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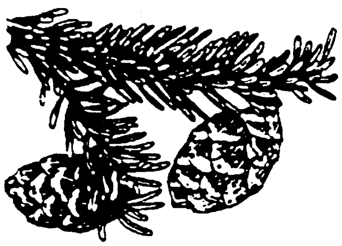

Tree Seed Working Group

NEWS BULLETIN

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SEED STORAGE

THIS ISSUE AT A GLANCE

CHAIR'S 'ARMCHAIR' REPORT

Hello, welcome to this issue on a very important topic – seed storage. Thank you to everyone who has contributed to this edition. For most of our species, seed storage is something we take for granted. All of our conifer species and most of our broadleaved species are considered to have orthodox seed storage behaviour and can therefore be dried to low moisture contents (< 10%, fresh weight basis) and stored at sub-freezing temperatures. This allows seedlots to be used over many years and even decades without large changes in quality. As an example, a *Pseudotsuga menziesii* var. *glauca* seedlot collected in 1959 had an 89% germination at that time and when retested in 2006 the seedlot achieved 86% germination after over 46 years of storage! We can quantify deterioration, but our estimates are probably conservative as the timing of collection, handling, processing of cones and fruits, and our understanding of biological processes have greatly improved over the lifespan of our oldest seedlots.

Seed storage also allows for many species and populations within species to be stored efficiently as *ex-situ* seed reserves for gene conservation purposes. This is especially important where *in situ* conservation is not possible. Expanding urbanization into some of our most pleasant climates in Canada is a major issue in the southern Ontario Carolinian forest and to a lesser extent the grand fir forests along the east coast of Vancouver Island. Several provinces (Alberta, British Columbia, Manitoba, and Ontario) already have *ex-situ* genetic conservation programs and an effort (CAFGRIS) is underway for these provinces to share this information allowing for a National assessment of the conservation of our genetic resources. I encourage all provinces to consider establishing seed banks for genetic conservation as this is a simple and efficient method of conserving the genetic variability of most of our tree species in addition to *in situ* efforts in parks and other types of protected areas.

Seed storage is a relatively simple exercise for orthodox species, but species producing recalcitrant seed are far more complicated. Recalcitrant seeds are present in the genera *Aesculus*, *Castanea*, *Juglans*, *Quercus*, and in some species of *Acer*. Seeds of these species can be stored, but storage must be at high moisture contents (> 30%, fresh weight basis) and at temperatures close to 0°C for temperate zone species. These conditions are also favourable to fungal growth and potentially the rapid deterioration of a seedlot if no preventive measures are taken. Elevated temperature and moisture content also makes premature germination a possibility. For tropical recalcitrant species, the situation is worsened as seed must be stored above 20°C exacerbating any potential for problems due to fungi or pre-germination. Seed storage of recalcitrant species, either for commercial use or for genetic conservation, is probably the single biggest challenge facing seed science and technology worldwide!

Seed storage generally refers to seed stored *ex situ* under some appropriate conditions to maintain viability. There are also two other *in situ* forms of seed storage: soil seed banks and canopy seed banks (Farmer 1997[1]). Canopy seed banks are present in *Pinus contorta*, *P. banksiana*, and *P. rigida* which possess serotinous cones and in *Picea mariana* where cones are semi-serotinous. Serotiny is hypothesized to have evolved with fire to allow seed to be dispersed at the time when a suitable substrate is available for germination and when much of the competition has been eliminated. In BC, the mountain pine beetle is influencing the canopy seed banks of *Pinus contorta* that are being killed by this pest epidemic. After tree death, the trees lose foliage, stands open up and enough heat is reaching cones to break the serotinous cone scale bonds and release the seed. In some stands the stem density of the canopy may still preclude seedling establishment, in other areas competition will preclude seedling establishment and in some areas natural regeneration is occurring. In all areas, the opportunity for collecting the multi-year canopy seed bank in *Pinus contorta* is being removed in large areas affected by the mountain pine beetle.

With a few exceptions soil seed banks generally do not contain tree seeds. Notable exceptions are *Prunus pensylvanica* and *Liriodendron tulipifera*, but other species are considered transient in that, given adequate conditions, the seeds may remain viable for up to three years, but they do not appear to be an important strategy for continuation of the species. Soil seed banks are more important for shrub species, herbs, and of course weed species.

Currently all past issues of the Tree Seed Working Group News Bulletin (back to 1983) are available online as pdf documents at the following link: http://www.for.gov.bc.ca/hti/treeseedcentre/tsc/tswg.htm . We hope to add functionality to allow readers to search for articles by author or subject matter to allow for easier use of these past News Bulletins. For the next News Bulletin we will open up the subject matter to an important topic that many jurisdictions are reviewing in light of climate change – Seed Transfer. The Christmas edition of the Tree Seed Working Group will be an overview of how seed transfer is considered and implemented today as well as some visions of what it will look like in the future. Have a great summer.

Dave Kolotelo
Chairperson

EDITOR'S NOTES

Seed storage is a topic that is near and dear to me. My chief interest is long term storage for gene conservation purposes but short term storage is just as important for seed centres servicing reforestation programs. Many factors impact seed storage ability and these have been discussed in a number of papers in the scientific literature. The bottom line is that seed is a living 'organism' and anything that affects its health will surely have a negative impact on it during storage. Conifer seed stores very well as does most hardwood seed however some hardwood species are defined as being recalcitrant and thus storing seed of these species is a challenge. Of interest is the fact that most of the hardwood species listed as endangered or threatened in Canada produce recalcitrant seed. Therefore, there is a research opportunity here to develop ways and means of storing germplasm of these species.

There are a number of papers in this issue that present data on the deterioration rate of seed from a variety of species. As well there is a paper presenting research on a technique to store bur oak germplasm. Also you will find several other articles on a variety of topics.

I hope that you have a great summer and that the Seed Gods will smile on you.

[1] Farmer, R.E., Jr. 1997. Seed Ecophysiology of Temperate and Boreal Zone Forest Trees. St. Lucie Press, Delray Beach, FL, USA. 253 p.

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Comments, suggestions, and contributions for the News Bulletin are welcomed by the Chairperson and Editor.

BROADLEAF SEED LONGEVITY

At the British Columbia provincial Tree Seed Centre there are four broadleaf tree species currently in our inventory: *Alnus crispa*, *Alnus rubra*, *Populus tremuloides*, and *Betula papyrifera*. All of these species are considered orthodox (seed can de dried and stored at sub-freezing temperatures) and are stored at -18°C at moisture contents under 10%. These species are considered non-dormant and tested dry – they are not imbibed or stratified before testing. Moisture, to initiate germination, is imbibed from the moistened media at the start of testing. A previous review of broadleaf storability was presented in Tree Seed Working Group News Bulletin volume 28 (http://www.for.gov.bc.ca/hti/publications/misc/HWTSWG28.pdf). This note is intended to provide an update on broadleaf tree seed longevity.

The deterioration rate was calculated as the current germination capacity (GC) minus the initial GC all divided by the time between tests. It is presented as the change in germination percent per year. It provides a basic linear estimate of deterioration and a simple method of comparing species and seedlots within species, especially when limited numbers of tests are available for seedlots over time. Comparisons of deterioration rate estimates performed in 1998 and 2007 and across species are presented in Table 1.

Populus tremuloides showed the highest deterioration rate, although the 2007 estimate (-3.1%/year) was less dramatic than -8.6%/year estimated in 1998. The more recent estimate is based on an average of nine years of storage compared to only about two years in 1998. Seedlots varied in deterioration rate from a low of -0.2% /year to a high of -18%/year. Removal of the fastest deteriorating seedlot (-18%/year) reduced the average deterioration to -1.0 % /year and only two seedlots were above this level (-1.3% and -3.0%). *Populus tremuloides* displays orthodox seed behaviour, but in general the genus *Populus* is described as having microbiotic or short life-span seeds. Under natural conditions the lifespan of *Populus* spp. seeds may only be a few weeks to months, but if seed is collected and immediately processed, dried, and stored at sub-freezing temperatures then seed can maintain high viability for more than ten years (Wyckoff and Zasada 2002).

Betula papyrifera seed displayed good storage as a slightly positive deterioration rate was estimated in 1998 and 2007. Seedlots varied from a deterioration of 1.16%/year to a gain of 3.25%/year. *Alnus crispa* also showed an average gain in germination over time and the highest deterioration rate was only -0.30%/year illustrating promising longevity for this species. These positive average deterioration estimates are not intended to imply that germination increases during storage. Sampling of different genotypes (within seedlot variability) over time is the most reasonable explanation for these estimated germination gains.

Alnus rubra, our most commercial broadleaf in BC, was estimated to have an average deterioration rate of -0.68%/year. Seedlots varied from a deterioration of -1.16 %/year to a gain of 3.25%/year. Estimates in 1998 indicated a small germination gain, but the 2007 estimates are based on averages of over three times as many seedlots and almost three additional years of storage.

The broadleaved species described here have generally good storability that falls within the range of BC conifers. There is inadequate knowledge concerning seed storage for other

Table 1. Comparison of broadleaf seed deterioration rate (DetRate = germination / year) estimates performed in 1998 and 2007[1] at the BCMOFR Tree Seed Centre.

Species	1998			2007		
	No. seedlots	DetRate (%/yr)	Ave. storage period (years)	No. seedlots	DetRate (%/yr)	Ave. storage period (years)
Alnus crispa	na	na	na	11	+0.27	9.0
Alnus rubra	5	+0.20	3.9	16	-0.68	6.8
Betula papyrifera	12	+0.20	6.0	23	+0.10	9.9
Populus tremuloides	8	-8.60	1.9	8	-3.10	8.7

[1] For ease of comparison, the 1998 deterioration rate values have changed sign to be consistent with 2007 values. Current methods present negative estimates as indicative of decreases in germination.

broadleaved BC tree species. The most problematic for seed storage is *Quercus garryana* which is considered recalcitrant (cannot be dried and stored at subfreezing temperatures) and *Acer macrophylum* which is considered intermediate. All other broadleaved trees in BC are considered orthodox, but no specific information on seed storage behaviour is available.

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CONIFER SEED LONGEVITY

There are over 5,500 conifer seedlots in the seed inventory located at the British Columbia Tree Seed Centre. This is the backbone of the BC reforestation program on Crown land. Maintaining up-to-date germination information is critical to ensure that seed is being used efficiently to produce seedlings and that seed owners have accurate inventories of the quality and quantity of seed they own. Repeated germination tests allow for an assessment of how quickly and to what degree germination changes over time – the deterioration rate. The average deterioration rate of species has been a key factor in assigning a germination retesting frequency, but it is not the only factor. Consideration of individual seedlot deterioration rate, historic use, seedlot size, feedback on past nursery and lab performance, and total number of tests able to be performed in a given year all influence if and when a seedlot is retested. This note provides an update on calculated average seed deterioration rate estimates for BC conifer species in relation to values calculated in 2002[1].

The deterioration rate was calculated as the current germination capacity (GC) minus the initial GC all divided by the time between tests. It is presented as the change in germination percent per year. The only limitations are 1) that the initial and current germination test types are the same and 2) that the storage duration is greater than 500 days[2]. If test types change, the deterioration rate will be calculated based on a subset of the entire life of a seedlot in long-term storage. The deterioration rate provides a basic linear estimate of deterioration and a simple method of comparing species and seedlots within species. Non-linear estimates, providing a more realistic description of deterioration, are problematic to construct due to the limited number of germination tests available per seedlot. A comparison of average species deterioration rate estimates for BC conifers performed in 2002 and 2007 is presented in Table 1.

[1] The 2002 article can be found at http://www.for.gov.bc.ca/hti/publications/misc/RTS%20TSWG36.pdf

[2] 660 seedlots were not included in the deterioration rate estimates as they had only one germination test performed or they were not in long-term storage for a minimum of 500 days between initial and current germination tests.

Table 1. Comparison of conifer seed deterioration rate (DetRate = germination / year) estimates performed in 2002 and 2007 at the BCMOFR Tree Seed Centre.

Species	2002			2007		
	No. seedlots	DetRate (%/yr)	Avg. storage period (yrs)	No. seedlots	DetRate (%/yr)	Avg. storage period (yrs)
Abies amabilis	165	0.06	7.8	159	-0.69	10.1
Abies grandis	49	-0.72	11.3	51	-0.99	13.4
Abies lasiocarpa	107	0.17	8.9	132	-0.93	9.7
Abies procera				18	-1.52	7.1
Chamaecyparis nootkatensis	35	-2.16	4.6	42	0.37	7.4
Larix occidentalis	173	-0.67	7.8	165	-0.49	11.2
Picea glauca / engelmannii complex	1233	-0.22	13.3	1004	-0.26	16.6
Picea sitchensis	194	0.03	14.7	163	-0.03	20.4
Picea X *lutzii*	50	-0.28	11.5	43	-0.23	15.0
Pinus contorta var. *contorta*	49	-0.15	8.5	52	0.01	11.3
Pinus contorta var. *latifolia*	1495	-0.08	8.5	1342	-0.05	11.7
Pinus monticola	95	-0.19	8.6	114	-0.21	7.8
Pinus ponderosa	150	-0.46	6.6	184	-0.34	8.2
Pseudotsuga menziesii var. *glauca*	609	-0.21	13.2	525	-0.22	16.6
Pseudotsuga menziesii var. *menziesii*	349	-0.10	11.7	194	-0.08	16.5
Thuja plicata	370	-1.24	8.5	268	-1.02	11.0
Tsuga heterophylla	366	-1.13	12.1	292	-1.03	15.6
Tsuga mertensiana	47	-0.46	14.1	47	-0.49	16.3
Total	5610			4795		

Only three species exhibited deterioration rates greater than 1%/year: *Abies procera* (Bp), *Thuja plicata* (Cw), and *Tsuga heterophylla* (Hw). The last two species have consistently shown the highest deterioration rates since 1997 and are prioritized for germination retesting. These are also considered the conifers with the lowest level of seed dormancy in BC. There is much less data on Bp deterioration with only 18 seedlots and an average of only seven years of storage history. In general, *Abies* spp. display relatively erratic germination, especially *Abies amabilis* and *A. lasiocarpa*, and this is reflected in the relatively large changes in deterioration rate estimates between 2002 and 2007. The species exhibiting the largest estimate change was *Chamaecyparis nootkatensis* which had a rather large -2.16 %/year estimate in 2002, but a gain of 0.37 in 2007. Although test procedures (soak, warm stratification, and cold stratification duration) have not changed, there has been increased emphasis on monitoring and maintaining a high (about 44%) moisture content during the warm stratification phase of the pre-treatment. This has generally resulted in improved germination and the prime factor explaining this large change in the deterioration rate estimate.

Several species show very slow (< -0.1%/year) deterioration: *Picea sitchensis*, *Pinus contorta* var. *contorta*, *Pinus contorta* var. *latifolia*, and *Pseudotsuga menziesii* var. *menziesii* with these species requiring less frequent germination retesting to provide up-to-date information. The remaining species' estimated deterioration rates fall within the range of -0.21 to -0.49% germination loss per year and show relatively little change from the 2002 estimates of deterioration rate.

Calculation of average species deterioration rates is the first step in the review and improvement of our germination retesting program at the Tree Seed Centre. Actual changes to retesting frequencies have not been finalized as I expect to restructure our retesting program to optimize the use of testing resources. Some of the possible changes being considered are described below:

1) **Seedlot Deterioration** – A key to this exercise was to move from a species specific deterioration rate to a seedlot specific deterioration rate in specifying the retest frequency of an individual seedlot. This will require some software enhancements and will likely chronologically follow an initial general adjustment to the species retest frequency. Complications include seedlots in

which only one test is available or that the storage duration is less than 500 days. The species retest frequency will probably default for these seedlots. The emphasis will be on identifying those seedlots for more frequent retesting which deteriorate faster than the species average and are being actively used.

2) **Historic Use** – Seedlots are currently prioritized for retesting if they were used in the previous sowing year and consideration is then given to when seedlots were last used. This prioritization will continue, but it will probably be automated with system improvements in the coming year. Initial thoughts are to look at seedlot use in three categories:
   a. Used in the last five sowing seasons
   b. Used in the last five to ten sowing seasons
   c Not used in the last ten sowing seasons

   Other options include the number of times a seedlot has been used or the number of trees produced over a given time frame. Different retest frequencies may be employed for each category with the possibility that seedlots not used in the last ten years will not receive germination updates until seed is expected to be used for sowing. The intent is to not use testing resources for seedlots that have a very low probability of being used in the upcoming sowing year.

3) **Seedlot Size** – Currently very small seedlots may not be retested as their impact on maintaining current information is limited and resources are better spent on other seedlots. Minimum size varies by species, but generally if seedlots do not have sufficient seeds to produce 10 000 seedlings a germination retest is not initiated.

4) **Genetic Class** – Seed orchard produced seed is a high priority for germination retesting. An initial look at deterioration rate by genetic class indicates lower levels of deterioration for seed orchard seed, but the comparison is confounded with seedlot age. Seed orchard seedlots are generally younger and are used faster than the inventory of natural stand seed, so this is the most likely explanation for these differences. Genetic class is also considered in relation to the proportion of seedlings requested for that class. For example, for interior spruce where 82% of the seedlings are produced from orchard seed, it is less important to test **all** natural stand seedlots than in a species like lodgepole pine where only 12% of the seedlings are derived from orchard seedlots. Genetic class useage by species is used to prioritize which species obtain the greatest investments in updating natural stand seedlots germination information.

5) **Germination Test Precision** – The deterioration of a seedlot over time is one parameter affecting the ability to provide an accurate estimate of the germination capacity. The other parameter affecting the germination precision is the variability present in germination tests among the four replicates. This has previously been presented as the precision of germination tests (see http://www.for.gov.bc.ca/hti/publications/misc/GCTSWG36.pdf) and can vary greatly between species and between seedlots within a species. This variability is easily quantified and should also be considered when assigning retest frequencies to individual seedlots.

The deterioration rate (of species and individual seedlots) is an important attribute for determining when seedlots should be retested. The linear deterioration rate is a simple statistic allowing for prioritization of species and seedlots, but it does not increase our knowledge concerning seed deterioration in the biological sense as germination is expected to be non-linear as seeds age. Retesting programs will also provide the data to develop these non-linear functions in the future with additional long-term data. The rate of deterioration is just one of the elements to be considered in determining the frequency that seedlots should be retested for germination.

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SEED LONGEVITY AT THE NATIONAL TREE SEED CENTRE

The National Tree Seed Centre is celebrating its 40th anniversary this year. The Centre was established at a time when reforestation programs were starting or expanding in Canada and there was need for research on seed processing, handling, and testing as well as for obtaining, storing, and providing seed of known origin and quality for research. From its modest beginning the Seed Centre has expanded its inventory to over 120 Canadian tree and shrub species with seed samples from across the range for many tree species. Seed from species with an orthodox storage behavior is stored at -20°C with a moisture content less than 8%. Germination testing is an integral component of the Seed Centre's program. The data are necessary in order to maintain up-to-date records for each seedlot and ultimately can provide seed storage ability profiles for the various species.

This issue of the Tree Seed News Bulletin provided the impetus to look at germination data for a number of species in order to estimate the rate of loss in germination. The statistic applied to the data was Deterioration Rate defined as: (current germination – initial germination) / interval, in years, between the two tests. This provides a number that describes the annual change in germination. The statistic implies that the change is linear which may not be the case. As data accumulate, there will be an opportunity to compare the deterioration rate among different age classes of seed for a species.

The Seed Centre stores seed from a number of hardwood species but sufficient data from tests conducted in a consistent manner presently exist for only nine species (Table 1). One observation about the species represented here is, with the exception of white elm (*Ulmus americana*), seed size is small with *Populus* seed being the smallest but despite its size largetooth aspen (*P. grandidentata*) seed has a low deterioration rate. *Alnus* and *Betula* species are members of the same family, Betulaceae. The deterioration rates vary among the species with yellow birch (*Betula alleghaniensis*) having the lowest (-0.44). One way to reduce deterioration rates of *Alnus* and *Betula* seed may be by improving seed cleaning techniques to remove partially filled seed using alcohol separation (Simpson and Daigle 2003). Partially filled seed may produce a countable germinant when initially tested but these seed probably deteriorate at a faster rate than completely filled seed thereby negatively biasing germination of a seedlot at a later time. Seedlots from these genera have been processed or upgraded using alcohol separation and when tested in the future will show if the deterioration rate differs between treated and untreated seed.

Table 1. Seed deterioration rates for nine hardwood species plus range in initial and current germination capacity and range of years seed was in storage at the National Tree Seed Centre.

Species	No. seedlots	DetRate (%GC/yr)	Initial GC range (%)	Current GC range (%)	Storage duration range (yrs)
Alnus incana spp. *rugosa*	40	-0.57	5.5 – 97.0	5.5 – 76.0	8 – 27
Alnus viridis spp. *crispa*	30	-2.11	14.0 – 97.5	12.0 – 61.5	14 – 19
Alnus viridis spp. *sinuata*	17	-0.99	15.5 – 88.2	6.5 – 87.0	7 – 19
Betula alleghaniensis	25	-0.44	11.5 – 91.0	24.0 – 85.0	7 – 32
Betula papyrifera	25	-1.40	15.5 – 86.0	4.0 – 65.0	3 – 16
Populus grandidentata	11	-1.46	92.2 – 99.5	17.0 – 100.0	8 – 22
Populus tremuloides	9	-3.46	28.0 – 98.2	10.0 – 78.0	6 – 29
Ulmus americana	7	-1.48	28.0 – 97.0	40.0 – 96.0	3 – 14

Conifer seed is characterized by having good storage ability. Most of the species evaluated had a deterioration rate less than -0.60%/year. Black spruce and jack pine (*Pinus banksiana*) had the lowest deterioration rates and this is probably a reflection of these species producing semi-serotinous and serotinous cones, respectively. This cone characteristic makes it imperative that seed remains viable for many years so that it can readily germinate when the cones open and release the seed. This is illustrated by seed from a jack pine seedlot that declined in germination from 90% just after collection to 80% 53 years later! This seed had not always been stored at -20°C. The deterioration rate for Sitka spruce (*Picea sitchensis*) does not conform to that found for the other spruce species. Some of the seedlots had exceptionally high deterioration rates that biased the species mean. White pine (*Pinus strobus*) had the highest deterioration rate of all the conifers and this is again a reflection of several poor quality seedlots. Eastern white cedar (*Thuja occidentalis*) demonstrated that its seed deteriorates very slowly when stored at -20°C which is contrary to what several provincial seed centres are experiencing.

Table 2. Seed deterioration rates for thirteen softwood species plus range in initial and current germination capacity and range of years seed was in storage at the National Tree Seed Centre.

Species	No. Seedlots	DetRate (%GC/yr)	Initial GC range (%)	Current GC range (%)	Storage duration range (yrs)
Abies balsamea	61	-1.06	25.7 – 90.8	7.5 – 80.0	8 – 36
Larix laricina	150	-1.51	1.0 – 98.5	0.1 – 96.0	8 – 36
Picea engelmannii	7	-0.57	42.0 – 98.0	55.0 – 96.5	6 – 13
Picea glauca	422	-0.47	60.5 – 100.0	7.5 – 100.0	8 – 29
Picea mariana	33	-0.13	82.5 – 99.8	73.0 – 100.0	11 – 26
Picea rubens	10	-0.38	35.9 – 100.0	28.0 – 99.5	16 – 40
Picea sitchensis	23	-1.96	39.2 – 98.8	0.5 – 91.5	7 – 34
Pinus banksiana	620	-0.19	14.0 – 100.0	3.0 – 100.0	10 – 53
Pinus contorta var. *latifolia*	72	-0.57	38.8 – 100.0	5.5 – 96.0	13 – 42
Pinus resinosa	22	-0.47	31.0 – 100.0	15.5 – 99.0	20 – 45
Pinus rigida	24	-0.32	68.5 – 100.0	45.5 – 100.0	14 – 15
Pinus strobus	16	-2.78	26.5 – 97.0	10.0 – 62.5	23 – 40
Thuja occidentalis	19	-0.36	6.5 – 88.8	4.0 – 94.0	3 – 14

Readers can compare these deterioration values with those presented by Dave Kolotelo, for the same species, in his two articles. Other factors such as time, year, and location of collection and seed handling and processing also impact seed deterioration in addition to seed moisture content and storage temperature. Generally speaking, seed with a high initial germination capacity deteriorates at a slower rate than seed with a low initial germination capacity. I would like other seed centres to apply this deterioration statistic to their data and present the results in a future issue of the News Bulletin. This would be useful in order to compare deterioration rates within a species among various seed centres.

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BUR OAK SEED STORAGE – DIFFICULT BUT MAYBE NOT IMPOSSIBLE

Oak seed is classified as recalcitrant due to its inability to tolerate desiccation below a relatively high minimum moisture content (http://www.nsl.fs.fed.us/wpsm/Quercus.pdf). This creates difficulty when trying to maintain long term viability with conventional storage regimes. Recalcitrant seeds show a common suite of traits that have co-evolved with desiccation sensitivity. Thus recalcitrant seeds are typically large (0.5g), have a thin seed coat, and are shed during wet periods (Pritchard et al. 2004, Daws et al. 2005, 2006).

Acorns of bur oak (*Qurecus macrocarpa*), a native white oak species in New Brunswick, have been collected over a number of years (Erb's Cove 45°57' N 65°92' W and Jemseg 45°84' N 66°11' W). These sites are isolated populations at the northeastern part of the species' range. Conventional storage involves fresh acorns being dried to ~40% moisture content and placed in mason jars maintained at 3 C. Using this method, acorns will remain viable from 1 up to 3 years (http://www.nsl.fs.fed.us/wpsm/Quercus.pdf).

To increase the storage capability of this species, moisture content and desiccation tolerance are the two key areas that determine the storage behaviour of the seed. In order to store recalcitrant seed for longer periods without deterioration, storage at lower temperatures (where the seed is maintained in a biologically inactive state) is a potential alternative method of storage. The main problem with this approach is that seed with a high moisture content suffers cell damage during the freeze-thaw process (Wendell, 1999).

A desiccation profile of Bur oak was established to determine when the seed reached a stable moisture content. The seed coat was removed and a small portion of tissue, including the embryonic axis (referred to as an explant), was placed in a Petri dish in a laminar air flow hood to air dry. Moisture content was determined for this tissue over different time intervals using a titrator. At each time interval additional explants were plated out on a growing medium and evaluated for growth. Figure 1 represents a typical desiccation profile. After 72 hr of desiccation the explants were dead and the moisture content remained stable at approximately 18%. This trend shows that bur oak embryonic axes can withstand some desiccation before losing viability.

Although the moisture content (18%) was high, a low temperature protocol for long term storage was investigated. Temperature profiles were established to determine when the freezing damage occurred in the tissue. As the temperature decreased in the explant there was a point of entropy where energy was released as ice crystals formed (Burke et al, 1976). A programmable freezer, that allows a stepwise control of the freezing rate, was used to examine the point at which this occurred. A program was set up to control the rate of decrease in temperature using a hypodermic needle probe inserted into the explant. Temperature was recorded at 5 sec intervals with a data logger. The control explant low temperature profile shows an increase in temperature occurring at -8 C (~ 40% moisture content) (Fig. 2a). An anti-vitrifying compound can be used to reduce the temperature at which heat release occurs. These compounds inhibit intra- and inter-cellular ice formation. PVS2 has been used extensively in cryopreservation with a number of different species and tissue with high moisture contents (Volk and Walters 2005). One of the main components of PVS2 is DMSO (dimethyl sulfoxide) that acts as an antifreeze thereby slowing the rate of freezing of the unbound water molecules in the tissue. A low temperature profile of an explant treated with PVS2 is shown in Fig. 2b. The addition of PVS2 resulted in an increase in freezing tolerance, as freezing in the tissue occurred at approximately -27°C. Thus the anti-vitrifying agent altered the point of entropy as it occurred at a much lower temperature (-27°C compared to -8°C). These results show promise for storage of bur oak embryonic axes at -20°C. Further experimentation is required to determine the feasibility of this approach for low temperature storage of bur oak embryonic axes.

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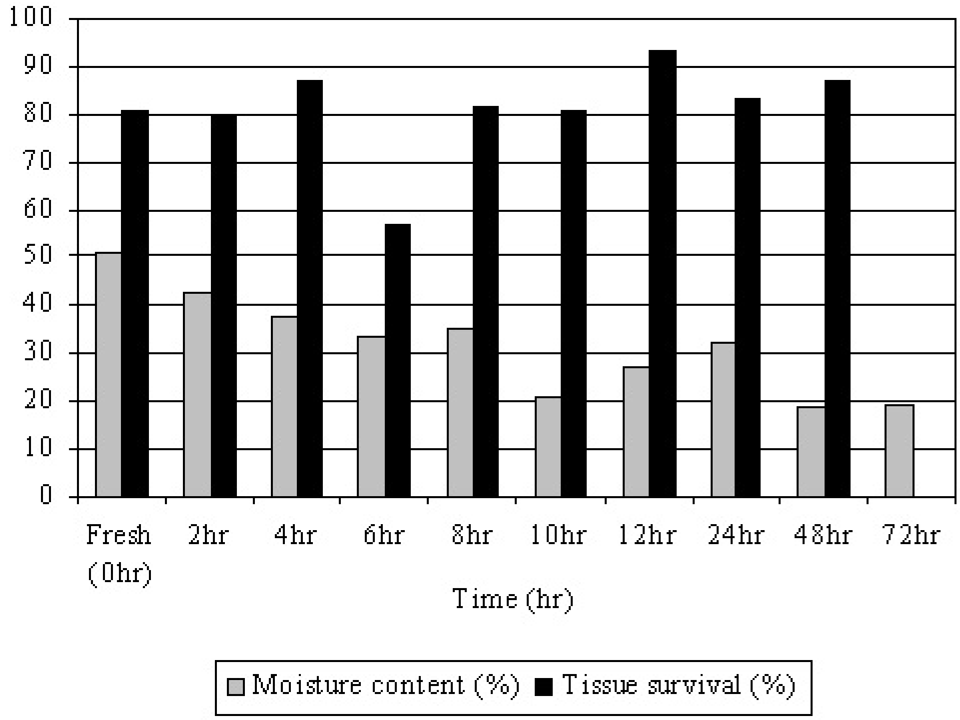

Figure 1. Bur Oak desiccation profile of explant moisture content and survival.

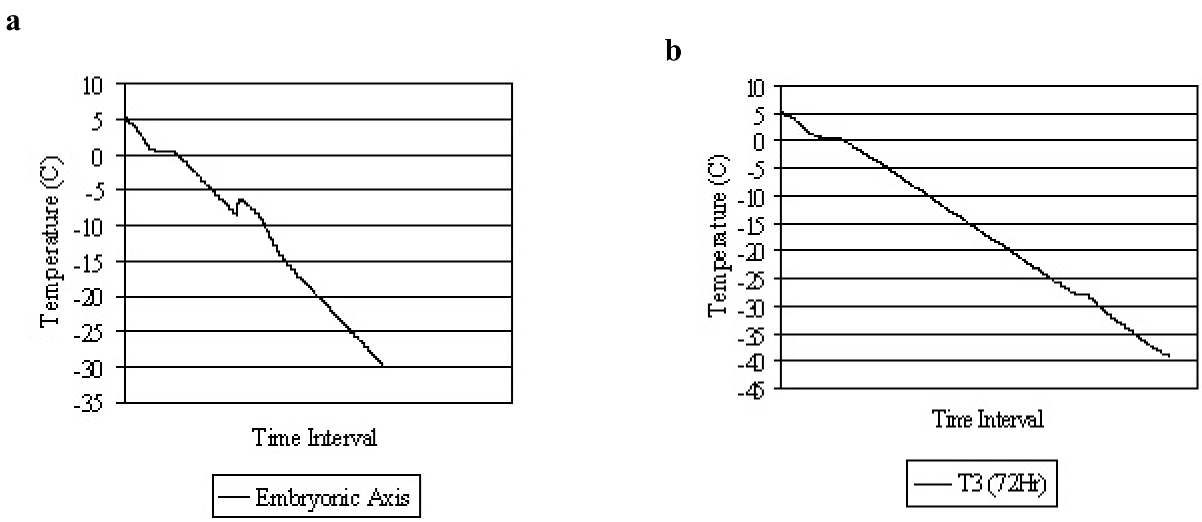

Figure 2. Bur Oak low temperature profile. a) Control, b) Treated with PVS2.

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RED SPRUCE: TO STRATIFY OR NOT!

There are questions that from time to time pop up and we've had a chance to look at certain elements in a more inquisitive manner - Seed Stratification and Seed Upgrading.

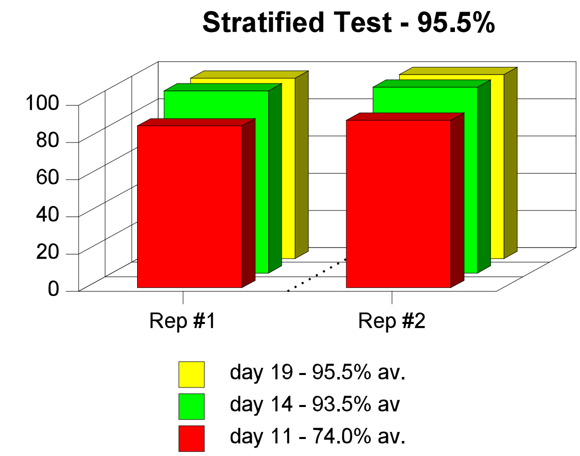
We all know the benefits of stratification but for some reason, we choose not to do it, because why?- it's seeding time and there is not time or historically it has not been done that way before!

An opportunity presented itself not so long ago, when the question of stratifying red spruce (*Picea rubens*) seed was raised. This is something that we feel needed to be shared. You'll see by the results charted below the difference stratification made and what it could mean to a nursery growing this crop.

This red spruce seedlot was received from the Atlantic Forest Seed Centre. The project was to establish how this seedlot could be enhanced. Simply described, it began with comparative testing; seed stratification for 21 days vs. seed that had not been stratified. Figures 1a and 1b highlight the real difference better than any words one could put to it, **"A picture is worth a thousand words".** While looking at the charts, look closely at the element of **"Vigour"** during the test, in relation to the overall germination capacity.

Following the initial germination testing, the seedlot was treated through the use of IDS protocols, which also included a Prevac treatment. Germination tests were conducted following stratification and the results are charted below for the individual fractions collected after the separation process (Fig. 2) and Table 1.

a



b

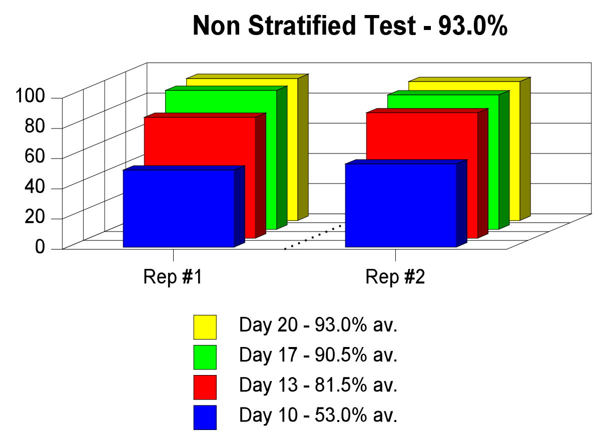
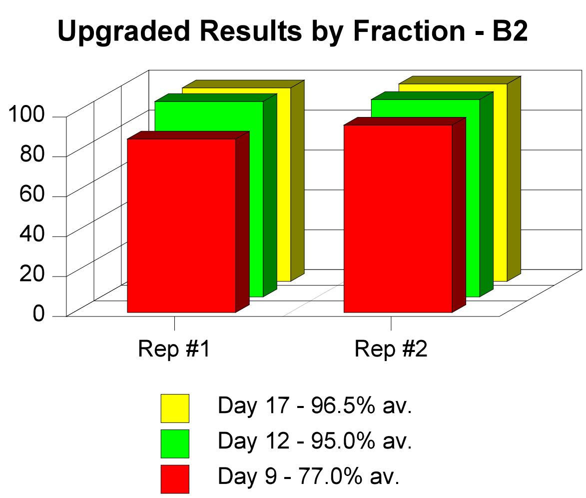
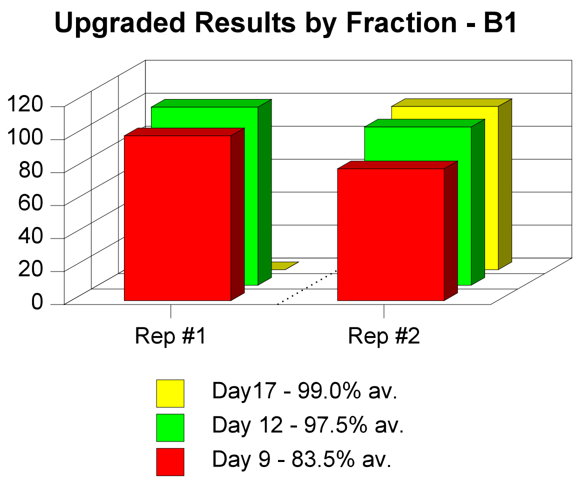


Figure 1. Initial germination tests on red spruce seed. a - stratified seed, b - non-stratified seed.

a



b



c

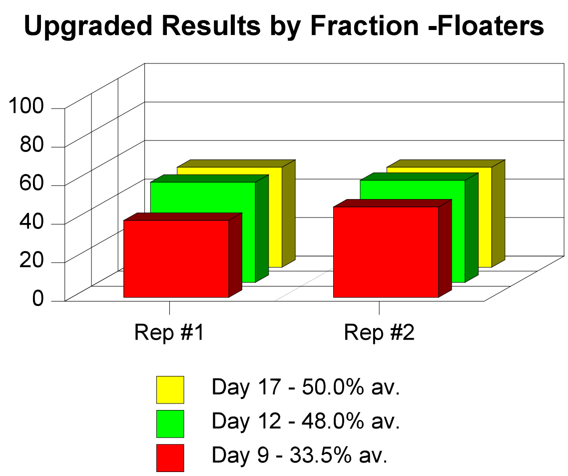


Figure 2. Germination of red spruce seed following IDS treatment. a - B1 fraction, b - B2 fraction, c - floaters.

Table 1. Upgrade summary by viable seed and weight

Fraction	Viable Seeds	Weight in grams
B1	265,886	833.449
B2	45,437	131.700
Floaters	4,614	24.282

Conjure up thoughts regarding simply stratifying your seed !! or alternately, seedlot upgrading !! Test it for yourself and observe the differences, these were ours.

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QUALITY OF RETURNED SEED

This article focuses on the quality of seed following the practice of stratified seed being dried back and stored for future use. This situation generally results from excess seed reaching the nursery. In British Columbia, seed that is withdrawn for growing seedlings on Crown land, but not used, must be returned to the Tree Seed Centre for evaluation and testing[1]. The returned seed program consists of an initial evaluation of seed quality and quantity. Requests that appear to have obvious seed deterioration, significant fungal contamination, debris contamination (other species of seeds, peat), where identity is in question or when the quantities do not justify further testing are discarded. Returned requests for the same seedlot, from the same nursery and stratified within a month of each other may be combined into common fractions to increase efficiencies of subsequent processing, testing, and registration. For portions with pregerminated and decorticated seed or minor fungal contamination the portion may be hand-cleaned, screened, and/or upgraded on the gravity table to remove these undesirable seeds.

As seed arrives in a variety of moisture conditions, the first step, after any required cleaning, is to dry the seed to storage moisture content. This is done at ambient conditions over several days with progress being monitored using non-destructive moisture meters. Once the seed is considered dry

[1] This is a requirement of the 'Chief Forester's Standards for Seed Use' for Crown land reforestation in British Columbia. http://www.for.bc/ca/code/cfstandards/pdf/CF_Seed_Standards.pdf

enough for long-term storage it is sampled for germination and moisture content testing. Once moisture content is confirmed to be less than 10% the seed is placed in our freezers at -18°C. An initial germination test is performed on the seed and an additional test is performed six months later. All testing is performed using the standard stratification regime for each species. Even if the original seed quality was maintained the seed is not re-mixed with the parent seedlot due to concerns with pathogens as well as the reduced vitality of the seed (even if it doesn't show up in germination tests).

This review looks at the results that we have had at the BC Tree Seed Centre with returned seed requests from the 2002 to the 2006 sowing season for lodgepole pine (*Pinus contorta*), interior spruce (*Picea glauca / engelmannii* complex*)* and Douglas-fir (*Pseudotsuga menziesii*[2]). The results are intended to illustrate that the practice allows for the re-use of previously stratified seed. This is not a static program and we continue to evaluate the best practices to increase seed-use efficiency.

The success of this activity is highly dependant on how the seed is treated at the nursery following sowing and how quickly it is returned to freezer storage. Optimal treatment includes drying the seed back to below 10% moisture content (once the nursery is confident that they have achieved expected germination), placing the seed under cool conditions, and shipping the seed to the Tree Seed Centre as soon as possible.

The results of our germination testing of returned seed are presented in Table 1. The average germination results on returned seed for all three species is quite high and exceeds the current result for Douglas-fir and many seed portions within the other two species. The average time since the last germination test was 2.2 years for lodgepole pine and 1.7 years for the other species. For all three species, the majority of seed was returned to the Tree Seed Centre within two to three months after shipping to the nurseries, although some seed was returned after up to eight months later without appreciable decline in quality.

Table 1. Germination capacity (GC) results for returned seed in comparison to available current lab tests and portion retesting after 6 months storage.

Species	No. portions	Current GC (%)	Returned seed GC (%)	Returned seed 6-month GC (%)	No. portions (6-month retest)
Pli[3]	275	94.8	94.9	95.1	219
Fd	123	92.4	93.0	93.0	79
Sx	103	93.5	92.4	92.4	72

[3] Pli = *Pinus contorta*, Fd = *Pseudotsuga menziesii*, Sx = *Picea glauca / engelmannii* complex

Our practice has been to retest returned seed after six months of freezer storage. Again, there was very little difference between these results and the original seedlot germination and initial testing of the returned seed. The sample size {No. portions (6-month retest)} was smaller for the 6-month retest as some portions were used for sowing before a second test could be completed.

The results indicate that the re-use of stratified seed of lodgepole pine, Douglas-fir, and interior spruce is possible. This program extends to other species, but there is no attempt to save *Abies* spp. requests due to the long stratification periods required, concern with resin vesicle damage, and inherent variability in germination exhibited by these species.

This is an operational program designed to maximize seed-use efficiency. The returned seed portions are registered as new seedlots for subsequent sowing. The preference is for nurseries or clients to reduce seed quantities ahead of stratification, so there is no returned seed, but not everyone chooses to participate in this activity. In 2007, approximately 6.5 million seedlings were produced from returned seed.

Although many of the comparisons indicate an increase in germination, these increases are quite small and not considered operationally significant. Sampling variability, seed upgrading at the nursery, and subsequent removal of germinated, decorticated or fungal contaminated seed at the Tree Seed Centre are the most reasonable explanations for these modest germination gains.

[2] Coastal and interior varieties are grouped together as Douglas-fir.

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CANADIAN FOREST GENETIC RESOURCES INFORMATION SYSTEM

The Canadian Forest Service (CFS) in collaboration with partners from the provinces, non-governmental organizations, and academia are developing a knowledge management system called CAFGRIS, (Canadian Forest Genetic Resources Information System) which will provide information concerning forest genetic resources. At this point in time a prototype was developed that addresses native tree species and threats to these species.

The purpose of CAFGRIS is to gather, integrate, and synthesize digital information, thereby generating new knowledge concerning native tree species and threats to these species. CAFGRIS is deployed through the National Forest Information System, which is a Canadian Council of Forest Ministers initiative. The prototype includes both spatial and non-spatial information. CAFGRIS, adheres to the Canadian Geospatial Data Infrastructure standards and principles, and international standards such as Open GeoSpatial Consortium. In doing so this has allowed us to access various data sources, which we would otherwise not be able to access, including US geospatial data (e.g., distribution maps). In addition, through the use of CFSNet infrastructure, we are able to ensure that the information is accessible, current, and authoritative.

Currently, the prototype contains information regarding the biology and ecology of native tree species and the threats to these tree species where applicable. Each tree species has a designation that was generated through a survey conducted by the CFS in 2003 to assess the conservation requirements of native tree species. Also official federal designations through the Species at Risk Act and provincial or territorial designations are included with supporting documentation. Information pertaining to *in situ* conservation is included, specifically the identification of protected areas and information pertaining to *ex situ* conservation is available on a per species basis. At this point in time we have access to the CFS National Tree Seed Centre data and we hope to be able to expand this to include data from other provincial *ex situ* collections.

CAFGRIS has a mapping component, where information can be displayed for single or multiple tree species. On the map symbols which depict the location where seed has been collected and stored by the CFS National Tree SeedCentre are geospatially displayed on this mapping component. When you click your mouse over the symbol, the seed collection site, you can obtain information concerning the seed collected at that site including whether bulk or single tree collections were made, age, and germination of the seed. There are a number of overlay options, which can be displayed on the map including protected areas, current burn areas, and ecozones. These are shown on the upper right side of the mapping page.

For each species there is text information available. For example, for butternut (*Juglans cinerea*), *ex situ* conservation is recommended based on the results of the 2003 survey. This species has an official federal designation of endangered and the COSEWIC (Committee on the Status of Endangered Wildlife in Canada) status report is available as a PDF file. Also in Ontario butternut is designated as endangered. The reasons for butternut being a species of concern, that is threats to this species, are listed followed by photographs.

CAFGRIS will be expanded in 2007–08 to develop the application necessary for accessing sources of information over the Internet, that pertain to: 1) assessment of the current status of native tree species by ecozones, 2) predict what the future status may be under a variety of climate change scenarios (using the climate change model Seedware), 3) assessment of the current conservation status of these species (including *ex*, *in* and inter *situ* conservation efforts), and 4) predict the future conservation requirements. In addition, the project will identify: 1) data deficiencies and uncertainties in the species' status and threats, 2) stimulate cooperative research efforts to obtain the information necessary to evaluate species status and threats, and 3) foster voluntary efforts to conserve the species before official species listing is warranted.

To access CAFGRIS: https://cfsnet.nfis.org/cafgris/index.html .

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CAREER OPPORTUNITY AT BC's PROVINCIAL TREE SEED CENTRE

In late August or early September, we will be recruiting a full-time Testing Technician at the BC Ministry of Forests and Range Tree Seed Centre. As one of two technicians reporting to the Testing Supervisor, this position conducts seed, cone, seedlot, and request assessment and testing throughout various TSC activities including cone and seed processing, registration, interim and long-term storage, preparation for use and distribution. The job description is currently being rewritten and the position's classification will be reviewed prior to posting. We are looking for an individual with the following qualifications: relevant past education and experience; strong teamwork, results focus and client service skills; and leadership potential. If you're interested in this opportunity or know someone who is, I'd be pleased to hear from you.

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UPCOMING MEETINGS

Seed Ecology II
Sep 9–13, 2007 Perth, Western Australia
www.seedecology2007.com.au

IUFRO Larix Symposium
Sep 16–21, 2007 Québec City, QC
http://www.mrn.gouv.qc.ca/carrefour/larix.asp

Canadian Poplar Council
Sep 16–21, 2007 Québec City, QC
http://www.mrn.gouv.qc.ca/carrefour/populus.asp

Carrefour de la recherche forestière
Sep 19–20, 2007 Québec City, QC
http://www.mrn.gouv.qc.ca/carrefour/

Forest Nursery Association of BC and the Western Forest & Conservation Nursery Assoc.
Sep 17–19, 2007 Sidney, BC
Contact: Evert Van Eerden ev.newgen@shaw.ca

Translational Seed Biology: From Model Systems to Crop Improvement
Sep 17–20, 2007 U. of California, Davis
http://www.plantsciences.ucdavis.edu/seedsymposium2007/index.htm

Canadian Tree Improvement Association
Aug 25–28, 2008 Québec City, QC
Contact: J. Beaulieu Jean.Beaulieu2@nrcan.gc.ca

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