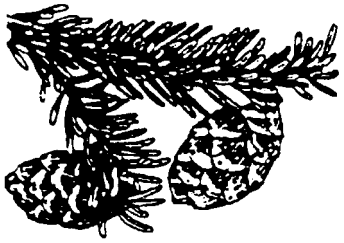
CANADIAN FOREST GENETICS ASSOCIATION
ASSOCIATION CANADIENNE DE GÉNÉTIQUE FORESTIÈRE



Tree Seed Working Group

NEWS BULLETIN

No. 60 December 2014

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CHAIR'S 'ARMCHAIR' REPORT

I'd like to first wish everyone the very best over the holiday season. Peace on Earth – good will to everyone. Thank you to all the contributors for our 60th News Bulletin! For those who haven't been involved in the TSWG for very long, this group was initiated and started producing the News Bulletin in 1983. On such 'birthdays' I think it is worthwhile to review the group's principal aim which is to promote tree seed science and technology through:
1) seed research from bud initiation to seed utilization,
2) identification of seed problems relating to tree improvement and forest management,
3) the exchange of information on seed related problems, and by
4) advising on implementation practices.

These words ring true to me today, are of equal or more importance to today's reforestation and conservation efforts, align with my job responsibilities, and are at the core of why I continue to support this working group. The aim boils down to simply promoting our highly specialized field and exchanging information on our challenges and successes in trying to move all of our programs forward. I encourage you all to make contributions to the News Bulletin – whether it be reporting some of your recent advancements or findings, a summary of meetings of interest or even questions to the group, they are all welcome. Both Dale and I are still committed to continuing this Bi-annual Newsbulletin for the foreseeable future, but we both are feeling stretched – your contributions certainly make our roles much easier - Thank you. All past editions of the Tree Seed Working Group News Bulletin can be found here:
http://www2.gov.bc.ca/gov/topic.page?id=4E4651B3A01448FAB6F39ACAD1C348C1

Note that all of our BC webpages have had their links changed and I probably have already forwarded your comments regarding the user-friendliness and aesthetics of this change. In terms of future Newsbulletins, I'll throw out a specific challenge to Ontario to become a more active participant in the Newsbulletin.

I'd like to send out a few "shout-outs", which seems to be the current term for acknowledgement, of some of our past and present contributors. I still speak occasionally with Ben Wang (our first Chair) who still keeps active with seed science and technology questions and can still be found many days at the skeleton-facility leftover from the glory days of the Petawawa National Forestry Institute. I also recently exchanged e-mails with Dr. Graham Powell (chair from 1988 to 1991) who has also been a very active 'retiree' and recently completed his second book "*Lives of birches, ironwood and maples, a comparative account of the trees indigenous in much of northeastern North America"* which is currently at the publishers – Congratulations. Also from New Brunswick, Kathy Tosh has slipped out the backdoor into retirement to enjoy life and to have more time to dedicate to her passion for golf. My first forestry-related job was actually working under Kathy Tosh and the late Guy Caron when they were doing their graduate studies under Dr. Powell – it certainly is a small community of practice. Heather Rooke, Manager of our facility recently met with Dr. Carole Leadem who forwarded a few seed-related reference boxes and is also staying active and contemplating a move south to the San Francisco Bay area to be closer to family (and the climate can't hurt either). Lately I seem to be the recipient of a variety of seed-related materials from retirees which often result in doubles-plus for my desk and our library collection. If anyone could use copies of older hardcopy tree seed science references, please let me know. I won't be putting together a list, but if there are subject areas of interest that would help me getting you what you want.

I'd also like to make a special "shout-out" to Diane Douglas who has been editing and producing our local BC TicTalk Newsletter and will be retiring at some point in the New Year. Diane has been bearing the extension torch for tree improvement in BC and has really helped get our message out with a variety of methods and at various levels – Thank you Diane. Diane's swan song TicTalk will be coming out soon and I'll provide a link in the next News Bulletin. It includes an article on pelleting from our newest seed technician Hester Williams, a seed inventory review reminder from Spencer Reitenbach, and a variety of articles outlining some of the current restoration activities with whitebark pine.

This is also an opportunity to put in my opinion on the value of extension, which seems to be the first area to feel the knee-jerk reaction of budget cuts, and our increasing need for it in the future. I see an ever increasing gap between science, with its general emphasis on biotechnology and *.omics tools, and the operational practitioner. I strongly believe we need more extension to bridge this widening gap and enable technology transfer to be driven by needs and not pipe dreams. If half the money we spend on GE^{3}LS (**G**enomics-related **E**thical, **E**nvironmental, **E**conomic, **L**egal and **S**ocial research) was dedicated to actual extension efforts we would be much further ahead in actually implementing these technologies to help in practical ways.

I'd like to thank those that returned a cone and seed processing survey that I sent to all known-by-me Canadian facilities. I'm hoping for a few more responses before summarizing and have recently sent out a reminder; so look for that in the July News Bulletin. This News Bulletin also contains an article from David Brownstein who provided a very interesting presentation to our staff on pre-World War II cone and seed processing in Canada and agreed to put some of the highlights into the News Bulletin. David also has put out a call for information related to this specific history topic that he has been working on for over a decade.

I would like to also promote our next Tree Seed Working Group workshop which will take place on August 17, 2015 in Fredericton, NB prior to the Canadian Forest Genetics Association (CFGA) meeting. Details can be found at the conference web site http://forestgenetics2015.ca. The current plan is to focus the workshop on the area of water activity and its applications to tree seed science and technology. I'm very excited by this technology and an overview of how we have been applying it in BC is presented by Meaghan Duke. There have been several articles on this technology lately and to borrow a quote from my Quebec friends and colleagues (News Bulletin # 55), I look forward to it changing our lives as well. Best of the season to all.

Dave Kolotelo
TSWG Chair

EDITOR'S NOTES

There are many different, disparate articles in this News Bulletin. Variety is the spice of life. Fabienne Colas and Josianne DeBlois have an

interesting article where they have determined germination test replicate tolerance differences when using replicates of less than 100 seed. Meaghan Duke has written about her experience and challenges with evaluating water activity of seed. Melissa Spearing writes about her experiences and thoughts with regards to a seed course that she took at the Millennium Seed Bank. Sean O'Shea presents interesting results from a pelletized seed trial. Hart Kunze provides a summary of the status of the clonal seed orchard site that he manages. Tannis Beardmore reports on a butternut conservation project to conserve germplasm of this endangered species. David Brownstein provides a brief glimpse into the past when cone harvesting first began in British Columbia for seed export to Europe and the evolution of artificial regeneration.

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

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

All issues of the News Bulletin are available at: http://www2.gov.bc.ca/gov/topic.page?id=4E4651B3A01448FAB6F39ACAD1C348C1

MAXIMUM TOLERATED DIFFERENCES BETWEEN GERMINATION TEST REPLICATES WITH LESS THAN 100 SEEDS

Introduction

Berthier Forest Tree Seed Centre (BTSC), the only forest tree seed centre in Quebec, stores all the seed required for the annual seedling production by Quebec forest nurseries. Germination tests are conducted for each seedlot after seed extraction and then periodically according to a retest frequency table specific to each species.

BTSC uses the standards of the International Seed Testing Association (ISTA 2009) to certify the quality of the tests it performs. The result of a germination test must be the mean of 4 replicates of 100 seeds. The maximum difference tolerated between replicates varies according to the calculated average germination percentage (Table 1, ISTA 2009). If the difference between replicates is smaller than the maximum tolerated, the germination percentage is valid. If the difference is greater than this value, the test must be repeated. The new germination percentage is calculated and compared to the one from the previous test. If the difference is within the tolerance (Table 1) then the germination percentage reported is the mean of the two tests.

Most germination tests at BTSC are carried out with 4 replicates of 100 seeds. However, for lots with smaller quantities of expensive seed, such as those obtained from controlled crosses or of hybrid larch and which are not subject to seedling production contracts with private nurseries, it is not justifiable to use 400 seeds for a germination test. In these cases, there are less than 100 seeds per replicate, but there are still 4 replicates. The ISTA standard only defines a maximum tolerated difference between replicates in the case of germination tests made with 4 replicates of 100 seeds. BTSC uses the same tolerance table for all germination tests, regardless of sample size. We constructed validation tables for the different cases that may arise at BTSC in order to ensure the reliability of the germination test results.

Table 1. Maximum tolerated difference between 4 replicates of 100 seeds in a germination test, according to the mean germination percentage obtained (two-tailed test with a 5% significance level)

Mean germination (%)	Maximum tolerated difference (%)
99 or 2	5
98 3	6
97 4	7
96 5	8
95 6	9
93-94 7-8	10
91-92 9-10	11
89-90 11-12	12
87-88 13-14	13
84- 86 15-17	14
81-83 18-20	15
78-80 21-23	16
73-77 24-28	17
67-72 29-34	18
56-66 35-45	19
51-55 46-50	20

Statistical Calculations Used to Determine the Maximum Tolerated Difference Between the Replicates for the ISTA Standard

The tolerance table recommended by ISTA (Table 1) was constructed using the equations developed by Miles (1963). These were used to calculate the maximum tolerated difference, assuming random variation only. The formula uses, among others, the standard deviation of the binomial distribution (*ó*) which is defined by the following equation:

$$\sigma = \sqrt{\frac{GP_{mean} \times (100 - GP_{mean})}{n}}$$

where GP_{mean} is the mean germination percentage of the *k* replicate of *n* seed, and

n is the number of seeds per replicate.

Using the standard deviation *ó*, it is possible to calculate the significant range *S*, which is the limit value beyond which we consider that the maximum difference between the replicates is too great for the germination test to be valid:

$$S = q_{1-\alpha/2}(k, \nu) \times \sigma$$

where $q_{1-á/2}(k,v)$ is the critical value (quantile) of the studentized range distribution[1] at a significance level *á* and for *k* replicates, assuming an infinite number of degrees of freedom *v* (two-tailed test), and

ó is the standard deviation of the binomial distribution.

Table 2 presents the critical values of the studentized range for 2 to 10 replicates, assuming an infinite number of degrees of freedom and a two-tailed test with a significance level of 5% (Pearson and Hartley 1954). These values can also be obtained by using the *probmc* function of SAS (2011).

Table 2. Critical values of the studentized range for 2 to 10 replicates, assuming an infinite number of degrees of freedom and a two-tailed test with a significance level of 5% (Pearson and Hartley 1954)

Number of replicates (*k*)	$q_{.,975}(k,\infty)$
2	3.17
3	3.68
4	3.98
5	4.20
6	4.36
7	4.49
8	4.60
9	4.70
10	4.78

[1] The studentized range is the difference between the highest (X_{max}) and lowest (X_{min}) values of a sample from a normal distribution. This difference is measured in standard deviation units of the sample: $(X_{max} - X_{min})/ó$.

To determine the maximum acceptable difference between replicates, it is necessary to determine the tolerated range *R*, which is smaller than the significant range *S*. *R* should correspond to the value of the real significant range, rounded down to the nearest integer. However, *S* values calculated with the second equation are not entirely accurate, since the critical values of the studentized range are determined on the basis of a normal distribution; yet in the calculation of *S*, we find *ó*, which is the standard deviation of a binomial distribution. To be conservative and take into account a possible error in the values of *S*, Miles (1963) used the following arbitrary rule: "The tolerated range *R* corresponds to the value of *S* rounded up to the nearest integer when the decimal part of *S* is greater than or equal to 0.8, and rounded down to the nearest integer when the decimal part of *S* is smaller than 0.8." To apply this rule, 0.2 is added in the equation below.

Moreover, since the ISTA rule states that the mean germination percentage reported must be rounded to the nearest integer, the maximum difference is calculated by replacing GP_{mean} by $(GP_{mean} - 0.5\ \%)$. The equation used for calculating the maximum range is therefore:

$$R = q_{0.975}(k,\infty) \times \sqrt{\frac{(GP_{mean} - 0.5) \times (100 - (GP_{mean} - 0.5))}{n}} + 0.2$$

where $q_{0.975}(k,\infty)$ is the 97.5th quantile of the studentized range distribution for *k* replicates, assuming an infinite number of degrees of freedom (two-tailed test, with a significance level of 5%),

GP_{mean} is the mean germination percentage of *k* replicates of *n* seeds, and

n is the number of seeds per replicate.

The value of *R* obtained by the above equation is rounded to the second decimal, then down to the nearest integer. The resulting value corresponds to the maximum tolerated difference.

It is important to note that ISTA provides users with a file that allows one to calculate germination percentages and to check their validity when 4 replicates of 100 seeds are used. The equation used in this file for the calculations is the same as the third equation.

Tables of Maximum Tolerated Differences

Tables 3 to 5 present the maximum tolerated differences between replicates of a germination test at a significance level of 5%, when using 4 replicates of 25, 50, or 75 seeds.

Please note that the complete version of this text is available here, in French, and presents the tables for values of *n* from 10 to 90.

Conclusion

ISTA standards for germination tests require the use of 4 replicates of 100 seeds. If, for some reason such as the scarcity or value of seeds, there are less than 100 seeds per replicate, we must bear in mind that results will be less accurate, since the maximum tolerated difference between replicates significantly increases when the number of seeds per replicate decreases. For example, if the mean germination percentage obtained is 95%, the maximum tolerated difference between replicates will be 9% for 4 replicates of 100 seeds, but will increase to 19% for 4 replicates of 25 seeds.

Acknowledgments

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Table 3. Maximum tolerated difference (%) between 4 replicates of 25 seeds in a germination test according to the mean germination (two-tailed test with a 5% significance level)

Mean germination (%)			Maximum tolerated difference (%)
99	or	2	9
98		3	12
97		4	14
96		5	16
95		6	18
94		7	19
93		8	21
92		9	22
91		10	23
90		11	24
89		12	25
88		13	26
87		14	27
86		15	28
84-85		16-17	29
83		18	30
81-82		19-20	31
79-80		21-22	32
77-78		23-24	33
75-76		25-26	34
73-74		27-28	35
70-72		29-31	36
67-69		32-34	37
62-66		35-39	38
52-61		40-49	39
51		50	40

Table 4. Maximum tolerated difference (%) between 4 replicates of 50 seeds in a germination test, according to the mean germination percentage (two-tailed test with a 5% significance level)

Mean germination (%)			Maximum tolerated difference (%)
99	or	2	7
98		3	8
97		4	10
96		5	11
95		6	13
94		7	14
92-93		8-9	15
91		10	16
90		11	17
88-89		12-13	18
87		14	19
85-86		15-16	20
83-84		17-18	21
80-82		19-21	22
78-79		22-23	23
75-77		24-26	24
71-74		27-30	25
66-70		31-35	26
59-65		36-42	27
51-58		43-50	28

Table 5. Maximum tolerated difference (%) between 4 replicates of 75 seeds in a germination test, according to the mean germination percentage (two-tailed test with a 5% significance level)

Mean germination (%)			Maximum tolerated difference (%)
99	or	2	5
98		3	7
97		4	8
96		5	9
95		6	10
94		7	11
93		8	12
91-92		9-10	13
89-90		11-12	14
87-88		13-14	15
85-86		15-16	16
83-84		17-18	17
80-82		19-21	18
76-79		22-25	19
72-75		26-29	20
67-71		30-34	21
57-66		35-44	22
51-56		45-50	23

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THE CURRENT ROLE OF WATER ACTIVITY AT THE BC TREE SEED CENTRE

Water activity is a relatively new technology to the Tree Seed Centre in British Columbia, but it already has an integral role in seed testing in Quebec and France. Research conducted in Quebec and France on water activity has been published in several articles in previous issues of the News Bulletin. I will provide some background information for those unacquainted with the subject.

A water activity meter (Fig. 1) is a quick, non-destructive tool for evaluating moisture in a sample of seed. The meter outputs a value between 0 and 1, which is equivalent to equilibrium relative humidity (eRH) if the meter and the sample are at the same temperature. It differs from a moisture content test in that it assesses free water rather than total water in a sample of seed. Free water is water that is biologically available for chemical reactions and/or deterioration via microorganisms. The amount of free water in a seed will depend on its relative composition of lipids, starches, and proteins which varies between species. Storing seed in the freezer with a water activity measurement that is either too high or too low will decrease the longevity of the seed. The ideal level ultimately depends on the species; however most now accept the concept of a universal value. At the Tree Seed Centre, we use a range of 0.35 ± 0.05, which is based on advice from Quebec (Colas et al. 2010), however Karrfalt (2010) has suggested a value closer to 0.30.

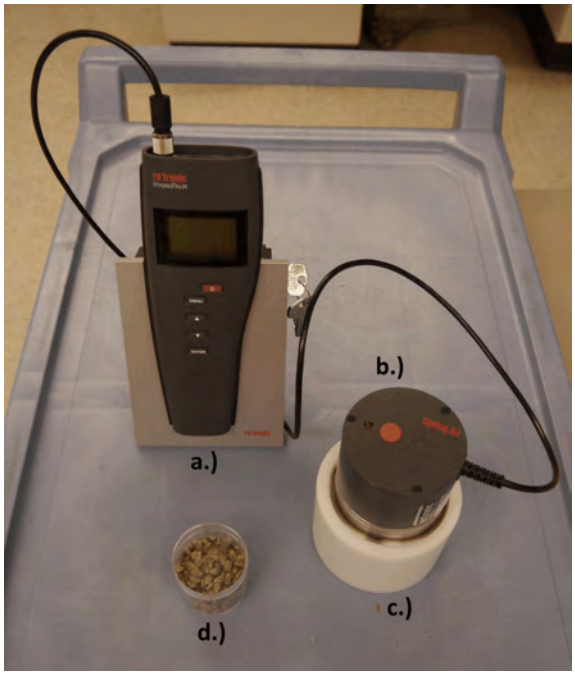

Figure 1. Water activity meter used at the BC Tree Seed Centre: a.) Rotronic HygroPalm AW1 meter, b.) AW-DIO probe, c.) sample holder, and d.) sample cup.

The Role of the Water Activity Meter at the Tree Seed Centre

Currently, we use the water activity meter primarily for testing seed collected for genetic conservation and research. The seed is often rare and valuable, thus a non-destructive method is imperative for determining if seed is dry enough to go in the freezer.

Collections intended for the seedbank arrive at the Tree Seed Centre grouped by population (noted by location), with each population containing, on average, a sample of 10 parent trees. Populations are stored in the cooler at 2°C to await an initial water activity assessment. If the water activity is too high, the seeds are dried back and retested prior to placement in the freezer at -18°C to maximize seed longevity.

My Routine

To test a population, one family is taken from the cooler and allowed to acclimate with the water activity meter in the lab. This is an important step, as both the meter and the sample must be at the same temperature in order to obtain an accurate reading. While waiting, one representative x-ray is taken of each population. Once the samples have reached room temperature an initial measurement of water activity is taken. To date, all samples have had a water activity above the specified range of 0.35 ± 0.05 and have needed additional drying. Seed is hygroscopic and its ability to dry is highly dependent on the environment it is in. If the surrounding air is holding less moisture than the seed then the seed will lose moisture and "dryback". However, when the surrounding air is holding more moisture than the seed, the opposite occurs, and the seed will absorb moisture. At the Tree Seed Centre, seed bank samples are dried by spreading them individually on accelerated aging (AA) trays (Fig. 2) and allowing them to air dry. These AA trays are primarily used in testing during stratification of yellow cedar (*Callitropsis nootkatensis*) and white pine (*Pinus monticola*), but they work quite nicely for my purposes since their size and design facilitate air flow.

Figure 2. Accelerated ageing dishes used for seed dryback. Each sample is spread individually on its own labelled dish and allowed to air dry.

The summer months proved quite difficult for drying seed. Relative humidity (RH) was quite high, averaging about 65 %, and it was not possible to simply air dry the seed in the lab. To work around this issue seed were dried in the Seed Centre's dedicated drying room. Warm air was pumped into the room. The warm air flowed over the seed and was then exhausted. Since warmer air can hold more moisture than cooler air, using the room seemed somewhat effective in removing moisture from the seed. Unfortunately, there is currently no way to control the humidity of the warm air pumped in and thus was of limied utility.

Another concern when using the drying room was the issue of the meter needing to be at the same temperature as the seed being tested. I tried taking the meter into the drying room and testing the seed in there, but a population of 10 samples that takes 10 minutes each to test meant staying in the drying room at 30°C for almost 2 hours, which was both uncomfortable and counterproductive. In the lab, other work can be done during the 10 minutes that it takes to do an assessment, but it is difficult to multitask in the drying room. The alternative was to test the seed in the lab. This also proved to be counterproductive due to the extra time needed to allow the seed to adjust to the lab (i.e., meter) temperature. This is when I first noted that seed were able to absorb moisture through the poly bags they were stored in, regardless of type, thickness, or whether it had a zip seal. Seed that had been dried back to the appropriate water activity range would rapidly reabsorb moisture in the lab, even though they were bagged, especially during the summer months when relative humidity was high. It was also noted that dried seed would gain moisture if placed in the cooler. To negate this, seed that had been dried back and tested was immediately placed in the freezer. Seed

placed in the freezer did not seem to absorb moisture.

Overall, seed dryback during the summer months was difficult and time consuming, but by November, ambient relativity humidity had decreased to approximately 30%. This simplified the dryback process since seed could be left in the lab to air dry. Dryback times seemed quite variable, but most of the variability in dryback times seemed dependent on the initial moisture of the seed and RH of the ambient air. To date, about 950 samples have been tested, dried back, retested, and moved to the freezer for long-term storage.

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SEED CONSERVATION TECHNIQUES TRAINING COURSE

Hello Canadian Forest Genetic Association and Tree Seed Working Group Members and readers. Many years ago I had a desire to work with seeds. Today I am a commercial nursery grower and horticulturist, focusing on native seed collection, genetic conservation, and phenology. Along the way I became a Certified Seed Collector in 2009 through the Forest Gene Conservation Association's (FGCA) Ontario's Natural Selections seed source certification program and now work with the FGCA on various conservation projects. Most recently I assisted in updating the *Seeds of Ontario Trees and Shrubs* manual through research, photography, and input from the Ontario Tree Seed Plant, Ontario Ministry of Natural Resources and Forestry (MNRF), nurseries, and experienced collectors. In my travels, I collect seed for nurseries, have collected *Fraxinus* spp. for the National Tree Seed Centre's (NTSC) conservation project, and grow butternut (*Juglans cinerea*) seedlings from DNA-tested seed trees for use in permited plantings under the MNRF's Endangered Species Act. The complex science of seed banking has fascinated me since my first tour of the Tree Seed Plant. During my years of study at the Niagara Parks School of Horticulture, I dreamed of working in a seed bank. This year, it came true.

In September 2014, I participated in a three-week Seed Conservation Techniques training course at Kew's Millennium Seed Bank (MSB) in England, sponsored by the Forest Gene Conservation Association and Ground Covers Unlimited, a commercial nursery where I work. The course offered a complete view into the workings of an international seed bank, from strategic frameworks such as the Convention on Biological Diversity (CBD) and Convention on International Trade in Endangered Species of Wild Fauna and Flora, prioritizing collections to processing and long-term utilization projects. Specific lectures I enjoyed most highlighted the scientific principles behind seed viability equations, seed-air moisture relations, genetic diversity sampling methods, seed bank design, and cryopreservation. Dr. Wolfgang Stuppy's presentation on seed morphology and evolution was as detailed as his scanning electron microscope photographs, yet a fun group activity dissecting various grocery store fruits brought his research to life. Other hands-on activities such as International Seed Testing Association-standardized germination testing methods, tetrazolium staining, and accelerated aging experiments cemented the practice beyond theory. Doing it is always better than reading about it. The course content is summarized in *Seed Conservation: Turning Science into Practice* (Smith et. al 2003); the first major review of non-domesticated seed conservation science since 1972 and possibly my most dog-eared book after Baskin and Baskin (2001)!

Living in the largest wild seed bank in the world was inspiring to say the least. I have seen a few germplasm facilities in my studies but it was

amazing to see all the best equipment available in one place. Keith Manger and John Adams, both experienced in testing all manner of field and lab technology, rationalized and instilled a need for a digital x-ray, indicating silica gel, hygrometers, trifoliate aluminum packets, and Munter sorbtion dryers to do the best conservation work. They led side-by-side comparisons measuring seed moisture content by oven drying method versus the ease of a Rotronic lab-based hygrometer, as well as digital x-rays to other quality assessment methods. From this, I quickly realized the implications of time, accuracy, and non-destructive testing for valuable seedlots. Everything from a £50 portable field kit to a £30,000 drying room was based on the same scientific principle: dry seed is safe seed. The value ratio is also a figure that will stick with me; 345 units of time/money to plan, collect, and process viable seed to 1 unit of time/money to safely store it for decades, if not hundreds of years. Seed banks are a cost-effective conservation strategy and should be a part of any orthodox species recovery effort here in Canada, as stipulated by Article 9 of the CBD.

The course was highly interactive among participants, who came from South Africa, Malawi, Ghana, British Virgin Islands, Puerto Rico, Australia, Azerbaijan, Israel, South Korea, and Thailand. The global perspective and experiences from other countries was as informative as meeting the MSB researchers themselves. There were representatives from gene banks, herbaria, universities, forestry research institutes, and botanical gardens; many with PhDs and high scientific credentials. It was a telling moment, however, when Dr. Tim Pearce, a seasoned field collector leading MSBP projects all over Africa, asked how many in the room had harvested seeds in the wild before. I was proud and shocked to be one of three, out of 15! It was reassuring to know you don't need to have a doctorate to collect seeds and that good research depends on collectors obtaining high quality seeds.

Despite our various practical experiences, there was commonality in our concerns for disappearing species. Many first-hand stories highlighted habitat loss, overharvesting by local peoples, urban expansion, agricultural clearing, desertification, and regions of high endemic diversity. Lack of staff and management resources was a common challenge in participants' agencies. For example, with 4,985 species on the International Union for the Conservation of Nature's Red List of conservation concern, the South African National Biodiversity Institute's limited staff can only manage 250 new collections a year and many species are at risk of extinction before collections might be made. I realized the number of endangered plant species in Canada is comparatively manageable, and that our temperate climate and large North American land base gives us a spatial and temporal advantage over smaller biomes. That said the MSBP's first target of 10% of the world's at-risk flora came primarily from arid and montane habitats. Reaching 25% of the world's flora means expanding collections to boreal, arctic, and coastal species at risk. Species that will have difficulty shifting ranges under climate change (see climate envelope projections from McKenney et al. 2007) were also on my mind during this discussion. So we have some time, but how much do we really need?

The course also presented case studies on how to value seed bank expertise, an important justification in the economic costs of seed banking. International projects such as the Useful Plants Project and Crop Wild Relatives program highlighted the need to target species used for food, medicine and future genetic breeding resources, often with the help of and for the financial benefit of local communities. In the course of six years, these projects gained more botanical data on some species than had been accumulated in the past 100 years. Restoration projects such as the UK National Tree Seed Project aims to store samples from each of the 24 seed zones for 150 orthodox native woody species. I saw parallels in our efforts with the Ontario tree seed zones, the FGCA's Seed Collection Area Network, and valuable living gene banks such as those at the University of Guelph Arboretum. I also observed a rather unique field gene bank dubbed the UK Native Seed Hub for producing seed of source-identified chalk grassland species that were unavailable in the UK's commercial trade. I helped collect seeds of five rare species one afternoon, a five-minute walk from the dormitories. It highlighted the value of our seed orchards in Ontario and Canada that bring together isolated breeding populations, helping secure a future for genetically diverse seed production.

I was the only representative in the course from a country not currently with a MSB Partnership agreement. But I can appreciate why. I feel we have the facilities, scientific minds, and eager hands in Canada to perform the same level of work that the MSB demonstrated. At the same time, given the survey of Canadian tree species of concern by Beardmore et al. (2006) and a notable 47 species still requiring some degree of *ex situ* conservation, *what's stopping us?* It's a big question, with some partial answers already, beginning with seed bank managers' financial constraints, "wish list" equipment upgrades, extra staff at facilities, and the need for better trained and better paid collectors. There are many challenges but I realize more than ever what tools

are available to understand and preserve what we have before it is too late. Until next time, thank you for this opportunity to share my passion. I would be happy to entertain comments and questions.

I would like to extend my gratitude to Barb Boysen of the FGCA, Dale Simpson of the NTSC, and Sean Fox of the University of Guelph Arboretum for their letters of support for my course application and all the seedy opportunities they have offered over the years. Also a hearty thanks to Brian Swaile, Ron Thayer, Al Foley, Dr. Dan McKenney, and Dennis Eveleigh for their encouragement and mentorship.

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PELLETIZED SEED TRIAL

At the New Brunswick Department of Natural Resources Kingsclear Tree Nursery the goal is to maximize occupancy in the trays. The nursery uses a Jiffy pellet system and two crops are seeded per season, one in March and the other in June. Recently we started using pelletized seed to try to increase seeding accuracy, thereby decreasing the number of voids and reducing the number of doubles which increases thinning costs. The pelletized seed is visible and easy to use in the seeding machine (Fig. 1). There is also no clogging of tubes from pitch and/or dirt which is found in clean seed to some degree. The people operating the machinecan easily see whether a cell is occupied or not.

Figure 1. Seeding machine using pelletized seed.

In 2013 trials were conducted using stratified, pelletized seed of white spruce (*Picea glauca*), black spruce (*P. mariana*), and red spruce (*P. rubens*). The white spruce did not perform well. The black and red spruce seed performed much better. As summarized in Table 1, pelletized seed had slightly higher germination, reduced number of doubles, and there were less voids in the trays.

Table 1. Tray comparisons for single-seeded pelletized black spruce seed versus single-seeded regular black spruce seed

Treatment	House	Voids/tray at seeding	% Voids	Total voids at germination	Doubles/tray at seeding	% Doubles	% Germination
None	38	12	2.7	39	29	6.6	85
None	39	19	4.3	30	11	2.5	93
None	40	19	4.3	44	14	3.2	90
None	41	11	2.5	25	29	6.6	99
None	42	15	3.4	35	11	2.5	93
None	43	15	3.4	38	5	1.1	90
None	44	23	5.2	34	6	1.4	91
None	45	23	5.2	38	7	1.6	96
	Mean	**17**	**3.9**	**35**	**14**	**3.2**	**92**
Pellet	46	5	1.1	16	1	0.2	96
Pellet	47	4	0.9	14	2	0.5	97
Pellet	48	1	0.2	11	5	1.1	97
	Mean	**3**	**0.8**	**14**	**3**	**0.6**	**97**

Using unpelletized seed can result in higher production costs due to the resulting increased number of empty cells (Table 2).

Table 2. Total number of black spruce seedlings produced at the nursery and cost of voids using unpelletized seed

		At seeding		At germination				
Total trays seeded	Total seedlings	Mean voids/tray	Total voids	Mean voids/tray	Total voids	Cost of voids at 12.5¢ each	Total seed used (kg)	Cost to pelletize seed ($500/kg)
19,010	6,253,300	8	152,080	17	323,170	$40,396	12.96	$6,480

If a portion of the seed was pelletized and the nursery could recoup some of the loss due to voids, this would result in substantial savings. If pelletized seed was used and there was a reduction of 25%, 50%, or 75% of the voids at seeding, substantial savings would be realized (Table 3). Results were similar for red spruce.

Table 3. Estimated savings by pelletizing seed and reducing the number of voids at seeding and germination

Percent reduction of voids from 152,080 (in Table 2)	No. seedlings saved at seeding	No. seedlings saved at germination	Value of seedlings saved	Total savings (minus cost of pelletizing)
25	38,020	80,793	$10,099	$3,619
50	76,040	161,585	$20,198	$13,718
75	114,060	242,378	$30,297	$23,817

In 2014, pelletized seed was used for 99% of the black spruce and 50% of the red spruce that was seeded. The seeding was tracked closely and there was a difference in seed line accuracy for voids compared to using untreated seed, 97% vs. 84%, respectively (Table 4).

Table 4. Difference in voids in 2014 between pelletized (P) single-seeded red spruce and black spruce compared to regular seed

Species	Total voids	Total pellets (cells) sampled	% Accuracy of voids	Total doubles	% accuracy of single-seeding
Red spruce (P)	26	5,280	99.5	194	95.8
Red spruce	100	4,400	97.7	777	80.1
Black spruce (P)	74	10,912	99.3	127	98.2
Black spruce	57	1,848	96.9	181	87.1

For the Kingsclear Nursery the cost of pelletizing seed directly resulted in a decrease in voids at seeding, a reduction in doubles, and an increase in seedlings at germination. The cost of pelletizing the seed is a worthwhile investment as it saves money by decreasing voids, reducing thinning costs, and ultimately producing trays of seedlings with higher occupancy.

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J.D. IRVING LIMITED TREE IMPROVEMENT PROGRAM UPDATE

Parkindale Seed Orchard

There are a total of 27,000 ramets, of various ages, established for seven species in our clonal orchard. First- and second-generation orchards of white spruce (*Picea glauca*) are at the seed production stage. There are also black spruce (*P. mariana*), first- and second-generation orchards with most of the seed coming from second-generation orchards. There are two categories of red spruce (*P. rubens*) orchards: first-generation comprised of clones from northern New Brunswick which produced seed for the first time in 2014, and first- and second-generation orchards of clones from Nova Scotia with all seed coming from the first-generation orchard. There are two Norway spruce (*P. abies*) orchards: a first-generation and a smaller weevil resistant orchard. Jack pine (*Pinus banksiana*) orchards are first- and second-generation with all seed coming from the second-generation block. The first-generation white pine (*P. strobus*) orchard has been

producing seed for 12 years. There are first-generation orchards of tamarack (*Larix laricina*) but this species is not used in reforestation programs at this time.

All first-generation orchards, with the exception of red spruce North and white pine, are 50% rogued. Seed production was fair in 2014 with a good crop in the white pine and smaller amounts in second-generation white spruce and red spruce North. All second-generation white spruce ramets are stem injected with gibberellic acid to stimulate more flowering and higher seed production.

Sussex Tree Nursery

We had a great year in our somatic seedling production program. Survival increased dramatically from 75 to 96% for white spruce and was also much better for Norway spruce (increased to 75% from less than 40% in previous years). All somatic seedlings are planted from lab dishes into mini plugs and surviving seedlings are later transplanted into Multipots (Fig. 1).

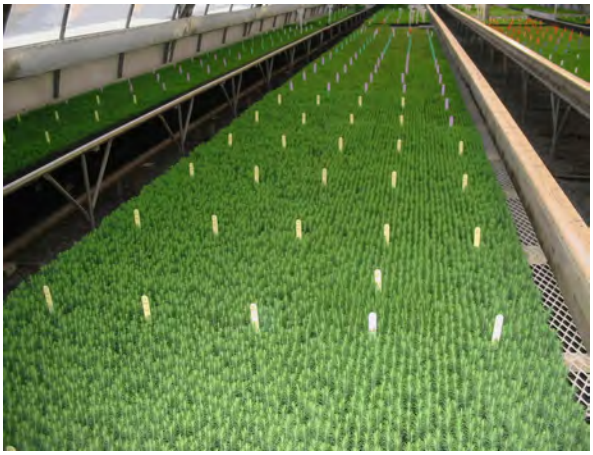

Figure 1. Spruce somatic seedling production.

We are presently building a new research lab at the Sussex Tree Nursery to scale up our somatic seedling production. The lab will be in operation in early 2015.

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BUTTERNUT *ex situ* CONSERVATION

The native range of butternut (*Juglans cinerea*) extends over the entire northeastern quarter of the United States, including many states immediately west of the Mississippi River, and into Canada. Butternut is more cold-tolerant than black walnut (*J. nigra*), and it grows as far north as the Upper Peninsula of Michigan, New Brunswick (NB), Nova Scotia, southern Quebec, and Ontario. In Canada, butternut is native to Ontario, Quebec, and NB. This species is being extirpated throughout most of its range in North America by a canker disease caused by the non-native fungal pathogen *Ophiognomonia clavigignenti-juglandacearum*. It has been over 45 years since the initial 1967 report of butternut canker on butternut in North America. Estimates of mortality caused by the canker are as high as 92% (Carlson and Guthmiller 1993, Phytopathology 83:1352). In Canada, the impact of the canker has led to butternut being listed as an Endangered species by SARA (Species at Risk Act).

Results identifying and testing putatively canker-resistant trees suggest that there may be no true resistance or tolerance to this virulent strain of canker (Ontario Recovery Strategy for Butternut 2013). The SARA Butternut Recovery Strategy states 'that recovery will largely depend on the identification of canker resistant-strains, the conservation of genetic material and a program to restore viable populations that can fulfill butternut's ecological function' (SARA Recovery Strategy for the Butternut in Canada 2010, Environment Canada). Given the possible uncertainty concerning resistance, the high mortality across butternut's native range, and the inability to conserve butternut *in situ*, we decided to focus on *ex situ* conservation for the future recovery of this species.

Our work is focussed on using *ex situ* conservation to capture and maintain genetic variation in seed banks. There is no butternut germplasm conserved in seed banks, since nuts can only be stored for a maximum of two years. An innovative technique developed by Beardmore and Vong 1998, Can. J. For. Res. 28:903–910 allows for part of the nut that can regenerate into a healthy tree (root-shoot axis) to be cryopreserved (stored at -196°C). In order for axes to tolerate -196°C, nuts must be stored for one year at 4°C, and then axes are isolated from these nuts, treated, and cryopreserved. Axes from nuts collected in the fall of 2014 will survive when cryopreserved a year later. Theoretically, cryopreserved material can be stored indefinitely and recent work shows that butternut axes cryopreserved for 10 years were fully germinable, supporting the use of cryopreservation for the

long-term *ex situ* conservation strategy. A range-wide study of butternut genetic diversity has shown that the NB populations are the most unique and genetically diverse ones in North America (report to NB Dept. Natural Resources by Dr. Romero-Severson 2012). Additionally, NB contains some of the last remaining uninfected butternut trees in North America.

As part of a three-year project, we have just completed our first year of work where we were able to collect over 13,000 nuts in NB. The root-shoot axis of these nuts will be cryopreserved and stored at the Natural Resources Canada, Canadian Forest Service's National Tree Seed Centre in Fredericton, NB. Reliable identification of butternut has been complicated by the presence of hybrid trees, which were propagated primarily for nut production, and naturally occurring hybrids and backcrosses between butternut and introduced Asian walnuts, primarily Japanese walnut (*J. ailantifolia*). Each tree that we have collected nuts from will be genotyped which will help us map the genetic diversity of this species in NB and identify any hybrids.

For the following two years, we will expand our collections and also hopefully collect nuts from Ontario and Quebec. The main expected result of the proposed work is the creation of a long-term *ex situ* conservation collection of butternut and development of guidelines for the use of this resource, and how it will support future restoration and recovery activities. This work is collaborative amongs multiple partners with Natural Resources Canada, Canadian Forest Service (Fredericton, NB) leading the effort. Our partners include NB Department of Natural Resources; Maliseet Nation Conservation Association; Department of National Defence, Canadian Forces Base Gagetown; Notre Dame University; Fundy Model Forest Network; Ontario Forest Gene Conservation Association and Canadian Wildlife Service.

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PRE-1939 BRITISH COLUMBIA CONIFER SEED COLLECTION

After the First World War, the British government appealed to Canada for tree seed to replant the devastated European countryside. To meet this need the provincial government of British Columbia (BC) established a system for cone harvesting, seed extraction, and overseas shipment. Though this was deemed appropriate for forests in Europe, the hand-planting of tree seedlings was considered neither economically feasible nor desirable as a method of forest regeneration at home. Initially the BC Forest Service's Research Division attempted to encourage the natural reproduction of commercially valuable species after logging, and researchers performed life-history studies on the assumption that logging within the forests' reproductive requirements would ensure a perpetual forest through natural regeneration. Rather than dictating how logging would proceed, research uncertainties were used by the logging industry to avoid the imposition of cutting restrictions. As BC exported thousands of pounds of tree seed overseas (Fig. 1), local harvesting technology evolved in ways that made natural forest regeneration on the coast impossible. Formerly undesirable, hand planting became imperative so the system meant for foreign reforestation was retooled for local use and set in action by 1940. Over twenty years in a series of imperceptible but steady shifts, provincial government policy towards forest regeneration was reversed by 180 degrees. The successful system that had reforested Belgium, England, and Scotland with BC tree seed was implemented at home, leading to the abandonment of the original policy of natural regeneration.

Figure 1. Seed extraction plant, New Westminster, British Columbia, 1922. (From Johnstone 1991)

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ATISC SEED CONSERVATION COURSE

The Alberta Tree Improvement & Seed Centre (ATISC) runs an annual 2-day Seed Conservation Course. The course covers topics right from seed assessment before harvest and correct seed handling, all the way through to understanding seed storage longevity, germination issues and techniques. There is also a cut testing lab to look at seed morphology and how this can inform germination testing plus a group germination problem solving exercise to help everyone feel more comfortable using their new knowledge in a practical way.

The course instructor is the Provincial Seed Specialist at ATISC. Lindsay gained an MSc in phytoremediation working with green alders and mycorrhizae on the Sudbury Barrens in Ontario and then spent five years as germination and longevity specialist at the Millennium Seed Bank, part of the Royal Botanic Gardens, Kew. She has taught both in-house and travelled to many countries around the world training others in seed conservation management and science.

Attendance for the course is free but limited and the dates are 2–3 February and 19–20 March, 2015. Attendance for only one day is not permitted. To sign up or if you have any questions or concerns about course content and applicability, please send an email to Lindsay Robb at lindsay.robb@gov.ab.ca

UPCOMING MEETINGS

ISTA Annual Meeting
June 15–18, 2015 Montevideo, Uruguay
http://www.seedtest.org/en/event-detail---0--0--0--53.html

Canadian Forest Genetics Association
August 17–20, 2015 Fredericton, NB
http://forestgenetics2015.ca

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