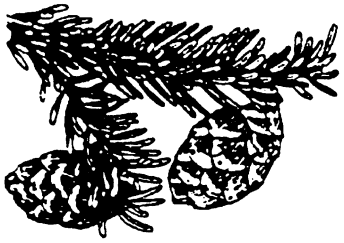
CANADIAN FOREST GENETICS ASSOCIATION
ASSOCIATION CANADIENNE DE GÉNÉTIQUE FORESTIÈRE

Tree Seed Working Group



NEWS BULLETIN

No. 50 December 2009

SEED TESTING

THIS ISSUE AT A GLANCE

Page	Article
3	Tree Seed Testing in Canada
4	How Can Seed Tests Help Valuate Seedlots?
6	Seed Testing at the Atlantic Forest Seed Centre
7	Overview of the Quebec Seed Testing Program
10	Seed Testing at the National Tree Seed Centre
12	J.D. Irving – Parkindale Seed Orchard
13	Scheduling Germination Tests
15	Water Activity – An Efficient Tool for Seed Testing
18	Quick Tests
20	Quick Testing Using Vaporous Contrast Agents, Chloroform - $CHCL_3$
23	Applicability of the Accelerated Ageing Technique in Predicting Storability of Lodgepole Pine (*Pinus contorta* var. *latifolia* Engelm.) Seed
26	Online Glossaries
26	Status of the OECD Tree Seed Certification Scheme
27	Integration and Use of Somatic Clones in Seed Orchard Management and the Production of a New Generation of Seeds With a High Genetic Value
31	Upcoming Meetings

CHAIR'S 'ARMCHAIR' REPORT

Welcome to the 50th edition of the Tree Seed Working Group News Bulletin. From its humble beginnings in 1983 (referred to as the 'fledgling TSWG' in Newsbulletin #1) there certainly has been growth as the News Bulletin now reaches over 250 people worldwide. What is probably more amazing is that this group has persisted that long. This can partly be attributed to the importance of our subject, but mostly reflects on the dedication of the chairpersons, editors, and contributors to the topic of tree seed. Thank you to everyone who has contributed to our working group and its Newsbulletin. It seems like I've been under a deluge of 50th celebrations with the BC Forest Service Tree Seed Centre celebrating last year, tree improvement (I mean forest genetics) currently celebrating 50 years in BC and now the 50th edition of the TSWG News Bulletin.

The theme of this News Bulletin is "Seed Testing" and I'd like to provide a brief overview of seed testing history and some additional links for those interested in more information. I generally think of seed testing in reference to two primary associations – Association of Official Seed Analysts (AOSA) and International Seed Testing Association (ISTA) that provide standardized rules, guidance, and a forum for the exchange of information. The following are timeline references I used to produce the relatively brief early seed testing history summary below:

AOSA http://www.aosaseed.com/docs/Collaboration_Report_1996.pdf
http://www.rngr.net/Publications/tpn/18/pdf.2005-05-13.1684309811/view

ISTA https://www.seedtest.org/upload/cms/user/S4.9.0950.Muschick1.pdf

Both associations trace the origins of seed testing to Dr. Friedrich Nobbe who founded the first seed testing lab in 1869. The lab was in Tharandt, Germany (about 14 km Southwest of Dresden) and

although initial testing was focused on agricultural seeds the town was also home to the oldest academy of forestry in Germany (formed in 1811). Initial questions focused on seed purity, specifically in relation to species purity, but evaluation of the potential of a seed to produce a healthy plant was also identified. In 1876 Dr. Nobbe produced the first Handbook of Seed Science that contained information on seed physiology, statistics, and the practical implementation of these principles. By this time there were about 32 seed testing stations including the first North American seed lab at the Connecticut Agricultural Experiment Station. By 1896 the number of seed testing labs had expanded to 199 in 19 different countries.

The first International Seed Testing Congress occurred in 1906 in Hamburg, Germany and that same year the first private seed lab opened in the United States. In 1908, the AOSA was formed and the first set of rules was printed by the USDA in 1918. In 1924, ISTA was formed and included the AOSA president on its first executive committee. The year 1924 also saw the formation of FIS which later became the International Seed Federation (ISF). This organization is an international forum for the seed industry and is more involved with representing the seed industry, dealing with issues of trade, and intellectual property. Congresses have occurred in Canada in Ottawa (1972) and Toronto (1992), and the next World Congress will be held in Calgary between May 31 and June 2, 2010 (see http://www.worldseed.org/isf/history.html for more information).

In 1928, ISTA produced their first set of rules and at the World Seed Congress a Committee on the examination of Tree Seeds (CFS) was set up. A review of history and method validation in these early years (1928–1934) is provided in the latest edition of the ISTA Newsbulletin (No. 138 – https://www.seedtest.org/upload/cms/user/STI_138_Oct_2009.pdf - see pages 33 to 36). The early rules were all focused on agronomic crops and it wasn't until 1953 that some important tree seeds were included in the ISTA rules and not until 1965 did they appear in AOSA rules. Does anyone know the year and location for a) the first Canadian seed testing laboratory and b) the first Canadian seed testing laboratory to test tree seed? Fame and fortune awaits the first to respond ☺ (see Ben Wang's article, page 3 in this bulletin)

The other organization that touches some of us in the tree seed trade is the Organisation for Economic Co-Operation and Development (OECD). Since 1958 the OECD has had a set of seed schemes that provide certification of quality and intended to facilitate international trade. See Dale's article on page that discusses the current status of the OECD Scheme in relation to the international tree seed trade.

I won't go into all the further developments of these organizations, but they are all dynamic and looking to stay relevant in our rapidly changing world. In terms of AOSA and ISTA there have been great efforts made to harmonize rules, but there are still some philosophical differences that reflect general continental attitudes towards genetically modified organisms (GMO's). You would be hard pressed to find mention of GMO material within AOSA, but a great deal of effort is being invested in GMO testing by ISTA.

This summer the CFGA meeting will occur from July 20–22 in Thunder Bay. The plan is to offer a Tree Seed Workshop, but Dale will not be able to attend and my attendance is in serious doubt, so we need your help. If you plan to attend, have ideas or would like to make a presentation, please contact Dale or myself. The News Bulletin is also a good way to advertise meetings or provide summaries and proceedings links, so please keep that in mind.

I'll leave you with our Tree Seed Centre testing mantra "**A test result is only as good as the sample taken**" – please keep that in mind. May you have a great holiday season with friends and loved ones.

Dave Kolotelo
TSWG Chairperson

EDITOR'S NOTES

Here we are celebrating another milestone of the Tree Seed Working Group. Last year we celebrated the 25th anniversary of the Working Group and now this issue marks the 50th News Bulletin. What a remarkable journey we have had over the past 26 yea**rs.**

This News Bulletin is the biggest yet and I am grateful to all who contributed articles. From Ben Wang's lead-off article it seems that tree seed testing has been happening in Canada for about 100 years. There are a number of contributions summarizing seed testing operations and procedures at various seed centres as well as a number of articles describing different types of tests that are conducted. Although germination testing protocols vary among seed testing labs it is important that the procedures are conducted in a consistent manner at each lab.

I wish everybody a Joyous Holiday Season and a healthy, prosperous New Year.

Dale Simpson
Editor

TREE SEED WORKING GROUP

Chairperson
Dave Kolotelo
BC Ministry of Forests and Range
Tree Seed Centre
18793 - 32nd Avenue
Surrey, BC V3S 0L5
Tel.: (604) 541-1683 x 2228
Fax.: (604) 541-1685
E-mail: Dave.Kolotelo@gov.bc.ca

Editor
Dale Simpson
Natural Resources Canada
Canadian Forest Service
Atlantic Forestry Centre
P.O. Box 4000
Fredericton, NB E3B 5P7
Tel.: (506) 452-3530
Fax.: (506) 452-3525
E-mail: Dale.Simpson@nrcan.gc.ca

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

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

TREE SEED TESTING IN CANADA

Testing of tree seed is an essential component of artificial regeneration. To evaluate the suitability of a seedlot for sowing, it is essential to determine its ability to germinate and develop healthy seedlings in the field. Testing of tree seed is usually concerned with the purity, germinability, and treatment effects. Test results reveal the suitability for sowing and as well as a value for comparison with other seedlots in trade.

To conduct tree seed testing, agencies require well trained personnel and essential equipment and facilities so that the test results can be reproducible. This is the reason why it is important to have seeds tested at officially recognized laboratories where testing procedures are standardized and conducted by trained staff.

Tree seed testing for purity and germination was started in Europe in the 1930s and was added to the rules for testing seeds of the Association of Official Seed Analysts in 1965 and revised in 1970 (Baldwin 1942; Bonner 1974). In Canada, the introduction of the Seeds Act and its regulations were legislated in 1905 (Doug Ashton, pers. comm). The Act has been revised subsequently, but forest tree seed was not included until 1977 when the definition of seed was broadened to cover forest tree seed (Edwards 1987). However, there were no national uniform testing standards established for tree seed and each forest agency had its own established rules, procedures, and interpretation of test results. Following the recommendation from a National Workshop on Tree Seed Production and Tree Improvement held in 1978 for obtaining delegated authority to certify and regulate tree seed under the Seeds Act, the Canadian Forest Service (CFS) sponsored four tree seed testing workshops across the country to promote the need for uniform tree seed testing methods. Those hand-on workshops were well attended and received. Furthermore, CFS published "Guidelines for grading and labeling forest tree seeds in Canada" (Edwards et al. 1988) and "Methods and procedures for testing tree seeds in Canada" (Edwards 1987).

Official tree seed testing was included in the accredited agricultural seed laboratories by ISTA, although there was no tree seed test required. From 1968–89, the National Tree Seed Centre was accredited as the ISTA laboratory for tree seed testing at the encouragement and guidance of Dr. Cuddy of Agriculture Canada. The Seed Laboratory at the CFS's Pacific Forestry Centre, Victoria, BC, was also accredited by ISTA for testing tree seed in the early 1970s to the 1990s. This was because ISTA certificates were required for any seed exported to Europe that was OECD certified.

Recently, with the exception of Agriculture Canada's ISTA accredited laboratory in Saskatoon for testing purity and germination, there is no accredited laboratory for testing tree seed in Canada. However, BC legislation requires tree seed collected for use on Crown Lands to be tested and stored in the BC Tree Seed Centre. In the most recently revised Alberta Sustainable Development's "Alberta Forest Genetic Resources Management and Conservation Standards", it specifies that tree seed testing must be done by a recognized tree seed testing laboratory.

When did tree seed testing begin in Canada? There is mention in an internal document that seed viability testing started in 1903 at the PFRA Shelterbelt Centre, Indian Head, SK (Bill Schroeder, pers. comm.). Seed testing started in 1909 at the provincial forest nursery, Berthier, QC (Normand Brault, pers. comm.). To reiterate Dave Kolotelo's plea, if anyone has information on early seed and/or tree seed testing in Canada please pass it along.

References

Baldwin, H.I. 1942. Forest tree seed of the North Temperate Regions. Chronica Botanica Company,Waltham, Mass., USA. 240 p.

Bonner, F.T. 1974. Seed testing. Pages 136–152. *In* G.S. Schopmeyer, tech. coord. Seeds of woody plants in the United States. USDA, For. Serv, Agric. Hndbk. 450.

Edwards, D.G.W. 1987. Methods and procedures for testing tree seeds in Canada. Can. For. Serv., For. Tech. Rep. 36. 31 p.

Edwards, D.G.W.; Pollard, D.F.W.; Wang, B.S.P. 1988. Guidelines for grading and labeling forest tree seed in Canada. For. Chron. 64:334–344.

Ben S.P. Wang
Natural Resources Canada
Canadian Wood Fibre Centre
Petawawa Research Forest
PO Box 2000
Chalk River, ON K0J 1J0
E-mail: bwang@nrcan.gc.ca

HOW CAN SEED TESTS HELP VALUATE SEEDLOTS?

This article will provide an overview on how the results of seed tests can help in the valuation of tree seeds. It is intended to provide both the seed owner and the seed buyer with a better understanding of their product. The discussion provided will be general in nature, but examples will be provided in reference to the current BC Ministry of Forests seed prices: http://www.for.gov.bc.ca/HTI/treeseedcentre/tsc/fees.htm#surplus
and in the context of our seed use legislation in British Columbia (Chief Foresters Standards for Seed Use): http://www.for.gov.bc.ca/code/cfstandards/html/
The most common and simplest valuation system is to sell seeds at a standard price per kilogram and the various adjustments to this based on seed testing results will be discussed.

The importance of seedlot moisture content for longevity of orthodox seed is rarely questioned and even though most people recognize that seed weight is influenced by moisture content, this variable seems absent from seed valuations. Our legislation states that for registration the moisture content (MC) must be between 4 and 9.9%, yet if we sell by seed weight the difference in moisture content is not accounted for. Does this really matter? Let's use Sitka spruce (*Picea sitchensis*) ($4000/kg) as an example. If a kilogram of seed was at 4% MC then the dry weight equivalent is 960 grams *vs.* 901 grams at 9.9 % MC. So, one kg can vary by as much as 59 grams of dry mass and for Sitka spruce this difference is equivalent to about $236/kg. The difference reaches its peak with western redcedar where the acceptable MC range can result in a $384/kg difference.

It is generally accepted that reduced MC will increase seed longevity, so the intent is certainly not to advocate moisture loading to increase the cost recovery of seed sales. It is simply one of the seedlot characteristics that varies and can easily be incorporated into pricing. MC also contributes to differences in seed yield when presented as kg of seed per hectolitre of cones and a similar correction factor to those discussed can also be employed to standardize yield reporting.

The results of seed weight and purity tests will be discussed together as they both influence the number of seeds per gram which is a variable we use in BC to calculate potential seedlings. The purity of a seedlot is simply the average proportion of a seedlot deemed to consist of pure seed. A purity of 99.0% implies that 990 grams out of one kg of seed is pure seed and the remaining ten grams consists of debris (inclusion of seeds of other species is quite rare, but also considered an impurity if applicable). The seed weight test is the average weight of 100 seeds (derived as the average of eight replicates) and is a method of quantifying seed mass.

To provide a more meaningful seed mass variable, seeds per gram (SPG) is used and calculated as the seedlot purity (%) divided by the average weight of 100 seeds. The higher SPG values represent the lightest seeds and vice versa. This is probably the largest and most important source of variation unaccounted for in seed valuation. I'll supply a few examples. For seed orchard produced coastal Douglas-fir, the SPG ranges from 106 to 68 indicating that there is a difference of 38 seeds

available per gram or 38 000 seeds per kg. For natural stand lodgepole pine, the range is much greater (504 to 253) resulting in a difference of 251 000 seeds per kg! These examples illustrate the extremes for effect, but clearly show that attention to SPG can result in a much lower (or higher), but more realistic cost per seed compared to a standard price per kg. Certainly one consideration is whether there is a practical advantage in the use of larger seeds. I am not convinced that an advantage exists in terms of plantation success, but there are contradictory results in the literature. A good review is provided by Sorenson and Campbell (1985) and some additional comments are provided by Kolotelo (2000). An alternate view, along with different references, is provided by Castro et al. (2008). If you believe that seed size is an important attribute to meet your objectives then you may be content paying more per seed based on a simple, unadjusted per kilogram cost.

Germination Capacity (GC) is the variable that has been used in seed valuation in British Columbia. Currently, if a seedlot is below the species average then a price adjustment is applied using the specific seedlot germination as a proportion of the species average germination [seedlot GC / species GC]. No cost adjustment is applied to above average germinating seedlots. The GC is the other variable along with SPG that is used in the calculation of potential seedlings and that may be a variable used to valuate seed. A simpler solution may be to use the GC and SPG variables independent of the relationship to potential seedlings. This could take the form of quantifying the **germinable seeds** represented as **(amount of seed [g]) X (SPG) X (GC/100)**. Instead of a basic seed price per kg of seed, this more encompassing variable would have seed pricing as a function of germinable seeds. A further refinement could be to account for the seedlot moisture content (as a decimal value in the equation) in terms of germinable seeds on an oven-dry (0%) or other standardized (i.e., 7%) MC. For oven-dry weight assessment the equation would expand to:
Germinable seeds based on dry weight = (amount of seed [g]) X (1-MC) X (SPG) X (GC/100)

This variable is intended to account for variability in seed attributes between seedlots. I believe the greatest benefit would be derived by integrating GC and SPG as these are the most variable results between seedlots. There is also variability within a seedlot and this has been quantified as the precision of germination tests and can be surprisingly high in some cases. Variability in SPG tests within a seedlot has not been similarly quantified, but as is common with our relatively wild tree species – variability should be the expectation.

In addition to the standard tests of seed characteristics, other variables may also aid in seed valuation. Fungal assay testing is one example, but valuation is complicated because the link between fungal occurrence (% contamination or infection) does not readily correspond to disease or loss of seedlings, but a quantification of potential risk. A seedlot with 10% *Fusarium* spp. contamination has greater risk than a seedlot with 2% contamination, but the relationship may not equate to five times the risk. These relationships have not been well documented and unique nursery conditions could play the largest role in the risk of seedling loss.

Other variables such as Genetic Worth for growth or disease resistance may play an important role in seed valuation, but these values are not the product of a seed testing lab. There are several seed testing results that may improve the quantification of seed value to more closely reflect the number of germinable seeds and adjust for differences in moisture content. Hopefully, this will promote further discussion on seed valuation and a better appreciation of how seed test results practically impact the commodity value of each unique seedlot.

References

Castro, J.; Reich, P.B.; Sanchez-Miranda, A.; Guerrero, J.D. 2008. Evidence that the negative relationship between seed mass and relative growth rate is not physiological but linked to species identity: a within-family analysis of Scots pine. Tree Physiol. 28:1077–1082.

Kolotelo, D. 2000. Differences in seed and seedling attributes between select (orchard produced) and standard (wild stand) seedlots. *In* N. Wilder, P. Byman and C. Hawkins, tech. co-ords. Proc. 20th Forest Nursery Assoc. BC Ann. Meet. 18–21 Sep 2008, Prince George, BC.

Sorenson, F.C.; Campbell, R.K. 1985. Effect of seed weight on height growth of Douglas-fir (*Pseudotsuga menziesii*) seedlings in a nursery. Can. J. For. Res. 28:418–426.

Dave Kolotelo

SEED TESTING AT THE ATLANTIC FOREST SEED CENTRE

The Atlantic Forest Seed Centre (AFSC) has been operating at the Kingsclear Forest Nursery, located outside Fredericton, NB since 1976. Over this time period many thousands of seedlots have been tested on a regular basis. Seed testing is an important component of the AFSC operations.

At the AFSC we extract and store seed for the NB Department of Natural Resources as well as for other clients across the Maritimes, Quebec, and Maine. Seed testing is conducted daily on seedlots during extraction and if the seed is stored at our facility we regularly test seed requiring stratification every 2–3 years (i.e., white spruce (*Picea glauca*) and balsam fir (*Abies balsamea*)) and every 5 years for seed not requiring stratification (i.e., black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*)). Some of the older seedlots such as a 1982 jack pine stand (98%) and a 1983 black spruce stand (95%) still have great germination after almost 30 years in storage.

Like many forest seed centres we follow the ISTA standards for seed testing. For germination testing a sample of 400 (4 x 100) seeds per seedlot is tested using a Petawawa germination box with filter paper. Seeds are placed on the filter paper using a vacuum head to ensure correct numbers and distribution. The trays are placed in one of our two germination cabinets set at 28°C for 10 hours and 20°C for 14 hours. Seeds are counted every 5–7 days until day 21. All results are entered into a computer system and the information shared with our clients.

Other testing includes seeds per gram, purity, and moisture content (MC). We aim for between 6–10% MC, depending on the species. For purity, if we want to remain friends with the nursery growers, we try to get as close to 99% as possible. To help us plan for orchard production we have kept track of yields over the past 20 years. The table below gives the average seeds/gram, yield (kg/hl), and average number of seed/hl by species. There is a wealth of information in our data base that can be utilized!

Table 1. Seed statistics for a number of tree species.

Species	Seeds/g	Kg/hl	Seeds/hl
Tamarack	395	0.751	296 645
White pine	59	0.984	58 056
Red pine	128	0.926	118 528
Jack pine	257	0.848	217 936
Norway spruce	104	1.141	118 664
Black spruce	638	0.658	419 804
Red spruce	337	1.076	362 612
White spruce	397	1.189	472 033
White cedar	771	1.402	639 600
Balsam fir	126	2.416	304 416

Over the years we have tried many experiments evaluating the impact that the duration of stratification has on germination. Three weeks stratification seems to work best for most species most of the time. The AFSC works closely with the nursery staff to identify any problems that arise due to germination problems and work to increase germination percentage and uniformity.

We have been fortunate to have dedicated long term staff at the AFSC that work very hard to ensure that the seed is extracted, stored and maintained with tender loving care…it is a living thing after all! To conclude, I would like to take this opportunity to thank all the folks that have worked so hard to ensure the success of the TSWG News Bulletin, especially Dave's and Dale's efforts over the last number of years. We have greatly benefited from all the information in the News Bulletin and congratulate you on your success.

Kathy Tosh
N.B. Dept. Natural Resources
Tree Improvement Centre
Kingsclear Tree Nursery
3732 Rte. 2 Hwy.
Island View, NB E3E 1G3
E-mail: Kathy.Tosh@gnb.ca

OVERVIEW OF THE QUEBEC SEED TESTING PROGRAM

In Quebec, the ministère des Ressources naturelles et de la Faune (MRNF, Québec Ministry of Natural Resources and Wildlife) manages the reforestation program from seed collection to planting. All the seeds required for seedling production are stored and tested at the tree seed centre in Berthier (Centre de semences forestières de Berthier, CSFB). About 4 billion seeds are stored at the CSFB. Every year, about 2500 hl of cones and hardwood fruit are harvested and treated at CSFB. In spring, seeds are sent to the 6 public and 15 private nurseries that produce the seedlings (between 150 to 180 million each year) for the MRNF.

All the seed quality tests are performed at the CSFB laboratory by two lab workers. Depending on the species, tests are in accordance with ISTA rules or the methods described by Edwards (1987). Since 2007, the evaluation of water activity was added to our list (for details see Baldet et al., page 15 in this bulletin).

The major conifer species are, in decreasing order, black spruce (*Picea mariana*), jack pine (*Pinus banksiana*), and white spruce (*Picea glauca*). Hardwood species include yellow birch (*Betula alleghaniensis*, Quebec's provincial tree), red oak (*Quercus rubra*), sugar maple (*Acer saccharum*), red ash (*Fraxinus pennsylvanica*), white ash (*Fraxinus americana*), black walnut (*Juglans nigra*), and black cherry (*Prunus serotina*).

Each year 2500 tests are performed at the CSFB, of which 1200 are germination tests. The results are compiled in the data base management system (Système Semences). This system is connected with PLANTS, the system built for tracking seedling production in the nurseries (public and private). Those two systems are the bases of the traceability system that is used for the reforestation program.

The list of quality tests performed at the CSFB for the major reforestation species is shown in Table 1 (softwood) and Table 2 (hardwood). For those interested in the complete list, please contact Normand.

The year the seed is extracted, all quality tests are performed on each seedlot. Depending of our frequency germination test table (in revision), only the germination test and water activity are done subsequently to evaluate the quality of our seedlots in storage.

Reference

Edwards, D.G.W. 1987. Methods and procedures for testing tree seeds in Canada. Can. For. Serv., For. Tech. Rep. 36. 31 p.

Normand Brault and Michèle Bettez
Ministère des Ressources naturelles et de la Faune
Direction générale des pépinières et des stations piscicoles
Centre de semences forestières de Berthier
1690, chemin Grande Côte
Berthierville, QC J0K 1A0
E-mail: normand.brault@mrnf.gouv.qc.ca

Fabienne Colas
Ministère des Ressources naturelles et de la Faune
Direction de la recherche forestière
2700, rue Einstein
Québec, QC G1P 3W8

Table 1. Seed quality tests performed at CSFB for major softwood species.

	CSFB Seed quality testing								
Species	AE	KSKG	PGNS	VGNS	PGS	VGS	PHUM	PP	RX	FUNGI
EPB, PIB SAB EPN, EPR, EPO MEL, MEE, MEH, MEJ, PIG, PIR,THO										

Species	
EPB	White spruce
EPN	Black spruce
EPO	Norway spruce
EPR	Red spruce
MEL	Eastern larch
MEE	Europeen larch
MEJ	Japanese larch
MEH	Hybrid larch
PIB	White pine
PIG	Jack pine
PIR	Red pine
SAB	Balsam fir
THO	Eastern white cedar

Tests	
AE	water activity
KSKG	Number of seeds /kg
PGNS	Non-stratified germination %
VGNS	Non-stratified vigour
PGS	Stratified germination %
VGS	Stratified vigour
PHUM	Humidity %
PP	Purity %
RX	X-ray interpretation
FUNGI	*Cylindrocadium, Phoma* and *Fusarium*

Table 2. Seed quality tests performed at CSFB for major hardwood species.

		CSFB seed quality testing										
Category	Species	AE	KSKG	KSHL	COUP	PGNS	VGNS	PGS	VGS	PHUM	PP	RX
Orthodox	ARG, BOJ, BOP, CAS CET,ERG, ERR,											
Orthodox	FRA, FRP											
Recalcitrant	CHR, CHG											
Recalcitrant	NON											

Species	
ARG	Common Sea Buckthorn
BOJ	Yellow Birch
BOP	Paper Birch
CET	Black Cherry
ERG	Manitoba Maple
ERR	Red Maple
ERS	Sugar Maple
FRA	White Ash
FRP	Red Ash
CAS	Siberean Peashrub
CHG	Bur Oak
CHR	Red Oak
NON	Black Walnut

Tests	
AE	water activity
KSKG	Number of seeds /kg
KSHL	Number of seeds /hl
PGNS	Non-stratified germination %
VGNS	Non-stratified vigour
PGS	Stratified germination %
VGS	Stratified vigour
PHUM	Humidity %
PP	Purity %
RX	X-ray interpretation
FUNGI	*Cylindrocadium, Phoma* and *Fusarium*
COUP	cut test

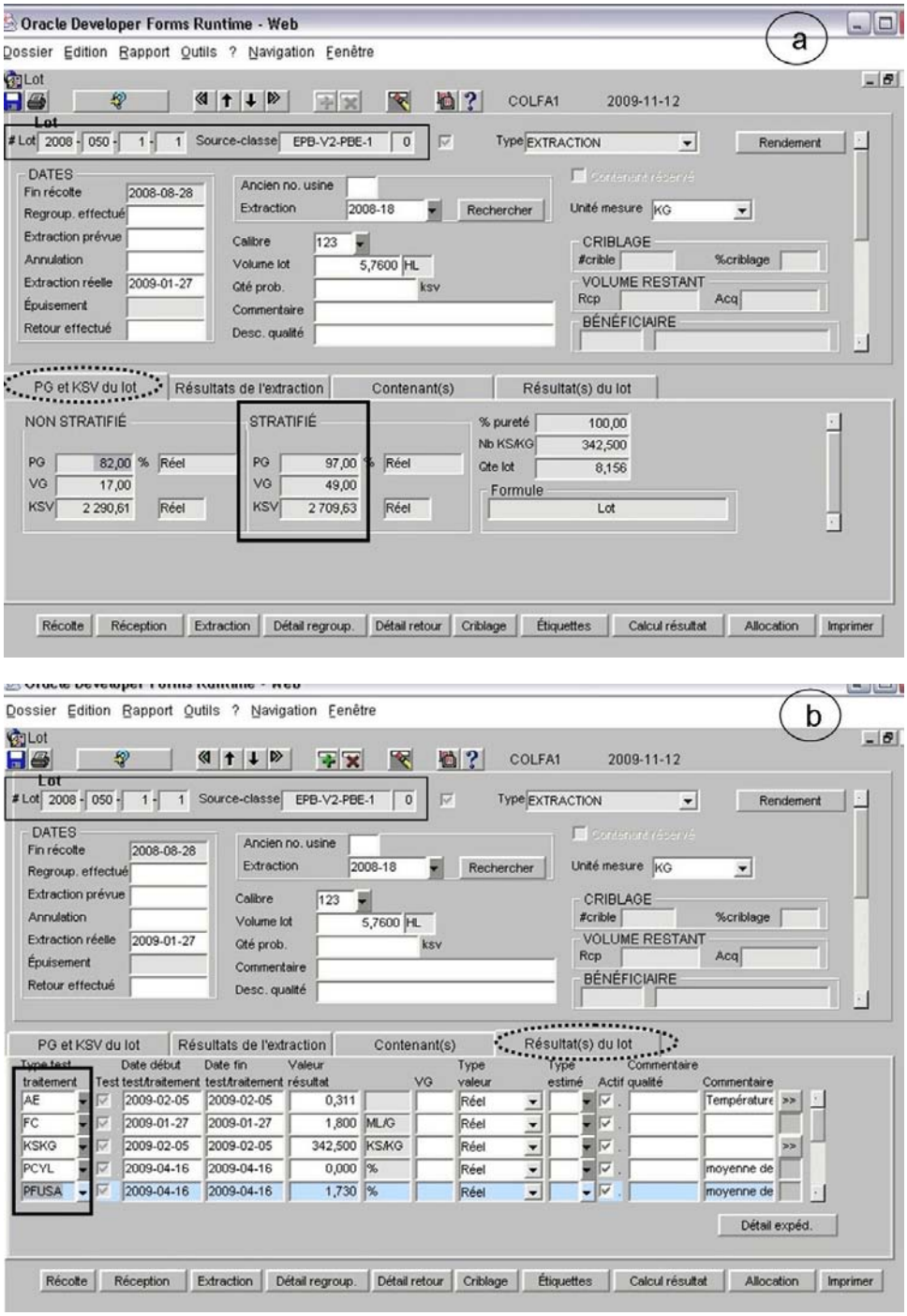


Figure 1. Screen capture of Systeme Semences; **a** - seedlot identification and germination (% and vigour) (PG) and thousands of viable seed (KSV), **b** - quality test results (see Table 1 legend for the translation).

SEED TESTING AT THE NATIONAL TREE SEED CENTRE

Seed testing is often the product of the organization doing the testing, the facilities available, and the resources available. The National Tree Seed Centre's (NTSC) mandate is twofold: to provide seed for scientific research and to store seed for genetic conservation. Consequently, seed quantities are small compared to other facilities, such as provincial or private seed centres, where stored seed is primarily used for growing seedlings for reforestation. Since most of the NTSC collections consist of a small quantity of seed (sometimes only a few grams) this has to be taken into account when testing is carried out.

Seed stored at the NTSC is collected by staff or is obtained through purchase or donation. Over the past 10 years, testing has been carried out on over 150 species of tree and shrub seed. The first test carried out on seed that comes into the lab is moisture content (MC). Two replicates of approximately 1–2 g each (for most species) are put in aluminum containers (diameter 5.0 cm) and placed in a forced draft oven at 103°C for 16 hours. The aluminum containers are stored in desiccators when they are not being used to prevent moisture uptake. MC is calculated using the formula (MC % = (Fresh Weight - Dry Weight)/Fresh Weight*100). The quantity of seed used (1–2 g) is considerably less that the 4.5 grams recommended by ISTA. This is due to the small size of most of the seedlots and not wanting to sacrifice valuable seed. The target MC for orthodox seed is 5–8 % and seed with MCs above 8 % are further dried prior to storage. MC is also checked when seed are re-tested. The average number of MC tests carried out by the NTSC over the past 10 years is 750 tests/year.

Once MC is within acceptable limits, the 1000-seed weight is determined. This is carried out by counting and weighing eight replicates of 100 seeds. When dealing with small seed (birches, poplars, willows) fewer replicates are performed. When the collected sample is small (less than 800 seeds), the total number of seed is counted, the total weight of the sample is determined, and the 1000-seed weight calculated. About 400, 1000-seed weights are carried out each year.

Germination tests are performed on all freshly collected seedlots as well as seedlots in storage that have not been tested for ten years. The recommendations for seed testing provided by ISTA and Association of Official Seed Analysts (AOSA) are used for many species and work very well. However, these recommendations do not succeed in germinating seed of some species. Experimentation has been conducted to determine the best method to use. Results from these experiments have indicated that an increase in the duration of the moist chilling treatment is often sufficient to alleviate dormancy and allow for full germination. We believe that this is due to the fact that most of our collections originate from northern provenances and we suspect that the rules were developed from tests that often used seed from sources originating from southern locations of species ranges. When more favorable germination protocols are developed they are henceforth used for seed testing and are passed along to researchers using the seed. At least 1000 germination tests are performed at the NTSC each year.

In most cases four replicates of 50 seeds are applied on moistened Versa-Pak™ in Petawawa germination boxes using a vacuum seed head (Fig. 1). When larger seed are being tested, the number of seed is usually reduced. Seed are given the appropriate treatment(s) and placed in Conviron G30 germination cabinets for 21–28 days at pre-determined temperature, photoperiod, and relative humidity. Germination conditions of 8 h light at 30°C followed by 16 h darkness at 20°C and a constant relative humidity of 85% are used for most species. The objective of our testing is to evaluate total germination of a seedlot. The first count is performed at day 14 and the final count at day 21 or 28. We are not concerned with the speed of germination which is reflected by assessing germination more frequently. A seed is considered germinated once it has achieved germination vigour class 3 class as described by Wang (1973) where the germinated seed has developed to the stage where cotyledons are visible and the hypocotyl and radicle are well developed. Seed are assessed as being: high vigour (class 3 described above), low vigour (normal germination but has not achieved vigour class 3 by the end of the germination period), and abnormal such as chlorophyll deficient, stubby root, decay, necrosis, reverse germination, etc. However, the various categories of abnormal germination are not recorded.

Viability tests (excised embryo and tetrazolium stain tests) are sometimes performed *in lieu* of standard germination tests. This usually occurs when the duration of a germination test is long as is the case with the ashes (*Fraxinus* spp.). ISTA recommends using 400 pure seeds and soaking seeds for 24–96 hours (depending on species) at temperatures not exceeding 25°C. Water should be changed twice daily to retard the growth of fungi or bacteria and the accumulation of seed exudates. We have found that soaking at 3°C for 96–120 hours works well for ash seed. After soaking, a scalpel is used to make a longitudinal excision

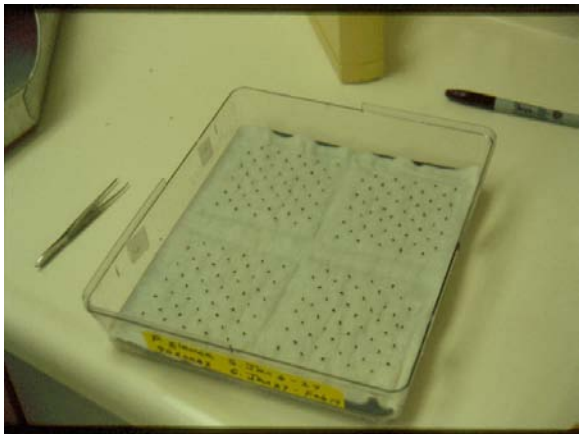


Figure 1. Seed on vacuum seed head (top) and four replicates of 50 seed each on moistened VersaPak™ (bottom).

through the seed coat and the seed is opened, the embryo removed, placed on VersaPak™, and incubated at 25°C for 14 days (Fig. 2).

Figure 2. Excision of black ash (*Fraxinus nigra*) seed using scalpel (bottom left) and red ash (*Fraxinus pensylvanica*) embryos after 14 days in germination cabinet (above).

References

AOSA 2002. Rules for testing seeds. Assoc. Off. Seed Anal. 166 p.

ISTA 2009. International rules for seed testing. Edition 2009/1. Int. Seed Test. Assoc., Basserdorf, Switzerland.

Wang 1973. Laboratory germination criteria for red pine (*Pinus resinosa* Ait.) seed. Proc. Assoc. Off. Seed Anal. 63: 94–101.

Bernard Daigle and Dale Simpson
Natural Resources Canada
Canadian Forest Service
Atlantic Forestry Centre
P.O. Box 4000
Fredericton, NB E3B 5P7
E-mail: bdaigle@nrcan.gc.ca

J.D. IRVING – PARKINDALE SEED ORCHARD

We had lots of cones on all our species at our clonal seed orchard at Parkindale. It came somewhat as a surprise as last year was fairly wet all summer. Cone collections began in the second-generation white spruce orchard on August 5 and we finished picking Norway spruce cones on October 9. The first-generation white spruce and Norway spruce cones were mostly collected using 45 foot boom lifts (Fig. 1). Some of the Norway spruce ramets were taller than the lifts could reach. The trees are just 20 years old, but we do NO top pruning. The orchard crew (2) will be busy extracting and cleaning seed for most of the winter. We are also custom seed cleaning for the P.E.I. Department of Environment, Energy and Forestry.

Cone Collection

The following quantities were collected:
White spruce, 1st generation (15 000 L); 365 000+ L left on the trees
White spruce, 2nd generation (1100 L); all cones picked
Red spruce, "southern clones" (4000 L); all cones picked
White pine (3600 L); all cones picked
Black spruce, 2nd generation (1800 L); 29 000+ L left on the trees
Norway spruce (38 000 L); picked 99%.

Seed Testing

We test all our freshly extracted and cleaned seed in January to March as soon as it arrives at the Sussex Tree Nursery. Stored seed is tested every 2 or 3 years, depending on age and possible use in the test year. We store approximately 1400 kg of seed at -15°C at the nursery in Sussex. The oldest seed we have in storage was collected in 1965.

Germination testing is done in a Conviron germinator. All seed is tested at 25°C for 21 or 28 days, depending on species. Light is provided for 18 hours/day. The following testing procedures are used. A sample of seed is taken from the storage container (fresh or stored). A total of 400 seed is tested from each seed container (could be more than one container with the same seed lot number). Four reps of 100 seed each are placed in four separate Petawawa germination boxes. A vacuum head is used to pick up 100 seed from the sample lot and place them in a 10x10 pattern on the germination paper in the box. Each of the reps

Figure 1. Collecting Norway spruce cones using a lift.

in a box is from a different seedlot. Water is added to the bottom of each germination box, after the four reps have been placed. The boxes are placed in the Conviron cabinet, making sure that each replicate is on a different shelf. All seedlots are checked for germination and water is added, as needed, once per week. Seed germination is recorded, on a tally sheet, each time it is checked. The results are totaled when the test is completed.

Germination of fresh seed averages: Black spruce – 98%, purity of 99.99%; white spruce – 95%, purity of 99.89%; red spruce – 98%, purity of 99.88%; Norway spruce – 99%, purity of 99.99%; jack pine – 98%, purity of 99.98%, and white pine – 86% (fresh) and 95% (moist chilled), purity of 99.95%.

We had some of our seed IDS tested at SKOGFORSK, in Sweden, in 2009. The quality of the seedlots, in most cases, could not be improved with the IDS treatment. White pine was the only seed with IDS improvement potential.

Hart Kunze
J.D. Irving, Limited
Sussex Tree Nursery
181 Aiton Road
Sussex, NB 4G 2V5
E-mail: Kunze.Hartmut@jdirving.com

SCHEDULING GERMINATION TESTS

Over the last four years the scheduling of germination tests at the BC Ministry of Forests Tree Seed Centre (TSC) has evolved in leaps and bounds. Gone are the days of germination counts so heavy that staff could not complete them in one day, tests scheduled for completion on holidays, and being understaffed to deal with the workload. These are now a thing of the past.

Testing staff at the TSC complete approximately 200 germination tests per month. In addition to the test preparation and germination counts, staff are also performing: seed moisture contents, cone moisture contents, seedlot purities, seed weight tests, seed x-rays, and seed withdrawals. Germination counts are done on Monday, Wednesday, and Friday and staff can perform 2300 counts per person per day. The various activities have led to a routine of specific tests and activities being performed in combination or alone on specific days of the week. For example: Tuesday - G31, G20, G10 are initiated, and seed withdrawals are performed; Wednesday - G32, G34, G64 tests are initiated and germination counts are performed. For a list of TSC germination test codes see http://www.for.gov.bc.ca/hti/treeseedcentre/tsc/tst1.htm page 46 and Appendix 1 for species codes. The combination of tests alone can mean that over 500 tests are completed in a month. All of these activities have led to the development of specific tools and steps to manage and create stable lab workloads.

When scheduling germination tests one must always be looking to the future. There are many different test types involved that have different stratification durations, soak times, and test durations. It is because of this that two specific tools were developed to support the method of scheduling tests. Scheduling of germination tests at the TSC has four main components.

1) Choosing the timeframe (usually a week).
2) Consulting the 'Do Not Start List' (an Excel spreadsheet).
3) The 'Germination Prediction Tool', a part of the Cone and Seed Processing System (CONSEP, the data management system used by the TSC. For more details on CONSEP please refer to TSWG News Bulletin 43, June 2006).
4) The availability of resources. This involves the use of a calendar indicating holidays, staff vacations, and scheduled flex leave days.

Timeframe

For planning purposes we begin with a particular block of time. This is usually a week but could be as short as a day.

Do Not Start List

The purpose of this tool is to ensure the best possible standard testing results. The output (MS Excel spreadsheet) provides a list of days when a specific test regime should not be started by identifying those days on which performing specific activities may cause a conflict. If a test type appears on this list on a day within the time frame one may find the test completing or going into a germinator on a statutory holiday. It sounds complex but read on and we will show how to use the tool based on the data from Fig. 1.

Input data Data entered include Table of Future Statutory Holidays and Testing Activity Steps that require staff to be present, expressed in days from the start of the activity. Examples: Move seed into germinator, last germination count day, move seed from warm stratification to cold stratification, testing dryback activity.

Methodology The tool scans the table of specific statutory holidays (input data 1) and subtracts the number of days for each testing activity step that requires staff to be present (input data 2). The output includes the Test Type Regime not to be started and the Activity Step that is in conflict. The result of this calculation is output as a Microsoft Excel dataset. Here is an example of the Do Not Start List calculation, based on Row 1 output data listed in Fig. 1: *"News Years day 2010"* minus *"30 Do_Not_Start_Days_Ahead"* = *"December2 2009, Into Germ G32 G34 - Into Cold Strat G52 - Dryback G64"*

A	B	C	D	E	F
Do-Not-Start-Day	Day_of_Week	REASON	Stat_Holiday	Stat_Date	Do_Not_Start_Days_Ahead
02-Dec-09	Wed	Into Germ G32 G34 Into Cold Strat G52 Dryback G64	New_Years_Day	01-Jan-10	30
03-Dec-09	Thu	End W1 Into Germ G10	Christmas_Day	25-Dec-09	22
03-Dec-09	Thu	Into Germ G20 G31	New_Years_Day	01-Jan-10	29

Figure 1. Do Not Start List, example output data.

Here is how we read the *Do Not Start List* output using the example data in Fig. 1:
1) Row 1: On Wednesday, December 2, 2009 do not start Test types G32 and G34 because they need to be placed into the germinator on New Years Day, 2010.
2) Row 1: On Wednesday, December 2, 2009 do not start Test type G52 because a move to cold stratification is needed to be performed on New Years Day, 2010.
3) Row 1: On Wednesday, December 2, 2009 do not start Test type G64 because a dryback procedure is needed on New Years Day, 2010.
4) Row 2: On Thursday, December 3, 2009 do not start Test type W1 because the last count day will be Christmas Day, 2009.
5) Row 2: On Thursday, December 3, 2009 do not start Test type G10 because a move into the germinator will be on Christmas Day, 2009.

This tool can easily be adapted to include any future days where staffing or timing will result in limited resources being available to complete those activities that should be completed on a specific schedule.

Germination Prediction Tool

The purpose of this tool is to provide an estimate of the expected daily workload by predicting future daily seed germination counts based on active and proposed tests. The tool also provides a "what if" function, allowing us to see how future germination counts would be affected by different testing start dates and workloads. This tool is one of the components of CONSEP and has two parts or screens. The first part is where the "what if" dates, species, test type, and number of tests are entered (Fig. 2) and the second part involves calculating and reporting the germination predictions.

Sample Set Note: Soak is Day 0 for What If

	Date:	Species:	Test Type:	Day Number:	Test Count:
	2009-12-03	PLI	G20	0	10
	2009-12-03	FDI	G10	0	10
	2009-12-03	HW	G31	0	5
▶					0

Figure 2. Germination prediction tool what if data entry screen.

Input data All germination tests currently in soak, stratification, or being counted plus all "what if" tests are entered in the tool. Ten-year average daily germination count data for each species, test type regime, and count day combination are entered.

Methodology For a future count day we match and count all Germination Tests in Progress (and the "what if" tests) to the Test Regime and Species combination of the 10 year-daily germination count average and multiply by the number of Germination Tests that match the above criteria. The tool also adjusts the prediction counts for test categories where the number of seeds is not standard.

Figure 3 is an example of the output from the Germination Prediction Tool. As you can see this screen lists the "what if" tests on the top and count predictions on the bottom. The Expected Count column provides the total number of normal germinated seeds that are expected to be counted. The other count columns are included in the total but are showing the counts for specific species or test categories that can affect the lab workload.

RUN TIME: 3:38:00 PM **Germ Count Prediction Results** USERID :

DATABASE:

What If

Start Date	Species	TestType	Day Number	Activity Count
2009-12-03	FDI	G10	0	10
2009-12-03	HW	G31	0	5
2009-12-03	PLI	G20	0	10

Weekday	Count Date	Expected Count	TRL Count	PLI Count	Non PLI Count	QA Count Non PLI
Wed	2009-12-02	5933	0	2522	3411	0
Fri	2009-12-04	5689	0	1940	3749	0
Mon	2009-12-07	3135	0	604	2531	0

Figure 3. Germination prediction tool, example output report.

Available Resources

With the Germination Count Predictions generated, the final step is to compare the expected count numbers to a staff calendar to compare resources. This includes the number of staff, the predicted number of germinants to be counted, and the other activities one may need to perform that day. If the number of counts per person is too high (more than 2300 per person) the number of tests may be changed or one may decide not to initiate a particular test type. The reverse could be done if the predicted counts per person are too low. In both cases one simply changes the activity count or deletes it altogether and regenerates the predictions.

Scheduling germination tests using this method and the tools that have been developed has minimized surprises, allowed for resource levelling, and better enabled task diversification to limit repetitive strain actions. Germination tests complete on time and in conflicts due to holidays (and resultant test date variability) have been minimized. The tasks in the TSC lab now flow with minimal conflicts. This method and the tools that have been developed have taken the guess work out of scheduling germination tests.

Laura Klade and Michael Postma
BC Ministry of Forests and Range
Tree Seed Centre
1879 - 32 Avenue
Surrey, BC
V3S 0L5
E-mail: Michael.postma@gov.bc.ca

WATER ACTIVITY -- AN EFFICIENT TOOL FOR SEED TESTING

Water activity (a_w) is a non-destructive, portable, and rapid technique currently used to assess the moisture of hygroscopic materials (e.g., agro-food, pharmaceuticals) and particularly, the one of intermediate products such as orthodox seeds (Baldet et al. 2007). This technique, as well as "equilibrium Relative Humidity" (eRH), is a reliable moisture indicator because it is directly related to the water potential (Ψ), which indicates the energy status of water in hygroscopic matrices. While gravimetric moisture content (GMC) quantifies only the total amount of water in a product, a_w qualifies the intensity of the bonds between water and other molecules (e.g., lipids, carbohydrates, or proteins) and therefore, it illustrates the availability and mobility of water

within a substance. Water activity has three main advantages: 1) this technique is rapid (less than 20 min per sample), 2) non-destructive (very useful for small samples of great value such as seeds for *ex situ* genetic conservation), and 3) easy to use (minimal training required).

Sorption isotherms to characterize the species

The Cemagref, a research centre in France, has already demonstrated the efficiency of a_w, and it is currently applied to the moisture management of major temperate forest orthodox seeds in French tree seed centres. Since 2007, in cooperation with the Cemagref, the ministère des Ressources naturelles et de la Faune du Québec decided to verify if a_w is also efficient with orthodox boreal species. In order to test the a_w efficiency with these species, we started to build sorption isotherms.

A sorption isotherm shows the resulting moisture content of a sample when exposed to different eRH or a_w values. It permits one to describe the moisture behaviour of a seedlot and to determine numerous thermodynamic parameters and particularly, the optimal a_w value for seed storage. This value is obtained from the inflexion point of the 3rd degree regression of isotherm data. More than 100 sorption isotherms were carried out and interpreted for a total of 9 orthodox species: *Abies balsamea, Fagus sylvatica, Fraxinus exelsior*, *Picea glauca, Picea mariana*, *Pinus banksiana*, *Pinus contorta, Pseudotsuga mensiezii*, and *Thuya plicata*. From 5 to 20 seedlots of different ages and provenances were used for these selected species. See Fig. 1 for the results obtained from *P. banksiana*. The seedlots were harvested from 1984 to 2006. Their germination capacity ranged from 78 to 94%. The estimated a_w of best stability is 0.38. The resulting GMC varies approximately from 6.5 to 8 %. The isotherm curves are separated, indicating significant intra-specific variation with different resulting GMCs for a given a_w. However they are parallel and this indicates a similar behaviour of water thermodynamics in these seedlots.

All our results follow the same pattern as those obtained in the past in France. Water activity of best stability is determined for each species and this value will be used to optimize seed processing in tree seed centres. It appears that every optimal storage value obtained for each orthodox species, always amounted to an a_w value of 0.35 (-140 MPa water potential). Consequently, we suggest that this a_w value (0.35) could be used as a universal target for moisture management of diverse orthodox reproductive material in gene banks where it is not operationally possible to determine

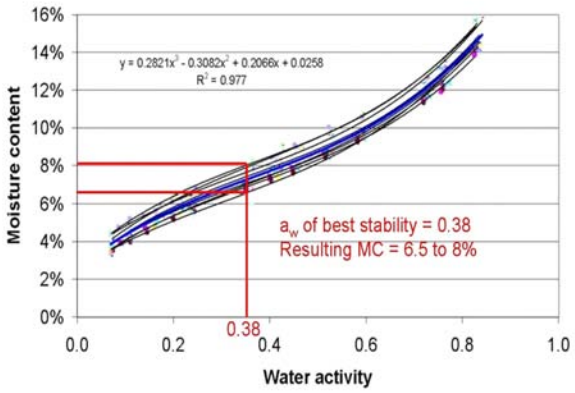


Figure 1. Sorption isotherms obtained with *Pinus banksiana* (jack pine) after 20 seedlots analysis.

optimal GMC prior to mid or long-term storage due to sample sizes or scarcity of seeds. This part of our work will be presented during the 29th Seed Symposium at the next ISTA Congress in Cologne, Germany (16–20 June 2010).

Water Activity is a Good Indicator for Seed Quality Control

Since 2007, a_w tests have been performed at the Berthier Tree Seed Centre (TSC) (see Brault et al., page 7 in this bulletin). In order to determine the optimal frequency of viability determination for the seedlots at the Berthier TSC, we have identified seedlots for which germination capacity was evaluated each year. Therefore, each container was opened once a year for sampling. The integration of a_w measurements allowed us to rapidly reveal the critical elevated a_w values on seedlots and, thereafter, we decided to evaluate GMC and observed a subsequent increase of GMC. We concluded that repeated opening of the seed containers can have a significant impact on the seed moisture content. Up to now, this moisture increase did not reduce germination capacity of the seedlots, but on a long-term basis, this could change. Water content is a good, non-destructive tool to monitor long-term seed storage.

A Seed or Pollen Dryer Monitored with Water Activity

We recently noticed that some a_w variation can occur during the storage of seeds, due to exogenous or maybe endogenous factors. So, how to deal with this? Is it better to do nothing or to plan an adjustment of a_w for the seedlots? Since the

seed samples are precious, there is no place for any mistake.

The Cemagref has developed a pollen or seed dryer that is monitored with a_w (Fig. 2). The pollen or seed dryer runs at room temperature on the basis of the "two pressure" saturation principle. First, compressed air is saturated with humidity in a tank under controlled pressure and thereafter, this saturated air is released at the desired pressure in order to induce a constant targeted value of a_w, which depends on the ratio between these two pressures. The samples are dried at a non-stressing room temperature without applying any thermal energy. A technical note will be published soon and it will allow you to find more details on how it works and how to build your own "a_w dryer". With this dryer, seed (or pollen) can be adjusted to the desired a_w without applying any heat. The concomitant use of a_w measurements and a_w dryer is a very useful tool combination to monitor and optimize the seed moisture status for long term conservation purposes.

Figure 2. Seed and/or pollen dryer monitored with a_w. 1- air pressure distribution, 2- saturation tank, 3- cabinet for seed or pollen equilibration.

Conclusion

Genetic diversity of forest reproductive materials, in combination with environmental effects, induces significant intra-specific phenotypic diversity of materials like pollen and seedlots. Thus, it becomes very difficult to predict the moisture behaviour of forest reproductive material and it complicates management procedures. Water activity assessment appears to be a reliable moisture monitoring technique for materials having high phenotypic variability. This results, for a given species, in reproducible moisture management procedures based on single recommended a_w values. Therefore, this a_w technique will be a useful tool for improving diversity conservation.

For those interested in water activity and its application in seed management and conservation, an ISTA workshop, dedicated to water activity will be hosted by the Cemagref in Montargis, France during the autumn of 2010.

Acknowledgements

A special thank to our colleague Jean Gagnon (DRF) for his revision and improvement of the text. Seeds of *P. contorta* and *T. plicata* were kindly provided by Dave Kolotelo

References

Baldet, P. 2006. Fiches de recommandations pour la technologie du pollen. Pages 371–396. *In* Reproduction sexuée des conifères et production de semences en vergers à graines. Quae, collection Synthèses, Paris, France.

Baldet, P.; Colas, F.; Bettez, M. 2007. Water activity: a new paradigm for seed and pollen management. Can. Tree Imp. Assoc., Tree Seed Work. Group Newsbull. 46:10–12.

Patrick Baldet
Cemagref
Unité de recherche Écosystèmes forestiers
Domaine des Barres
45290 Nogent-sur-Vernisson, France
E-mail: patrick.baldet@cemagref.fr

Fabienne Colas
Ministère des Ressources naturelles et de la Faune (MRNF)
Direction de la recherche forestière
2700 rue Einstein
Québec, QC G1P 3W8

Michèle Bettez
MRNF
Direction générale des pépinières et des stations piscicoles
Centre de semences forestières de Berthier
1690, chemin Grande Côte
Berthier, QC J0K 1A0

QUICK TESTS

Most people generally think of estimating the ability of seedlots to produce plants through germination tests, but a variety of quick tests are available. Are they useful and for what? Here is my perspective. If you have additional information, please consider submitting an article for our next News Bulletin.

It should be clear that germination tests provide an estimate of germinability using a specific dormancy-breaking pretreatment and specific germination conditions. Quick tests on the other hand estimate viability, or proportion of living seeds, in a sample independant of the pretreatment or environmental conditions provided. The viability will always be higher or equal to the germinability of a seedlot. The difference is generally attributed to dormancy – seeds that are viable, but cannot germinate with the pretreatment and conditions provided. The advantage of quick tests is obviously the reduced time needed to get results, but they also provide an estimate of the maximum potential germination of a seedlot. The disadvantage is that actual nursery germination may differ greatly due to dormancy (and how well our pretreatments overcome dormancy) and the environmental conditions of the nursery.

I will discuss four quick test methods in this article: Hydrogen peroxide, Tetrazolium, X-ray, and Cutting tests. At our facility we have some, but limited experience with the first two, use x-rays as more of a documentation of seed anatomy, and cannot imagine working efficiently at our facility without cutting tests. The first three techniques (plus a modification of the x-ray test) are well described with many references in a publication from Dr. Carole Leadem "Quick Tests for Tree Seed Viability" from 1984 that can be found and downloaded at:
http://www.for.gov.bc.ca/hfd/pubs/Docs/Mr/Lmr018.htm
I won't review the methodology, but will try and summarize some of the highlights of the methods in the Table below and provide some comments on each method.

Table 1. Comparison of five methods to evaluate seed viability.

Method	Time	Seed cutting method	Chemicals required
Hydrogen Peroxide	7 days	Clipping at radical end avoiding radicle	Hydrogen Peroxide (1%)
Tetrazolium	8 to 24 hours	Cut seed parallel to embryo	Tetrazolium Chloride (1%)
X-ray	Instantaneous to weeks	NONE	NONE
X-Ray Contrast	Instantaneous to weeks	NONE	Trichloromethane (Chloroform)
Cutting	Instantaneous to days	Bisect seed in two equal parts longitudinally	NONE

Hydrogen Peroxide

The hydrogen peroxide (H_2O_2) test is relatively simple to perform and assess, relatively quick, and is the only test that actually measures growth to estimate viability. As with all of the quick tests requiring some form of seed cutting, the smaller the seed the more challenging it is to perform the cut properly. This is even more important with the H_2O_2 test as any damage to the radical can impact its growth and the reliability of the estimate. In Leadem (1984), references are provided on the observation that hydrogen peroxide can substitute for stratification and the explanation generally offered is that this is due to the resulting increased oxygen concentration. I am unaware of recent studies substantiating this and although some BC nurseries use hydrogen peroxide as a sterilant of surface borne contaminants (most prominently *Fusarium* spp.), there really isn't a clean comparison between unstratified + H_2O_2 treated seed and stratified seed. It may be a useful tool (last resort) when there is no time for stratification, but in general it would be a much more expensive treatment than cold stratification.

Tetrazolium

The Tetrazolium (TZ) test is probably the most well known quick test and is used extensively for a wide variety of agronomic crops, especially grasses. For species with deep dormancy (requiring long pretreatments) the TZ test is often

used in seed valuation in Europe. There is also general support for this method as both AOSA and ISTA have guidebooks dedicated to TZ testing, although emphasis is on agronomic crops. Test methodology is relatively simple, but it is important that the seed is cut parallel to the embryo so that staining intensity is related to embryo 'health ' and not the distance the stain must travel. Interpretation can be difficult due to improper cuts, difficulty in classifying partially stained embryos, and the complication of recently damaged seeds showing greater staining intensity (Leadem 1984).

I don't see TZ testing as a very useful predictive tool to estimate viability given the time required, variability exhibited , subjectivity in interpreting the partially stained embryos, and the fact that the condition of the nutritive tissue is totally ignored. I do think that it can play a key role in providing information of a general nature as to whether seeds are alive (viable) or dead due to some type of insult. An actual example relates to some hemlock seed that was stratifying in a fridge at a nursery. The fridge 'failed' and the imbibed seeds were exposed to subfreezing temperatures of -7°C. The question was are the seed still viable or do I need to stratify a new quantity of seed? A great use for TZ testing, and yes the seed were still viable and able to produce a crop. Noland and Mohammed (1997) advocated the use of fluorescein diacetate as an alternate viability stain for tree roots and seeds.

X-Ray

At our facility, x-rays are used as a means of documenting seed anatomy when seedlots receive their initial suite of tests prior to being placed into long-term storage. We x-ray the fourth germination replicate (100 seeds) as a standard part of new seedlot testing. We do not classify individual seeds, but use the x-ray as a possible means of explaining poor seedlot performance and assessing the probability of upgrading the seedlot using more modern processing methods than were originally used.

Characteristics that are easily seen and considered useful are extent of mechanical damage (indicating PRE-VAC may be an efficient upgrading technique), proportion of insect-filled seeds (most commonly *Megastigmus* spp.), proportion and degree of embryo immaturity, and proportion of empty seeds remaining. I have not used any contrast agents and cannot comment on their operational use (see Kim Creasy's article, page 20 of this bulletin). The one limitation of x-ray analysis is that the properties of imbibed seeds are more difficult to assess and these tests are best performed on dry (<10% moisture content) seeds.

Cutting

I saved the best for last – cutting tests. We don't routinely use these as a final seedlot test reported to clients, but they play a key role in pre-collection evaluations (to assist in quantifying embryo maturity and megagametophyte condition), evaluations on cone receipt (to identify problems and provide a 'ballpark' estimate of viable seed yield), at seed extraction (to confirm viable seed are not retained in cones before discarding), and during various processing phases (to ensure viable seed are not being discarded). In final cleaning, cutting tests are the main criteria used to determine which seed fractions are retained, discarded or should be considered as another seedlot with reduced viability. The advantage of cutting tests is that results are available instantaneously following seed bisection and decisions can be made immediately or information forwarded to clients. The disadvantage is that it is difficult to produce a rigorous set of interpretative instructions or a photo manual due to the large number of species and conditions in which the test is performed. At our facility we often use the phrase 'Art and Science" in relation to cone and seed processing and this is an appropriate description of cutting test methodology. The relevant variables in a cut test are discussed below.

Several items in a cutting test can vary depending on the seed development phase, moisture content, proportion of questionable seed, and any *a priori* information regarding collection or post-collection handling. Cutting tests can be viewed as having a supporting and decision-making role rather than a quantitative one in seedlot construction. Sample size can be adjusted in relation to the specific situation, but a minimum sample at final cleaning is 5 replicates of ten seeds. Sample sizes can be increased if unusual characteristics are seen or there is a larger occurrence of immaturity, megametophyte discolouration or deterioration. The classification and any photo guide can be difficult as the observed characteristics can be strongly influenced by moisture content of the material, ability to accurately bisect the seed, magnification, and even type of light source used. Cutting tests can be performed in the field using a 10X to 15X hand lens, but best performed under a dissecting microscope with a ring light that can provide magnification up to 30X.

Any seed bisection provides some information on the internal contents of a seed, but a longitudinal section through the most narrow dimension provides the most embryo and megagametophyte tissue to base your classification upon. If the product appears to have a high proportion of viable seed (or obviously deteriorated seeds) then cutting tests based on dry seed (<10% moisture content) may suffice, but to identify more subtle forms of deterioration we generally consider imbibed cutting tests to be superior. Dry seeds are more difficult to cut, but allow decisions to be made immediately. Duration of imbibition can vary by species or sample condition with some samples requiring 48 or more hours.

A simplified version of a cutting test flowchart can be found on page 8 of the Seed Handling Guidebook (http://www.for.gov.bc.ca/hti/publications/misc/seed_handling_guidebook_hi.pdf) indicating one of many possible roadmaps in characterizing bisected seeds. Cutting tests are similar to other viability tests in that it is generally easy to identify viable seeds and non-viable seeds, but the seeds in the grey-zone in between can be extremely difficult to classify. A simplification of the individual seed assessments would be a quick assignment of rotten (or severely deteriorated) seeds, immature seeds, and empty seeds to their respective categories – that's the easy part. The remaining classification is based on the appearance of the embryo in terms of consistency and colour (which can be quite variable between and within species) and the megagametophyte consistency and colour which should be firm and light cream to white in imbibed seed. Moisture content plays a key role in assessing colour differences and is probably the largest obstacle to standardizing the tests. An example would be that the colour and degree of shrinkage in viable dry seeds would be indications of a problem if seen in an imbibed seed.

A further optional step in confirming viability estimates is to put the cut seeds into a covered germination dish for several days to observe if any photrophic behaviour such as greening of the embryo or physical bending toward light occurs. These have been referred to as incubation tests, although even less documentation is available regarding these compared to cutting tests. They are an additional tool that can be incorporated into the quick test to try and maximize information obtained.

All of these viability tests require some amount of calibration to make them useful for tree seeds which generally have higher levels of variability compared to other plants. These quick viability tests are extremely valuable in making decisions from when to collect to what fractions to combine in a seedlot. I'm still convinced that even with its non-standardized shortcomings the cutting test is still the most useful viability estimation tool for efficient operational decision-making. This review has made me re-consider the usage of some of these other viability tests and that we should have a certain degree of competence in all of them as they can be useful additions to our suite of diagnostic tools. I'm very curious how other facilities approach estimating viability under operational conditions and hope others will contribute on this subject in the future. No one said excellence was going to be easy!

References

Leadem, C.L. 1984. Quick tests for tree seed viability. BC Min. For., Res. Br., Land Manage. Hndbk. No. 18. 45 p.

Noland, T.L.; Mohammed, G.H. 1997. Fluoroscein diacetate as a viability stain for tree roots and seeds. New For. 14:221–232.

Dave Kolotelo

QUICK TESTING USING VAPOROUS CONTRAST AGENTS, Chloroform - $CHCL_3$

Vaporous Contrast testing by no means replaces direct germination testing. This kind of quick testing provides us with immediate answers without the wait. It's a tool one can utilize in targeting a concern of unusually low germination in a seedlot or when germination estimates are required ahead of the actual time consuming completion of a direct germination test.

To facilitate quick delivery of test results for international trade, ISTA has, since 1974, adopted a general directive that seeds of any species requiring more than an 8-week germination test period should be tested by alternative methods. With the current trend of increasing demand for seed testing and decreasing resources, rapid tests for tree seed viability are promising (Bhodthipuks 1994).

Many rapid testing methods have been developed and widely used for temperate zone species and some of them, such as tetrazolium staining (TZ), excised embryo, and x-radiography tests, have

been incorporated into the current international rules (Bhodthipuks 1994).

Two important restrictions have to be remembered when using aqueous agents or vaporous contrast agents in seed testing:
1) There is no universal contrast agent - as yet - that can be used successfully in all species for the prediction of the germinability of seeds (Simak 1980).
2) A contrast agent does not necessarily reveal all kinds of physiological damage to a seed. Consequently the contrast agent medium acts in a seed sample selectively, depending on the cause of death (Simak 1980).

The biological basis for using a vaporous contrast agent such as $CHCL_3$, is that it "highlights" damaged tissues, but is unable to pass through the semi-permeable membranes of healthy living cells. This vaporous contrast agent, $CHCL_3$, absorbs the radiation and accumulates in damaged tissues, causing an increased contrast in the radiographic image - a highlight in the damaged area of the individual seed (Bhodthipuks 1994).

X-radiography and more specifically X-ray contrast (XC) was a very useful tool used in searching out a method to detect the instance of mechanical damage in the processing of jack pine (*Pinus banksiana*) seed in early 1990 at the Ontario Tree Seed Plant, after a major facility retrofit. Gary Scheer and Ben Wang, Canadian Forest Service, provided the technical direction and laboratory facilities to implement and complete the project work.

Protocol method

This is the actual set-up procedure for administering the vaporous contrast agent, Chloroform ($CHCL_3$). All work should be done under a fume hood when using $CHCL_3$.
- 100 seeds randomly sampled are collected from specific sample points throughout the extraction/processing system,
- dry seed samples, <12% moisture content, are placed in 100 ml beakers,
- 250 ml beaker, half filled with cotton batten, is inverted to cover the 100 ml beaker,
- 15 ml of $CHCL_3$ are uniformly placed on the cotton batten just prior to inverting the larger beaker over the smaller beaker. Ensure CHCL3 has been absorbed by the cotton batten so no dripping occurs,
- in the closed beaker configuration, the heavy vapours of the $CHCL_3$ settle downward to the bottom and settle uniformly over the seeds,
- the beakers are left in this configuration for 2½ hours, then x-ray radiographed,
- after the treatment the seed should be x-ray radiographed within 1 to 2 hours as the $CHCL_3$ disappears from the seeds after about 6 hours (Simak 1980).

X-Ray Method

Ready pack II Kodak Industrex M film is used with a film exposure of 3ma, 15kv for 80 sec. Ensure the seeds are spread evenly over the film to simplify the visual evaluations.

X-Ray Evaluation

The x-ray radiographs of the seeds were evaluated for mechanical damage according to their appearance on the film. Seeds that are circled (Figs. 1 and 2), appearing bright, are damaged. The seeds were classified into two simple categories - good and deteriorated. Deteriorated being damaged seeds highlighted by the $CHCL_3$ vapor.

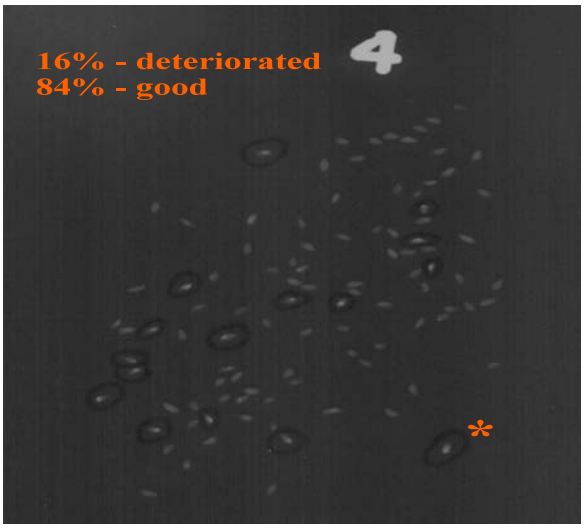


Figure 1. X-Ray of $CHCL_3$ contrast; damaged seed circled

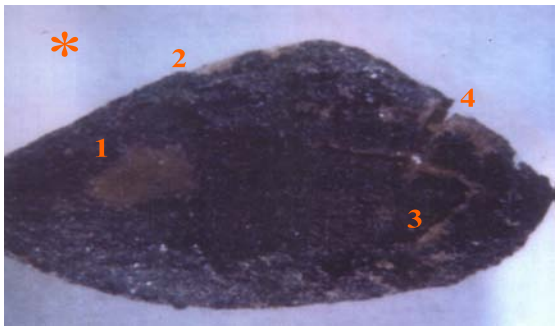


Figure 2. * Highlighted seed; 1) abrasion; 2) chip; 3) crack; 4) seed breakage

Project Conclusions

The study concluded that the poor seed germination was due to extensive mechanical damage, occurring during the extraction phase of the overall cleaning process.

The chloroform tests revealed vital results and information, which isolated and linked the seed damage to the new kiln design.

The study also established that minor mechanical damage caused by equipment used for seed cleaning and transport, had virtually no impact and could not be identified as a contributing factor in the abnormally low germination of jack pine seed (Creasey 1991).

Summary

The project work simply summarized for this note, was, at the time, very intensive and methodically conducted due to the magnitude of its impact. The collaboration with colleagues and the co-operation between other seed and research centres, especially the folks at Canadian Forest Service, Ontario Forest Research Institute, and Smoky Lake, Alberta enabled corrective measures to be administered and implemented.

The work done with the vapour contrast $CHCL_3$ technology is an easy procedure that enables mechanically damaged seed to be directly identified, whether it be for a large or small scale operation. It's a timeless technology and something not to be forgotten about or over looked. Reinventing the wheel is not necessary.

Table 1 demonstrates the difference between $CHCL_3$ results and germination testing. Vapour contrast technology highlights "numerous types" of seed coat damage including abrasions, cracks, chips, and seed breakage. Some damage will have an immediate impact on germination (cracks, chips, and seed breakage). Other damage highlighted, ie, abrasions, will probably have repercussions over time relating to germination capacity and vigour.

Table 1. Comparison of chloroform and germination test results

$CHCL_3$ test	Germination test
19.2%	84%
20.9%	88%
22.3%	83%
28.0%	93%
34.0%	85%

Visual assessment of tested $CHCL_3$ samples could more specifically categorize the seed/seedcoat damage, thus making $CHCL_3$ results more comparable to direct germination testing. See Fig. 3. In the damage assessment categories, seed coat abrasion can be the most ambiguous relation to germination capacity. The $CHCL_3$ contrasts abrasion due to tissue damage.

New technology always keeps us on our toes and we need to be ever mindful to maybe, just maybe, expect the unexpected. It's not necessarily about what has happened but instead our response to what happens next. Hopefully this note has provided some insight regarding a solution to an unexpected chain of events.

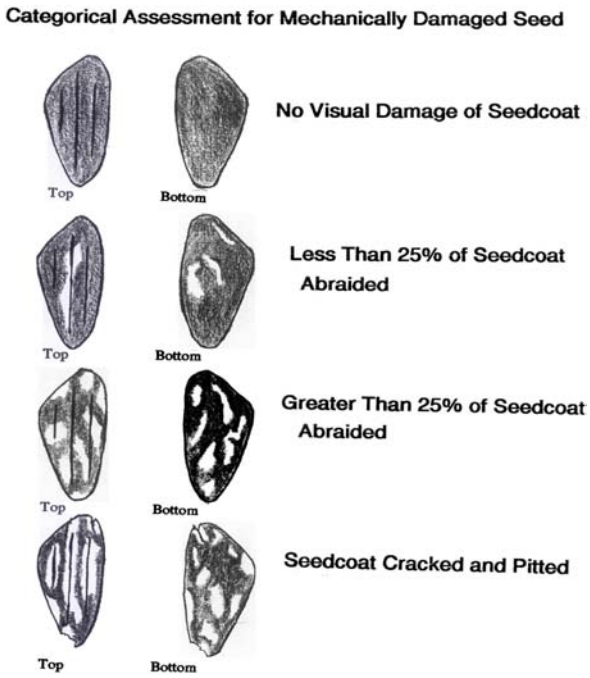


Figure 3. Seed coat damage assessment for seed exposed to $CHCL_3$.

References

Bhodthipuks J.; Pukittayacamee P.; Saelim S.; Wang B.S.P.; Yu S.L. 1994. Rapid viability testing of tropical tree seed. Asean Forest Tree Centre Project, Training Course Proceedings No.4.

Creasey, K.R. 1995. Jack pine study history. Ont. Min. Nat. Res.

Simak, M. 1980. X-Radiography in research and testing of forest tree seeds. Sver. Lant., Instit. for Skogs., Rap. NR 3. Swedish Univ. Agric. Sci., Dep. Silviculture, Umea, Sweden.

Kim and Wendy Creasey
Nature's Common Elements
P.O. Box 29003
Barrie, ON L4N 7W7
Tel: (705) 323-9098
E-mail: nces@look.ca

APPLICABILITY OF THE ACCELERATED AGEING TECHNIQUE IN PREDICTING STORABILITY OF LODGEPOLE PINE (*Pinus contorta* var. *latifolia* Engelm.) SEED

Introduction

Seed ageing is a natural, irreversible metabolic process. The rate of ageing, however, depends on the inherent seed longevity of the species, seed quality prior to storage, and the influence of the storage environment. One method for predicting relative seed storability of agricultural crop seeds, the accelerated ageing technique, was developed by Delouche and Baskin (1973). This technique has been used widely and has been standardized as a method for vigour testing in the handbooks of both the ISTA (Hampton and TeKrony 1995) and Association of Official Seed Analysts.

Accelerated ageing has been used for evaluating tree seed storage potential for a number of species: jack pine (*Pinus banksiana)* and red oak (*Quercus rubra*) (Pitel 1982); longleaf pine (*P. palustris*) and slash pine (*P. elliottii*) (Branche et al. 1988); white spruce (*Picea glauca*) and lodgepole pine (*P. contorta* var. *latifolia*) (Wang et al. 1990); Sitka spruce (*P. sitchensis*) (Chaisrisri et al. 1993); and Norway spruce (*P. abies*) (Tomaszewski et al. 2000). Results from these applications seem to be reasonable but there has been no proof to confirm whether the technique is reliable. In the mid-1980s the National Tree Seed Centre, then located at the Petawawa National Forestry Institute, Chalk River, Ontario was requested to evaluate the storability of 22 reference seedlots of lodgepole pine collected in Alberta. Results of the accelerated ageing technique were presented in a poster displayed at the 22nd meeting of the Canadian Tree Improvement Association in Edmonton, Alberta in 1989 (Wang et al. 1990).

Fortunately, there is an opportunity to compare the results from the accelerated ageing tests with the seedlots which have been stored at -80°C and germination tested periodically for 24 years from 1981 to 2005. It is a rare occasion to confirm the validity of the accelerated ageing technique for predicting the storage potential of tree seed. The results of the two accelerated ageing durations used in 1989 were compared to germination test results from 2005.

Methods and Materials

Eight lodgepole pine seedlots were collected across the Province of Alberta in 1976, 1977, and 1978. The seeds were stored in sealed containers,

immediately after processing, at 2°C until September 1986 when they were moved to -18°C. The seeds were transferred to -80°C ultra-low storage in 1988. The accelerated ageing was conducted in 1989 at the former Petawawa National Forestry Institute using a Controlled Environment germinator (Conviron, Winnipeg, MB) where four replications of 50 seeds each were exposed to 40°C and 98% relative humidity for periods of 3, 10, and 21 days. Immediately following each time period, four replications of 50 seeds each were germinated on Kimpak germination medium moistened with 120 ml of de-ionized water in Petawawa germination boxes (Wang and Ackerman 1983). Another four replications of 50 seeds each were placed on Kimpak in Petawawa germination boxes and moist chilled at 2–4°C for three weeks before they were germinated. All seeds were germinated for 21 days at 20–30°C alternating temperatures with 8 hours light at 30°C and 16 hours in darkness. Germination progress was checked every other day and seeds were considered germinated when they developed into vigour class 1–3 (Wang 1976). At the end of the germination test, un-germinated seeds were cut to examine whether they were fresh, empty, or dead.

Germination tests were conducted annually or biannually by Alberta Sustainable Forestry at the Seed Centre located at Smoky Lake using samples of seed removed from storage. Germination tests were conducted following the same methods employed at Petawawa using moist, chilled seed.

Results

Germination test results presented are from tests using moist, chilled seed. At the time of storage, germination averaged 94.9% and ranged from 84 to 100 % (Table 1). Increased durations of accelerated aging treatments reduced seed germination. Mean germination for the seedlots subjected to 3 days of high temperature and humidity was 92.3% and ranged from 72 to 99%. After 10 days of treatment, average germination decreased to 61.3% and ranged from 12 to 93% and further decreased after 21 days to 38.5% ranging from 6 to 92%. After 24 years in storage mean germination was 85.4% and ranged from 61.0 to 92.0%.

Table 1. Mean germination (%) of 8 lodgepole pine seedlots before storage, after being subjected to three durations of accelerated aging (AA), and after 24 years in storage at -80°C.

Seedlot	Initial Germ.	Germ. 3 days AA	Germ. 10 days AA	Germ. 21 days AA	Germ. 24 years
0585	99	96	93	92	94
0676	100	96	83	68	99
0949	91	89	72	32	85
1014	99	98	86	46	99
1165	84	94	64	42	61
1402	96	94	30	7	75
1445	98	99	50	6	88
1607	92	72	12	15	82
Mean	94.9	92.3	61.3	38.5	85.4

Accelerated ageing treatments were only successful in several instances in predicting future seed quality. Seedlots 0585, 0676 and 1014 had high initial germination, the higher or highest germination after each accelerated aging treatment and the highest germination after 24 years in storage (Table 1). There was no consistent pattern for the other seedlots. With the exception of seedlot 1607, the 3 day accelerated aging treatment did not have an impact on germination. Accelerated aging did not impact the germination of seedlot 0585 but had a dramatic impact on seedlots 1402, 1445, and 1607.

The data were subjected to a correlation analysis. Seed germination prior to storage was not correlated with the accelerated ageing treatments but significantly correlated with germination after 24 years storage (Table 2). Germination of seed aged for 10 days was significantly correlated with the 21-day treatment and was most highly correlated with 24 years storage than the other aging treatments.

Table 2. Pearson correlation coefficients for germination of lodgepole pine seed among accelerated aging treatments and storage durations.

	3 days AA	10 days AA	21 days AA	24 years
Initial germ.	0.39	0.31	0.27	0.87*
3 days AA		0.67	0.31	0.24
10 days AA			0.82**	0.50
21 days AA				0.44

Significance level: * = 1%, ** = 5%

Discussion

An accelerated aging treatment of 3 days did not, on average, reduce germination of moist chilled seed. Aging for 10 and 21 days did decrease germination and this varied substantially among seedlots indicating that there is genetic variation in the response to this treatment. Krakowski and El-Kassaby (2005) reported that germination of lodgepole pine seed decreased following 12 days of accelerated aging. They also found variation among the seedlots in their response to the aging treatments.

Seed germination of any of the accelerated aging treatments was not well correlated with germination after 24 years storage. On the other hand, germination of the seed before it was stored was significantly correlated with 24-year storage. Based on these results with these seedlots accelerated aging failed to predict the storage potential. Delouche and Baskin (1973) state that seedlots that maintain germination during accelerated aging also maintain germination in storage. This is best exemplified by seedlot 0585 and to a lesser extent by seedlots 0676 and 1014.

Seed used for the accelerated aging tests had been stored at 2°C and -18°C for 10–13 years before -80°C storage. It is possible that this had an impact on their response to the aging treatments. With these findings, we would like to suggest that further research is required for future application of the accelerated ageing technique on predicting storability of tree seed.

References

Branche, C.A.; Elam, W.W.; Hodge, J.D.; Bonner, F.T.; Marquez, A.C. 1988. Accelerated aging of selected tree seeds. Pages 327–334. *In* J. Worrall, J. Loo-Dinkins and D.P. Lester, eds. Proc. 10th N.A. For. Biol. Workshop: Physiology and Genetics of Reforestation, 20–22 July 1988, Vancouver, BC.

Chaisurisri, K.; Edwards, D.G.W.; El-Kassaby, Y.A. 1993. Accelerated aging of Sitka spruce seeds. Silvae Genet. 42:303–308.

Delouche, J.C.; Baskin, C.C. 1973. Accelerated aging techniques for predicting the relative storability of seed lots. Seed Sci. Technol. 1:427–452.

Hampton, J.G.; TeKrony, D.M. 1995. Handbook of vigour test methods. Int. Seed Test. Assoc., Zurich, Switzerland. 117 p.

Krakowski, J.; El-Kassaby, Y.A. 2005. Lodgepole pine and white spruce germination: effects of stratification and simulated aging. Silvae Genet. 54:138–144.

Pitel, J.A. 1982. Accelerated aging studies of seeds of jack pine (*Pinus banksiana* Lamb.) and red oak (*Quercus rubra* L.). Pages 40–54. *In* B.S.P. Wang and J.A. Pitel, comps. and eds. Proc. Int. Symp. For. Tree Seed Storage. 23–27 Sep 1980, Petawawa Nat. For. Inst., Chalk River, ON.

Tomaszewski, M.; Janson, L.; Zaleski, A. 2000. DNA, RNA and free nucleotides content in the Norway spruce and Scots pine seeds of different resistance to the accelerated aging test. Prace Inst. Badawczego Lesnictwa, Series A, 3 (900):5–15.

Wang, B.S.P.; Ackerman, F. 1983. A new germination box for tree seed testing. Environ. Can., Can. For. Serv., Petawawa Nat. For. Inst., Inform. Rep. PI-X-27, 15 p.

Wang, B.S.P. 1976. Dormancy and laboratory germination criteria of white spruce seed. Pages 179–187. *In* Proc. 2nd IUFRO Symp. Physiol. Seed Germ. Japan.

Wang, B.S.P.; Pitel, J.A.; Schilf, J.M. 1990. Accelerated aging is effective to predict seed storage potential. Page 95. In F.C. Yeh, J.I. Klein and S. Magnussen, eds. Proc. 22nd Meet. Can. Tree Imp. Assoc., Part 2. 14–18 Aug 1989, Edmonton, AB.

Ben S.P. Wang
Natural Resources Canada
Canadian Wood Fibre Centre
Petawawa Research Forest
PO Box 2000
Chalk River, ON K0J 1J0
E-mail: bwang@nrcan.gc.ca

Corrine Andriuk
Alberta Sustainable Resources Development
Alberta Tree Improvement and Seed Centre
PO Box 750
Smoky Lake, AB T0A 3C0

Dale Simpson
Natural Resources Canada
Canadian Forest Service
Atlantic Forestry Centre
PO Box 4000
Fredericton, NB E3B 5P7

ONLINE GLOSSARIES

If you've ever had trouble finding the definition of a technical term, perhaps these glossaries may be helpful. If you have another favorite online glossary, please forward and we'll include it in our next News Bulletin.

Forest Genetics Glossary
http://www.esf.edu/for/maynard/GENE_GLOSSERY.html

Glossary Forest Genetics and Tree Improvement
http://abtreegene.com/glossary.html

Multilingual Glossary Forest Genetic Resources
English, French, German and Spanish
http://iufro-archive.boku.ac.at/silvavoc/glossary/1_0en.html

Glossary of Seed Biology and Technology
http://en.sl.life.ku.dk/upload/tn59_001.pdf

Glossary of Seed Germination Terms for Tree Seed Workers
http://www.srs.fs.usda.gov/pubs/gtr/gtr_so049.pdf

Dave Kolotelo

STATUS OF THE OECD TREE SEED CERTIFICATION SCHEME

The Organization for Economic Cooperation and Development (OECD) published regulations in 1974 for the collection and trade of forest tree seed. The purpose of the Scheme was to encourage the production and use of seeds, parts of plants, and plants that were collected, transported, processed, raised, and distributed so that they were uniformly identified and assigned to a category. The four categories were: Source-identified (minimum standard), Selected, Untested Seed Orchards, and Tested (OECD, 1974). Canada has been a member of the Scheme since 1970 and joined at a time when there was much demand from European countries for seed from Canadian west coast species such as Douglas-fir (*Pseudotsuga menziesii*) and Sitka spruce (*Picea sitchensis*). In the early 1990s work began to re-write and update the Scheme in order to account for, among other things, new ways to produce reproductive material such as via biotechnology. The inclusion of genetically modified material led to much discussion which resulted in the new Scheme not being accepted by all member countries. After 10 years a compromise was reached in 2006 where the Scheme was reduced to the two lower categories with the understanding that the two higher categories would be added when appropriate text is crafted.

At the time it was recognized that the Scheme was restrictive as it only applied to the two lower categories. Many countries had increasing quantities of seed from seed orchards that they wished to market. Over the past two years work has been progressing on developing a proposal for a category called "Qualified". Qualified material is defined as having been selected at the individual and/or provenance level but genetic testing may or may not have been initiated or completed. The category includes seed orchards which are defined as plantations of selected clones, families, or provenances which are isolated or managed to avoid or reduce pollination from outside sources and managed to produce frequent, abundant, and easily harvested crops of seed (OECD 2009). The proposal was presented at the annual OECD

meeting held in Rwanda in early October where is was accepted. The proposal will be distributed to member countries for their approval. If the approval is unanimous then a recommendation will be forward to OECD Council for adoption.

If this goes ahead then this will allow seed orchard seed to be certified again and it will be another step in the process to have a complete Scheme. The highest category, Tested, will deal with the genetically modified material issue.

References

OECD. 1974. OECD Scheme for the control of forest reproductive material moving in international trade. Org. Econ. Co-op. Dev., Paris, France. 28 p.

OECD. 2009. Draft summary record of the annual meeting of the OECD forest seed and plant scheme. TAD/CA/F/M(2009)2. Paris., France. 22 p.

Dale Simpson
Natural Resources Canada
Canadian Forest Service
Atlantic Forestry Centre
P.O. Box 4000
Fredericton, NB E3B 5P7
E-mail: dsimpson@nrcan.gc.ca

INTEGRATION AND USE OF SOMATIC CLONES IN SEED ORCHARD MANAGEMENT AND THE PRODUCTION OF A NEW GENERATION OF SEEDS WITH A HIGH GENETIC VALUE

Introduction

The production of seeds of high morpho-physiological quality from superior genotypes is among the priorities of the ministère des Ressources naturelles et de la Faune du Québec (MRNF) (Québec Ministry of Natural Resources and Wildlife). Presently, more than 80% of the seeds of the commercial species used for reforestation in the province of Quebec are collected from first- or second-generation seed orchards.

In an effort to accelerate the selection of highly productive genetic material, the MRNF has been integrating somatic embryogenesis (SE), on an operational scale, into the different steps of the reforestation and genetic improvement framework for plantations of the principal commercial species since 2004 (Lamhamedi et al. 2006, Tremblay and Lamhamedi 2006). The objective of this integration is to continuously introduce the most recent selections originating from genetic improvement programs. This will not only maximize the production of high quality genetic material, but also significantly increase forest productivity.

The first research projects involving SE of black spruce (*Picea mariana*) emphasized the optimization of the different steps of SE, such as cryogenics (Cheliak and Klimaszewska 1991, Bomal and Tremblay 2000, Tremblay et al. 2005), *ex vitro* acclimation in the absence of mist (Lamhamedi et al. 2003), as well as the outplanting of clonal tests (Tremblay and Lamhamedi 2006). Subsequent studies showed that SE clones of different North American forest species performed equally well under controlled environment, nursery conditions (Lamhamedi et al. 2000, Park 2002) and in planting trials (Grossnickle and Folk 2007, Dean 2008). Recent results show that somatic clones (SC) of Norway spruce (*Picea abies*) can be used as stock plants for the production of high quality seedlings by mass cutting propagation (Lamhamedi and Tousignant 2009). With a view towards exploiting the full potential of SE techniques for multi-varietal forestry, which emphasizes controlled crossing of elite SC, it appears necessary to ensure that the clones are capable of producing male and female flowers as well as cones full of high quality seeds that will germinate and produce seedlings.

Relative to other North American forest species, notably white spruce (*Picea glauca*) which flowers after 15 to 25 years of growth (Leadem et al. 1997), black spruce begins to produce flowers at about 10 years of age (Youngblood and Safford 2008). However, operational cone harvest cannot begin until the trees are 15 years old (Smith and Greenwood 1995). Our knowledge about the direct effect of SE techniques on the reproduction of forest species is very limited, notably with respect to the precocious or late appearance and formation of male and female flowers, the degree of receptivity of female flowers, and the formation of cones by SC.

A research project was initiated by the MRNF in 2005. The general objectives of the study consist of: 1) evaluating that the flowers produced by black spruce SC are normal, thus permitting the production of quality pollen, seeds and subsequently, seedlings, that meet the 25 norms and morpho-physiological criteria for planting stock in Québec and 2) demonstrating that the pollen produced by SC leads to the production of quality seeds.

Material and Methods

A demonstration clonal test, consisting of 16 black spruce SC was established in August 1997 on the grounds of the Duchesnay Experimental Station, 25 km northwest of Quebec City (Lat: 46º51'05'', Long: 71º38'37''). The first female flowers were observed in 2001, only four years after planting. In 2003, the SC bearing female cones were pollinated (Fig. 1) to produce seeds. The pollen (polymix) that was used came from a first-generation seed orchard. The seeds were characterized according to international norms (ISTA 2009; mass, dimensions, germination) (Fig. 2). Large-sized seedlings were produced from these seeds at the Saint-Modeste forest nursery (Lower Saint Lawrence region; Fig. 3). The concentrations of mineral nutrients (N, P, K, Ca, and Mg) were determined for the shoot and root tissues. Electrical conductivity and the concentration of mineral nutrients in the substrate were also determined, following the procedure described by Lamhamedi et al. (2003). A plantation of these seedlings was established at the Duchesnay Experimental Station in the spring of 2007 (Fig. 4).

Figure 1. Female black spruce cones produced by clone M240 (Colas et Lamhamedi, DRF)

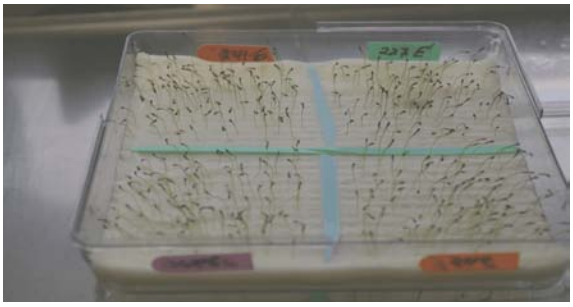


Figure 2. Black spruce seedlings during a germination test in a germination box (Colas, DRF)

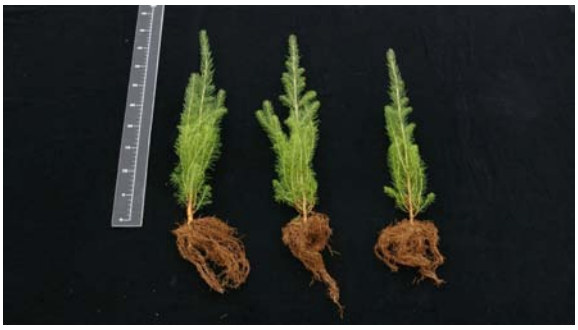


Figure 3. 2+0 seedlings from seeds produced by somatic clone M237. The seedlings were produced in 25-350A containers at the Saint-Modeste forest nursery (Lamhamedi, DRF)

Figure 4. General view of the plantation at Duchesnay after three growing seasons. The seedlings were grown from seeds produced by somatic clones. (Colas, DRF)

To prove that the quality of the pollen produced by the SC was identical to that produced by conventional seedlings, we carried out controlled crossings in 2009 between six black spruce SC grown in two clonal tests; the Duchesnay plantation and another plantation that had been plantled at the Saint-Modeste nursery in 2001. The pollen from the six somatic clones was

collected in 2008 and its viability determined (Fig. 5). The pollen was stored in our pollen bank as described by Colas and Mercier (2000). The cones were harvested (Fig. 6) and the seeds are presently been characterized.

Figure 5. Pollen grains from clone M228 during the *in vitro* germination test on a Brewbaker and Kwack (1963) medium. (Colas, DRF)

Figure 6. M227 cones pollinated by pollen from clone M286 (Colas et Lamhamedi, DRF)

Results and Discussion

Flowering of the black spruce SC began four years after planting for some trees. In all cases, once initiated, flowering continued in subsequent years, indicating that the trees had attained sexual maturity (Zimmerman 1972), even if it was precocious. We also observed, in different somatic clonal tests, the appearance of female flowers and cone formation on somatic clones of hybrid larch (*Larix* x *marschlinsii*) (Fig. 7) and white spruce at ages three and six years, respectively. Different hypotheses may be put forward to explain the precocious sexual maturity observed in SC. Is it due to accelerated aging resulting from SE? Are somatic clones grown under particularly stressful conditions which favour precocious flowering? Does the developmental pathway of SE favour the premature stimulation of the genes responsible for flowering? These different hypotheses are discussed by the authors in a recent publication (Colas and Lamhamedi 2010).

Figure 7. Hybrid larch female cones produced by a somatic clone after only three years (two years in a nursery and one year in plantation). Receptive cones in spring (top); different size of mature cones (bottom). (Lapointe, DRF).

Analysis of the results showed that the seeds from the SC are of very high quality and meet the international criteria pertaining to seed germination. The viability of SC pollen and the pollen tubes produced during *in vitro* germination tests are comparable to those produced by conventional seedlings (Fig. 5).

Morpho-physiological analyses of seedlings grown in a nursery from seeds produced by black spruce SC show that the seedlings are of similar quality to those produced from seed orchard seeds. This demonstrates the possibility of using these high genetic quality seeds for the production of seedlings as well as stock plants for mass cutting propagation.

Our results illustrate, for the first time, that black spruce clones, produced by SE, produce female flowers at an early age and high quality cones and seeds within six years of planting, without the need for intensive culture (no fertilizer application or flower induction treatments). The cones produced seeds with a germination rate similar to seeds from seed orchards. The seedlings grown from the SC seeds had, after two growing seasons in a nursery, similar morpho-physiological characteristics to those grown from seed orchard seeds. These seedlings also exhibited good growth and survival after outplanting.

Conclusion

The capacity of SC to produce pollen and seeds conveys an added value to the SE method. These seedlings will no longer be solely used for multi-varietal plantations, but also as seed trees and to produce high genetic quality pollen. The exceptional genetic quality of SC will rapidly increase the genetic gains of the improvement programs for our commercial species, thus resulting in highly productive plantations. The operational integration of SE in seed orchard management for the production of seeds with exceptional genetic gain will provide a solid scientific foundation for highly productive multivarietal forestry while maintaining the genetic diversity of the target forest species. Moreover, with respect to an article that was recently accepted for publication (Colas and Lamhamedi 2010), the authors confirmed the uniqueness of our approach and the importance of our work for genetic improvement programs

Refrences

Bomal, C.; Tremblay, F.-M. 2000. Dried cryopreserved somatic embryos of two *Picea* species provide suitable material for direct plantlet regeneration and germplasm storage. Ann. Bot. 86:177–182.

Brewbaker, J.L.; Kwack, B.H. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot. 50(9):859–865.

Cheliak, W.M.; Klimaszewska, K. 1991. Genetic variation in somatic embryogenic response in open-pollinated families of black spruce. Theor. Appl. Gen. 82:185–190.

Colas, F.; Lamhamedi, M.S. 2010. Intégration potentielle de l'embryogenèse somatique dans l'aménagement des vergers à graines : floraison précoce et production de graines et de plants par des clones somatiques d'épinette noire (*Picea mariana* (Mill.) BSP). Can. J. For. Res. (accepted)

Colas, F.; Mercier, S. 2000. Évaluation et maintien de la viabilité des pollens utilisés dans le programme d'amélioration de arbres. Gouvernement du Québec, Ministère des Ressources naturelles, Forêt Québec, Direction de la Recherche Forestière. Mémoire de recherche forestière 135. 78 p. http://www.mrnf.gouv.qc.ca/publications/forets/connaissances/recherche/Colas-Fabienne/Memoire135.pdf

Dean, C.A. 2008. Genetic parameters of somatic clones of coastal Douglas-fir at 51/2 years across Washington and Oregon, USA. Silvae Genet. 57(4-5):269–275.

Grossnickle, S.; Folk, R. 2007. Field performance potential of a somatic interior spruce seedlot. New For. 34(1):51–72. http://dx.doi.org/10.1007/s11056-006-9037-0

ISTA. 2009. International rules for seed testing. Int. Seed Test. Assoc., Bassersdorf, Switzerland.

Lamhamedi, M.S.; Chamberland, H.; Bernier, P.Y.; Tremblay, F.M. 2000. Clonal variation in morphology, growth, physiology, anatomy and ultrastructure of container-grown white spruce somatic seedlings. Tree Physiol. 20:869–880. http://www.mrn.gouv.qc.ca/publications/forets/connaissances/recherche/Lamhamedi-Mohammed/Tree-physiology-20-869-880.pdf

Lamhamedi, M.S.; Chamberland, H.; Tremblay, F.M. 2003. Epidermal transpiration, ultrastructural characteristics and net photosynthesis of white spruce somatic seedlings in response to *in vitro* acclimatization. Physiol. Plant. 18:544–561.

http://www.mrnf.gouv.qc.ca/publications/forets/connaissances/recherche/Lamhamedi-Mohammed/Physiologia-Plantarum-118.pdf

Lamhamedi, M.S.; Colas, F.; Tousignant, D.; Tremblay, L.; Rainville, A.; Guay, J.-Y.; Ouellette, C.; Rioux, M. 2006. Intégration de l'embryogenèse somatique dans la filière de plants forestiers du Québec. *In* 4e Atelier sur la production de plants forestiers au

Québec. Min. Ress. nat. Faune, Sainte-Foy, Qc. 1 p.

Leadem, C.L.; Gillies, S.L.; Yearsley, H.K.; Sit, V.; Spittlehouse, D.L.; Burton, P.J. 1997. Field studies of seed biology. F. Aitken, ed. B.C. Min. For., Res. Br., Victoria, BC. 196 p.

Park, Y.-S. 2002. Implementation of conifer somatic embryogenesis in clonal forestry: technical requirements and deployment considerations. Ann. For. Sci. 59(5-6):651–656.

Smith, R.; Greenwood, M. 1995. Effects of gibberellin $A_{4/7}$, root pruning and cytokinins on seed and pollen cone production in black spruce (*Picea mariana*). Tree Physiol. 15(7/8):457–465.

Tremblay, F.-M.; Iraqi, D.; El Meskaoui, A. 2005. Protocol of somatic embryogenesis: Black spruce (*Picea mariana* (Mill.) B.S.P.). Pages 59–68. *In* S.M. Jain and P.K. Gupta, eds. Protocol for somatic embryogenesis in woody plants. Springer, Dordrecht, Netherlands.

Tremblay, L.; Lamhamedi, M.S. 2006. Embryogenèse somatique au ministère des Ressources naturelles et de la Faune du Québec : Du laboratoire au site de plantation. Des plants et des hommes 9:6-11. http://www.mrnf.gouv.qc.ca/publications/forets/connaissances/recherche/Lamhamedi-Mohammed/DesPlants-des-Hommes-9-3-6-11.pdf

Youngblood, A.; Safford, L.O. 2008. *Picea* A. Dietr. spruce. Pages 793–806. *In* F.T. Bonner and R.P. Karrfalt, eds. The woody plant seed manual. USDA, For. Serv., Agric. Hndbk 727.

Zimmerman, R.H. 1972. Juvenility and flowering in woody plants : a review. HortSci. 7(5):447–455.

Fabienne Colas and Mohammed S. Lamhamedi
Ministère des Ressources naturelles et de la Faune
Direction de la recherche forestière
2700, rue Einstein
Québec, QC G1P 3W8
E-mail: fabienne.colas@mrnf.gouv.qc.ca

UPCOMING MEETINGS

Seed Ecology III - Seeds and Change
Jun 20–24, 2010 Salt Lake City, Utah
www:seedecology 3.org
Contact: admin@seedecology3.org

Canadian Forest Genetics Association
July 20–22, 2010 Thunder Bay, ON
www.fgo.ca
Contact: Kathie Brosemer
kbrosemer@fgo.ca

Recent Advances in Seed Research and *ex situ* Conservation
Aug 15–21, 2010 Taipei, Taiwan
Contact: Tannis Beardmore
tbeardmo@nrcam.gc.ca

ISTA Workhop: Water Activity Measurement Applied to Seed Testing
Oct, 2010 Montargis, France
Contact: Patrick Baldet
patrick.baldet@cemagref.fr

RECENT PUBLICATIONS

Chmielarz, P. 2009. Cryopreservation of dormant orthodox seeds of forest trees: mazzard cherry (*Prunus avium* L.). Ann. For. Sci. 66(4):405.

Elsner, W.K.; Jorgenson, J.C. 2009. White spruce seedling (*Picea glauca*) discovered north of the Brooks Range along Alaska's Dalton Highway. Arctic 62(3):342–344.

Forbes, K.; Beardmore, T. 2009. Seed storage potential for dwarf birch (*Betula glandulosa* Michx). Propag. Orn. Plant. 9(3):143–150.

Funda, T.; Lstiburek, M.; Lachout, P.; Klapste, J.; El-Kassaby, Y.A. 2009. Optimization of combined genetic gain and diversity for collection and deployment of seed orchard crops. Tree Gen. Genome. 5(4):583–593.

Garcia, R.; Siepielski, A.M.; Benkman, C.W. 2009. Cone and seed trait variation in whitebark pine (*Pinus albicaulis*; Pinaceae) and the potential for phenotypic selection. Am. J. Bot. 96(5):1050–1054.

Grenier, M.; Sirois, L. 2009. Reproductive development and seed ripening in *Betula papyrifera* along an altitudinal thermal gradient in eastern Appalachia (Canada). Botany 87(5):492–500.

Hsia, J.F.; Francl, K.E. 2009. Postdispersal sugar maple (*Acer saccharum*) seed predation by small mammals in a northern hardwood forest. Am. Midl. Nat. 162(2):213–223.

Johnstone, J.; Boby, L.; Tissier, E.; Mack, M.; Verbyla, D.; Walker, X. 2009. Postfire seed rain of black spruce, a semiserotinous conifer, in forests of interior Alaska. Can. J. For. Res. 39(8):1575–1588.

Kalemba, E.M.; Janowiak, F.; Pukacka, S. 2009. Desiccation tolerance acquisition in developing beech (*Fagus sylvatica* L.) seeds: the contribution of dehydrin-like protein. Trees 23(2):305–315.

Marsico, T.D.; Hellmann, J.J.; Romero-Severson, J. 2009. Patterns of seed dispersal and pollen flow in *Quercus garryana* (Fagaceae) following post-glacial climatic changes. J. Biogeog. 36(5):929–941.

Masaka, K.; Yamada, K. 2009. Variation in germination character of *Robinia pseudoacacia* L. (Leguminosae) seeds at individual tree level. J. For. Res. 14(3):167–177.

Milbau, A.; Graae, B.J.; Shevtsova, A.; Nijs, I. 2009. Effects of a warmer climate on seed germination in the subarctic. Ann. Bot. 104(2):287–296.

Phartyal, S.S.; Godefroid, S.; Koedam, N. 2009. Seed development and germination ecophysiology of the invasive tree *Prunus serotina* (Rosaceae) in a temperate forest in Western Europe. Plant Ecol. 204(2):285–294.

Probert, R.J.; Daws, M.I.; Hay, F.R. 2009. Ecological correlates of *ex situ* seed longevity: a comparative study on 195 species. Ann. Bot. 104(1):57–69.