This section focuses on the long-term storage of 'dry' conifer seeds, and presents some information on the storage of stratified seeds in relation to sowing. Species can generally be categorized as 'orthodox' or 'recalcitrant' in seed storage behaviour, although some authorities also include an intermediate class. Orthodox seeds, which includes conifers, can be dried to low moisture contents (<10%) and stored under sub-freezing temperatures for years, decades, or longer. **Recalcitrant** seeds cannot be dried down to low moisture contents and generally have very short life expectations (days, weeks, or months). The *Quercus* spp. (oaks) are an example of north-temperate recalcitrant species, although most recalcitrant species are tropical or sub-tropical in origin.

Long-term Storage

Long-term storability is generally quite good with conifer tree seeds, relative to many other orthodox plant species. It is generally accepted for orthodox species that storability, or the speed of deterioration, is related to storage temperature, storage moisture content, species, and initial seed quality. Storability is increased at low moisture contents and low temperatures and greatest for seeds with high initial seed quality. Harrington (1963) proposed a general rule of thumb which stated that the time for 50% of seeds to die (P50) was doubled for every 5°C reduction in temperature or 1% reduction in moisture level. As an example, if it takes 60 years to reach P50 at -20°C with a moisture level of 7%, the P50 age would be 30 years if the storage temperature was -10°C

Storability is increased at low moisture contents and low temperatures and greatest for seeds with high initial seed quality or if the moisture content was 8%. Much more sophisticated models have been developed, but they have mainly concentrated on agricultural crops. See the following references for a more thorough review of current research into storability (Roos 1989; Walters 1998).

At the BC Ministry of Forests Tree Seed Centre seeds are stored at -18°C and must be

between 4.9 and 9.9% moisture content for registration. Seeds are stored in a plastic bag which has had all excess air expelled and then placed into a waxed cardboard box (Figure 62). An inventory location is placed on the cardboard box and the seedlot registration number placed inside and outside the plastic bag as well as on the box Figure 63). The freezer used for long-term storage should be secure from vandalism or fire and have a backup generator in case of power outage. Concerns have been raised that an increase in moisture content may occur when storage bags are opened under warmer conditions for seed withdrawal. A study of changes in moisture content Orthodox seeds can be dried to low moisture contents (<10%) and stored under sub-freezing temperatures for years, decades

during freezer storage indicated that the average change was approximately 0.1% per year, but that two species (Amabilis fir and western larch) had double this rate of moisture gain (Prabhu 1994). Seedlots of these two species should have moisture content retested after 10 years in storage.

Seeds may be stored in plastic, metal, or glass containers, but they should be airtight to avoid increases in moisture or infection from pests. A study in Scots pine using 14 kg plastic containers indicated that it takes 36 hours for a seed mass to warm from -16°C to an ambient temperature of 22°C (Sahlén and Bergsten 1982). This duration would be less in a smaller container (e.g., 7 kg bags used in BC), but it does illustrate that temperature change is not rapid. In Alberta the freezers are placed underground and the following advantages are stated: protection from fire, savings in maintenance costs, and savings in energy requirements through the natural insulation of soil around the building. After a mechanical



Figure 62 Long-term storage of tree seeds in polyethylene bags.



Figure 63 Long-term freezer storage facilities at the BC Ministry of Forests Tree Seed Centre.

failure of 72 hours, the temperature inside the freezers had only increased by 10°C (Altmann and Lafleur 1982).

There have been several reviews on the storability of conifer tree seeds (Barton 1954; Holmes and Buszewicz 1958; Wang 1974). The oldest tests are probably extremely conservative due to an incomplete understanding of the importance of moisture and temperature control and through improvements in tree seed collection and processing over time. Storability results of up to 50 years showed very slight changes in germination for slash pine although shortleaf pine deteriorated from 68 to 25% during the same period (Barnett and Pesacreta 1993).

In BC, some seedlots have been freezer-stored for more than 30 years. Several of these are presented in Figure 64 and include some of our oldest seedlots of coastal Douglas-fir, western hemlock, Sitka spruce, and western redcedar. Storability in coastal Douglas-fir is fairly good, but one can see that individual seedlots have unique storability patterns.



Figure 64 Long-term storability patterns at the BC Ministry of Forests Tree Seed Centre in seedlots of a) coastal Douglas-fir (Fdc); b) western hemlock (Hw); c) Sitka spruce (SS); and d) western redcedar (Cw).

While western hemlock seedlots A and B have an equivalent germination capacity going into freezer storage, after 20 years of storage their GC differs by approximately 40%! Initial seedlot quality is not always a good predictor of rate of deterioration.

In Sitka spruce some seedlots show an initially low GC, but over time increases in germination do occur. This is a perplexing issue and one for which we do not have an adequate explanation. Although controversial, it has been hypothesized that seedlots can become less dormant after prolonged freezer storage. However, Sitka spruce does not have deep dormancy and this does not adequately explain the large increases in germination found in some of the illustrated seedlots. Western redcedar is presented to illustrate the rapid deterioration (decrease in germination) that can occur and to show that some western redcedar seedlots can perform well after prolonged periods of freezer storage. These examples from our oldest seedlots illustrate that while species vary in storability, a greater source of variability may be the

The deterioration rate was calculated on a seedlot basis as the initial germination minus the current germination divided by the time in storage individual seedlot and how it has been handled leading up to freezer storage. Deterioration is not fully understood and, due to the long time periods involved, there have been few controlled experiments on seed deterioration in conifers.

The average species deterioration of tree seeds was quantified in BC to rank species and assign retesting frequencies. The deterioration rate was calculated on a seedlot basis

as the initial germination minus the current germination divided by the time in storage based on a consistent test type and presented as change in germination capacity (GC) per year. The results and recommended retest frequencies for BC conifers are presented in Table 7. Western redcedar showed the highest deterioration rate (1.44%/year) among our species and is therefore retested more frequently than others. Some species exhibit positive deterioration rates and these values probably reflect sampling variation, improvements in seed testing, and possibly changes in dormancy due to storage rather than increased seed quality during storage. Seedlots that deteriorate more rapidly than the species average may be tested more frequently.

The actual cause of deterioration is not completely understood, but has attracted a great deal of research (mainly for agricultural crops and gene conservation purposes). It is generally accepted that seeds will first lose their **vigour** or ability to germinate under sub-optimal conditions, then lose the ability to germinate normally, and eventually die and not germinate at all. Suggested theories on seed deterioration include depletion of food reserves, alteration of chemical composition, membrane alteration, enzyme alteration, and genetic damage (Roos 1989).

Gene Conservation

Seed storage facilities can also play an important role in the conservation of genetic resources through the maintenance of a seed bank. This seed bank should contain a representative sample of the natural populations for the tree species of interest and be maintained strictly for conservation purposes. This form of gene conservation complements, but does not replace the need to have adequate areas of land dedicated to gene conservation within natural ecosystems. One of the biggest advantages of seed banks is efficiency as one handful (100 grams) of interior spruce seed can be composed of approximately 50 000 unique **genotypes**!

 Table 7
 Deterioration rate estimates and germination retest frequencies for BC conifers

Species	Deterioration rate (∆%/yr)	Retest frequency (months)
Amabilis fir	-0.78	24
Grand fir	-0.24	24
Subalpine fir	+0.67	24
Western redcedar	-1.44	18
Coastal Douglas-fir	+0.03	39
Interior Douglas-fir	-0.07	39
Mountain hemlock	-0.36	24
Western hemlock	-1.22	20
Western larch	-1.06	22
Coastal lodgepole pine	+0.08	42
Interior lodgepole pine	-0.01	36
Western white pine	-1.03	30
Ponderosa pine	-0.28	30
Sitka spruce	+0.10	42
Interior spruce	-0.07	36
Sitka x interior spruce hybrid	-0.25	30
Yellow-cedar	+0.46	36

Storing Stratified Seed

At the nursery, prior to sowing, seeds should be maintained under stratification conditions $(2-5^{\circ}C)$ and given adequate aeration by opening the top of the bag when seeds arrive. These conditions will result in an extension of stratification, which may be beneficial, but seedlots of lower quality should

be sown as soon as possible. Storage of stratified seeds occurs when sowing dates are delayed (and pretreatment has already been initiated) or following sowing with seeds remaining. If seeds are stored at high moisture contents for an extended period, germination or a reduction in viability can occur (Barnett 1974; Danielson and Tanaka 1978). If seeds are dried, unwanted germination can be avoided, storage can be

Orthodox seeds should not be stored under sub-freezing temperatures at elevated moisture contents

greatly extended, and the pretreatment benefits (removal of dormancy) may be retained. Orthodox seeds should not be stored under sub-freezing temperatures at elevated moisture contents (above 14% moisture content), as ice crystals can form within the seeds, damaging contents and reducing germination (Barnett 1974; Bewley and Black 1994). In Douglas-fir, drying from 45 to 35% moisture content removes moisture from the seed coat, but drying to 25% reduced moisture content in the seed coat, megagametophyte, and embryo (De Matos Malavasi et al. 1985). The moisture level for drying will probably vary by species, as those with more storage reserves (e.g., ponderosa pine or Douglas-fir) will probably store better than those with limited reserves

> (e.g., western redcedar or western hemlock). For loblolly pine, ponderosa pine, and Douglas-fir drying seeds to between 21 and 26% moisture content allowed the seeds to be stored for between 9 and 10 months without a significant reduction in germination. Seeds with a low GC will not retain viability as well as those with high GC values (Danielson and Tanaka 1978; Belcher 1982).

The BC Ministry of Forests Tree Seed Centre does not normally dry sowing requests if the sow date has been delayed by three weeks or less, but will dry sowing requests to approximately 20% moisture content if the delay is longer. If seed quality is poor the seed owner or nursery may decide to discard the seeds and initiate a new sowing request based on the revised timelines. Guidelines for the return of extra seeds from sowing requests are included in the seed sowing section.