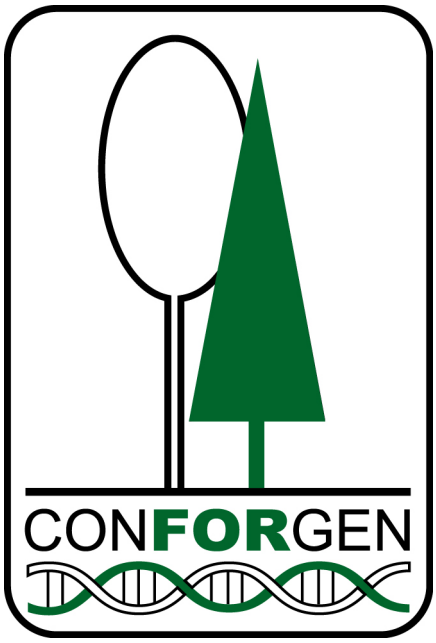
Guidelines for Collecting and Storing Seed for *ex situ* Conservation



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Storage of seed is an efficient means of conserving genetic variation as a large quantity of material can be stored in a relatively small space. However there are limitations, the most important of which is that not all seeds can be stored. Species that produce recalcitrant seed are particularly problematic because the seed can neither be dried nor placed in frozen storage. There are a number of Canadian tree species that produce recalcitrant seed (e.g., oaks). In contrast, seed from conifer species and most temperate deciduous species is classified as orthodox and can be dried and stored frozen where it will remain viable for many decades.

An *ex situ* gene conservation program should attempt to preserve the maximum among-population (and within-population) variation, with the goal of preserving evolutionary potential rather than individual genes (McIlwrick et al. 2000). Seed represents the next generation as it is a product of the trees currently growing in the forest. Thus it contains new and unique combinations of genes.

The purpose of these guidelines is to present minimum standards for collecting and storing seed for *ex situ* conservation.

Factors Impacting Genetic Diversity

Species with widespread distributions, out-crossing breeding systems, and widely dispersed seeds tend to have more genetic diversity within populations and less variation among populations as a result of gene flow. Conversely, endemic species, those with small ranges or disjunct populations, have the least genetic diversity. Endemic species and species with limited gene movement should have smaller effective population sizes and should experience greater losses of genetic variation through drift. Thus populations of such species are expected to have lower overall genetic diversity; however, they have more genetic diversity among populations than more widespread species (Hamrick et al 1992).

Most of our tree species are wind pollinated. This results in relatively homogeneous populations within a region (Krakowski 2011). However, there are factors that come into play such as differences in phenology among trees and the existence of "neighbourhoods" which may cause trees to be related to varying degrees. These factors can result in a reduction in the amount of genetic variation in seed collected in one location but this can be mitigated by making collections throughout the region. Collecting from multiple populations increases the likelihood of capturing low frequency alleles which could have potentially major beneficial effects, e.g., disease resistance).

Gene flow is more limited for species that are insect pollinated. For species where seed is dispersed by wildlife there can be less genetic diversity among populations because the seed may be spread over a large area (Krakowski 2011). This is dependent on the animal involved. For example, Clark's nutcracker (*Nucifraga columbiana*) can disperse and cache whitebark pine (*Pinus albicaulis*) seed over distances of 30 km (Lorenz et al. 2011) while squirrels cache seed close to the source.

Collection

Generally, the highest proportion of genetic variation occurs within populations or stands. In order to capture as much of this variation as possible seed must be collected from a reasonably large number of individuals. The word "reasonably" is used because of the manner in which seed is collected. For example, following a harvesting operation, it is easy to collect seed from a large number of trees whereas fewer trees are sampled when they have to be climbed.

Within each region or seed zone, stands should be sampled on sites that represent the environmental variation found within the region/zone. A stand should contain at least 5000 trees. This is important to ensure there is a sufficient number of unrelated, randomly mating trees. Failing this, trees can be sampled over a geographical area provided the ecological conditions they are growing in are similar and isolated trees are not sampled.

Mature seeds and cones should be collected in heavy crop years when pollen production is high in order to ensure high out-crossing and good physiological quality of the seed. Seeds and cones must be collected as close as possible to maturation and prior to natural dispersal. Inspect seeds and cones before collection to avoid collecting those that are insect damaged. Damage can vary from tree to tree.

Collect seed from at least 20 trees spaced at least 50 m apart and preferably 100+ m apart. Seed quality varies among trees and sometimes seed may need to be discarded from one or more trees due to low quality. High quality seed stores longer. It is preferable to keep the collections separate by individual tree. Keeping the seeds separate by tree provides more options for using the seeds in the future because these are of greater value in breeding work and genetic studies and can facilitate restoration work (Knight et al. 2010). As well, seed quality deteriorates over time at different rates among trees due to maternal effects. In contrast, bulked seed collections should/can be made when this is the most practical option. Equal quantities of seed or cones should be collected from 30−50 trees.

The following guidelines for quantities of seed to collect are based on seed weight.

Large seed (1000-seed weight > 15 g)
2000 seed per single tree
5000 − 10000 seed for a bulk

Small seed (1000-seed weight < 15 g)
5000 seed per single tree
50000 seed for a bulk

See ENSCONET (2009a) for additional considerations and guidelines for collecting seed.

Storage

Maintaining seed viability is critical to ensure that seed is available to users and is genetically representative of the population from which it was acquired (FAO 2014). Basic data such as moisture content, 1000-seed weight (or equivalent) and germination should be collected for each seedlot. Seed moisture content and storage temperature are key factors for long-term storage. Orthodox seed can be safely dried to a moisture content of 3−7% which is equivalent to the international standard that seed should be dried to equilibrium in a controlled environment of 5−20°C and 10−25% relative humidity (FAO 2014).

Seed must be stored in hermetically-sealed containers. The containers should be labelled inside and outside and placed in sub-zero storage conditions. The international standard for storage temperature is -18±3°C (FAO 2014). There is always a risk that the seal of the storage container will fail and moisture will infiltrate the container. An option, for clear containers (e.g., glass), is to place inside a sachet containing self-indicating silica gel, equilibrated to the seed drying environment. A change in the color of the silica gel indicates that moisture has infiltrated the container. If this occurs, the seed should be re-conditioned and placed in another container.

It is important that a sufficient quantity of seed is placed in storage to allow for future testing/monitoring and be available for research or other projects such as restoration or *ex situ* plantations. The quantity of seed stored depends on the species and the means of collection. The volume of the storage container is also a consideration with respect to the number of seed stored. Seed should completely fill the storage container to reduce oxidation and ageing. Small seed from individual-tree collections is best stored in vials and the vials placed in a larger container with a hermetic seal. The vials should be completely filled to limit the amount of air.

Monitoring

Viability is the most important metric used to monitor seed quality over time with germination tests being the most common method. Seed should be germination tested prior to storage in order to establish a baseline value. It is important that seed being stored for *ex situ* conservation be disturbed as infrequently as is possible but at the same time viability needs to be monitored. For species producing seed that stores well (50+ years), the testing interval should be 10−15 years. For species producing seed that stores well for less than 50 years the testing interval should be 10 years. For species that little is known about long-term storability, small samples taken from the primary seedlots can be stored under the same conditions and tested every 5 years. The primary seedlots should be tested every 10−15 years, depending on results from the samples. These testing intervals are a guide. They can be adjusted according to data obtained from germination tests.

The number of seed used for testing is also a consideration. It is important not to waste seed for germination testing. A maximum of 200 seed is sufficient and 50 seed is considered acceptable (ENSCONET 2009b) with the test set up using two or four replicates depending on the number of seed tested. The number of low vigor and abnormal germinants (stubby, stunted, necrotic radicle; stunted hypocotyl/epicotyl) should be recorded because an increasing proportion is often an early indication that deterioration is occurring. Performing a cut test on ungerminated seed allows for the determination of the number of empty and damaged seed in the sample allowing for germination to be expressed in terms of the number of seed capable of germinating (ENSCONET 2009b).

See ENSCONET (2009c) for additional suggestions and recommendations with regard to germination testing.

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