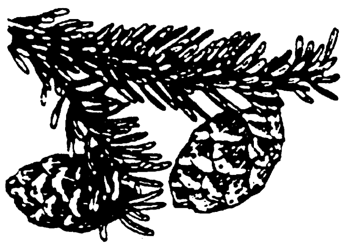
CANADIAN FOREST GENETICS ASSOCIATION
ASSOCIATION CANADIENNE DE GÉNÉTIQUE FORESTIÈRE

Tree Seed Working Group



NEWS BULLETIN

No. 52 December 2010

GENETIC CONSERVATION

THIS ISSUE AT A GLANCE

CHAIR'S 'ARMCHAIR' REPORT

Hello and Happy Holidays to one and all. It has been an exciting time in the BC Forest Service as last month we were in the midst of implementing a re-organization (with downsizing) only to be provided with a new re-organization plan (without downsizing). This one was quite significant and resulted in the separation of the BC Forest Service into two different Ministries: Ministry of Forests, Mines and Lands (FML) and Ministry of Natural Resource Operations (MNRO). A very significant change was the dissection of the Research Branch as an entity and its functions being divided among the above ministries as well as the Ministry of Environment. Of significance, to this audience, is the addition of the Forest Genetics section to the Tree Improvement Branch. This makes sense from the standpoint of streamlining the tree improvement delivery system, but is causing some anxiety within the research community. It is too early to tell how everything will work out. The latest re-organization was also primarily the work of our Premier who is stepping down, so we may even have a new direction with a new Premier in February. We live in very interesting times indeed.

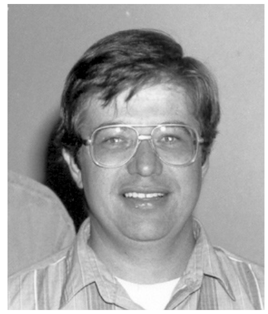
The Canadian Forest Genetics Association (CFGA) meeting is scheduled for August 16 to 18 in Thunder Bay, Ontario with a Tree Seed Workshop preceding the meeting on August 15th (see http://www.cfga-acgf.com/ for more information). The forum is basically open at this point, so **if you have any ideas or suggestions please forward them to Dale or myself**. The theme of our last workshop in Quebec City, 2008 was 'Preserve and Multiply Forest Genetic Resources'. A theme helps focus the workshop, but an open forum or a more basic review of seed characteristics may be appropriate. We are also considering a field trip to SeedTek which is a company that offers seed evaluation and upgrading services.

In the area of Genetic Conservation, tree seed can play a vital role. Protected areas are generally considered King in genetic conservation as they conserve the biodiversity of all the organisms and their interactions in an ecosystem. There are always threats to Kings with climate change and natural disaster events being good reasons not to put all of our eggs in one basket. A quantity of tree seed dedicated to genetic conservation provides insurance for the diversity of our trees which are the dominant life form in many of our ecosystems. A seed bank (or *ex situ* reserve) is an extremely efficient and cheap means of conserving genetic diversity. A handful (110 grams) of white spruce seed can conserve 50 000 unique genotypes. These can be maintained very cheaply and for long durations in below freezing conditions. I encourage all jurisdictions to invest in maintaining a separate seed bank for genetic conservation. Those wanting to know more about genetic conservation and how you can contribute should attend the CON**FOR**GEN workshop on August 19th, 2011 after the CFGA meeting in Thunder Bay. Additional details on Seed Storage can be found in News Bulletin # 45 (http://www.for.gov.bc.ca/hti/publications/tswg/TSWGNewsbulletin45.pdf).

I'd like to bring to your attention two books that I think would be of interest. The first is by a former chair of our Committee, **Dr. Graham Powell**, who has produced "**Lives of Conifers: A Comparative Account of the Coniferous Trees Indigenous to Northeastern North America".** A few additional details can be found from this link and hopefully you can find or order it from a bookseller near you. http://www.amazon.ca/Lives-Conifers-Comparative-Coniferous-Northeastern/dp/1550418696/ref=sr_1_1?s=books&ie=UTF8&qid=1291664675&sr=1-1 .
A great reference for those dealing with Northeastern conifers or anyone wanting to update themselves on conifer life history in general.

The second book is by **Dr. Claire Williams** and is titled **"Conifer Reproductive Biology"**. The book provides an evolutionarily focused treatment of the subject matter and a sneak peak into the Foreword, Table of Contents, and some of the initial and concluding pages are available at the following link http://www.amazon.ca/Conifer-Reproductive-Biology-Claire-Williams/dp/1402096011/ref=sr_1_1?ie=UTF8&qid=1291668902&sr=1-1# . This is a good reference with emphasis on Pines. And I don't receive anything from Amazon for these links ☺.

I am saddened to inform you of the passing of Dr. Peter de Groot on October 22, 2010. Peter was a longstanding supporter of our organization and had led the Cone and Seed Insect Working Party of our group. Those that knew Peter are well aware of the depth of this loss to the scientific and humanitarian communities.



I was always impressed by Peter's ability to provide large contributions at both the scientific and practical levels. His ability to put into practice "Seek first to understand… then be understood" speaks volumes for Peter's patience, humility, and desire to continually improve all he touched. My deepest sympathies to his family and loved ones.

I hope to see as many of you as possible at the CFGA meeting this summer. There will be no particular theme for the next Newsbulletin. I think we will have a theme for every second edition, but your contributions on any tree seed related topics are most welcome. Have a Safe and Merry Holiday Season.

Dave Kolotelo
TSWG Chair

EDITOR'S NOTES

Conservation of forest genetic resources is becoming increasingly important as a result of all the forces impacting our environment and the forests. A number of years ago a geneticist remarked that we should be strategic in implementing genetic conservation programs. I think that this is a point well taken. I do think there can be various levels (intensities) of conservation. Programs need to be implemented now for species that are being seriously impacted by one or more agents. The article by Donna Palamarek on what the province of Alberta is doing is an example. At the other end there are species with large ranges that are not being threatened now but certainly climate change will have an impact over time. For these species efforts should be directed at a broad scale to sample and conserve some of the genetic variation that is currently present. This may prove to be valuable in the future.

There are some genetic conservation activities being conducted across Canada and no doubt this type of work will expand with time. We need to be as proactive as possible. I think you will find all the articles in this issue dealing with 'genetic conservation' interesting and encouraging. See the "Selected References" section of New Bulletin # 45 for articles on seed storage and longevity.

I wish each one of you a Merry Christmas and best wishes for a prosperous New Year!

Dale Simpson
Editor

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

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

ONE SIZE DOESN'T FIT ALL: *Ex situ* GENETIC CONSERVATION TARGETS VARY WITH LIFE HISTORY TRAITS

How many seeds are enough? True to form, any biologist can take a simple question and end up with a complicated answer! For *ex situ* genetic conservation, the objective is to capture as much genotypic variability as possible for a target population in each seed collection. In British Columbia (BC), there is legislation governing collection distances for operational seedlots (Province of BC 2004), but not for conservation collections. Over the years, the trend in conservation collections has been to increase the size and number of accessions since a species' life history influences the amount and distribution of population genetic variation (Hamrick and Godt 1996).

Dispersal and Gene Flow

Commercial species in BC are typically outcrossing conifers that are wind-pollinated and have wind-dispersed seeds. Wind-mediated gene flow creates relatively homogeneous populations within a region (in which ideal conditions support one panmictic population adapted to that area), such as a seed planning unit, and ensures that collections throughout that region should capture most of the alleles. But for species whose seeds are dispersed by wildlife, or are pollinated by insects, the genetic patterns differ, changing the collection strategy. Seed dispersal may be less skewed towards the parent tree and spread over a wider area, resulting in less genetic differentiation among populations compared to wind-dispersed species. This effectively creates larger breeding zones. Pollen flow may be less biased towards prevailing wind direction (e.g., Rogers et al. 1999) and be spread with a wider and more radial or normal distribution pattern, again possibly homogenizing populations over a wider area. So to capture the same distribution of genotypes in a population, seed collections could be made from fewer populations, or across a wider area than for a wind-dispersed species.

A collection of 10 well-spaced trees is considered the minimum number of parents to ensure an effective population size that conserves most of the population variation in an area (see Roberds and Bishir 1997; Russell and Yanchuk 2005). Trees should be 30 m apart, but 50 m or more is preferable, as long as they lie within one elevation band (depending on the species, but usually 200–300 m). More trees is better, and the exact number depends on tradeoffs between acquisition costs and seed yield.

Mating System and Clonality

The mating system of a species needs to be considered. Most commercial species have been studied, but for many others little is known about their most basic biology. Capacity for inbreeding and fitness of inbred progeny can be influenced by many factors. Some species, like western redcedar (*Thuja plicata*), have few or no deterrents to selfing in natural populations and have marginal inbreeding depression. Many species in the Pinaceae are primarily outcrossers, where selfed progeny rarely survive in nature. Some angiosperms have evolved self-incompatibility genes. Species often lie along this continuum of mixed mating with some degree of relatedness within a stand or population, reflected in genetic substructuring so that adjacent trees are more closely related than distant ones. Population density affects selfing rate – where there are more potential mates, most species tend to have higher outcrossing, with increasing inbreeding as distance between unrelated individuals grows (Gapare and Aitken 2005; Mimura and Aitken 2007). Even within individual trees, higher outcrossing has been found in upper crowns than lower crowns (El-Kassaby et al. 1986; Chaisurisri et al. 1994). Species with clonal growth habits likely tolerate some inbreeding (Rogers et al. 1999; Ally et al. 2010).

For tree seed collections, this means that seed should be collected from well-spaced stems that are less likely to be related and therefore more likely to capture the full diversity of alleles. This also means that only a single stem should be collected from a clump, as it often is impossible to determine on site if a clump is comprised of separate individuals.

Habitat Niche and Population Density

A species' ecological amplitude (in general, the range of edaphic conditions it is adapted to) affects sampling and planning for conservation – a generalist species is robust to spatial sampling designs at most scales, while a specialist needs a finer scale sampling plan. This becomes important for the logistics and cost planning of the collection. For a sparsely distributed species it might be difficult to locate enough mature or female individuals to collect from to represent an ecological unit or stand. For species with an uncommon niche, it may be hard to find enough well-spaced individuals to make a collection if the population is concentrated in a small site, running the risk of collecting from one group of related individuals at the expense of broader population sampling. Genetic diversity also differs between populations in the core of a species' range versus those at the periphery (Gapare et al. 2008).

Capturing Rare Alleles

To capture rare alleles (< 5% frequency), there are two options. The most robust is to make a larger collection to increase the statistical likelihood of capturing a rare allele. The other option is to target environmentally heterogeneous habitats and populations on the margin of a species' range, based on the assumption that environmental variability is associated with greater genetic variability. The effectiveness of this latter option depends on the species. One could argue that most low-frequency alleles are either deleterious or have no known advantage, but we simply do not know the effects they may have in future climates, and this argument gains weight if they have not been expressed in the phenotype (which may of course comprise effects of many genes and genotype-by-environment interactions).

Some good guidance on numbers required for conservation is provided by Yanchuk (2001), who calculated conservation thresholds for different types of inherited traits (Table 1). A phenotype is assumed here to be a composite reflection of the effects of multiple alleles with quantitative expression, such as early bud burst or increased pest resistance. To conserve a trait influenced by a single recessive allele, one would need to collect seed from over 50 times more individuals than one influenced by a single dominant allele. An allele with low copy number is likely to be lost simply due to genetic drift, so these figures provide solid guidelines for conservation planning. In BC, conservation collections at the Tree Seed Centre aim for at least 1000 viable seeds per population, collected from at least 10 well-spaced trees as described above. Estimates by Krakowski et al. (2009) use a threshold of 3 populations from a given region to ensure both adequate population size and redundancy in the event of a catastrophe. This is somewhat lower than the 5000 recommended, but in a typical wild population the contributing pollen and seed parents of 1000 out-crossed seeds represent well over 5000 individuals. This may not be the case where inbreeding is prevalent.

Table 1. Number of individuals in a population or seed collection needed to capture a desired number of copies of a trait with a 95% confidence level (from Yanchuk 2001).

Phenotype (quantitative trait)		Dominant (binomial) allele		Recessive (binomial) allele	
5 copies	20 copies	5 copies	20 copies	5 copies	20 copies
181	554	89	275	~5000	~15000

Collecting to Meet Your Needs Most Effectively

What does this all mean for a planned seed collection? Plans need to be customized to accommodate the life history traits of each species. Consult the literature on your species and find out about its distribution, mating system, ecological amplitude, and reproductive biology. Design your sampling plan to meet your objective – spacing between trees, seeds per tree, number of trees per stand, and so on. Make sure you collect enough seed in each population including an adequate number of individuals, seeds per individual, and populations per region. Don't hesitate to collect more – it's always cheaper and easier to add some seeds now than to re-collect later.

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ONE SIZE DOESN'T FIT ALL: SEED CHARACTERISTICS

The genetic architecture of a species will have an impact on the sampling structure required to capture a species' genetic diversity. The species' seed characteristics will have a large influence on the efficiency, effectiveness, and durability of *ex situ* seed collections (seed banks). Although *ex situ* conservation can refer to clone banks, progeny tests, and tissue cultures it is specifically seed which I will discuss. A primary benefit of seed banks is the efficiency of storage. For a species like white spruce (*Picea glauca*) a small handful of seed (110 g) can represent over 50 000 unique genotypes! This efficiency allows for very large numbers of genotypes to be conserved relatively cheaply within a small space.

A primary determinant of durability relates to a species' seed storage characteristics. All of our north temperate conifers are considered **orthodox** species meaning their seeds can be dried to low moisture contents (< 10%) and can be stored at sub-freezing temperatures. This is an incredible advantage with our major tree species. In British Columbia (BC), seed that is over 50 years old is still being used successfully for operational reforestation. Some conifers have been described as having a functional storage potential (germination > 60%) of 100 years (Simpson et al. 2004). This estimate is probably realistic given the increased knowledge we have today concerning collection timing, post-collection handling, processing, and storage of tree seed compared to when our oldest seedlots were originally collected.

It is good news for orthodox species, but some tree species in Canada are considered **recalcitrant** and cannot be dried or stored at sub-freezing temperatures. This does not mean that short-term storage is not possible with these recalcitrant species. The most prominent recalcitrant genus is *Quercus* with all species exhibiting recalcitrant seed storage behaviour. Even though longevity of seed storage is limited with *Quercus*, acorns have been successfully stored for up to three years with high moisture contents at temperatures just above freezing (see Bonner 2008 for a full review). Other notable recalcitrant Canadian trees are silver maple (*Acer saccharinum*) and butternut (*Juglans cinerea*). For butternut, the embryonic axis with some cotyledon tissue dried to below 4.8% moisture content showed potential for storage at -196°C (Beardmore and Vong 1998). A few familiar ornamentals are also recalcitrant; sycamore maple (Acer pseudoplatanus) and the buckeye (*Aesculus)* and chestnut (*Castanea*) genera. Have I left out any Canadian tree species?

Some species are considered orthodox even though their longevity in storage is not very long-term. Good examples are species in the genera *Populus* and *Salix* which have seeds that have been reported to have short lifespans under natural conditions, but whose seeds can be stored for years if properly dried and stored at subfreezing temperatures. Even with conifers there is variability in seed longevity. In BC, western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), and our true firs (*Abies* spp.) display the most rapid seed deterioration. I don't think it is a coincidence that these are also species which contain resin vesicles in their seed coats. The role of these resin vesicles is unclear and very little research has been performed on them considering the ecological and economic importance of these species. Damage to these structures has generally resulted in reduced germination (Gunia and Simak 1968; Kitzmiller et al. 1973). Is the reduced storage experiences with these species due to damage to resin vesicles or simply due to the presence of these structures? I'm quite amazed that these mysterious structures have not received greater attention given their importance to quality seed production.

Within orthodox species, there can also be a wide range in seed deterioration between individual seedlots. It is often thought that the higher the initial germination the better the storage potential. This has some validity as high initial germination is often the result of good collection timing, proper handling, and efficient, careful processing. On the other hand some 'shortcuts' in any of these activities may not have an impact immediately, but after some period of storage. The results of these shortcuts may also show up when seed is germinated under non-optimal germination conditions. Below average germination speed or vigour may be an initial clue that a seedlot will not have a long lifespan. Estimates of seed deterioration to calculate the number of viable seeds (conservation collections) or germination retest frequencies (operational collections) are currently based on a species average change in germination. My challenge is to refine this to an individual seedlot basis and this is problematic with new seedlots which only have one germination test result.

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Dave Kolotelo
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ALBERTA PINE SEED CONSERVATION PROGRAM IN RESPONSE TO MOUNTAIN PINE BEETLE

The mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (MPB) is the most destructive insect pest attacking mature pine forests in western North America today. Alberta Sustainable Resource Development (ASRD) has been monitoring MPB in Alberta since 1977. According to the ASRD's Mountain Pine Beetle Management Strategy 2007, MPB has spread to west-central and north-western Alberta through range expansion due to a warming climate since the 1990s. A catastrophic long distance dispersal event took place in the summer of 2006 and again in 2009 which resulted in epidemic infestation of lodgepole pine (*Pinus contorta* Dougl. ex Loud var. *latifolia* Engelm.) and hybrid pine stands in northwestern Alberta. ASRD declared MPB a pest emergency on April 11, 2007 and developed and implemented an aggressive control program to reduce and contain beetle damage and salvage heavily infested stands.

The guiding policy for MPB management in Alberta is the Mountain Pine Beetle Management Strategy which outlines management responsibilities for required activities among ASRD, industry, Federal agencies, municipalities, and land owners. Genetics and tree improvement and related research plantings require a different level of program. To address those needs, in 2007 the Genetics and Tree Improvement program, under the Alberta Tree Improvement and Seed Centre (ATISC), developed an action plan for MPB management. In addition to other objectives, one of the main goals was to carry out genetic conservation seed collections to advance the genetics programs for lodgepole pine, jack pine (*P. banksiana* Lamb.), whitebark pine (*P. albicaulis* Engelm.), and limber pine (*P. flexilis* James). Below are the highlights of those seed conservation activities from 2007 to 2010. This program is ongoing and the numbers below do not include collections made in the latter part of 2010.

Although limber pine and whitebark pine are not commercially important species, emphasis was placed on seed collections for these two species as they were listed by Alberta as endangered in September 2009. While the cause of population decline for these two species is primarily due to white pine blister rust (*Cronartium ribicola*), additional decline is occurring due to MPB attack, management practices, and climate change. Seed collections from these two species will be used as a conservation and restoration measure as well as for eventual species recovery work such as pest resistance screening and pest resistance breeding activities. A provincial species recovery team, consisting of specialists from various backgrounds, is currently drafting a recovery plan for both whitebark and limber pine in Alberta.

Whitebark and limber pine collections are costly as many of the stands are accessed by helicopter only and cone crops must be caged prior to harvest to prevent predation by Clark's Nutcracker (*Nucifraga columbiana*) which necessitates visiting each collection site at least twice. Over the past three years, 154 single tree and 1 bulk whitebark pine collections and 122 single tree and 3 bulk limber pine collections have been made. Because seed is still quite rare and valuable, any germinants obtained during viability testing were transplanted into seedling styroblocks. Currently there are 603 limber pine seedlings and 460 whitebark pine seedlings growing at ATISC. These seedlings are to be used for outplanting in select locations and for a pilot grafting program.

Although other native pine species in the province are currently classified as "Not at Risk", conservation seed collections were seen as necessary to conserve unique regional populations of these species (e.g., known disease resistance, high genetic growth potential, and low cone serotiny populations) and where MPB activity is high and populations are at risk of extirpation. Selection of populations for conservation seed collections was based on regional representation and knowledge of genetic variation related to climate, geography, and ecosystems. Collections have been prioritized based on the regional MPB threat.

To date, there have been 39 targeted regional lodgepole pine bulk conservation population collections. All were successful with the exception of two collections in the subalpine along the continental divide in Willmore Wilderness Park where low cone serotiny prevented using standard lodgepole pine seed collection procedures. Each bulk collection was made from a minimum of 30 representative trees per population to adequately capture genetic variation. In total, 37 lodgepole pine cone bulk collections representing 28 seed zones have been completed as part of the ATISC MPB program. Reconnaissance surveys are continuing with collections in the subalpine to try and determine the extent of low serotiny populations.

In addition to the bulk collections, single tree collections were occasionally made in the same stands. Single trees were selected based on their initial assessment as being phenotypically superior (above average height, straight stems, natural pruning). Cones, scions, and a single wood disc were collected from each tree and shipped to ATISC for processing. Approximately 10 scions were grafted for each parent tree. All successful grafts will be established in the lodgepole pine clone bank at ATISC as a conservation measure. Cones were collected from 67 individual lodgepole pine trees.

Additional limber pine, whitebark pine, and lodgepole pine conservation collections are being planned for the 2010/11 season. These collections will be prioritized on the basis of MPB risk of attack as determined by stand susceptibility, MPB management zonation, cone availability, and various surveys.

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CONSERVING FOREST GENETIC RESOURCES BY SEED STORAGE

Forest gene conservation is necessary for protecting genetic diversity of trees in reserved populations where they can respond to natural evolutionary processes. Both *in situ* and *ex situ* populations are crucial to the conservation of the overall resource (PNW 2003). This important issue has been discussed for some time but little action has been taken. At the 7th Session of the FAO Panel of Experts on Forest Gene Resources it was recommended that an updated review document be prepared on the potential role of *ex situ* conservation as a complement to the more frequently applied *in situ* conservation strategy. To follow-up that recommendation, FAO engaged researchers at the Petawawa National Forestry Institute to prepare a review on *ex situ* conservation strategies using storage of seeds, pollen, and *in vitro* cultures of perennial woody plant species and a report was prepared and published (Wang et al. 1993). *Ex situ* conservation of genetic resources using seed storage has been initiated by the Canadian Forest Service's National Tree Seed Centre in Fredericton, N.B., and the tree seed centres operated by the provinces of Alberta, British Columbia, and Manitoba. However, there are no standards established for sampling, collecting, storage, etc. For agricultural seed, the FAO/IPGRI (1994) Genebank Standards recommends drying seeds to low moisture contents (3–7% fresh weight, depending on the species) and storing them in hermetically sealed containers at low temperature, preferably -18°C and monitoring their viability regularly. Regeneration is required when germination is below 85% of the original value. Although Walters et al. (2004) showed there was a decline in germination of seed stored in liquid nitrogen, low-moisture seeds stored in sealed containers at -20°C can be expected to last up to 200 years. Unlike agricultural seeds, tree seed cannot be easily regenerated due to the long juvenile growth period of trees.

For efficient conservation of forest genetic resources using seed storage, we need to develop standards and guidelines for sampling populations, collecting seed, and storing seed. In this article, we propose some guidelines for discussion.

Sampling

The objective is to obtain as much of the genetic variation as possible that exists in a population. The sampling effort increases substantially as one seeks to obtain an increasing proportion of the genetic variation present, particularly rare alleles. Thus there is a trade-off between obtaining a sufficient sample of the genes and the practicality of collecting the seed. The mating system is something to be considered as well (see Jodi Krakowski's article on page 3 for further elaboration on this and other items). The stand has to be large enough to contain a sufficient number of trees (minimum 20 ha). Failing this, trees can be sampled over a geographical area provided the ecological conditions they are growing in are similar and isolated trees are not sampled. Seed or cones should be collected from a minimum of 15–20 trees that are spaced 100 m apart. The seed can be kept separate by individual tree. When the opportunity arises to make a bulk collection, collect from 30–50 trees spaced 50–100 m apart and mix the seed/cones together. Attempt to collect equal quantities from each tree.

Seed/Cone Collection

Seeds and cones should be collected in heavy crop years when pollen production is high to capture the genetic diversity of the population and to ensure good physiological quality of the seed. Seed must be collected when it is fully mature. When making single-tree collections care must be taken to ensure they are kept separate. Collected seeds or cones need to be handled carefully in order to maximize seed quality (Wang 2001).

Seed Storage

Seed should be stored dry and frozen. For maximum longevity, the most recent up-to-date results suggest that true orthodox seeds (e.g., black spruce (*Picea mariana*), white spruce (*P. glauca*), jack pine (*Pinus banksiana*)) can be expected to store for 100 years in sealed containers when stored at -20°C with a moisture content of 5-8% (fresh basis) (Simpson et al. 2004). The stored samples have to be monitored periodically (10–20 years) and another *ex situ* conservation strategy (e.g., using some seed to establish *ex situ* plantations) may need to be considered when germination has declined to 60% of the original value, depending upon tree species.

Quantity of Seed to Store

It is important that a sufficient amount of seed is placed in storage to allow for future testing/monitoring and be available for research or other projects such as *ex situ* plantations. The quantity of seed available, to some extent, depends on the species and the means of collection. The following are proposed:

Large seed (1000-seed weight > 20 g)
2000 seed per single tree
5000–10 000 seed for a bulk

Small seed (1000-seed weight < 20 g)
5000 seed per single tree
50 000 seed for a bulk

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IUFRO TREE SEED SYMPOSIUM

The IUFRO Tree Seed Working Group had their symposium in Taipei, Taiwan, August 16–18, 2010. The meeting was hosted by the Taiwan Forestry Research Institute. The symposium theme was "**Recent Advances in Seed Research and *ex situ* Conservation**". This is the first meeting this group has had which focused on *ex situ* conservation. *Ex situ* conservation has probably never been as important as it is today, with many species in danger of extinction, threatened by habitat transformation, over-exploitation, alien invasive species, pollution, and climate change. The disappearance of such vital and large amounts of biodiversity poses one of the greatest challenges for the world community.

The symposium was well attended with representation from 17 countries. Proceedings from the conference have been published and should be available electronically within a month on the IUFRO Tree Seed Working Group web site (http://www.iufro.org/science/divisions/division-2/20000/20900/20903/publications-and-references/). All of the presentations were excellent and it was very interesting to see the diverse array of topics on *ex situ* conservation.

A series of presentations reviewed *ex situ* conservation activities and challenges in Canada, Mexico, Taiwan and also in a selection of African, Asian, and Latin American countries. The Canadian review (Dr. Tannis Beardmore, Canadian Forest Service, Fredericton, Canada) discussed the storage behaviour of seeds produced by our native tree species and national-level *ex situ* conservation efforts. For Canadian tree species, 57% produce orthodox seeds, 23% produce questionable orthodox seeds, 13% produce recalcitrant seeds, and 1% produce questionable recalcitrant seeds and seed of unknown or uncertain storage type. The impact of being able to store the seed of 57% of our native trees reliably, in the long term, was discussed. Dr. Javier Lopez-Upton (Colegio de Postgraduados en Ciencias Agricolas, Texcoco, Mexico) discussed the challenges in Mexico associated with *ex situ* conservation. Mexico has more than 3000 native tree species, with 1000 species endemic to the country. Mexico is currently developing a system to secure the germplasm of aquatic, agricultural, forest, livestock, and microbial species, through a national genetic resources banking system. Dr. Loo (CGIAR, Rome, Italy) spoke about the pressures on forest tree species in Africa, Asia, and Latin America and the role that *ex situ* conservation has, and how essential it is for these efforts to be integrated into other conservation means.

The Key Note presentation "Biogeography and Phylogeny of Seed Dormancy and Nondormancy in Trees" was presented by Dr. Carol Baskin (University of Kentucky, Kentucky, USA). This was a very interesting presentation that focused on germination ecology and dormancy, specifically on the five classes of dormancy, and how these classes of dormancy (and also nondormancy) are distributed geographically and phylogenetically among trees worldwide. Seed dormancy data were compiled for 5084 species of trees, which are found growing in 13 major vegetation zones on earth. These data were used to evaluate the world biogeography and phylogenetic position of the five classes of dormancy and nondormancy. Dr. Carol and Jerry Baskin are compiling a database for the world biogeography of seed dormancy in trees. This should be an excellent resource once it is completed and made accessible.

Highlights of a few of the other presentations:

- Dr. Christina Walters (USDA-ARS National Center for Genetic Resources Preservation, Fort Collins, Colorado, USA) talked about "Seed Longevity and Deterioration in Orthodox Seeds: A Perspective Based on Structural Stability of Visco-Elastic Materials". Dr. Walters spoke about seed deterioration as a problem of maintaining structural stability within the cytoplasmic matrix of seed cells as they dry and while they are stored. She discussed concepts about visco-elastic behavior in seeds, which can be classified as orthodox, recalcitrant, and intermediate. She presented compelling evidence for considering ageing as a reaction that links structural and chemical stability in seeds, where moisture, temperature, and seed quality interact to control the rate at which seeds age.

- Dr. Costas Thanos (Department of Botany, University of Athens, Athens, Greece) spoke on the "Timing of Seed Germination and Life History of Trees: Case Studies from Greece". His talk focused on mechanisms involved with the post-winter timing of germination, including shifting the reproductive cycle so seeds are produced and dispersed promptly as germinating seeds at the end of the winter (*Pinus nigra*), and how a long exposure to freezing temperatures under snow cover can lead to germination before snow melt (*Aesculus hippocastanum).*

- Dr. Hugh Pritchard (Kew Gardens. Wakehurst Place, Ardingly, West Sussex, United Kingdom) spoke on "*Ex situ* Conservation Issues Associated with Recalcitrant Seeds". His talk addressed the impacts of ageing, in particular the role of oxidative stress and associated processes that result in cells losing viability in recalcitrant seeds.

- Mr. Ben Wang (Natural Resources Canada, Canadian Forest Service, Petawawa, Ontario, Canada) spoke on the " Moist Chilling and Dormancy of Eastern White Pine (*Pinus strobus* L.) Seeds". Mr. Wang presented his work on the analysis of data on the dormancy and germination of eastern white pine (*Pinus strobus* L.) from its natural distribution in Canada. This research demonstrated that dormancy in eastern white pine varied among individual trees, and between stands (populations), seed years, latitudes, and geographic locations. Also, pretreatments with 28 days of moist chilling were found to release dormancy and enhanced germination regardless of the seed source.

- Dr. Milan Mataruga (Faculty of Forestry, University Banja Luka, Banja Luka, Bosnia and Herzegovina) spoke on the "Dynamics of Imbibition, Seed Germination, and Seedling Development of Austrian Pine (*Pinus nigra* Arnold) from Populations Growing in Contrasting Habitats of Southeastern Europe". Dr. Mataruga talked about his research assessing numerous physiological and morphometric parameters of seed collected from different provenances in either very hard rocky conditions or in favorable habitats. He provided evidence that there was some degree of adaptation to environmental conditions although the variability was very high within each provenance's habitat types.

- Dr. Alvin Yanchuk (British Columbia Ministry of Forests and Range, Victoria, British Columbia, Canada) spoke on the "The Role and Future Challenges of *Ex Situ* Gene Conservation Approaches for Forest Tree Genetic Resources". Dr. Yanchuk's talk discussed the role and value of *ex situ* seed collections in a changing climate and how one should carefully re-examine *ex situ* priorities and collections, considering such factors as how seed are collected, stored, evaluated, and deployed to mitigate potential climate-change impacts.

- Dr. Marcelino Siladan (Forestry and Environment Research Division, Los Banos, Laguna, the Philippines) spoke on the "Assessment of Seed Distribution, Dissemination, and Diffusion Pathways of Priority Tree Plantation Species in the Philippines". This talk discussed the impact of the origins and historical movement of seeds on the current state of the country's seeds sources, origins and systems of seed distribution, and dissemination of priority plantation tree species in the Philippines.

Lastly, Taiwan was a wonderful venue for the symposium and many thanks to the chair of the organizing committee Dr. Ching-Te Chien, Taiwan Forestry Research Institute, who hosted an excellent meeting.

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WATER ACTIVITY MEASUREMENT APPLIED TO SEED TESTING: ASSESSMENT OF THE ISTA WORKSHOP

Cemagref in France and DRF in Québec have been cooperating since 2006 in a joint R&D project related to water activity (a_w) measurement applied to seed moisture quantification and testing. They have demonstrated the effectiveness of this technique for moisture management of forest tree seed and pollen. The Franco-Canadian team presented their results during the ISTA

Forest Tree and Shrub Seeds (FTS) Workshop in 2008 in Péri, Italy. Regarding the results presented at this workshop and more recently at the 29th ISTA Congress in June 2010 in Cologne, Germany, Cemagref was requested, in conjunction with DRF, to organize the first ISTA workshop dedicated exclusively to water activity measurement applied to seed testing.

The workshop, entitled "Water Activity Measurement Applied to Seed Testing", held October 13–15, was organised by Cemagref on behalf of the Moisture (MOI), FTS, and Storage (STO) ISTA Technical Committees. The workshop took place in Montargis, France.

This workshop aimed to evaluate the potential role of water activity measurement as a new ISTA rule to quantify seed moisture. The event welcomed 20 participants and 6 lecturers coming from Brazil, Canada, Croatia, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Italy, Netherlands, Spain, Sweden, and USA. About half of the participants were involved with tree seeds with agricultural crops represented by the remainder. Two thirds of the participants were ISTA members or people working in ISTA certified seed laboratories. The workshop combined lectures, a demonstration, and training session as well as social events in the Loire Valley and Montargis municipality.

P. Baldet and F. Mariette (Cemagref) and F. Colas (DRF) participated as lecturers and presented their collaborative work in order to demonstrate, in particular, the significant intra-and inter-specific variability of the relation between water activity and moisture content of a given sample at a given moisture state. These results demonstrated the complementarities of the two techniques to describe moisture in seedlots as a commercial basis for moisture content and a biological aspect for a_w. P. Baldet and F. Mariette presented the concepts of a_w, eRH (equilibrium relative humidity), and water mobility described with NMR (nuclear magnetic resonance) methodology. Fabienne Colas presented the operational applicability and implementation of water activity in seed centres, the construction and interpretation of sorption isotherms, and the use of a_w in forest diversity management programs. The three chairmen of the ISTA committees (Mr. Fabio Gorian, FTS; Mr. Harry Nijënstein, MOI; and Mr. Hugh Pritchard, STO) presented the needs, expectations, and experiences of the technical committees directly concerned with moisture management of seeds.

The first conclusions of the workshop demonstrated the real potential of a_w to become a new ISTA rule for seed moisture quantification. It has been particularly noted that a_w is a non-destructive, rapid, and easy to run test. These operational advantages show the way to a successful development of this technique among laboratories world-wide involved with agricultural, forest, or ornamental seeds. The official report on the workshop will be available on the ISTA website (www.seedtest.org).

The group in front of the Sully castle (14th century). Photo by Sonia Launay, Cemagref.

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CANADIAN FOREST GENETICS ASSOCIATION 2011 CONFERENCE

The Organizing Committee is pleased to invite you to attend the 32nd CFGA Conference to be held on August 15–19, 2011, at Lakehead University in Thunder Bay, Ontario. The conference theme is: **Forest Genetics and Tree Improvement: New Knowledge, Challenges and Strategies**.

Following the tradition of the CFGA, the conference will be comprised of presentations by invited and volunteer speakers on recent advancements of research and operations in forest genetic resource management and utilization.

The main CFGA conference will be held from Tuesday, August 16 to Thursday, August 18. The CFGA Tree Seed Working Group Workshop will be held on Monday, August 15. The forum on Conservation of Forest Genetic Resources (CON**FOR**GEN) will be held on Friday, August 19

Please plan to attend and share the latest information with friends and colleagues from Canada and around the world.

Abstracts are invited for oral presentations and posters. A one-page abstract (up to 500 words) for each oral presentation/poster should be submitted electronically by email to Paul Charrette no later than April 15, 2011.

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INTERNATIONAL SEED FEDERATION (ISF) – TREE AND SHRUB SEED GROUP

The ISF Tree and Shrub Seed Group is a group of national associations of tree and shrub seed companies, and individual tree and shrub seed companies. Currently the group has 40 members from 27 countries. The group was founded in 1978 and has met 33 times in 20 different countries.

The meetings take place once a year in one of the member countries and consist of a one day discussion on technical topics that are of relevance to the tree seed traders. The discussions are followed by interesting presentations by experts in the tree seed sector. Subsequently 1 or 2 days of excursions are organized to tree or forest related locations. The group has visited tree seed nurseries, research labs, model forests, and reforestation sites, among others. The group is dynamic and maintains an active discussion site throughout the year.

The 2011 meeting will take place in Madrid, Spain with the 2012 meeting planned for Chile. Here are some excerpts from the minutes of the latest meeting, held in Ithaca, NY on August 23, 2010, that are relevant to tree seed.

Pests and Diseases

In the United Kingdom, *Phytophthora rhymorum* is spreading to *Larix leptolepis* strands and in France there is a new beetle attacking Pine from the South to the North (the Bostrichid beetle on *Pinus pinaster*).

It would be good if there was a harmonized protocol for seeds of *Prunus* species coming from Europe. Currently a review and acceptance of the protocol needs to be done to certify that the resulting plants are certified virus indexed. If one imports *Prunus avium* from Europe one can get seed into the US but one cannot claim that the resulting seedlings are certified virus free. Protocols for testing of the mother trees need to be determined that will be acceptable to the US Department of Agriculture (USDA), and the USDA will need to know that the protocol for each country is acceptable to them.

The participants expressed concern about the export restrictions caused by *Gibberella circinatum*. A participant reported that many samples of *Pinus taeda* seed had been imported and all had the fungus. In France there is currently a push to import *Pinus taeda* to replace *Pinus pinaster* that was destroyed by storms. Possibly because they need the seed so badly they can push to get the quarantine lifted.

Another participant mentioned that imported seedlots of Douglas-fir (*Pseudotsuga menziesii*) had been tested and found free of this fungus, however, later on the disease was found in the nursery. So it was possible that one could not determine if there is fungus in the seedlot or not. Currently for European Union (EU) companies it is not possible to re-export *Pinus* and *Pseudotsuga* species and it would be necessary to amend this situation. It was proposed to contact the phytosanitary officials in Brussels to start the process. The main problem here would be to change the quarantine regulations, and sometimes it takes up to 5 or 7 years to change such a regulation.

It was proposed that ISF should investigate how to start trading in *Pinus* species again. In the recent past, the ISF Secretariat had investigated the possibility to kill this fungus on pine seeds, but the problem was that no infected seedlots could be found. Analyses had indicated that the fungus was found many times in seedlots but the fungus was dead. Therefore it would be necessary to determine if the fungus is alive or dead.

All agreed that the problem was not scientific, but political with the EU acting to prevent the fungus from coming in. It was felt that there was no logic between science and legislation. The EU has been over-reacting for trade issues. After discussion, it was agreed that this would be brought forward at the upcoming OECD (Organization for Economic Cooperation and Development) meeting in Paris. A good resource for an overview of the diseases is a report from the European Plant Protection Organization (EPPO). Two topics relating to the OECD were discussed; the Harmonized Supplier Document and the import of seed from the US and Canada under the qualified category. The OECD is working on these matters and the Chairman will send a short report after the OECD meeting September 28–30 in Paris.

A question was raised whether US companies have trouble getting seeds in the US, especially *Abies*. In response it was replied that occasionally *Megastigmus* is found in the seeds. In the past there used to be a heat treatment, however, this is not applied anymore. In addition it was mentioned that many fungi can be treated with a heat treatment, however sometimes such a heat treatment does kill too many seeds.

Future Activities

Some of the general comments that were made were: In the past there was more trade going on and the seed lots were bigger. The trade of oak seeds and imports of Douglas-fir seeds were both less. The Phytosanitary Regulations on import and export, and also the fact that many countries were requesting only seeds from native sources, has lead to a decrease of the international seed trade.

Grants had been offered to companies that were using locally collected seeds. It had started with forest species and now local shrubs need to be used as well. It is difficult for seed companies to switch origins for grants. Germany decided that by 2020 it will only allow German plants from German collected seeds.

DNA fingerprints will be used to determine origin of seeds; the same technique that is used for forest species. There are almost no reforestation programs in Europe, only natural regeneration is going on. It was proposed that ISF should develop a vision for the next 5–10 years.

It would be interesting to conduct research into using non-native provenances in the EU. The energy crisis and the demand for wood and biomass are becoming more important. In certain countries money was available from the government for *Salix* and *Populus*, but these would come from cuttings, and therefore would not help the seed industry much. However in some countries, direct sowing is beginning to replace the use of cuttings. The matter of biomass could help with the current trade problem, because local provenance issues are not as important. But it would also be necessary for the seed trade to survive on the local market demand for seeds.

It was stated that business of all members had decreased around 30% in the past years, which was partly due to the lack of reforestation and the requirements to use only native material. With global warming it is even more important to bring new genetic material into the forest. The cost to regenerate a forest is so high that there is no money for planting. Concerns were expressed that if there would be no change then there would be less and less tree seed companies left. ISF should influence the local authorities in Europe and the USA.

The effects of climate change were also discussed, indicating that if the transition in climate change was smooth, nature could handle itself, but if the transition was fast, then new sources would need to be introduced. As a result of climate change it might very well be that authorities realize that they are required to plant trees from other sources, rather than just native sources. Species need to be managed without thinking about borders. It would be more important to think about the entire ranges of species.

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EUROPEAN SEED KILNS CONFERENCE

The International Conference of the European Seed Kilns was held in Verona, Italy, 9–12 September 2010. The theme was **Climate Changing and Tree Seed Production**.

The papers are available for download at: http://www.iskc.eu/programma-en.html

MEXICO TREE SEED CENTRE

The vision is a tree seed centre based on all the good science of generations of foresters, researchers, technologists, and inventors, delivering a top flight service to forest nurseries in Mexico and beyond.

My colleague, Ing. Pedro Hernandez Provensal, has an expanding forest nursery business and has won some contracts to supply seedlings to the government forest agency CONAFOR. He needs seed and there is no source other than local collections made by community groups. Seeding is scheduled for December to mid February in order to produce stock for planting in June/July 2011.

The location, Uruapan, Michoacan, is a volcanic, mountainous area in central Mexico with natural forests of *Pinus montezumae*, *P. michoacana*, and *P. pseudostrobus* suffering degradation through selective logging. The seed source is probably genetically degraded. Cone collection starting with *P. montezumae* is already being conducted and will conclude with *P. pseudostrobus* in January. Adolfo Soto puts out the word to the communities requesting bags of cones. He "knows" where each community is collecting but no records are kept. Adolfo complains that some collectors, working for other agents, have collected *P. pseudostrobus* too early and the crop is not abundant this year.

These observations are confined to one small area but it appears there is a need to improve seedlot identification and definition of source such as tree quality. Then there's the matter of cone maturity. The bags of cones are hauled to Adolfo's processing site by each community or family and stacked in the shade for drying (Fig. 1). This is not a maturation process step. Some collections are delivered without bags or the bags were needed for immediate reuse (Fig. 2).

Figure 1. Adolfo's cone storage area.

Figure 2. Cones without bags.

To dry the cones, Adolfo spreads them on sheets of poly, in the sun. At this time of the year rainfall is uncommon (but it happened last year). *P. montezumae* and *P. michoacana* cones open in 5 days but *P. pseudostrobus* will take 3 weeks. Drying is ordered by collection date but collections are not handled as separate seedlots, other than by species (Fig. 3).

Figure 3. Cone drying area.

Seed extraction is simple. Two cones are struck against each other, by hand, to release the seed onto the poly sheet. Cones are discarded and the winged seed and debris collected for immediate cleaning. The winged seed is immersed in cold water for 5 minutes. This causes the seed to separate from the wing and the heavy seed sinks. All floating material is discarded which Adolfo estimates is about 30% by weight. The seed and some debris (resin, gravel, pieces of cone scales) is lifted from the water and dried, in the shade, with the help of a fan, for a period of 2 hours.

Adolfo sells this seed for MXN $700 to $750/kg (approximately US $60/kg). Seedling prices are about MXN $2.10 each (US $0.17 each).

Pedro will be using a precision seeder, sowing into Copperblock 77/170's. He needs a secure supply of high quality seed and, in order to deliver an outstanding product, he needs seed from known provenances.

With all that, why not create the Mexico Tree Seed Centre and also help other nurseries in Mexico and beyond?

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TREES ARE SPECIES AT RISK

Since June 5, 2003 the Federal government has had a Species at Risk Act (SARA). The act was a response to the Canadian Biodiversity Strategy in response to the United Nations Convention on Biological Diversity. The purpose is to prevent indigenous species from becoming extinct, providing for the recovery of challenged species, and to encourage management options preventing other species from becoming at risk. The SARA also establishes the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as an independent body of experts to assess the level of risk. The process is basically that COSEWIC will assess species and assign a level of risk followed by a public consultation process and then a decision on whether to list a species and how to categorize it. A large amount of additional background material is supplied at the SARA Registry home page: http://sararegistry.gc.ca. Under the definitions provided, the term wildlife includes vascular plants.

In November 2010, a "Consultation on Amending the List of Species under the Species at Risk Act: Terrestrial Species" was produced calling for public input. The document can be obtained from either of these pages, in English and French respectively: http://sararegistry.gc.ca/involved/consultation/default_e.cfm or http://sararegistry.gc.ca/involved/consultation/default_f.cfm

Comments may be submitted by e-mail to the SARA public registry at: SARAregistry@ec.gc.ca or by regular mail to: Director General, Canadian Wildlife Service, Environment Canada, Ottawa, Ontario, K1A 0H3. The consultation is open until February 4, 2011. Of specific interest with trees are the proposed addition of whitebark pine (*Pinus albicaulis*) as an endangered species and the confirmation of cucumber tree (*Magnolia acuminata*) as an endangered species. **If you have any involvement with those species, please let your thoughts be known through the process!**

I thought it would be useful to list the tree species (my definition) that currently reside on the SARA list and their status.

Common Name	Scientific name	COSEWIC Status	SARA Status
American chestnut	*Castanea dentata*	Endangered	Endangered
Butternut	*Juglans cinerea*	Endangered	Endangered
Cherry Birch	*Betula lenta*	Endangered	Endangered
Cucumber tree	*Magnolia acuminata*	Endangered	Endangered
Eastern Flowering Dogwood	*Cornus florida*	Endangered	Endangered
Red Mulberry	*Morus rubra*	Endangered	Endangered
Shumard Oak	*Quercus shumardii*	Endangered	Endangered
Blue ash	*Fraxinus quadrangulata*	Special Concern	Special Concern
Whitebark pine	*Pinus albicaulis*	Endangered	Not Listed

David Kolotelo

MULTI-DISCIPLINARY PROJECT ON THE BIOLOGY OF INVASION – GRADUATE POSITIONS POSSIBLE

The goal of the project is to identify the molecular, biological, and ecological mechanisms of parasitism. In particular, we will study the invasion of forests by *Megastigmus*, a genus of seed parasites. These insects are aggressive invaders that cause massive seed losses. They also provide sites for other insects that carry fungal pathogens that, in turn, are responsible for disease epidemics. When accidentally introduced to western Europe from western North America they spread into most forest ecosystems of France.

Researchers Patrick von Aderkas, Jürgen Ehlting (University of Victoria's Centre for Forest Biology) and Steve Perlman (Department of Biology) received funding from a Franco-Canadian Partnership Grant (NSERC Strategic Partnerships Program with Agence National de Recherche Programme Blanc International). The other members of the team include Jean-Noël Candau of the Canadian Forestry Service (Sault Ste-Marie) and Marie-Anne Auger-Rozenberg, Alain Roques, Thomas Boivin, and Lionel Roques of the French Institut National de la Recherche Agronomique.

Specific projects include: a) transcriptomics of the Douglas-fir – *Megastigmus* association, b) microbial associates of *Megastigmus*, c) modeling dynamics of *Megastigmus* invasion, d) systematics of *Megastigmus*, and e) histology of conifer ovule and wasp development.

There are seven graduate positions and one post-doctoral position available. For more information or to find out how to apply see http://web.uvic.ca/~macbi/en/ or http://web.uvic.ca/~macbi/fr/.

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UPCOMING MEETINGS

10th Conference of the International Society for Seed Science
"Seed Science in the 21st Century"
April 10–15, 2011   Salvador - Costa do Sauipe, Brazil

ISTA Annual Meeting 2011
June 13–16, 2011
Tsukuba, Japan
Visit: www.seedtest.org/AM11

32nd Canadian Forest Genetics Association Meeting
"Forest Genetics and Tree Improvement: New Knowledge, Challenges and Strategies"
Aug 16–18, 2011 Thunder Bay, Ontario
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