 60 g are dried on blotter paper as forced air can dry these requests very quickly resulting in uneven drying or removal of internal moisture. A quick alternative to air drying is to surface dry seeds in a spin-drier (Gosling et al. 1994). Surface drying is carried out to optimize the moisture content for stratification, remove surface moisture to limit fungal growth, increase the speed at which oxygen may reach the embryo, and help the seeds to flow freely to assist mechanical sowing. When moisture is present on the seed coat the seeds stick together, complicating mechanical sowing. During surface drying it is important that only water from the surface of the seeds is removed and that drying does not remove internal moisture. This methodology is similar to the "target moisture content" prechill described by Jones and Gosling (1994) although the target level is reached through surface drying, gauged by visual and tactile cues. Tactile cues include seeds flowing freely through ones fingers and visually seeds are uniformly lighter in colour and duller in appearance as surface moisture is removed (see Figure 69).

Surface drying is carried out to optimize the moisture content for stratification, remove surface moisture to limit fungal growth, increase the speed at which oxygen may reach the embryo, and help the seeds to flow freely to assist mechanical sowing

Surface drying following at least partial stratification is advocated by some because maximum moisture content may not be obtained during the hydration period (Edwards 1996). Tanaka et al. (1986) do not recommend surface drying before stratification as germination speed may be reduced, but in this case a standard drying regime (25°C for 30 min.) was used without regard to drying rates. This resulted in the moisture content of surface dry seeds ranging between 26 and 41% for Engelmann spruce and lodgepole pine. If an inadequate amount of moisture is imbibed during the soak period either increase the soak duration or make moisture available during stratification.

Figure 68 Surface drying seeds a) illustrating a drying room that utilizes vents which draw moisture off the seed coat and b) a close-up of the manual movement of the seeds required for attaining uniform surface drying.

Controlling the moisture content during stratification can virtually eliminate the problem of pregermination during stratification, which has plagued many north-temperate conifers (Edwards 1986; Jones and Gosling 1994; Jensen 1996).

Excess moisture may also increase the bulking-up of seed-borne pathogens such as *Calosypha fulgens* which favours cool, moist conditions (Sutherland 1981). Higher moisture

Figure 69 A comparison between surface moist (dark) and surface dry (light) seeds in interior spruce.

contents during stratification increases the respiration rate and this may be detrimental to subsequent seed performance due to the accelerated use of storage reserves (Leadem 1993). The seeds may use all of their reserves in respiration and other metabolic activities before they germinate and become self-sufficient. This concern is probably more an issue when sub-optimal conditions (e.g., temperature) or sowing delays occur. The issue of sowing delays deserved and received a great deal of attention when bareroot sowing was widespread in BC, but currently almost all crops are grown in containers and sowing delays because of weather conditions at the nursery are rare. If sowing delays are greater than three weeks the seeds should be dried to 20% moisture content to reduce metabolism and the nursery instructed to soak seeds for 24 hours prior to sowing. Clients are encouraged to update and maintain correct sowing dates on SPAR to allow for seed preparation to proceed as efficiently as possible.

The results of moisture content testing after stratification as part of the BC Tree Seed Centre Quality Assurance program (at time of shipping) are presented in **Table 8**. For example, in interior lodgepole pine 95% of the sampled requests had a moisture content between 30.0 and 30.6% (30.3 ± 0.3) over the past eight seasons. The Tree Seed Centre general target for stratified moisture content has been 30% and this coincides well with lodgepole pine and spruce, which account for

Excess moisture may also increase the bulking-up of seedborne pathogens

approximately 75% of the sowing requests each season. The moisture contents in **Table 8** are intended to quantify the differences in hydrated moisture content among species and are considered estimates of internal seed moisture content. It is suggested that our species fall into three classes for moisture content of seeds with fully hydrated internal components (embryo and megagametophyte):

Moisture Content	Species
Low [<30%]	Sitka spruce, western hemlock, and ponderosa pine
Medium [30 to 32%]	Interior spruce, interior lodgepole pine, coastal lodgepole pine, and Sitka × interior spruce hybrid
High [>32%]	Coastal Douglas-fir, Amabilis fir, mountain hemlock, western white pine, grand fir, interior Douglas-fir, western larch, and subalpine fir

At the BC Ministry of Forests Tree Seed Centre surface drying is not carried out on Amabilis fir, subalpine fir, Noble fir, western white pine, and yellow-cedar. However, free water between seeds within the seed mass is drained in these species. The *Abies* spp. are generally dried back after four

Table 8 Stratification moisture contents, number of requests, and confidence intervals for sowing requests at time of shipping sampled between 1992 and 2001

Species	Number of requests	Mean MC (%)	95% confidence interval (± value)
Amabilis fir	103	32.9	0.6
Grand fir	39	34.0	1.1
Subalpine fir	97	35.3	1.0
Coastal Douglas-fir	120	32.7	0.5
Interior Douglas-fir	148	35.0	0.5
Mountain hemlock	28	32.9	1.3
Western hemlock	102	26.7	0.7
Western larch	152	35.0	0.8
Coastal lodgepole pine	35	30.6	0.6
interior lodgepole pine	269	30.3	0.3
Western white pine	105	33.9	0.5
Ponderosa pine	96	27.8	0.4
Sitka spruce	55	25.8	1.2
Interior spruce	327	29.8	0.4
Sitka × interior spruce hybrid	38	30.2	1.0
	1714	**31.5**	

weeks stratification and given an additional eight weeks stratification similar to the stratification-redry treatment which has been shown to be advantageous (Edwards 1986; Edwards 1996; Leadem 1986; Tanaka and Edwards 1986). Tactile and visual cues are also used to gauge the dryback procedure and average dryback moisture content results are shown in **Table 9**. The other two species are not surface dried because of concerns with structures restricting water uptake to the megagametophyte and embryo (Hoff 1987; Tillman-Sutela and Kauppi 1998). Although yellow-cedar still receives a period of warm stratification the elimination of this procedure in western white pine greatly improved the quality of seeds shipped as mould buildup and pregermination were virtually eliminated (**Figure 70**).

Figure 70 Problems associated with the former protocol of using warm stratification in western white pine: mould build-up and pregermination of non-dormant seeds in a seedlot.

Moisture content can be estimated non-destructively by weighing the stratified seeds and using target moisture content (knowing the initial request weight and storage moisture content) calculations. For example, if a sowing request is for 1351 grams at 7.9% MC and it weighs 1840 grams after surface drying, estimate the moisture content as follows:

Determine the ovendry weight of the request by using this MC equation:

ovendry weight = fresh weight * (1 – moisture content)
ovendry weight = 1351 * (1 – 0.079)
ovendry weight = 1244 g

Knowing the ovendry weight, calculate the moisture content at any weight using this MC equation:

$$\textbf{moisture content} = \frac{\text{fresh weight} - \text{ovendry weight}}{\text{fresh weight}}$$

moisture content = (1841 – 1244)/1841
moisture content = 0.324 or 32.4%

If unsure about the moisture status of a seedlot, or as part of a quality assurance program, use this simple method to

Table 9 The number of dryback procedures and average moisture contents for *Abies* spp. after dryback

Species	Dryback (#)	Mean MC (%)
Amabilis fir	211	32.2
Subalpine fir	91	33.4
Noble fir	7	33.8

determine MC non-destructively. Non-destructive moisture meters are available, but they perform poorly at moisture contents above about 12–15%.

Stratification

In BC the technique used in cold stratification is referred to as 'naked stratification' as no media is included with the seeds during moist chilling. The term stratification was originally coined to describe the layering or 'stratification' of seeds between layers of moistened material. Material that has been used in stratification includes moistened cloth, sand, and peat moss. Sphagnum moss has been used in cold stratification as it has a high water-holding capacity and good aeration, and studies have also indicated reductions in the incidence of damping-off (Hess 1996; Wang et al. 1998). Moist chilling in polyethylene bags was initially investigated with loblolly pine (Hosner et al. 1959) and referred to as 'storage.' Both stratification and storage of moist seeds under cool conditions have been used to describe naked stratification, but due to its brevity, the term stratification has survived despite the lack of actual 'stratification' in the procedure.

Tactile and visual cues are used to gauge the dryback procedure

After surface drying the seeds are placed into a premoistened **polyethylene** bag of appropriate size for the request, a label is attached and the top is tied to keep the top of the bag open approximately 3 cm. Polyethylene bags up to 4 mil (0.102 mm) allow for some oxygen and carbon dioxide exchange, but it is recommended to have the top of the bag open to allow for additional aeration (**Figure 71**). In some facilities air is piped into the bag through a plastic tube. For sowing requests of all species except western white pine, a maximum of 3000 grams is placed into each poly-bag. For example, if a sowing request is 4800 grams two bags, and two labels, will be used and they will both contain 2400 grams of seeds. Due to difficulties in obtaining

In BC the technique used in cold stratification is referred to as 'naked stratification' as no media is included with the seeds during moist chilling

Figure 71 Seeds in stratification: a) a view of sowing requests in stratification and b) illustrating the open bag for increasing oxygen exchange.

high germination with western white pine the maximum bag size is set at 1000 grams to allow for additional aeration and closer observation of the seeds during stratification. Optimization of stratification methods are currently being investigated for western white pine. During stratification monitoring of seed condition is important and may be accompanied by the gentle massaging of the bags or rotating the seeds to ensure anaerobic conditions do not build up in part of the bag, especially with species requiring long stratification (**Figure 72**).

Seeds are shipped to nurseries following the appropriate pretreatment, unless the nursery indicates otherwise. Bags of seeds are closed to avoid contamination during transport. Open them again upon arrival. Stratified seeds are fragile and must be handled with care. Seeds are shipped overnight, by courier, in expanded polystyrene containers with insulating packing material covering the seeds and ice packs placed on top to help maintain a cool temperature during shipping (**Figure 73**). With each shipment the seeds shipping label is attached indicating the simple steps for properly handling seeds at the nursery (**Figure 74**).

Bags of seeds are closed to avoid contamination during transport. Open them again upon arrival

Pelleting

Pelleting is primarily used in forestry to aid in the sowing of small, light, winged, or irregularly shaped seeds. In BC all western redcedar seeds are pelleted with a mixture of diatamaceous earth and binders that are slowly bound to the seeds with a water mist in a rotating drum (**Figure 75**). Western redcedar is not soaked or stratified prior to sowing thus allowing the pelleting process to be performed on dry seeds. Some nurseries are also requesting that red alder (*Alnus rubra* Bong.) seed be pelleted. Imbibed seeds can also be pelleted, but the shelf life will be greatly reduced. The disadvantage of pelleting is the reduction in the germination rate as the radicle must penetrate the pellet as well as the seed coat. Western redcedar has limited megagametophyte reserves, and under suboptimal conditions, one risks these reserves being used before germination is complete. Germinate pelletized western redcedar as quickly as possible, but do not allow the pellet to dry out as it may become "cemented" to the seed, restricting cotyledon emergence. The delay that the pellet imposes on the seeds has been estimated at four days for western redcedar. Pelleting is performed solely to be able to efficiently sow the seeds into containers.

Pelleting was shown to have a negative impact on germination in white spruce, jack pine, and red pine, but did not influence germination in black spruce. If black spruce was sown at sub-optimal germination temperatures, germination was adversely affected by pelleting. Pelleted black spruce can be stored for three years without adverse effect on germination (Fraser and Adams 1980).

Figure 72 Monitoring of seeds during cold stratification.

Figure 73 Contents of a seed shipment a) including ice packs and insulating material and b) a seed shipment awaiting pickup in the cooler.

Other Seed Treatments

These other seed treatments may have their place in the conifer seed handling system, but experience with their use is limited thus far. These techniques have flowed from agricultural use and are mainly aimed at imbibing seeds to a moisture content that allows germination to progress without radicle emergence. Several differences between sowing in agricultural crops and conifers are worth noting. Conifers are generally soaked and stratified prior to sowing while most agricultural seeds are sown dry. Agricultural crops are mainly sown into fields with relatively unpredictable germination conditions while conifers are mainly sown in greenhouses allowing much greater control of environmental conditions. Agricultural seeds are genetically quite uniform within a variety while conifer seeds are still very diverse. The optimization of any tree seed treatments will be more complicated than agricultural crops because of this level of diversity. These differences indicate that while these latter seed treatments are well suited for agricultural crops, a great deal of additional work is required for them to be used operationally with conifers.

Figure 74 Shipping label attached to sowing requests indicating proper treatment of seeds upon arrival.

The following are 'newer' techniques used to prepare seeds for germination. Seed priming is used to describe a wide variety of methods to partially hydrate seeds to a moisture content that initiates germination processes (biochemical reactions), but does not allow radicle emergence to occur. These methods have been used mainly with agricultural crops, but some experimental work with conifers is occurring. A brief definition of common methods and their many synonyms is presented to clarify the nomenclature present in the literature. For additional information on priming, refer to the excellent reviews by Heydecker and Coolbear (1977) and Welbaum et al. (1998).

Hydro-priming (also hardening[5]) involves controlling the amount of water entering seeds through a reduction in soaking time, exposure to low temperature, or treatment under high humidity conditions. This is similar to the target moisture content concept (Jones and Gosling 1994), although with many agricultural crops moist chilling is not performed.

Osmotic priming (also osmotic conditioning) involves the use of an **osmoticum**, such as **polyethylene glycol** to control moisture uptake through the reduction in the **water potential** of the solution (see page 85 for a more complete explanation of water potential). An osmoticum with a high molecular weight is favoured as it does not penetrate the seeds. Seeds

5 In some references hardening is used to describe repeated wetting and drying cycles that have been associated with drought resistance. A good review is provided in Heydecker and Coolbear (1977).

Figure 75 Pelleting of western redcedar.

are usually washed and dried back to the previous storage moisture content. The disadvantages of this method are the need to determine the optimum relationship between osmotica used, concentration, and temperature, which will probably vary by species and possibly seedlot. There is also a need to dispose of the priming solutions, which has proved to be operationally problematic.

Solid matrix priming (SMP) involves the use of a solid carrier with the seeds and water which are incubated together to generate a negative water potential. Examples of solid carriers include vermiculite, sand, calcited clay, and spagnum moss. After incubation the mixture is dried and seeds separated from the media using conventional seed processing equipment. Although many studies have indicated the superiority of SMP over osmotic priming there is still a large amount of optimization to be performed beforehand and some procedures are patented, but disposal is generally considered an easier activity with SMP.

Agricultural seeds are genetically quite uniform within a variety while conifer seeds are still very diverse

Drum priming (also invigoration) involves the use of a horizontal rotating drum into which water vapour is released until a predetermined level of hydration is reached. Drum priming is gaining in popularity over the above methods as large quantities of seeds can be treated operationally and there are no associated waste materials as found in the above two methods.

Biopriming is a method that may be combined with any of the above priming treatments and involves the introduction of beneficial organisms to reduce disease incidence or improve other aspects of germination. Fungicides may also be applied in combination with biopriming.

Fluid drilling refers to various means in which pre-germinated seeds are sown either in a protective gel or delivered to the soil with an amount of water. This is not strictly a priming treatment, but a sowing or delivery system. The gel or water delivered with the seeds may also contain nutrients, plant growth regulators, or pesticides (Gray 1981).

The topic of hydropriming or simply soaking seeds often raises the issue of aeration and whether this needs to be incorporated into the procedure. For Ocala sand pine it was shown that germination improved by using a 24 hour aerated soak (Outcalt 1991). In a trial with BC species the effects of aeration were not consistent across species. The practice produced improvements in western hemlock, western redcedar, and older seedlots of pine and spruce. Both coastal and interior Douglas-fir were negatively affected by aeration. Even within species the effectiveness was seedlot specific indicating difficulties in applying the procedure on an operational scale (Boomhower 1995).

Osmotic priming has been tried with several conifers with varying degrees of success. Huang (1989) found that all treatments enhanced seedling uniformity and reduced the number of abnormal seedlings, but many treatments resulted in decreased germination capacity for lodgepole pine and white spruce. Unfortunately no comparisons with stratification were included. Reduction in germination capacity, but improvements in germination speed were found in *Pinus brutia* var. *eldarica*. The authors state 'other invigoration treatments such as stratification or controlled hydration may offer greater benefits in nursery production at lower cost' (Khalil et al. 1997, page 24). In northern Fennoscandia osmotic priming was used to replace physiological ripening of Scots pine as complete seed maturation often does not occur at these latitudes. Improvements in germination capacity and seed vigour occurred when seeds were collected after anatomical maturity, but before physiological maturity (Sahlén and Wiklund 1995).

Solid matrix priming was investigated in loblolly pine using clay as the solid carrier. Increases in germination rate and synchrony were observed, but only slight improvements in germination capacity were observed. The optimum treatment reduced differences in germination vigour among families (Wu et al. 1999). Membrane tube invigoration (similar to drum priming) was shown to be far superior to osmotic priming for white spruce and jack pine. The invigoration regime produced faster germination, without sacrificing capacity, compared to prechilling, but all seedlots chosen were considered non-dormant (Downie et al. 1993).