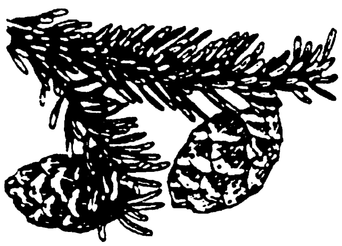
CANADIAN FOREST GENETICS ASSOCIATION
ASSOCIATION CANADIENNE DE GÉNÉTIQUE FORESTIÈRE

Tree Seed Working Group



NEWS BULLETIN

No. 58 December 2013

FROM SEED COLLECTING TO SEED STORAGE

THIS ISSUE AT A GLANCE

CHAIR'S 'ARMCHAIR' REPORT

Season's Greetings – wishing everyone the best over the holiday season. Iva and I will be spending Christmas Day aboard an airplane in preparation for some battery recharging time in Southeast Asia. I was fortunate to recently attend and talk at the Quebec Tree Seed workshop ("Les Semences Forestiéres; Un Maillon Clé des Plantations de Grande Valeur") on December 3rd and 4th in Berthierville, Quebec. It was attended by 150 participants! Presentations will be available for viewing on-line in the near future but I thought that I would touch on a few topics/ ideas/learnings from my personal perspective.

It was of great value to see how a different facility approaches common 'processing' activities with a different spin even when we both have the same equipment available. There are just so many unique value-added adjustments that the written word cannot capture effectively and seeing, touching, and having a dialogue are critical elements for 'real' information exchange in this highly specialized field. This observation lead to some discussion on the potential to have a dedicated forum or meeting focused on exchanging information related to 'processing' methodologies and learning from each other's experiences as this level of detail rarely gets published. It's like a full circle. When I took over chairing the TSWG in 2000 one of my first tasks was disbanding the Cone and Seed Insect (CSIWP) and the Tree Seed Processing and Testing (TSPTWP) working parties as they were not active and seemed to be subjects that could be easily covered under our TSWG umbrella. I'd be interested in the opinions of others on whether such a dedicated session would be valuable?, how often it should occur (realistically – lack of consistent organizational support across the country was really the downfall of the TSPTWP), and finally what your information exchange priorities are? I expect they will vary by facility. In the meantime resurrecting the listserver or exchanging ideas in a small distribution list may get things started.

I'm converted to accepting the benefits of water activity as a standard measure for determining seed moisture status to ensure seed longevity is maximized. I'm also excited by the incorporation of water activity (or equilibrium RH%) measures to guide cone and seed drying practices. We are currently testing hundreds of our small genetic conservation samples for freezer storage using water activity. The technology is also becoming more readily available at much lower costs with some units under $500. I'm not quite ready to give up our destructive tests as the seedlot specific moisture content result allows us to calculate the dry weight of our seeds and target specific moisture contents for stratification based on the weight of a sowing request. Maybe water activity could be useful there also, but it would require a lot of effort to get us to where we are right now with the moisture content targeting methods we use in BC for our deeply dormant species. There are lots of other water activity areas to pursue first.

Before the start of our "stratification season" this past fall I performed a couple of germination reviews for our two most valuable species with 'deep' seed dormancy mechanisms. Links are provided below to these reviews for western white pine (*Pinus monticola*) – (http://www.for.gov.bc.ca/hti/treeseedcentre/tsc/files/PW_Germination_Review_2013.pdf) and yellow cypress (*Callitropsis nootkatensis*) (http://www.for.gov.bc.ca/hti/treeseedcentre/tsc/files/YC_Germination_Review_2013.pdf). The results for western white pine indicated that nurseries performing hydrogen peroxide treatments experienced much lower germination falldowns. This was interesting and prompted me to look deeper into this sanitation practice and the abundant literature indicating its use as a germination enhancement tool across a wide variety of species.

The theme for this Newsbulletin is "From Seed Collecting to Seed Storage" and I appreciate the contributions submitted on the topic. In our hot dry Okanagan Valley in which many of our interior seed orchards are located there have been poor seed yields this past year. The extremely hot and dry weather during July and August are suspected as the primary factors. Is this an extreme year or are we seeing a trend we will not be able to easily battle – it's a question that causes some to lose some sleep. At the Tree Seed Centre we are specifically interested in the post-collection handling conditions provided to cones – at our facility, but also prior to that at the seed orchards or sites where natural stand cone collections are stored. This has been shown to be an important step in the production of high quality seedlots and we hope to put on a workshop at our facility next July to review important considerations and hopefully institute some common monitoring practices. It is still very much in the planning stage, but I was quite happy with the use of our cone processing area for the last TSWG workshop. There is also a BC Seed Orchard Association (BCSOA) meeting June 18 to 21 in Salmon Arm, BC which is also still in the planning phase.

I'd also like to pay tribute and offer condolences to the family of Joe Toth who passed away this past October at the young age of 42. Joe was the machine operator at Prince George Tree Improvement Station and contributed his skills in design and fabrication to improve the efficiency of that facility. He was the 'glue guy' in more ways than one and will be dearly missed by his colleagues, friends and family.

Dave Kolotelo
TSWG Chair

EDITOR'S NOTES

It has been quite some time since we have had a theme issue of the News Bulletin. Several of the articles relate to the theme. Michele Fullarton talks about clonal variation in cone and seed yields while Mary Myers writes about some of the techniques employed for collecting and storing shrub and hardwood seed. Fabienne Colas and Michèle Bettez provide an excellent overview of seed handling practices in Quebec. Gary Giampa has an interesting article about a means of evaluating cone ripeness which can make collecting more efficient. Lindsay Robb presents interesting results from a germination trial of limber pine seed. Barb Boysen's review of seed management issues and programs in southcentral Ontario is informative. Dave Kolotelo writes about the positive impacts of hydrogen peroxide on seed germination. Dale Simpson's article supports his previous ones about the use of ethanol to upgrade birch seedlots.

I hope that each one of you had a Merry Christmas and were able to take some time to reflect on the meaning of the season and to recharge the batteries. I wish you all best wishes for a prosperous New Year!

Dale Simpson
Editor

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

All issues of the News Bulletin are available at: http://www.for.gov.bc.ca/hti/treeseedcentre/tsc/tswg.htm

CLONAL COLLECTION OF SECOND-GENERATION BLACK SPRUCE CONES

In 2012, the second-generation black spruce (*Picea mariana*) seed orchards at DNR's Wheelers Cove location had an excellent cone crop due to $GA_{4/7}$ application in 2011. These two orchards were established in 1996 and 1997 and the total area is 3.6 ha, with a total of 2,191 grafts after rogueing. Since we had a sufficient seed inventory of second-generation black spruce, we decided to collect the cones by clone. The plan is to build up the seed inventory and to allow orchard mixes to be made by using equal quantities of seed per clone to maximize the genetic quality and diversity of the seedlings used for reforestation. Currently, the orchard bulk collections contain a mix of all the clones in unknown proportions. We focused on the best clones in the orchard using 20-year breeding values (BV) for volume from progeny tests. It was our first attempt at collecting this way. Maps were generated using EXCEL, which highlighted the location of all the ramets of one clone per map. All of the ramets in the orchard are tagged (Fig.1), so the cone-pickers were able to easily find the trees.

Figure 1. Clone identification tag

The cone pickers worked through the orchard in groups working on one clone per group. If there were only a few cones on the tree, it was skipped. The pickers used fourteen and sixteen foot tripod ladders to do the collections. As each burlap bag was filled (24 L/bag) it was carefully labeled and all cones for a particular clone were grouped together, hung in cone sheds, extracted, and the seed tested and stored by clone.

The results illustrate the large amount of variation experienced for all the characteristics evaluated (Table1). The quantity of cones collected per clone ranged from 154 to 552 L with an average of 329 L. Seed production ranged from a low of .38 kg to close to ten times this amount at 2.95 kg. These trees were topped during the collection and had been topped lightly in the past (1 or 1½ years removed) during previous collections. High cone production by a clone did not necessarily translate into high seed production. For example clone 618 produced the most cones but had the lowest seed set and the smallest seed. The seed production efficiency of clone 559, however, was more than 12 times that of clone 618 (Fig.2). Seed germination was very high, with germination exceeding 95% for 21 of the 24 clones.

Table 1. Cone and seed data by clone.

Clone	Cone volume (L)	Seed quantity (kg)	Wt. 1000 seed (g)	No. seeds/g	Germination (%)
506	387	2.24	1.52	658	96.00
513	280	2.03	1.48	676	96.00
514	334	1.87	1.22	820	97.50
527	186	1.68	1.36	735	96.25
531	312	2.46	1.34	746	98.75
534	156	1.21	1.80	556	92.75
536	350	2.83	1.42	704	93.75
544	348	1.06	1.50	667	98.75
546	404	1.72	1.30	769	98.25
555	414	2.20	1.68	595	93.00
559	182	2.03	1.76	568	98.25
560	408	1.75	1.30	769	95.25
561	534	0.46	1.12	893	95.25
564	240	1.09	1.22	820	98.00
582	452	0.78	1.14	877	98.50
610	344	2.21	1.28	781	98.25
611	360	1.45	1.32	758	97.75
618	552	0.38	1.04	962	96.00
626	288	2.00	1.34	746	98.75
636	315	2.95	1.68	595	99.75
638	154	1.14	1.60	625	96.50
639	334	1.36	1.46	685	95.75
647	208	1.96	1.38	725	98.00
656	356	2.10	1.36	735	96.75

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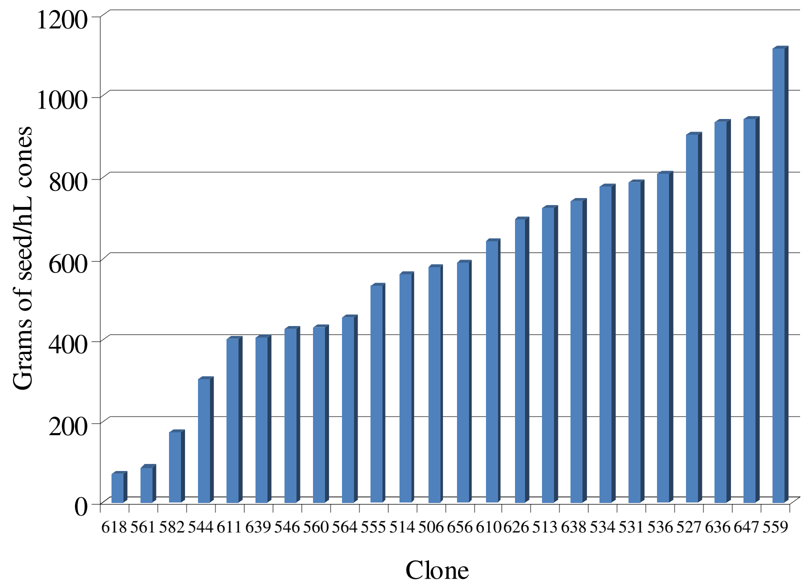

Figure 2. Seed production efficiency of 24 clones in an 18-year-old second-generation black spruce seed orchard.

The clonal contribution in a bulk mix of orchard seed could result in a large imbalance in the diversity and genetic gain (Fig. 3).

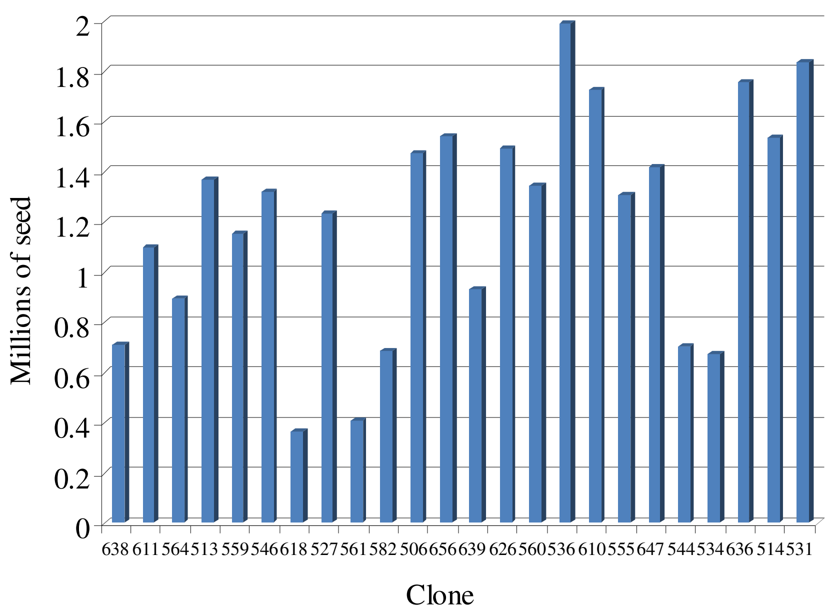

Figure 3. Seed production by clone sorted by breeding value (volume at 20 years) in ascending order.

Controlling the mix provides an opportunity to attain the highest gain possible by simply balancing the proportion of seed from the clones. Figure 3 illustrates that some of the higher breeding value clones aren't necessarily producing the most seed. It will be interesting to see how the results will vary from year to year at a clonal level. In conclusion, the seed mix when harvesting cones in bulk from an orchard collection will be uneven, depending on the clonal contribution. Collecting by clone gives you the ability to increase the gain by simply changing the proportions of seed in the mix.

We will try collecting by clone again, but make some changes in our procedure. We found that the pickers spent a lot of time moving ladders, taking time from the actual collections. In the future, we will collect cones from all trees in each row leaving a bag at each tree to be collected and sorted by Tree Improvement staff. In this way, maps will not be needed. It should be a more cost-effective way to collect cones. The main reason for the increase in cost was the time spent moving ladders around and looking for the next tree to collect from.

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SEED HANDLING CHALLENGES ON PRINCE EDWARD ISLAND

The Prince Edward Island provincial tree nursery has grown conifer seedlings for reforestation since it's establishment in the 1970s up until the early 1990s. At that time, a hardwood planting program was added to increase species diversity to the plantation landscape. With the increased activity of watershed improvement groups in the early 2000s, there has also been a demand for native shrubs to improve the quality of riparian zones, in order to protect streams and provide food for wildlife. The number of species grown at the nursery in 2013 has now reached a total of 45 (15 conifer, 13 hardwood, and 17 native shrubs). This variety of species has created a challenge for the nursery in seed collection, handling, and germination.

Conifer seed is collected mainly from first-generation seed orchards with a few smaller collections made from wild stands. Seed extraction is carried out at a facility in New Brunswick where the seed is cleaned and tested for germination. It is stored at the nursery at -6°C. Collection of deciduous tree seed is more challenging. As with the conifers, seed collection is timed to the optimum growing degree days for each species. Collection areas for hardwood seeds are spread across the Island and were selected based on parent tree quality and accessibility. Picking the seed is accomplished using a 3-ton truck with an aerial lift which requires reasonably good access. Seed is picked by hand and after collection, the hardwood seed is spread on drying trays.

Collection of native shrub fruit has been made easier over the years by the establishment of shrub beds at the nursery. With the shrubs easily accessible, we are able to access the fruit at optimum ripeness with the biggest challenge being competition from birds. Processing the shrub seed follows several approaches. Seed within fleshy fruit is generally extracted by crushing the soft outer tissue and washing the seed through a sieve. Shrubs such as bayberry (*Myrica pensylvanica*) have a very waxy coating on the surface of the fruit which is removed by grinding it against a rough surface until the seed is exposed. In the case of staghorn sumac (*Rhus typhina*), after the seed is removed from the fruit clusters, the seed must go through several hot water soaks until the seed is doubled in size before it is ready for stratification.

Following collection and cleaning in the fall, all the hardwood and shrub seeds (with the exception of oak) are placed on moist peat moss in paperpot trays (Fig. 1) and depending on the requirements of the species may go through a warm period and/or a cold period. The best results for cold stratification have been achieved by putting the trays outside with the overwintering seedling crop which is stored under plastic for the winter. The paperpot trays are brought into a covered shelterhouse in the spring and as the seed begins to germinate, the germinants are transplanted into multiport trays.

Figure 1. Hardwood seed in moist peat moss.

Figure 2. Red oak acorns in storage bag.

Red oak (*Quercus rubra*) is the exception to this process. It is the provincial tree of PEI and is in high demand by private landowners and watershed groups. During a good seed year we will collect up to 450 kg of acorns. Handling and storage of this particular crop is unique from all the others due to the size of the seed which must be stored above freezing and is only viable until the spring after collection. The acorns are usually picked before they fall from the trees because ground collections tend to have a high infestation of acorn weevil (*Curculio* spp.). The green acorns, with caps still attached, are spread on drying trays to ripen and when they turn brown the caps and litter are removed. The acorns are then put into tubs of water with a 10% solution of bleach which allows the empties to float to the top and also serves to disinfect the surface of the acorns to prevent fungal growth. The acorns are put into 4.5 kg mesh bags which are then placed into poly bags with a short piece of hose fastened in the closure to allow gases to escape (Fig. 2). In February, the acorns are taken out of the cooler and the mesh bag is again immersed in the same bleach solution, air dried, and put back into the cooler. In early April, the acorns are removed from the cooler and those that are already showing signs of germination (acorn cracked with radicle emergence) are planted directly into multiport # 15 trays. The remainder are spread over the surface of moist peat moss in paperpot trays, covered with white plastic, and placed in a warm greenhouse. As these germinate over time they are picked out and transplanted.

Attempting to grow as many as 45 different species, each with a unique seed handling and growing regime, has been a challenge over the last 10 years. Many of the techniques we use have been developed and improved upon through trial and error. With this approach, we are open to growing new species to better meet the needs of our clients.

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FROM SEED COLLECTION TO SEED STORAGE: A QUEBEC OVERVIEW

In Quebec, the Ministère des Ressources naturelles (MRN) is the sole manager and provider of forest tree seeds. About 120 million seedlings are planted each year (97% softwoods), which corresponds to about 300 million seeds shipped to nurseries for seedling production.

Development of a seed orchard network began in the early 1980's. Now, a total of 89 seed orchards are managed for 12 reforestation species; mainly black spruce (*Picea mariana*) (28 orchards), white spruce (*P. glauca*) (24), and jack pine (*Pinus* *banksiana*) (16). The orchards are distributed in the different ecological zones (Fig. 1) and specific seed deployment zones were defined by the geneticists of the Direction de la recherche forestière (Research branch).

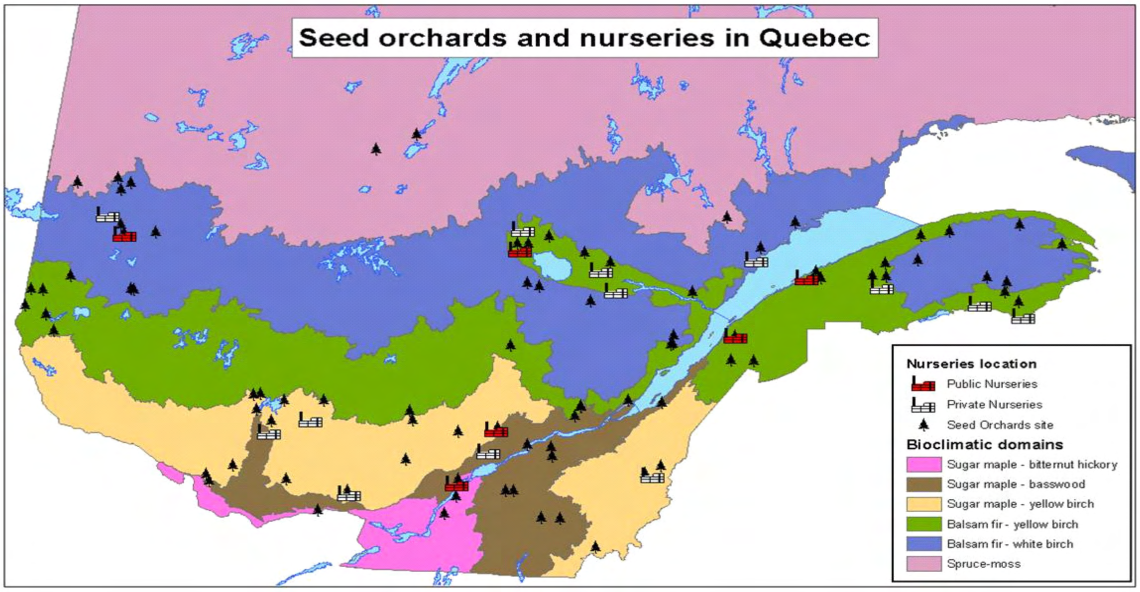

Figure 1. Distribution of seed orchards (all species) and nurseries in Quebec.

An indication of the levels of genetic gain for the various reforestation species was presented by Savary (2008). Gain ranges from 0 for natural sources to >16% for second-generation orchards.

The DGPSPF (Direction générale de production de semences et de plants forestiers) determined the species that can be used for reforestation. The list represents a total of 27 species (13 softwoods and 12 hardwoods) plus poplar and willow (Table 1).

Table 1. Species offered for reforestation program in 2014

Softwoods		Hardwoods	
White spruce	*Picea glauca*	Yellow birch	*Betula alleghaniensis*
Black spruce	*Picea mariana*	Paper birch	*Betula papyrifera*
Norway spruce	*Picea abies*	Black cherry	*Prunus serotina*
Red spruce	*Picea rubens*	Ash-leaved maple	*Acer negundo*
Eastern larch	*Larix laricina*	Red maple	*Acer rubra*
European larch	*Laric decidua*	Sugar maple	*Acer saccharum*
Japanese larch	*Larix keampferi*	White ash	*Fraxinus americana*
Hybrid larch	*Larix spp.*	Green ash	*Fraxinus pennsylvanica*
White pine	*Pinus strobus*	Siberean pea-tree	*Caragana arborescens*
Jack pine	*Pinus banksiana*	Bur oak	*Quercus macrocarpa*
Red pine	*Pinus resinosa*	Red oak	*Quercus rubra*
Balsam fir	*Abies balsamea*	Black walnut	*Juglans nigra*
Eastern white cedar	*Thuja occidentalis*	Poplar and willow	*Populus* spp. and *Salix* spp.

The data for the management of the sources and the seedlots are compiled in the SEMENCES system, an Oracle database. This system assures the traceability of the lot from collection to production to distribution from the nursery.

Extraction and storage of the seedlots are done at MRN's Berthier Tree Seed Centre (BTSC). Information on annual harvest needs and seed treatment are in Table 2.

Table 2. Average annual harvest and treatment for softwoods and hardwoods at the Berthier Tree Seed Centre

	Softwoods	Hardwoods
Average annual harvest needs (hL)	1 600	280
Average annual treatment volume (hL)	1 390	350
Average extracted seeds (millions)	305	5

Seed Collection

Seed and cone collection are planned to fulfill the needs of the reforestation program. During winter, branches from softwood orchards are forced to estimate cone production. Although not precise, it gives a good indication and helps to plan the harvest. The harvest is primarly done in seed orchards, on standing trees. Safety rules and procedures are applied to avoid accidents. Use of personal protective equipment is required. A guide outlining safe seed collection methods was produced (Assap and MRN 2008; in French only). After collection, cones are placed in square, stacking plastic racks (dimensions: 100 cm x 80 cm x 15 cm). Each lot (cone or seeds) is inspected upon arrival at the BTSC. Criteria include lot quality (cones without rocks or needles) and the correct identification of the lot (number, origin, etc). If quality is not good or the information incomplete, a non-conformity certificate is issued. This is a new procedure which was introduced with the ISO certification. See Bettez and Colas (2012) for more information on ISO certification.

With the exception of white pine, cones are kept in an unheated warehouse until extraction. White pine cones are stored in the BTSC at room temperature. This allows the cones to dry rapidly and to facilitate seed extraction.

Seed Extraction and Testing

Procedures for seed extraction vary from species to species such as kiln temperature and duration, de-winging duration, etc. However, the different steps employed during extraction are similar for the softwoods (Fig. 2). For hardwoods, each species has its own prescription. Please contact Michèle Bettez for further information.

For each step, targets were established for ISO certification. If the data obtained do not meet the target, a non- conformity certificate is issued and the treatment is adjusted. For example, if water activity of the newly extracted seedlot is higher than 0.4, it must be reduced to be between 0.3 and 0.4.

Since 2002, extensive work has been done to improve softwood seed germination. The BTSC adopted the *gentle* method which reduces seed damage during extraction which has resulted in a substantial improvement in germination (Table 3). For details on the quality tests done at the BTSC, see Brault et al (2009).

Table 3. Seed germination(%) improvement since 2002

Species	2002	2012
White spruce	78	96
Black spruce	95	98
Eastern white pine	81	96
Jack pine	88	94
Average	85	96

Seedlot Storage

BTSC has 3 cold rooms for seed storage. Orthodox seeds are kept at – 3°C and recalcitrant seeds at + 3°C (Table 4). Water activity was introduced as a new quality test in 2008 for orthodox species and is also used to monitor final seed drying.

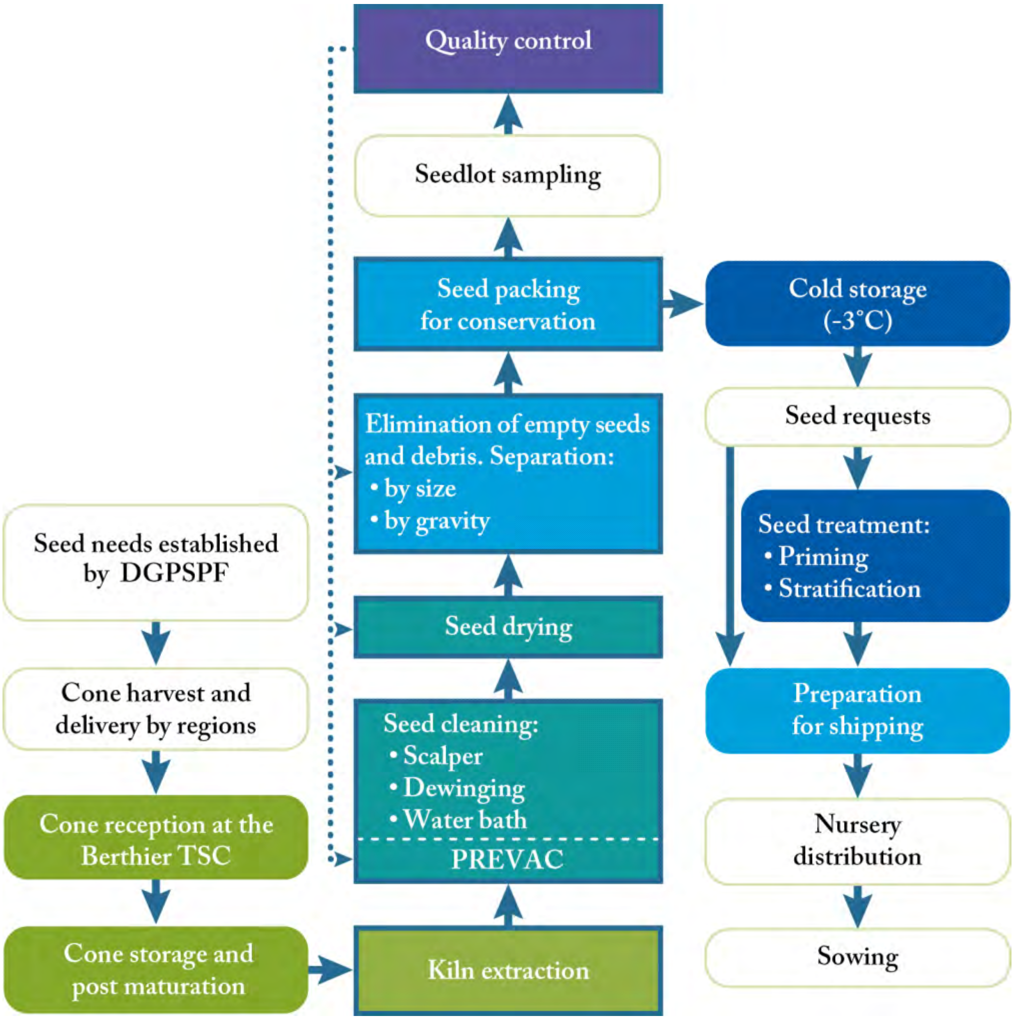

Figure 2: Seed treatment process at the Berthier Tree Seed Centre.

Table 4. Storage conditions for seed of the different types of species at the Berthier Tree Seed Centre

	Orthodox softwoods and hardwoods	Recalcitrant hardwoods
Temperature	- 3 °C	+3 °C
Container	20 litre rigid polyethylene	Bag or polyethylene bucket
Moisture content	< 10 %	> 35 %
Water activity	0.33 to 0.38	n/a
Duration	Softwoods > 10 years Hardwoods 2 to 10 years	< 6 months

The seed bank inventory is quite important (Table 5). Recently, obsolete seedlots (poor genetic quality, poor germination) were eliminated. This allowed for the closure of a cold room thus making important energy savings.

Table 5. Summary of the seed bank inventory

	Softwoods	Hardwoods
Sources/class	418	73
Seedlots	917	128
Total weight (kg)	9 700	920
Mean germination (%)	91	73
Quantity (viable seeds, millions)	3 900	24
Improved seeds (%)	44	

Advantages of ISO Certification

The BTSC is certified ISO 9001:2008 since 2011. A lot of work is necessary to maintain this certification, but it has many advantages, and not only for the BTSC.

For the BTSC

1. Introduction of better practices:
   a. The staff produce reports when the product is off-target → cause analysis → methods adjustment.
   b. Reduction of product non-conformity.
   c. Better control of the process → better quality thanks to the participation and mobilization of the staff.
2. Having validated, recorded and accessible processes ensures traceability information for the collective memory of the organization.

For the Clients (Nurseries)

1. Insurance that they receive a quality product that is part of a continuous improvement process.
2. Thanks to the annual survey on the quality of services received, clients become part of the continuous improvement process

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USING THE "AXIS TEST" TO DETERMINE CONE RIPENESS

The Province of British Columbia operates two conifer seed orchard facilities near Vernon, BC. The local climate is generally very hot and dry in the summer months when cones are developing. Cones mature early and can fairly quickly progress from an unripe condition to reflexed and shedding seed. Individual trees within each orchard exhibit a wide range of ripening behaviour. Equipment and labor logistics combined with a very short harvest season force us to concentrate our efforts on trees that are closest to seed shed. It is also worth noting that cones picked too early can have seed quality issues. Cones need to be collected at just the right time. Since we have thousands of trees which must be prioritized for collection, an efficient method of assessing cone ripeness is essential to ensure good seed quality and minimize losses.

The definitive test for assessing seed maturity – the seed cutting test where embryo length and megagametophyte condition are examined – is too finicky and time consuming to be practical in an operational field situation. Cone flotation tests and cone moisture content readings are other possible methods of judging cone ripeness, but, are also too clumsy to use in the orchard. The outside appearance and/or "feel" of a cone are not dependable indicators of ripeness. What is required is a quick, reliable method to judge cone maturity in the field.

The method we have developed for determining the ripeness of western larch (*Larix occidentalis*), interior spruce (*Picea glauca* x *engelmannii*) and Douglas-fir (*Pseudotsuga menziesii*) cones involves examining the condition of the cone axis. The axis can be quickly exposed by cutting the cone in half with a pair of pruners. If the axis appears brown and dried out then the cone is ready to be picked. A desiccated axis indicates that the tree is no longer "supporting" the cone and therefore the cone is getting ready to shed its' seed. If the axis still contains moisture (run the tip of the pruner blade down the axis and look for droplets) then there is little danger of the cone opening anytime soon. The tree is still supplying this cone with moisture and nutrients. Unfortunately this method does not work so well on pine species. Pine cones are too tough to safely cut in half with a pair of pruners.

The axis field test is reliable, easy to teach, and requires no special equipment. Workers can move quickly through the orchard making accurate judgement calls on cone readiness and setting harvesting priorities. I hope that you find this method useful.

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GERMINATION TESTING LIMBER PINE (*Pinus flexilis*)

Introduction

The germination method previously used for limber pine (*Pinus flexilis*) at the Alberta Tree Improvement & Seed Centre (ATISC) was based on methods obtained from other jurisdictions and had never been tested for suitability on Alberta seed. The Alberta Seed Testing Standards currently list the test as: pre-chill at 2–5°C for 21 days / unstratified and stratified tests / germinated at 25°C for 21 days. The full method also specifies that seed receive a 48 hr running or aerated water soak before being naked stratified with rinsing every 7 days in an attempt to reduce fungal growth. Using this method, the average germination of limber pine at ATISC in the past was 62%. Given the results of recent fresh seed cut tests, this lower than expected result is likely to be due in part to lower quality collections but could also be a result of less than ideal germination methods.

Limber pine is endangered in Alberta, mainly due to white pine blister rust (*Cronartium ribicola*), mountain pine beetle (*Dendroctonus ponderosae*), and limited natural regeneration. As climate change is expected to intensify the species decline, Alberta is initiating a limber pine conservation program with current efforts including the collection and storage of seed for resistance screening and outplanting. Limber pine seed has only been consistently collected and stored at ATISC within the last ten years and there is no information on seed longevity in storage. Therefore, finding the optimum germination method is important to managing the collections in the future and this research was designed in an attempt to find the simplest method for germination that produced the best result, by encompassing the most population genetic variability, for the propagation of limber pine seedlings.

Seedlots

Bulk seed collections from four provenances were used. Two collections were from the northern part of the limber pine range in Alberta and two from the southern part (Table 1).

Table 1. Collection and storage information on four limber pine provenances used in the trial

Accession no.	Collection location	Date collected	No. trees sampled
5423	Porcupine Hills	25-Aug-2003	30
6189	Windy Point	28-Aug-2009	41
6394	Panther	09-Sep-2010	58
6448	Sentinel Creek	13-Sep-2010	60

Method

Four cold stratification durations were tested: 28, 42, 56, and 70 days. For each period, germination tests consisting of two reps of 50 seeds each were set up in Petawawa Germination Boxes on moist Kimpak™ according to standard germination testing protocols and placed in 2°C. No soaking, rinsing or sterilisation was employed.

After the seeds were stratified accordingly, they were moved to a germination temperature of 25°C or 15°C. Standard tree seed germination protocols call for 25°C but since this is a high elevation species, a lower temperature was included in an attempt to more closely reflect soil temperatures *in situ*. Seeds were considered 'germinated' when the radicle was at least the same length as the seed (approximately 1 cm). Germinated seed were counted and removed every seven days. The tests were ended after 28 days and the remaining seeds were cut tested to determine the number of empty seeds. Abnormal germinants were included in the germination calculation.

To enable appropriate analysis and comparison, empty seeds were removed from the data. Germination rate (average days to germination) and total germination were calculated and analysed using an ANOVA on transformed data. All statistical analyses were performed using Genstat 12.0.

Fresh cut tests were also performed on a random 25-seed sample from each collection in an effort to distinguish seed quality issues from germination performance.

Results

There was a significant 2-way interaction between the accession and the stratification time used (Table 2). Three collections showed a significant increase in germination with lengthened stratification duration (Fig. 1), with an average increase of 15% between 28 and 70 days. However, the collection from Sentinel did not show any significant increase or decrease.

Table 2. Results for parametric 3-way ANOVA testing for differences in percent seed germination and interaction among groups for four limber pine accessions. df = degrees of freedom, eta = proportion of variance explained by the effect, ** indicates significance at the $p<0.05$ level

Source	df	eta
Accession	3	0.51**
Stratification Time	3	0.14**
Temperature	1	
Accession (Stratification)	9	0.16**
Accession (Temperature)	3	
Stratification (Temperature)	3	
Accession x Stratification x Temperature	9	

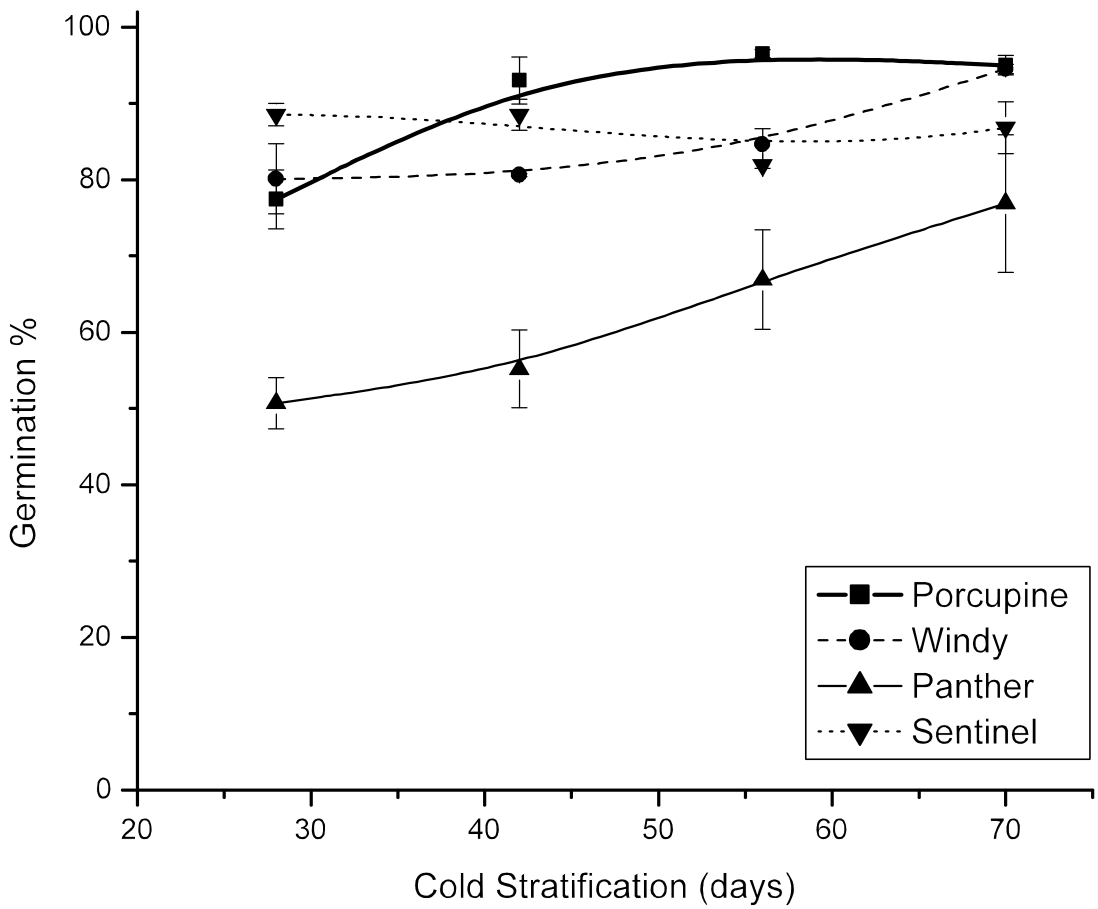

Figure 1. Total germination (± S.E.) of limber pine seed from four accessions for each of four stratification durations.

There was a 2-way interaction between stratification time and germination temperature on the germination rate (Table 3). All four collections germinated faster at 25°C than at 15°C; however, lengthening the stratification time significantly decreased the rate of germination for those seeds germinated at 15°C (Fig. 2) to rates comparable with 25°C. There was no significant effect of stratification time on rate for seeds germinated at 25°C.

The differences in maximum germination percentage achieved for each collection could not be linked to the age of the collection, i.e., this cannot be easily attributed to a loss in vigour. However, there was a distinct trend with the quality of the collection, as determined by a fresh 25-seed cut test (Table 4). As the overall maturity of the collection increased, the maximum germination achieved also increased. The differences in germination rate between accessions did not appear to follow any trend with age or seed quality.

Table 3. Results for parametric 3-way ANOVA testing for differences in seed germination rate and interaction among groups for four limber pine accessions. df = degrees of freedom, eta = proportion of variance explained by the effect, ** indicates significance at the $p<0.05$ level

Source	df	eta
Accession	3	0.37**
Stratification	3	0.07**
Temperature	1	0.16**
Accession (Stratification)	9	
Accession (Temperature)	3	
Stratification (Temperature)	3	0.11**
Accession x Stratification x Temperature	9	

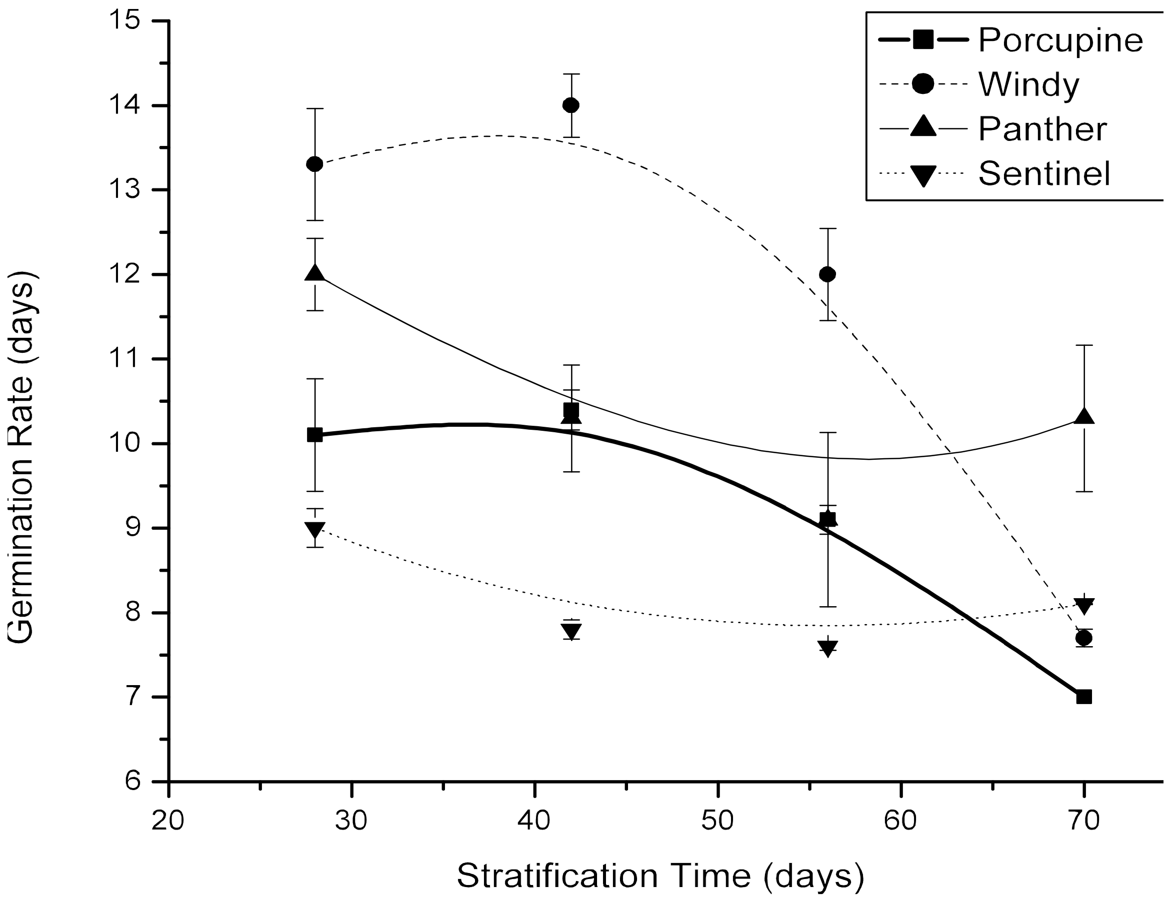

Figure 2. Germination rate (± S.E.) at 15°C of limber pine seed from four accessions for each of four stratification durations.

Table 4. Maximum germination, minimum germination rate, and seed quality for four limber pine provenances

Accession	Maximum germination (%)	Minimum germination rate (days)	Seed with embryos <75% of embryo cavity (%)
Porcupine Hills	96.5	7.29	0
Windy Point	94.5	9.49	14.3
Sentinel Creek	86.8	7.74	31.8
Panther	76.9	9.65	50

Conclusions

Increasing the duration of stratification did not affect the rate of germination but did increase the total germination in three of the four accessions by an average of 15%. Seed germination of the fourth accession (Panther) was not improved by longer stratification but was also not decreased. Therefore, changing the stratification time to 70 days in the Alberta Seed Testing Standards could mean an average 15% increase in total germination.

It should be noted that the current Alberta Seed Testing Standards method was also tested; however, the germination results were so low (5.3–28%) that these tests were not included in the results and it was assumed that an unknown anomaly affected the tests. It is also possible that the type of naked stratification used in the current method is not easily consistently reliable at keeping small numbers of these large seeds fully imbibed during stratification and this may have caused the low results. The Alberta Tree Improvement & Seed Centre will continue to stratify all species by keeping them in direct contact with a moistened growing medium (e.g., Kimpak™, sand) unless this proves impractical. This practice ensures full imbibition of seeds such as limber pine, which can take more than 48 hrs in water to fully imbibe (personal experience). It also provides the seeds with the free water necessary during imbibition and stratification for some DNA, membrane, and protein repair to take place, which could otherwise result in a loss of vigour.

Given the difficulty and expense of limber pine seed collection, there is a strong argument for the collection of high quality seed and the use of longer stratification times to produce 'higher value' seedlings that encompass a greater proportion of a given population by including both non-dormant and highly dormant seeds. Therefore, the next update of the Alberta Seed Testing Standards will include the new 70-day cold stratification treatment for the testing of limber pine seeds.

Update

Since the completion of this germination trial in 2012, the new testing method has been used successfully in other limber pine research conducted during 2012/13 regarding *in situ* and *ex situ* maturation of limber pine seeds with respect to germination and seed longevity. The results of this research were presented at an SER conference in Wisconsin in October 2013 and the work will be published in 2014. Currently, a whitebark pine (*Pinus albicaulis*) germination trial is underway and a similar maturation/longevity trial using whitebark is also being planned for fall 2014.

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MANAGING TREE SEED IN SOUTHCENTRAL ONTARIO – A DIVERSITY OF SPECIES AND PARTNERS

In southcentral Ontario, beginning in the late 1990s, many local, regional, and provincial groups have collaborated to increase private land tree planting efforts. And the stakes are high. Our southern forests are remnants of themselves after decades of development, invasive alien species, and climate change. The Forest Gene Conservation Association (FGCA), supported by the Ontario Ministry of Natural Resources (OMNR), has worked with reforestation partners to ensure basic genetic resource management principles are respected; that high quality, locally adapted seed of native species is used.

Ensuring that appropriate seed is continuously available facilitates the practice of these principles. It is a complex undertaking that the OMNR once handled in-house – from provincial planning through to local operations. After the MNR's exit from tree planting in the mid 1990's, many local partners continued to deliver small operational programs. But few to none had the expertise, resources or mandates to take on the planning needed to ensure supplies of appropriate seed. Therefore tree planting efforts largely sourced stock based on price and availability and not site, species or seed source.

Since 1998 the FGCA has made efforts to train seed collectors and promote adapted seed sources and native species within the ***Ontario's Natural Selections*** voluntary seed source certification program. But it wasn't until the OMNR provided funding for the long term 50 Million Tree Program (MTP) that things began to change and the delivery partner Trees Ontario (TO) is now going beyond expectations. In assuming the challenging planning role, they are helping the many other partners, from funders, to seed collectors, growers, local planting agents, and landowners to 'do the right thing'.

This planning role is being outlined in a comprehensive, living document, the **Trees Ontario Seed and Stock Management Plan** (SSMP - completion in March 2014). Its goal is to make sure that every tree planting stakeholder realizes that high quality, source identified seed and stock is the foundation for any successful afforestation program.

The SSMP focusses on the next five years, with references for long term capacity building (i.e., partnership development), succession planning (i.e., knowledge transfer), and job creation. The SSMP will be updated annually to reflect changes in stakeholder involvement, Trees Ontario governance, as well as the likely significant effects of climate change and invasive alien species which could lead to changes in species and seed sources.

Each element within the SSMP is addressed regarding Coordination and Administration, Short Term Stakeholder Capacity, and Long Term Stakeholder Capacity. Some detail of three plan elements is presented below.

Forecasting Long Term Stock Demand

There will always be biological constraints imposed upon planting plans. These constraints must be overcome through planning and good communication among stakeholders. Assuming a seed crop is available for the desired species and seed zone, it can take from one to often four years, beginning from seed collection through to seedling production, before a Planting Delivery Agent (PDA) can plant a tree. Unfortunately most landowners do not inquire about tree planting or begin planning a planting project with a local PDA until the year before planting. By this time the stock they will plant has already been grown. Two to five years previously, private growers had to speculate on species, seed zone, and stock type to produce, largely according to historical demand, seed availability, and pricing.

Currently TO is working to increase local stakeholder forecasts of stock needs (based on local soils, competing land uses, delivery capacity, etc.), to assist the TO Seed and Stock Coordinator to determine the long term planting stock demands by species and seed zones. This lessens the current risk involved in any one grower's speculation process. Involving local partners in estimating planting site availability and then seed crop and stock forecasting will allow those partners to look ahead and be more realistic regarding stock availability, and also to take ownership of the stock produced based on their forecasts.

Seed Collection Area Network (SCAN)

Seed Collection Area (SCA) management is a strategy developed to more efficiently forecast and collect high quality seed of known source. It was initiated by the OMNR in the 1980s and included white pine (*Pinus strobus*) seed orchards as well as high quality stands of other species, but was largely abandoned by the mid-1990s. The FGCA renewed management of the white pine orchards in 2000. In 2010 the SCAN was embarked upon by OMNR's Climate Change Initiative, the FGCA and TO partners and now includes the orchards, old OMNR SCAs, and new areas across southern Ontario's seed zones.

<u>SCAN Objectives:</u>

1. To manage high quality seed collection areas, which can provide a consistent supply of seed for the local seed zone, with less reliance on general collections.
2. To transfer seed management expertise and capacity to existing and new partners.

3. To support climate change adaptation by monitoring short- and long-term seed production.

SCAN now includes hundreds of sites representing many species across Southern Ontario's seed zones that meet the following criteria:
1. The stand size and quality ensures a good genetic base for frequent and high quality seed.
2. The stand contributes significantly to the amount of seed by species and seed zone needed annually to support afforestation programs.
3. Where seed banking is not a viable strategy, more SCAs will be located and managed.

Once fully established, SCAN management will involve landowners, seed collectors, growers and planting agents. Many others from arborists to school groups have expressed interest in monitoring and reporting flower and seed production. To protect property owners involved with the information collected through SCAN, TO staff will distribute the necessary information on a case-by-case basis.

Individual Site Management

The intensive management of the eight white pine seed orchards on Crown land has been coordinated by the FGCA in partnership with local groups and partly funded by the 50 MTP Seed Management budget and cone sale revenues. Management of other areas will depend on the landowners' interest and resources.

Seed Collector Access

Getting seed collected from the SCAN sites is the priority but it can take many forms. The landowner may decide to collect and sell the seed, or may grant limited access to certain collectors. TO can play an important role in advising the landowners of various options

Seed Collection

Seed collection is largely conducted by independent contractors for whom collection is only a seasonal source of income (See reference: *The Status of Seed Collection Serving Private Land Reforestation in Southcentral Region* February 2002). Some PDAs and growers also collect seed. Some focus on a few species while others collect many. There is considerable expertise involved regarding species, access to private land, crop maturity and viability, and markets.

Assigning Targets and Standards

The TO SSC, the Ontario Tree Seed Plant (OTSP), and growers all work directly with collectors to assign targets. Any one collector may have contracts with several buyers. Though buyers vary in how much they want and when, standards for seedlots must be consistent to ensure the seed is mature, viable, of high quality, and source-identified. Unless a collector has substantial experience and a proven track record, buyers are asking that collectors be Certified Seed Collectors (CSC) to ensure they have been taught appropriate methods for collecting, handling, and documenting seed. New CSCs are initially provided with small contracts to see if they can deliver. Targets are given by July 31 for many species and collectors are advised of targets for recalcitrant species.

Markets and Prices

The OTSP sets a base rate for seed by species which is mirrored by TO. However in any given year other buyers including growers, the forest industry or American seed buyers can increase prices. Some markets such as the OTSP may have lower prices but provide free shipping. Constant communication with collectors and the use of contracts can help ensure seed is collected when good crops are available.

Training and Mentoring Collectors

Efforts are continually made to keep the seed collection training curriculum current and to a high standard. Currently there are two workshops targeting seed collectors that were developed as part of the FGCA's *Ontario's Natural Selections* Seed Source Certification Program:
Certified Seed Collector Workshop – 3 days
Seed Collector Mentoring Workshop – 1 day

The text for the course will be a revised *Seeds of Ontario Trees & Shrubs* (Fig. 1) supported by the OMNR's Ontario Tree Seed Plant, which will be available in early spring 2014.

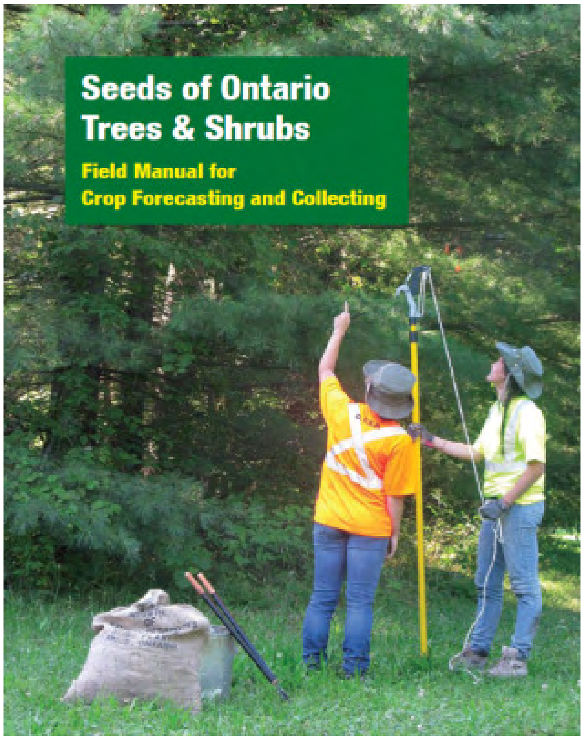

Figure 1. New seed crop forecasting and collecting manual.

Maintain Stable Level of Collectors by Seed Zone

Constant communication among all stakeholders is needed to help collectors fill their contracts. This requires up to date contact information and crop forecasts for the species and zones they are interested in collecting. Collectors will be more successful if they can be directed to locations with bumper crops or good but localised crops. Even a bumper crop can be lost to insects or drought, so information on how a crop is developing is important to communicate to collectors and buyers.

Every year can be unique in some way whether it is crop size or maturation timing or the effects of drought or insects on its collectability. TO and the OTSP deal with many calls each year. Having more local expertise to advise forecasters and collectors can help. The seed knowledge of TO's Field Advisors (FAs) can be increased, and opportunities may exist for retiring collectors to informally mentor new collectors.

Many species are delivered directly to growers for immediate sowing. The OTSP manages a good system of seed delivery. Making it easier for collectors to deliver seed can increase collection efforts as well as help maintain seed quality. In areas that are distant from courier service or growers, field depots run by TO or local PDA's can help.

Managing tree seed in southcentral Ontario is challenging in terms of the number and diversity of species and partners. Though working with many partners is often the more challenging part, the up side is the increasing number of people who understand the complexity and value of high quality seed. This can only result in better results for reforestation efforts, and a better future for southern Ontario.

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HYDROGEN PEROXIDE REVISITED

Hydrogen peroxide (H_2O_2) is our recommended seed sanitation method for nurseries to incorporate for reducing seed-borne contaminants (e.g., *Fusarium* spp.) on the surface of seeds. I think of this as the second line of defense from seed stratified at our facility as the seed will already have gone through a running water soak which has been shown to reduce seed-borne contaminants. A review of seed sanitation and hydrogen peroxide is provided in the Seed Handling Guidebook (Kolotelo et al. 2001; Pages 61 to 68 and in Appendix 4, page 102). The focus of this article will be to provide some additional hydrogen peroxide references not included there and to focus on the other identified benefits of hydrogen peroxide use like increased germination and 'vigour'. It seemed like a good opportunity to raise awareness of the benefits of the treatment and advocate for nurseries to consider performing some small scale trials to determine if operational benefits like increased germination can be realized. The finding that western white pine (*Pinus monticola*) seed treated with hydrogen peroxide at the nursery (see Armchair report) reduced the germination decline and the operational use of H_2O_2 in Quebec for the species they stratify has prompted this note.

The initial work by Dr. Ching (1959) illustrated that for Douglas-fir (*Pseudotsuga menziesii*) a 1% H_2O_2 soak for 36 to 48 hours increased the germination rate. It was shown that the uptake of oxygen and water was increased with a H_2O_2 treatment and suggested that this increased the conversion rate of fatty seed storage compounds to carbohydrates (the Glyoxylate pathway). There authors noted the high elevation seedlot tested seemed to respond more dramatically to the activation treatment. These details were not unexpected as the use of hydrogen peroxide as a viability quick test was published prior to these detailed findings (Ching and Parker 1958). For those interested in more information on viability quick tests, Dr. Carole Leadem (1984) produced a review and instructions for several quick tests.

One objective of this article was to provide an update of more recent findings with the use of H_2O_2 on tree seed. I will utilize the same format as Table 1 in the Seed Handling Guidebook and qualify that it's an entry into additional literature, not necessarily an exhaustive review – I'm certainly interested in other references you may have found.

Table 1. Study details on the beneficial effect of hydrogen peroxide treatments on tree seed

Species	H_2O_2 (%) concentration	Duration (hr)	Timing	Results	Reference
Pinus roxburghii	1%	24 hours	Pre-sowing Replaces water soak	17% increase in germination, 10 day reduction in germination period. <u>No stratification comparison</u>.	Ghildiyal et al. 2007
Pinus palustris	30% and 3%	15 min to 3 hours and 4 to 48 hours	Pre-stratification	14% germination increase and 96% reduction in fungal infection	Barnett and Varela 2004
Abies amabilis, A. lasiocarpa, and A. *grandis*	3%	0.5 to 4 hours	Post-soak / Pre-stratification	Results seedlot specific – no significant germination gains	Kolotelo 2003
Pseudotsuga menziesii, Pinus ponderosa, P. lambertiana	1%	48 hours	Pre-sowing = field trial	*Pseudotsuga* – 24% germination increase after 10 weeks; other species no significant gains	Stein 1965
Pinus elliottii	1%	2 to 4 hours	Pre-stratification Replaces water soak	Can reduce stratification needs from 30 to 15 days. Operationally not considered cost effective.	Forrest 1964
Fagus orientalis	1%, 2%, 3% and 30%	12, 24 hours and 15, 30 minutes	Pre-stratification	12% increase in germination and increase in speed	Rezaei et al. 2012
Tectona grandis	0.5 to 5.0% 1.5%	12 hours	Pre-sowing	18% germination gain open and 37% gain in misting chamber	Masilamani and Dharmalingam 1999

Although there have been many investigations with H_2O_2 on tree seeds there has not been a better explanation of mechanisms specific to tree seeds since 1959. The remaining text draws on experience with H_2O_2 treatments on other plants and the progress-to-date in understanding the extent of benefits and underlying mechanisms.

Hydrogen peroxide is considered an Active or Reactive Oxygen Species (AOS or ROS) and as a peroxide is characterized by a single Oxygen-Oxygen bond. Oxidation is defined as the interaction of oxygen and other substances or more technically the loss of at least one electron when two substances interact. Hydrogen peroxide is fat soluble and able to diffuse across cellular membranes. Hydrogen peroxide and superoxide (O_2^-) are considered weaker oxidizing agents compared to the hydroxyl radical (OH). The AOS molecules have generally been considered toxic and have been shown to be involved in seed ageing, cell injury, disturbances in seed development and germination, and dessication related damage in recalcitrant species (Bailly 2004). This research review also highlights the evidence illustrating AOS' as a signalling molecule involved in a wide variety of responses to various stimuli. The theory is that there is a balance between nullifying AOS molecules through enzymatic reactions (e.g., catalase) and allowing a certain amount to be retained to elicit various mechanisms for stress tolerance and pathogen resistance. Of specific interest to pathologists is the role of AOS in plant defense (Mehdy 1994) and especially the role of H_2O_2 in the plant's hypersensitive disease resistance response (Levine et al. 1994).

Looking further away from conifers there has been a great deal of effort to implement hydrogen peroxide into operational seed germination practices. For tree seeds, H_2O_2 concentrations of up to 30% have been used on Douglas-fir (*Pseudotsuga menziesii*) and southern pine seed without detrimental impact, but damage has been observed in agricultural crops at much lower concentrations. For triploid watermelon seeds, H_2O_2 concentrations above 2% produced radicle damage (Duval and NeSmith 2000) and in aged corn 3% H_2O_2 caused stunted root growth compared to a 0.15% solution (Liu et al. 2012). This dilute 0.15% solution was considered the optimum level for providing increased oxygen for germination and resulted in the doubling of oxygen consumption compared to untreated seeds. For barley, maize, haricot, vegetable marrow, garden radish, and carrot a H_2O_2 treatment increased germination rate and accelerated plant growth. For barley and maize, large gains of 19% and 32% germination were found in old seeds with newer seeds showing more modest, but consistent germination gains (Narimov 2000).

The effect of hydrogen peroxide appears to go beyond the sanitation and immediate germination benefits. There is evidence that treatment effects continue in the favourable performance of hydrogen peroxide treated seeds. For wheat seedlings, seed treatments with H_2O_2 ranging from 20 to 140 mM increased germination (80mM optimal), but also increased seedling growth and under drought stress the seedlings had reduced membrane damage and enhanced antioxidant expression (He et al. 2009). In barley, H_2O_2 treatment was very successful in overcoming germination delays attributed to increased levels of temperature and salinity (Kursat and Kabar 2010).

Hydrogen peroxide has also been used as a scarification agent with thin coated species, like *Ribes cereum* and it was very interesting that increased germination (6 to 12%) did not result in an increase in the number of seeds germinating during stratification which is a problem with this species (Rosner et al. 2003). Other studies have stimulated germination and seedling development with a low dose oxidative stress and that this was induced by hydrogen peroxide or low dose ionizing radiation (Korystov and Narimov 1997). Could the reported increases in germination through bleach treating conifer seeds (Wenny and Dumroese 1987) also be associated with this low dose oxidative stress in a manner similar to hydrogen peroxide?

As indicated this is not an exhaustive review, but a good entry point into the literature on hydrogen peroxide. I'll try and condense the information presented here and what we already knew to present some general concepts for tree seeds.

1. Hydrogen peroxide is not an inexpensive seed treatment, but there is ample evidence in tree seeds and agriculture crops that large benefits can result, especially with contaminated or marginal seeds or seeds which are being germinated under sub-optimal conditions. Some trials have shown very low concentrations (0.15%) to be able to produce significant gains in germination in agricultural species. There are hydrogen peroxide benefits beyond sanitation and those benefits may arise from much lower concentrations making the treatment a more cost-effective option.

2. There is very little evidence that hydrogen peroxide treatments can be detrimental to tree seeds. Hydrogen peroxide concentrations of 30% have been used operationally with Douglas-fir and southern pines without any negative effects. A 30% solution is also used to sterilize a wide

variety of tree species for Fungal Assay testing and germination is often seen following this treatment. Caution is advised when using hydrogen peroxide with species possessing resin vesicles as the result is not as predictable.

3. Gains in disease control and germination capacity are relatively easy to quantify, but unless seed treatment studies are continued into the seedling development phase then additional benefits such as seedling growth and abiotic stress tolerance cannot be quantified. These studies have been conducted on agricultural crops and in some cases the treatment is optimized for the specific variety and this may not be practical with our diverse and numerous seedlots.

I would be interested in your experiences testing or introducing hydrogen peroxide treatments into your crop cycle for sanitation, increased germination or 'vigouration' of your crops.

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IMPROVING THE QUALITY OF WHITE BIRCH (*Betula papyrifera*) SEED. III

This is the third and final installment of a series of articles dealing with improving the quality of white birch (*Betula papyrifera*) seed. It is very difficult to remove dead, damaged, and empty white birch seed using air aspiration because the seed are winged and light. A trial conducted at the National Tree Seed Centre using aspiration to remove debris and empty seed demonstrated that germination of de-winged seed increased to 74% compared to winged seed at 36% (Daigle and Simpson 2001). After 10 years in storage at -20°C, germination of the de-winged seed was still high (Simpson and Daigle 2011) demonstrating that de-winging did not negatively impact germination.

One challenge with air aspiration is preventing the removal of lighter filled seed. The Seed Centre uses floatation in absolute ethanol to separate filled from empty seed in *Larix*, *Picea*, *Pinus*, and *Tsuga*. Since this technique had also been used for birch (Björkroth 1973) it was decided to try it. There was a substantial improvement in germination with de-winged seed ranging from 0 to 73% and de-winged + alcohol separated seed ranging from 41 to 97% (Simpson and Daigle 2003). A supplementary test to evaluate the impact of the duration of seed in alcohol showed that germination did not decrease (Simpson and Daigle 2003).

The impact of alcohol separation on the storability of white birch seed was evaluated on 21 seedlots collected from five sites by germination testing seed that had been de-winged + alcohol separated and stored for 10 years at -20°C and comparing the results with those obtained prior to storage.

Methods

A sample of winged seed from each seedlot was retained for testing prior to storage. The remaining seed were placed in a cotton bag and gently rubbed to break off the wings. The contents were then transferred onto a fine mesh sieve to separate the crushed wing debris from the seed. This process was repeated several times until seed were completely de-winged. The de-winged seed were carefully blown in an aspirator to remove light debris as well as some empty and insect damaged seed. Following this, the seed were immersed in absolute ethanol and stirred to ensure any heavy seed had an opportunity to sink. When most of the seed had completed sinking in the column (15 seconds) the "sinkers" were collected in a strainer and rinsed under running tap water for 15 to 30 seconds. The wet seed were laid on coffee filters to dry for 24 hours.

Germination tests were set up by sowing 4 replicates of 50 seed each on moistened Kimpak™ in Petawawa Germination Boxes. The boxes were placed in a germination cabinet for 21 days and subjected to diurnal cycles of 20°C and darkness for 16 hours followed by 30°C and light for 8 hours. Relative humidity was maintained at 85%. Germination was assessed at 14 and 21 days. Germinants, classified as high vigor (cotyledons green and separated with a well developed radicle and hypocotyl), were removed at each assessment time. After 10 years of storage, germination tests were repeated following the same procedures as above.

Results and Discussion

Mean germination of the seed improved from 34 to 83% prior to storage by de-winging plus alcohol separation (Table 1). Germination of treated seedlots ranged from 45 to 97%. After 10 years in storage at -20°C, germination of the treated seed was 3% higher than that prior to storage and ranged from 54 to 97%. Germination of these treated seedlots prior to and after storage was slightly less than that reported by Simpson and Daigle (2012) for different seedlots. Seed quality was extremely low for collections from Site C but de-winging + alcohol separation dramatically increased germination (Table 1). In fact, if this treatment had not been applied these seedlots would have been discarded. When these seedlots are excluded from the dataset, mean germination of winged seed increases to 43% and that of treated seed to 89% prior to storage and to 91% after 10 years in storage.

Prior to storage, treated seed did not produce any low vigor germinants. After storage 1% of the germinants were classified as low vigor. A low vigor germinant is one that has not fully developed and often the cotyledons have not separated. As well, about 0.5% of the germinants were abnormal which was predominantly due to decay caused by mold.

These results continue to confirm those previously published (Simpson and Daigle 2011; 2012) that using absolute ethanol to remove dead and empty de-winged white birch seed substantially improved germination and that the alcohol treatment did not damage the seed as is evidenced by high germination both before and after 10 years in storage. An additional benefit from dewinging white birch seed is the reduction in volume thus requiring smaller containers to store the seed.

Table 1. Germination (%) of twenty-one winged and de-winged plus alcohol separated white birch seedlots, collected at five sites, before and after ten years of storage at -20°C

		Germination before storage		Germination after 10 years
Seedlot	Site	Winged	De-winged + alcohol	De-winged + alcohol
95	A	38.0	91.5	80.0
96	A	1.0	93.5	90.5
97	A	29.5	86.5	84.5
99	B	58.5	92.5	94.0
100	B	54.0	94.0	92.0
102	B	57.5	96.5	96.5
106	C	0.0	71.0	73.0
107	C	4.5	45.0	54.0
108	C	1.0	55.0	67.0
109	C	4.0	85.0	80.5
110	C	4.0	66.0	82.0
114	D	72.5	95.0	96.0
115	D	71.5	92.0	95.0
116	D	47.5	65.5	81.0
117	D	24.5	90.5	89.5
118	D	54.5	90.5	97.0
119	E	40.5	87.5	95.5
120	E	29.0	88.5	96.5
121	E	47.5	78.5	87.5
122	E	60.0	87.0	86.5
123	E	7.5	97.0	94.0
Mean		33.7	83.3	86.3

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ALBERTA SEED CONSERVATION COURSE

This is an annual 2-day course held at the Alberta Tree Improvement & Seed Centre (Smoky Lake – 2 hours north east of Edmonton). The course is directed towards anyone in industry and academia who collects, handles or stores seeds. Topics include: seed-air moisture relations, assessment/collection/seed handling, seed longevity and storage, seed storage behaviour, and germination and dormancy. There will be a hands-on seed cut testing lab and a paper-based germination problem solving tutorial. Limited to 12 participants but the course may run twice if there is enough interest. Proposed time frame is between 17th February and 14th March.

If you are interested, please contact Lindsay Robb: Lindsay.Robb@gov.ab.ca

UPCOMING MEETINGS

ISTA Workshop on Tree and Shrub Seed from the Mediterranean Basin
May 19–21, 2014 Madrid, Spain
www.seedtest.org/workshops
Contact: joelle@montaraz.com

AOSA/SCST Annual Meeting
May 31 – June 5, 2014 Fargo, North Dakota
www.aosaseed.com/2014-AOSA-annual-meeting.htm

ISTA Annual Meeting
June 16–19, 2014 Edinburgh, United Kingdom
www.seedtest.org/AM14

BC Seed Orchard Association
June 18–20, 2014 Salmon Arm, BC

RECENT PUBLICATIONS

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Popova, E.V.; Kim, D.H.; Han, S.H.; Moltchanova, E.; Pritchard, H.W.; Hong, Y.P. 2013. Systematic overestimation of Salicaceae seed survival using radicle emergence in response to drying and storage: implications for *ex situ* seed banking. Acta Physiologiae Plantarum 35(10):3015–3025.

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