

# 34<sup>th</sup> Canadian Forest Genetics Association Conference



## **International Union of Forest Research Organizations**

Unit 2.09.01 Seed Orchards

Unit 2.04.01 Population, Ecological and Conservation Genetics

### **Joint Conference**

Part 1 Minutes and Members Reports

Part 2 Symposium



August 17-20, 2015

Fredericton, New Brunswick, Canada

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Canadian Forest Genetics Association. Meeting (34<sup>th</sup>: 2015 : Fredericton, NB)

Proceedings of the Thirty-Fourth Meeting of the Canadian Forest Genetics Association : Tree Breeding Silviculture Growth and Yield

Includes preliminary text in French.

Contents : Part 1. Minutes and Member's Reports. Part 2. Symposium.

1. Forest genetics – Congresses.
2. Trees – Breeding – Congresses.
3. Forest genetics – Canada – Congresses.
- I. Atlantic Forestry Centre.
- II. Title: Proceedings of the Thirty-fourth Meeting of the Canadian Forest Genetics Association

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l'Association canadienne de génétique forestière. Conférence (34<sup>th</sup> : 2015 : Fredericton, NB)

Compte rendu de la trente-quatrième réunion de l'Association canadienne de génétique forestière: croissance et rendement de la sylviculture d'amélioration génétique des arbres

Comprend des textes préliminaires en français.

Sommaire : 1ère Partie. Procès-verbaux et rapports des membres. 2e Partie. Colloque.

1. Génétiques forestières – Congrès.
2. Arbres – Amélioration – Congrès.

3. Génétiques forestières – Canada – Congrès.

I. Centre de foresterie de l'Atlantique.

II. Titre.: Comptes rendus du trente-troisième congrès de l'Association canadienne de génétique forestière

PROCEEDINGS  
OF THE  
THIRTY-FOURTH MEETING  
OF THE  
**CANADIAN FOREST GENETICS  
ASSOCIATION**  
  
**PART 1**

**Minutes and members' reports**

Fredericton, New Brunswick  
August 17 -20, 2015

Editor  
K. Forbes

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These Proceedings are available at: [www.cfga-acgf.com](http://www.cfga-acgf.com)

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Canadian Forest Genetics Association  
2019

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University of New Brunswick – Faculty of Natural Resource Management  
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COMPTES RENDUS  
DU  
TRENTE-QUATRE CONGRÈS  
DE  
**L' ASSOCIATION CANADIENNE  
GÉNÉTIQUE FORESTIÈRE**

**1<sup>re</sup> PARTIE**

**Procès-verbaux et rapports des membres**

Frédéricton Nouveau-Brunswick  
17 au 20 août 2015

Rédacteur  
K. Forbes

Les demandes de renseignements peuvent être adressées aux auteurs ou à Kathleen Forbes, Rédacteur, ACGF, Ressources naturelles Canada, Service canadien des forêts-Atlantique, C.P. 4000, Fredericton, Nouveau-Brunswick, Canada, E3B 5P7. Courriel : [Kathleen.forbes@canada.ca](mailto:Kathleen.forbes@canada.ca)

Ces comptes rendus sont disponibles à : [www.cfga-acgf.com](http://www.cfga-acgf.com)

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2019

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# IN MEMORIUM

## Tribute to Dr. Narinder Dhir

Dr. Narinder Dhir led the Alberta provincial tree improvement programs for 33 years and was a prominent member of the Canadian tree improvement community.

Narinder Dhir was born in India where he completed his undergraduate and graduate studies in agriculture. His M.Sc. thesis (1966) in agricultural botany at the Indian Agricultural Research Institute was on "Cytogenetical Studies in Indian Vetiver". In 1971 he received his M.Sc. in forestry from the University of Minnesota. His thesis was entitled "Study of seedlings and nuclear characteristics of *Pinus resinosa*". Three years later he graduated with the Ph.D. degree in forestry from the same university. The title of his Ph.D. thesis was "A comparative study of inter- and intra-provenance crosses of eastern cottonwood (*Populus deltoides*)".

Following his graduation from the University of Minnesota, Narinder moved to Canada where he briefly worked as a Postdoctoral Fellow with the Canadian Forest Service at the Petawawa National Forestry Institute in Ontario. In 1975 he was hired by the Timber Management Branch of the Alberta provincial government. His task was to set up and lead the provincial tree improvement programs. That was a huge challenge for a recent graduate without any roots in Alberta. At that time he had no staff and no facilities. But he did very well and over the next 33 years he steadily built extensive tree improvement programs in the province. He established Alberta Tree Improvement and Seed Center at Smoky Lake which currently employs 23 people and temporary staff. At the center there are seed orchards, field tests, the provincial seed storage facility which holds all seed for reforestation on public land in Alberta, clone banks, labs, greenhouses and administrative buildings. During his tenure the Alberta Forest Genetics Council was established to promote cooperation with the industry, the Alberta Standards for Tree Improvement were initiated which regulate management of forest genetic resources on provincial land, and the Forest Genetics Association of Alberta was formed to address the needs of smaller forest companies lacking the resources to carry out tree improvement on their own. This work was in addition to establishing numerous species, provenance and progeny trials, clone banks and seed orchards. During his career Narinder went through several economic "boom and bust" cycles yet always managed to maintain and grow the programs even during hard times.

As a manager, Narinder took great interest in the professional development of his staff. He encouraged and made possible for some of his staff to pursue education in forest genetics at the university and college level. Under his leadership, Alberta government supported numerous research projects at the University of Alberta that have led to many M.Sc. and Ph.D. graduates who are currently active in academia, research and tree breeding in Canada and overseas.

Narinder was an avid reader of scientific books, journal articles, reports and magazines. He would not pass a new forest genetics or tree improvement book without purchasing it and including it in his library. Even after his retirement in 2008, Narinder kept track of new publications in forest genetics and readily shared his knowledge and expertise with his former co-workers. Narinder Dhir died in Edmonton, Alberta in April 2014.



## CFG/ACGF ACTIVE MEMBERS

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Victoria, BC

## BUSINESS MEETING MINUTES

## **CFGA/ACGF 34<sup>th</sup> Business Meeting Minutes**

Michele Fullarton chaired the 34<sup>th</sup> Business Meeting of the CFGA/ACGF held at Fredericton, New Brunswick on Tuesday August 18, 2015. Twelve Active Members were present.

### **398 Minutes of the 33<sup>rd</sup> Meeting** (as distributed August 6, 2015)

Motion: That the minutes of the 33<sup>rd</sup> Business Meeting be approved as published.  
Moved by: Om Rajora  
Seconded by: Yill Sung Park

Carried.

### **399 Membership**

#### **399.1 Honorary Membership**

The names of four nominees were presented as follows:

Leonard Barnhardt  
Jean Beaulieu  
Howard Frame  
Kathy Tosh

Motion: That the nominated Honorary Members be duly elected.  
Moved by: Yill Sung Park  
Seconded by: Om Rajora

Carried.

#### **399.2 Active Membership**

The names of six nominees were presented as follows:

Kathleen Forbes Fredericton, NB	Natural Resources Canada
Patrick Lenz Quebec, QC	Natural Resources Canada
Andrew McCartney Sussex, NB	J.D. Irving, Limited
Shona Millican Sussex, NB	J.D. Irving, Limited



Lindsay Robb  
Smoky Lake, AB

Agriculture and Forestry

Stefan Schrieber  
Edmonton, AB

University of Alberta

Motion: That the nominated Active Members be duly elected.  
Moved by: Jean Beaulieu  
Seconded by: Om Rajora

Carried.

#### **400 Chair's Report**

The 34<sup>th</sup> meeting of the Association was held in Fredericton, NB. The meeting was jointly hosted by the three Maritime Provinces: New Brunswick, Nova Scotia, and Prince Edward Island. The conference was joint with two IUFRO Units: Population, Ecological and Conservation (Unit 2.04.01) and Seed Orchards (Unit 2.09.01). We considered the event a success with 60 registrants.

The Organizing Committee consisted of the following individuals:

Michele Fullarton: Agenda, Field trip, Logistics, Finances

Tom Ng: Logistics, Finances

Om Rajora: Unit 2.04.01

Shona Millican: Field trip, Brochures

Greg Adams: Invited speakers

Yill Sung Park: Invited speakers

Yuhui Weng: Volunteer papers and Posters

Tannis Beardmore and Kathleen Forbes: Registration

Dale Simpson: Agenda, Registration, Student sponsorship

Dave Steeves: Nova Scotia Rep, Agenda

Mary Myers: PEI rep, Logistics

Terry Gauthier: PEI rep, Logistics

Support was provided by seven sponsoring agencies, a critical component of the meeting to fulfill our financial obligations.

Michele Fullarton  
Chair

Jean Beaulieu expressed his thanks to the organizing committee for having a good conference.

#### **401 Treasurer's Report**

Michele Fullarton tabled the financial statement for the period of June 2013 to June 2015 for information and acceptance by the membership (see Attachment #1). The statement shows a balance of \$ 20,643.68.

Motion: That the financial statement be accepted as presented.  
Moved by: Yill Sung Park  
Seconded by: Dave Kolotelo

Carried.

#### **402 Financial Contributions**

J.D. Irving , Limited  
Jiffy  
New Brunswick Department of Natural Resources  
Nova Scotia Department of Natural Resources  
Natural Resources Canada  
Prince Edward Island Department of Forests, Fish and Wildlife  
University of New Brunswick

Motion: That the CFGA/ACGF executive of the 34<sup>th</sup> meeting express our sincere appreciation to these contributors.  
Moved by: Dave Kolotelo  
Seconded by: Kathy Tosh

Carried.

#### **403 Editor's Report**

The proceedings of the 33<sup>rd</sup> meeting have not been completed. When they are uploaded to the Association's web site an announcement will be sent to all Active members, Honorary members, and registrants. As well, a copy of the proceedings on DVD will be sent to Canadian universities and other libraries.

Membership consists of: 18 Honorary, a net decrease of 1 since the last meeting  
80 Active, a net decrease of 6 since the last meeting  
59 Canadian libraries, a net decrease of 2 since the last meeting  
12 libraries do not receive the Proceedings because they can access them on the Association's web site

Dale Simpson

#### **Education Committee**

Requests to nominate a student for sponsorship to the Forest Genetics 2015 meeting were sent to all seven Canadian universities with accredited undergraduate forestry programs. Student nominations were received from five universities with sponsored students and affiliations indicated below.

Jiejie Wang	University of New Brunswick
Juliana Sena	Université Laval

Emma Kneil	University of Alberta
Jiayin Song	University of British Columbia
Tim Owen	University of Northern British Columbia

There was a joint evaluation committee consisting of Yill Sung Park, Tannis Beardmore, Sally John, and Dale Simpson. All students were eligible for all awards, but no student could win more than one award were criteria we used.

The CFGA Gene Namkoong award (\$300) is presented to a student for the best poster. The winner was Juliana Senda from Université Laval for her poster “Adjusting to Water Stress: Dehydrins Genes in White Spruce”.

The Carl Heimburger Award (\$500) is presented to a student for the best oral presentation. The winner was Adriana Suarez-Gonzalez from University of British Columbia for her presentation “Evidence that allelic introgression from *Populus balsamifera* (balsam poplar) underlies local adaptation in *P. trichocarpa* (black cottonwood)”.

Dale Simpson

## Working Group Reports

### Tree Seed Working Group

The Tree Seed Working Group continues to publish a biannual Newsbulletin and both Dale Simpson and I graciously thank all those who have contributed articles. Since our last meeting we produced editions 58, 59, 60, and 61. Past editions of the Newsbulletin dating back to 1983 can be found here: <http://www2.gov.bc.ca/gov/content/industry/forestry/managing-our-forest-resources/tree-seed/tree-seed-centre/cone-seed-improvement-program/tree-seed-working-group>

We try to encourage Newsbulletin participation by having a lottery at each workshop for those who have contributed articles since the last TSWG workshop. This year’s grand prize winners were Randy Ford and Al Foley who won a copy of “Lives of Conifers – A comparative account of the coniferous trees” by Graham Powell. Graham served as chair of this group from 19871 to 1991 and it was great to have him in the audience for the workshop.

The 2015 workshop had a theme entitled “ **Development and Application of Water Activity for Monitoring Seed Moisture Content**” which we think is an important technology for improving the conservation and storage of our precious seed and pollen resources. The following speakers provided their perspectives and jurisdictional activities in this area:

<b>Bob Karrfalt</b> , Director of the USDA Forest Service National Seed Laboratory	The practical applications of water activity to improve the efficiency of seed management systems
<b>Lindsay Robb</b> , Alberta Provincial Seed Specialist	Using water activity in tree seed banking and its implementation in Alberta
<b>Fabienne Colas</b> , Biologist/Seed Scientist - Quebec government Research Branch	The application of water activity to the management of pollen and seeds in Quebec

Dave Kolotelo also provided a talk on “Seed viability prediction options” as a review of tools available for assessing seed viability during collection and processing activities. To round out the workshop we had a visual tour or “Cross Canada seed centre check-up” of several Canadian Tree Seed Centres to complement what we would later see at Natural Resources Canada, Kingsclear and JD Irving. Fabienne presented a tour of the Quebec facility, Al Foley, manager of the Ontario Tree Seed Plant provided an overview of their facility in Angus; Lindsay Robb gave a visual tour of the Alberta Tree Improvement and Seed Centre facility and Dave provided an overview of the BC Tree Seed Centre. I thought that this was a good initiative that provided a good visual overview of how different facilities approach the same task of seed extraction and purification in addition to outlining the other activities and initiatives they are involved in. Last, but not least I’d like to thank the CFGA organizing committee for support with our refreshment break, tour transportation, our raffle prizes and for putting on a great meeting in Fredericton.

Thank you to Dale Simpson, our workshop speakers, attendees and Newsbulletin contributors for helping to continue to keep this working group alive and kicking.

Dave Kolotelo  
Chair

### **Old Business**

none

### **New Business**

none

### **Future Meetings**

#### **Location of the 2017 Meeting**

Andy Benowicz confirmed that the next meeting will be in Alberta.

#### **Location of the 2019 Meeting**

Quebec, QC.

#### **Location of the 2021 Meeting**

Ontario, possibly.

It was recommended that organizing committees should consider inviting other organizations such as IUFRO to join for a join for a joint conference in order to attract participants from a larger geographic area.

### **Adjournment**

Motion: That the 34<sup>th</sup> business meeting of the CFGA/ACGF be adjourned.  
Moved by: Dave Kolotelo

**CFGa/ACGF****Financial Statement  
June 2013 – June 2015****= June 30, 2013**

nce \$ 6, 146.23

**ldings \$ 6, 146.23**

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hequing account) \$ 1.11  
13 – Advance \$ 15,000.00  
– Surplus \$ 5,560.67

**bits \$ 20, 561.78**

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wards (CFGa 2013) \$ 800.00  
for CFGa 2015 \$ 5,000.00  
hosting and annual domain fees) \$ 273.33

**bits \$ 6,073.33**

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**= June 30, 2015**

nce \$ 20,634.68

**ldings \$ 20,634.68**

## ACTIVITY REPORTS FROM ACTIVE MEMBERS

## NEW BRUNSWICK TREE IMPROVEMENT UPDATE

**Michele Fullarton and Yuhui Weng  
c/o NB Dept. Natural Resources  
Kingsclear Provincial Forest Nursery  
3732 Route 102  
Island View, New Brunswick  
E3E 1G3**

**Keywords:** Breeding, second generation, GA4/7, third generation, seed orchard, black spruce, jack pine, white spruce, red spruce, Norway spruce, tamarack, NBTIC, tray mixes

The New Brunswick Tree Improvement Council (NBTIC) was formed in 1976. It is comprised of industrial, scientific and government agencies, and the objective of the Council is to provide well adapted, improved local seed for the reforestation of Crown land. The Department of Natural Resources (DNR) coordinates the NBTIC activities, and is responsible for the breeding and growing of test material. The Seedling Production section of DNR is currently growing 21 million seedlings annually for planting, with all the seed coming from seed orchards. The Tree Improvement program in NB continues to focus on the main reforestation species, white spruce (*Picea glauca* [Moench] Voss) black spruce (*Picea mariana* [Mill.] B.S.P.), red spruce (*Picea rubens* Sarg.), Norway spruce (*Picea abies* (L.) Karst) and white pine (*Pinus strobus* L.). Other species include balsam fir (*Abies balsamea* (L.) Mill.), jack pine (*Pinus banksiana* Lamb.) and tamarack (*Larix laricina* [Mill.] Karst.).

### SEED ORCHARDS AND SEED PRODUCTION

Orchard establishment for NBTIC members currently stands at 304 ha comprising of 7 coniferous species (Figure 1).

These orchards are meeting the reforestation demands for Crown land. Seed production in 2014 was poor, with just under 35 million seeds, with the majority of that collected from white spruce and white pine orchards. The seed collected from DNR's black spruce 2<sup>nd</sup> generation orchards was done by clone once again. This will give us the opportunity to provide a better clonal mix to the nursery, increasing volume gains in plantations at an acceptable diversity level. In 2015, indications in the orchard are that we will have a large cone collection in our high value second generation white spruce orchards. These orchards were injected with GA 4/7 in 2014.

### TREE BREEDING, TESTING AND SELECTION

Polycrossing of second generation black spruce and jack pine selections was completed in 2007. Third generation jack pine selections and further breeding are on hold due to a reduction in demand for this species in the reforestation program, although 40 selections were made to establish a 3<sup>rd</sup> generation orchard. DNR continues to follow the breeding strategies for black spruce and jack pine that were adopted in 1993. Clones have been uniformly deployed to breeding groups, and breeding is conducted in a positive assortative mating design. A total of 23 sub-lines have been established for the black spruce and 21 for jack pine. The full-sib testing is completed for black spruce, with one more test series to plant in jack pine. The black spruce breeding strategy is under review and will be modified for advanced generations. The second generation white spruce breeding for the past three years has been doing supplemental mass pollination on orchard clones to establish tests to evaluate clones for future rogueing. Pair-mating started in 2013 using an updated breeding strategy.

In the NBTIC program, three hundred and fifty-five tests have been planted on 400 ha located around the province. In 2014, a white spruce realized gain test series was established, which included an operational collection of second generation orchard seed. In 2015, the last series of black spruce 2<sup>nd</sup> generation full-sib progeny tests was



established. This completes the breeding for the black spruce second generation population. There have been no new selections made for any species in 2014 and 2015. Our focus right now is on completing the 2<sup>nd</sup> generation breeding and testing.

## **NBTIC DATA ANALYSIS AND RESEARCH**

The data analyst position has continued to be funded by the NBTIC. A total of 9 NBTIC test series were analyzed in 2013 and 2014 which included 1 half-sib progeny test, 1 clonal test, 5 full-sib progeny tests and 2 gain tests. Results of these analyses suggest that genetic gains were reasonably high. Genetic selection from the full-sib progeny tests (selecting 10%) would result in about 5~10% gains in 10-yr height for black spruce and in 12-yr height for jack pine. The realized gain tests confirmed that gains for the seedlots collected from the existing seed orchards were high. For example, a seedlot collected from a 2<sup>nd</sup> generation clonal seed orchard of black spruce achieved 40% more volume at age 10 years than the unimproved stand checklots. For white spruce, a seedlot collected from a 1<sup>st</sup> generation clonal seed orchard had a realized gain of 22% in 20-yr volume compared to the stand checklots. In addition to data analysis, data analyst was involved in a few research projects during this period, focusing on balancing genetic gain and diversity for reforestation, understanding benefits in carbon credits from tree improvement activities, and analyzing economics of a white spruce seed orchard. Results suggest that planting growth-improved seedlots would greatly enhance biomass productivity and C sequestration of plantations. Using real data, the benefit-cost analysis of the 1<sup>st</sup> generation white spruce clonal seed orchard located at Lower Queensbury, NB, showed that establishment and management of the orchard was an excellent investment overall.

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## NATIONAL TREE SEED CENTRE

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The mission of the National Tree Seed Centre is to safeguard Canada's forest genetic resources in the face of climate change and other threats by acquiring, evaluating, preserving, and providing a national collection of forest genetic resources to assist in securing the forest biological diversity that underpins the sustainable development of Canada's forests. The Centre aims to store seed of tree and shrub species from throughout their ranges in Canada. On-going activities include collecting and processing seed; conducting tests such as germination, moisture content, and 1000-seed weight; and setting up and evaluating research trials. This report covers activities during 2013 and 2014.

In order to expand the geographic sampling for species and to add new species to the inventory seed is obtained by collecting, donation, and purchase. Collecting is focused on obtaining seed from natural populations thereby capturing the natural genetic variation that is present within species. Most of the collection effort is focused on collecting seed from a minimum of 15 trees in a population and keeping the collections separate by tree. Many of the accessions donated or purchased are bulk collections from natural populations. Seed is stored at -20°C primarily in two categories: Seed Bank where seed is available for research and Gene Conservation. During the two-year period 5 seedlots from 4 species were collected and 244 seedlots from 14 species were donated or purchased. Almost 6,900 seedlots from 148 species exist in Seed Bank. The Gene Conservation collection also continued to grow. Seed in this collection conserves natural genetic variation through collections from species or populations that are unique or threatened as well as samples from seedlots that were collected for Seed Bank. The collection grew as a result of ash collections donated from Ontario and Quebec. As well, 15 *Pinus albicaulis* seedlots from three National Parks in Alberta and British Columbia were donated for back-up storage. About 4,950 seedlots from 50 species are stored in Gene Conservation. The Seed Centre also has a cryogenic facility. Most of the material stored is tissue arising from somatic embryogenesis but a conservation program, led by Tannis Beardmore, has started for butternut (*Juglans cinerea*). Butternut is listed nationally as endangered due to butternut canker caused by an exotic disease (*Sirococcus clavignenti-juglandacearum*). Butternut seed are recalcitrant however the embryonic axis is tolerant to desiccation and freezing. Axes from 25 collections made in New Brunswick were stored in liquid nitrogen. The program has funding for three years and more collections will be made.

A program for the conservation of ash seed has been led by the Seed Centre since 2004 due to the threat imposed by emerald ash borer (*Agilus planipennis*) that is expanding its range in Canada. There is an opportunity to collect and store seed to conserve the genetic variability of native ash species so that the material will be available for research and/or restoration planting after the insect infestation has passed through. There was a good seed crop in 2013 that resulted in over 140 collections donated from Ontario and Quebec. About 800 seedlots from five species are in storage.

Germination testing of seedlots in storage is an integral part of the Seed Centre's annual activities. For most species, germination tests are conducted every ten years. About 800 tests were completed during the two-year period. Testing is conducted using Petawawa Germination Boxes. Generally, 4 reps of 50 seed are tested in a box. For larger seed such as *Fraxinus* and *Acer* 25 seed per rep is tested. The data not only provide an indication of the

quality of each seedlot but when combined with previous test data they provide an indication of the long-term storage potential for a species. This is particularly important when seed is being stored long term for genetic conservation purposes.

Operational research is an important activity. Evaluating germination test data is one aspect as discussed above. Another activity is setting up germination trials to evaluate seed treatments to determine which one promotes maximum germination. A series of trials were set up for *Prunus pennsylvanica*, *P. serotina*, and *P. virginiana* var. *virginiana* evaluating various combinations and durations of moist incubation and moist chilling. These results provided the opportunity to refine the treatments for *P. virginiana* var. *virginiana* to maximize germination for nurseries growing seedlings for reclamation of oil sand mining sites in Alberta. A combination of nine treatments that included incubation in the dark (21°C) (2, 4, 6 weeks) and chilling in the dark (4°C) (16, 20, 24 weeks) were used. Following treatments, seed were transferred to germination boxes and placed in a germination cabinet (25°C for 12 h with light, 20°C for 12 h in dark, 85% relative humidity). The 2-week moist incubation + 20-week moist chilling treatment was most effective in maximizing germination. After 21 days in a germination cabinet, total germination ranged from 19–85%. Variation in germination was a reflection of seedlot quality. After being placed in the germination cabinet 99% of the seed that germinated did so within seven days.

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## Reclamation and reforestation of highly disturbed riparian zones using native willows

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**Keywords:** Biomass production, forest restoration, mine reclamation, native North American willows, *Salix* species

Forested land that has been highly disturbed by human activities such as mining can disrupt important ecological services that humans depend on. The productivity—or capacity to produce biomass—and the ecological services that were once provided must be restored as quickly as possible. Riparian zones are areas of special concern because water quality is an important ecological service that has become a main focus for regulatory efforts on former mine sites. Willows are naturally adapted to wetlands and riparian zones and can be used to mitigate erosion and siltation of water courses. They can also serve as natural filters for pollutants and heavy metals. As relatively short-lived species that invade and occupy disturbed sites for short periods during early stages of natural succession, willows may also be useful as nurse crops for the restoration of native forest cover. Our investigations of the usefulness of eight of the more than 108 native North America willows have shown that willows are promising for both biomass production and land reclamation on highly disturbed, low-fertility sites.

Our goal was to assess some common native willows for rapid revegetation of highly disturbed areas, including a former coal mine site in New Brunswick (NB), with the ultimate aim of promoting a more rapid natural succession to forest cover over areas currently being disturbed by oil sands mining in western Canada. We compared genetic variation within and among natural populations of willows for survival, biomass production, and various growth traits on disturbed coal mine sites and have selected a set of superior willow clones from several different species for distribution to land management agencies interested in site reclamation.

The species being tested include both tree and shrub willows adapted to a wide range of moisture conditions, thereby providing land managers with a range of plant forms adapted to a range of different site types. A representative sample of clones from each of these willows was collected from natural populations in Ontario (ON), Quebec (QC), and New Brunswick (NB) for establishment in common-garden field tests on the infertile shale rock overburden that dominates the Salmon Harbour coal mine site near Fredericton, NB (Lat. 46°07' N; Long. 66°05' W) on a property operated by NB Coal Ltd., a subsidiary of the local electric power utility, NB Power.

Field tests were established with 20 cm long, rootless, stem cuttings collected during the winter months from vigorous 1- and/or 2-year-old plants originating in natural populations in ON, QC and NB. Two-year-old coppice regrowth was harvested for measurements of biomass yield and coppice structure. A common-garden experiment established in 2008, which contained 20 clones from seven species collected directly from natural populations, showed significant differences in survival, biomass yield, and number of stems per coppice, with *Salix eriocephala* (ERI) showing the best survival at approximately 70%. Species with poor rooting ability, such as *S. bebbiana* (BEB) and *S. humilis* (HUM), had the lowest survival at approximately 3% and 10%, respectively, 6 years after establishment. The greatest number of coppice stems per plant and greatest biomass production were also produced by ERI, whereas *S.*

*amygdaloides* (AMY) and *S. nigra* (NIG) produced the least green biomass and among the lowest number of stems per coppice. However, these survival results contrasted quite sharply with those demonstrated by clones that had already undergone an initial round of selection based on prior field testing at the Montreal Botanical Gardens (MBG) for survival and growth. Clones selected from five species established in two MBG field tests showed significantly higher rooting success and thus higher survival. Clones of *S. interior* (INT) showed the best overall performance. Both ERI and INT had the greatest and most consistent rate of rooting success and survival under field conditions.

Throughout the northern hemisphere, willows are natural colonizers of highly disturbed, infertile sites and are capable of fixing their own nitrogen based on microbial associations in the stems. Site fertility does not appear to be a major limiting factor for willows established on rocky sites with very little soil organic matter, nitrogen, or soil development. Willows are also ideally adapted for rapid invasion and colonization of mine sites because of their abundant annual seed production and effective long-distance seed dispersal by wind. Among the willows tested, BEB and *S. discolor* (DIS) were common natural colonizers of these abandoned NB coal mine sites. All the shrub willows described here (BEB, DIS, ERI, HUM, and INT) flowered and produced viable seeds in the second growing season following establishment as stem cuttings. By establishing a series of small patches of several dozen individuals on wetter areas, it may be possible to quickly capture available, suitable sites via abundant natural seed dispersal with these highly fecund shrub willows. We estimated that a single 2-year-old coppice plant of ERI can produce several million seeds annually. The main requirements for willow establishment from seed are exposed mineral soil, a constant moisture supply during seed germination and early seedling development, and full sunlight for developing seedlings.

On barren, infertile, shale overburden, several of the willow species tested were able to grow reasonably well from unrooted stem cuttings when root development took place quickly enough to sustain early seedling growth during seasonal periods of moisture availability. However, these willows showed large variation in their ability to produce roots from rootless, dormant stem cuttings. Willows naturally adapted to riparian or wetland habitats, such as ERI, INT, AMY, and NIG, showed much better rooting ability than the non-riparian, upland willows BEB, DIS, and HUM. Variation in rooting ability is a key factor in survival and growth, and rooting success may be the most important criterion for determining which species are best for land reclamation on highly disturbed, low fertility sites. The most consistent and rapid root development from rootless dormant stem cuttings was demonstrated by ERI and INT.

A unique feature of INT is its ability to form multi-stemmed colonies that develop from rhizomes and the extensive, shallow root network from which new stems arise via suckering. Six years after establishment, many of the INT clones have already spread 3–4 m from the original ortet (mother plant or stem cutting) and are developing into small, multi-stemmed colonies. This spreading and stem suckering habit from rhizomes forms stem colonies capable of rapidly capturing a site to mitigate erosion and surface runoff. This colony-formation habit can be especially valuable for stabilizing stream banks and maintaining water quality.

Reclamation of mine sites with the aim of eventually restoring natural forest cover has proven difficult on coal mines across the Appalachian region of eastern North America because it is often difficult to establish trees directly on exposed, highly disturbed areas, and simply sowing grasses may delay the process of natural succession to forest cover by further inhibiting tree seedling establishment. Natural primary succession often takes many years, and restoration of the native forest cover can take many decades. However, both ERI and INT grew very well on these mine sites and both species have features that recommend them as useful, productive, and cost-effective species for reclamation and restoration on highly disturbed sites based on their rapid rooting ability, high survival, biomass productivity, and plant morphology. One of our longer-term objectives in testing native willows on former coal mine sites was to

develop a cost-effective protocol for using willows to both capture (revegetate) a site and prevent invasion by grasses and/or ericaceous shrubs detrimental to establishment of trees and to prepare a more fertile soil for forest succession. Willows are normally a short-lived species and could serve as a compatible nurse crop for the artificial introduction of longer-lived or more commercially valuable tree species, thereby shortening the decades-long natural succession process required to reestablish forest cover. Ultimately, our aim is to develop a “best practices plant system” to help restore not only coal mine sites, but also the large areas disturbed by oil sands mining in western Canada.

## Summary

The best clones of *Salix eriocephala* and *S. interior* had over 95% survival and produced approximately 5–6 Mt ha<sup>-1</sup> fresh mass from 2-year-old coppice growth on harsh, low fertility mine sites. Of the eight willow species being tested, we currently recommend these two species for use in mine reclamation efforts because they grew best overall and had the highest survival rates. Despite poor average rooting ability in *S. bebbiana*, *S. discolor*, and *S. humilis*, surviving genotypes of these species showed good subsequent survival and growth, and further selection for improved rooting ability in these species is warranted.

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## RECENT ADVANCES IN TREE REPRODUCTION, SEED AND SEEDLING PRODUCTION IN QUÉBEC

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**Keywords:** climate change, clonal forestry, indoor orchard, pollen, seed, *Larix* sp, *Picea glauca*, water activity, tree improvement program.

The Direction de la recherche forestière works in close collaboration with the Direction générale de la production des semences et de plants forestiers to solve operational problems that can or actually occur in seeds and seedling operational production.

### SEEDS

#### Workshop

A Workshop was organised at the Berthier Tree Seed Centre (BTSC) on December 3-4<sup>th</sup> 2013 (*Les semences forestières: un maillon clé des plantations de grande valeur*). 140 persons visited the Seed Centre and attended the conferences which presented the numerous steps linking genetic improvement to seedling production. A portrait of the forest context and of the forest seed production and uses in British Columbia was also presented. See the website<sup>1</sup> for the complete program, abstracts and presentations (in French). Since the BTSC is ISO 9001:2008

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<sup>1</sup> <http://www.mffp.gouv.qc.ca/forets/connaissances/recherche/atelier-conference-decembre-2013/accueil.asp>

certified, great work was done to establish standard procedures for each step of the work done at the TSC. This is excellent for the “organization memory” especially when people retire.

#### Water Activity

Seed water status is a key element for their conservation. Water activity ( $A_w$ ) measurement characterizes this status and its possible evolution more precisely than water content.  $A_w$  is a useful tool that has been used for several years in Quebec now. An adapted dryer ruled by  $a_w$  allows to stabilise the small sample of pollen and seeds (see Baldet and Colas 2013 for details). This stabilisation can be made for new lots but also for lots where  $A_w$  has risen during conservation. This allows to prolong their conservation. For operational seedlots, the BTSC adapted its drying line to monitor seeds moisture with  $A_w$  only.

Thanks to  $A_w$  which is non-destructive, we highlighted an increase of the seeds water status in conservation. This can be caused by the porosity of the container and its repeated openings. The development of a new container continues with the [CTMP](#) (Centre de technologie minérale et de plasturgie, Thetford-Mines, Québec). A less permeable polymer is tested, the results will be available at the end of 2015.

#### Larix Indoor Seed Orchard

Berthier Tree Seed Centre (BTSC) has the unique indoor seed orchard for the production of hybrid larch seeds (*Larix x marschlinsii*). This orchard has 3 200 grafts of 20 clones of European larch (*Larix decidua*) and 3 250 grafts of 19 clones of Japanese larch (*Larix kaempferi*). For each species, the clones are recommended by the improvement program of larch. A problem of stunted seedlings was detected in some hybrid larch seedling production. A study was conducted that concluded that some genetic combinations cause this problem (see Colas and Lamhamedi 2013 for details).

## GENETICS AND CLIMATE CHANGE

#### How will Superior White Spruce Families grow in the Future?

About 20 million white spruce (*Picea glauca*) seedlings are planted annually in Quebec. To meet the needs for improved seeds, first-generation seed orchards were established from 1983 to 1991, and the establishment of second-generation seed orchards began in the late 1990s. Seed trees in these second-generation seed orchards were selected from superior full-sib families. Tree improvement programs aim to identify and to select families that are well-adapted to present and future growing conditions. To gain insight into the stability of the family genetic response to climate change, we subjected white spruce seedlings from 60 full-sib families to a combination of two temperature regimes and two atmospheric CO<sub>2</sub> concentrations during two growing seasons. Warmer temperatures and a higher CO<sub>2</sub> concentration had a positive effect on some growth variables. Moreover, the absence of significant family  $\times$  treatment interactions suggests that the relative performance of selected families would not be altered by the anticipated climate change under the conditions of high water availability used in this experiment. Among physiological traits, only instantaneous water use efficiency responded positively to higher CO<sub>2</sub>, with a 51% increase that was consistent across families.



## CLONAL FORESTRY

What is the best way to deploy superior white spruce clones?

In 2018, the first white spruce cuttings to be harvested on stockplants produced through somatic embryogenesis will be deployed in Quebec. These clones have been selected on the basis of field measurements taken in clonal tests established from 2007 to 2016 on two different sites. We plan to deploy them as multiclonal varieties composed of a mixture of several clones. Though clonal tests provide a means to assess and compare the performance of various clones in a controlled environment, they give only a partial portrait of the behavior of clones established in forest plantations. In a context of operational deployment, one can ask whether the behavior and yield of a clone planted in a mix with others remains the same as when it is planted « alone » in a monoclonal plantation. This question can be addressed by comparing the performance of clones deployed both in a monoclonal and a multiclonal plantation on a same forest site. In 2014, to achieve this goal, the Direction générale de la production de semences et de plants forestiers (DGSPF) of the Ministère des Forêts, de la Faune et des Parcs (MFFP) began establishing a series of field tests which will be evaluated over an 8-year period in several administrative regions, plantations sites and ecological regions.

## ACKNOWLEDGMENT

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# **TREE IMPROVEMENT PROGRESS AT THE DIRECTION DE LA RECHERCHE FORESTIÈRE**

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## **INTRODUCTION**

The goal of the MFFP breeding programmes is to increase yield of high quality wood for major reforestation species while maintaining a high level of diversity, help to reduce the pressure on natural forests, select varieties better adapted to expected local climate conditions in the coming decades and help in the implementation of appropriate in situ or ex situ measures to conserve forest genetic resources.

## **WHITE SPRUCE TREE IMPROVEMENT**

André Rainville

Measures collected on white spruce seedlings from somatic origin during two growing seasons in the nursery and four years in clonal tests showed that early selection based on nursery performance is possible. At a research scale, clones selected for their high performance in the nursery could be the only one sent for further evaluation in clonal test, thus reducing testing costs, while for operational reforestation, a subset of these clones, representative of a sufficient genetic diversity, could be made available in a shorter period of time (Wahid et al. 2013).

A genomic selection project, led by Laval University to which participates the MFFP, showed that it is possible to predict individual genetic values for growth and wood quality with a precision of 90% compared to traditional methods, thus reducing the length and cost of tree breeding cycles (Beaulieu et al. 2014).

## CLIMATE CHANGE ADAPTATION MEASURES

André Rainville

In a context of climate change, new transfer models were developed; they predict future merchantable volume of white and black spruce plantations in Quebec under the effect of climate change. (Rainville et al. 2014, 2015). The establishment of 9 new tests in the last three years will give the opportunity to confirm and even improve the white spruce transfer model and, as a second objective, evaluate realized genetic gain from improved material. First measures collected on this material, in the nursery as well as in the tests, gave an evaluation of physiological and morphological reaction of transferred provenances (Carles et al. 2015).

## LARCH TREE IMPROVEMENT

Martin Perron

The second cycle of tamarack (*Larix Laricina* [Du Roi] K. Koch) improvement will be based on a low-intensity and low-cost breeding strategy, because of the low demand for tamarack seedlings for reforestation in Quebec. Therefore in 2013, an old tamarack progeny trial (3.5 ha) was intensively thinned for the second time based on the 31-year measurement. This genetic thinning leaves the 800 best of the original 11 500 trees. This progeny trial will now be used as propagation and breeding populations for the next cycle of improvement in eastern Quebec. This selection will produce a predicted mean gain of 12 dm<sup>3</sup> per tree (6%) (Perron 2014a, b). After good flowering years, we plan to collect seeds (open-pollinated mating) on the best 240 parents and eventually establish two progeny trials. For the western Quebec improvement zone, measurement and thinning will be completed in the coming years.

For hybrid larch, approximately 70% of the planned crosses to establish the base population of the second breeding cycle have been completed and the first two small progeny trials were established in 2012-2013.

## JACK PINE TREE IMPROVEMENT

Mireille Desponts

Second-generation controlled crosses are still ongoing for two jack pine breeding populations. In 2013, a population for the southern part of boreal forest was completed. Breeding work to create a population for the northern part of the territory will begin in 2015.

## BLACK SPRUCE TREE IMPROVEMENT

Mireille Desponts

In spring 2014, two full-sib second-generation progeny trials were established in the northeastern black spruce zone. Controlled crosses have been underway since 2012 to create another population for the south and central territories. The latter population will show high genetic diversity and include numerous provenances from a large territory between the 45° and 49° North latitudes, to allow experimentation on climate change adaptation.

The assessment of genetic parameters for wood properties in our black spruce breeding populations has progressed in 2013-2015. In situ measurements in two half-sib populations from boreal and mixedwood zones indicate that modulus of elasticity (MoE) is variable and highly heritable. Selection for growth, density and MoE in two clonal trials was completed in 2014 and will guide a seed orchard thinning. The clonal heritability (repeatability) estimate for MoE is also high. Selection for improvement of height growth, density and MoE could be profitable for all traits. In 2013, samplings and growth measurements of full-sib families in two 21-year-old experimental plantations were completed in collaboration with Université Laval, to evaluate the feasibility of genomic selection for mechanical wood traits such as wood density and cellulose microfibril angle (MFA). Preliminary results of phenotypic measures indicate that genetic selection for MFA could be as profitable as MoE, a closely-related trait.

## NORWAY SPRUCE TREE IMPROVEMENT

Marie-Josée Mottet

Analysis of genetic tests established in 2002 showed that some provenances of Norway spruce (NS) are well adapted to the colder climatic conditions of the balsam fir-white birch bioclimatic domain. In these tests, growth of NS was comparable to that of white spruce. Since NS is now mainly planted in Quebec's northern Lac Saint-Jean region, 3 provenance-progeny tests were established in 2015 in order to recommend well-adapted provenances for the future.

Ten-year results obtained from full- and half-sib tests confirm that white pine weevil (WPW) resistance is under moderate to strong genetic control for NS (Mottet et al. 2014 and 2015). Future measurements in these tests will be useful for developing genomic selection methods for growth traits, wood characteristics and WPW resistance.

The Escourt seed orchard comprised of resistant or tolerant genotypes has been producing seeds since 2014.

## HYBRID POPLAR IMPROVEMENT PROGRAM

Pierre Périnet and Marie-Josée Mottet

For the 2013 to 2015 period, selection of advanced-generation parents of *Populus* species for both intra- and interspecific hybridization is still ongoing in the Québec hybrid poplar program. In 2013, 57 selected trees of *P. deltoides* (males and females) from the Litchfield trial, Ottawa Valley region, were collected and successfully propagated through cuttings. Field-collected branches with staminate inflorescences from 17 trees were forced in greenhouse for pollen production. Pollen was stored for long-term conservation at -29° C.

In spring 2015, 55 families of T×T and M×T, obtained from the 2012 breeding, were planted in progeny trials. Additional seeds from these progenies, stored in the seed bank collection since 2012, were used for greenhouse mass production of seedlings during winter 2015. Plants will be established in the field for further evaluation, especially to achieve clonal selection for biomass and bio-energy production.

Several hybrid poplar populations from the 1996, 1998, and 2004 breeding programs are still under evaluation through many clonal tests established in different regions for local adaptation, vigor, and *Sphaerulina* (formerly *Septoria*) resistance. Promising clones of M×T, M×B, M×D, and M×N are expected soon to be released from these programs.

Spore trapping method has been developed to better understand the epidemiology of *Sphaerulina* spp. and measure the effect of mitigation strategies in plantation.

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## FORESTRY GENOMICS AND GENETICS RESEARCH AT UNIVERSITÉ LAVAL

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### INTRODUCTION

This report summarizes our efforts and results obtained in forestry and environmental genomics and genetics over the 2013-2015 period. The last two years have been crucial for the completion of the SMarTForests project - Spruce Marker Technologies for Sustainable Forestry, under the Large-Scale Applied Genomics Program (LSARP) of Genome Canada and the additional financial support of Génome Québec, Genome BC and Genome Alberta. The project has been led by J. Mackay (Univ. Laval), J. Bohlmann (Univ. of British Columbia), A. Yanchuk (BC Forests) and J. Bousquet (Univ. Laval), and it also involved other research scientists from Univ. Laval (N. Gélinas), the Univ. of British Columbia (K. Ritland, S. Jones, G. Bull), the Univ. of Alberta (J. Cooke), the Canadian Wood Fibre Centre - CWFC (J. Beaulieu), Natural Resources Canada - RNCAN (N. Isabel, A. Séguin), the Ministère des forêts, de la faune et des parcs du Québec - MFFPQ (M. Perron, A. Rainville and M. Despons), BC Forests (A. Yanchuk) and several collaborators worldwide. The emphasis has been put on expanding spruce genomic resources by sequencing the genome of *Picea glauca* and resequencing its transcriptome to inventory gene SNPs, and developing genomic prediction systems for assisting breeding operations including transcriptome-wide association studies and genomic selection proof-of-concept demonstrations in *Picea glauca* and *Picea mariana*. The characters considered are diverse and include pest resistance, wood quality, growth and phenology. In the following pages, some of these achievements will be presented.

The large-scale applied project FastTRAC – Fast Tests for Rating and Amelioration of Conifers, has also been launched in the Spring 2015 under the Genomics Applied Partnership Program (GAPP) of Genome Canada and the additional financial support of Génome Québec, Forest Products Innovation (FPI), CWFC, MFFPQ, J.D. Irving Ltd (JDI), RNCAN, the New Brunswick Tree Improvement Council (NBTIC) and the New Brunswick Department of Economic Development. The project is lead by J. Bousquet from Univ. Laval and G. Smith from FPI-CWFC, and it also involves scientists and practitioners from Univ. Laval (J. Beaulieu, N. Gélinas), FPI-CWFC (F. Robichaud and Y.S. Park), MFFPQ (M. Campagna, M. Perron, A. Rainville and M.-J. Mottet), JDI (G. Adams and A. McCarthy), NRCAN (N. Isabel and K. Klimaszewska) and NBTIC (M. Fullerton). Its goal is to bring GS at the operational scale of *P. glauca* and *Picea abies* advanced-breeding programs. Growth, wood quality and Norway spruce resistance to weevil attacks are the targeted traits of GS predictive systems.

Under the leadership of J. Bousquet, the Canada Senior Research Chair in Forest and Environmental Genomics established in 2001 has been successfully renewed for a third mandate of seven years, from 2015 to 2022. It was the first Canada research chair to have been successfully renewed for a third period.

Under the European Union Framework Program and the leadership of several European research organizations and of M.T. Cervera (INIA, Spain), the ProCoGen (Promoting Conifer Genomics) large-scale project has also been pursuing its activities where J. Mackay and J. Bousquet are involved in collaborative research including comparative and functional genomics and genome mapping of European conifers.

During the last two years, the group has included N. Pavy as senior research associate and several postdoctoral researchers: B. Hornoy, J. Prunier, G. Parent, G. De Lafontaine, A. Azaiez, A. Atangana, G.F. Vodouhe (now assistant prof. at the Univ. of Benin), N. Dhital (now for the Saskatchewan Forest Dept.), P. Lenz (now for CWFC) and T. Doerksen (now for BC Forests). Many graduate students have been involved in the diverse research projects, including at the MSc level F. Comté, G. Germanos, B. Marcos, M.B. Nadeau (now CEO of Viridis Terra Innovations Co.), R. Serra (now agroforestry specialist for Québec Federation of Forestry Cooperatives), G. Tessier, E. Yusufu Kachaka (now lecturer at the Univ. of Kinshasa), E. Warren; and, at the PhD level, G. Adoumandjali, A.M. Andrianandrasana, B. Cinget, B. Dufils, K. Jordaan, E. Kimpembe Ndinga, M. Lamara, T. Lubalega, C. Mendez-Espinosa, A. Ntumba Tshinyama, F. Omondi, E. Raherison, D. Ratahiriarisoa, A. Sahli, J. Stival Sena, J. Umba, J.-P. Verta (now postdoc in Tübingen, Germany), and G. Wani (now postdoc at the Univ. of Kashmir).

As this period ends, J. MacKay is also preparing to leave Univ. Laval for a newly created position at the Univ. of Oxford in the United Kingdom. I. Porth from UBC will be integrating his position in functional genomics during the Summer of 2015. J. Beaulieu also retired from the Canadian Forest Service in 2014 and given that he has been collaborating with the group for many years, he is now a regular member of the Canada Research Chair in Forest and Environmental Genomics and as invited professor at the Department of Wood and Forest Sciences of Univ. Laval

#### PHYLOGEOGRAPHY AND GENETIC DIVERSITY AT THE GENOME LEVEL (LEADER JEAN BOUSQUET)

Over the last two years, as part of our ongoing work of the phylogeographical atlas of conifers, several additional range-wide phylogeographical studies have been completed. The phylogeographical study of the cosmopolitan *Abies balsamea* using cpDNA and mtDNA markers (Cinget et al. 2015a and PhD thesis of B. Cinget) has revealed new glacial refugia in central North America and has confirmed controversial refugia on the northeastern coast of North America. Contrary to other largely distributed boreal conifers, there was a large congruence between cpDNA and mtDNA geographical structures in spite of different transmission of chloroplast and mitochondrial genomes in the Pinaceae (respectively paternally and maternally), indicating limited cpDNA gene flow in balsam fir compared to other boreal conifers such as jack pine, white spruce or black spruce. Reduced pollen gene flow could be due to the morphology of *Abies* large pollen grains and/or recurrent spruce budworm epidemics that afflict the demography of the species. The phylogeography of *Larix laricina* using cpDNA and mtDNA markers (M.Sc. thesis of E. Warren, manuscript in revision) has also been completed, revealing geographic patterns largely congruent with that of other widespread boreal conifers, with well delineated eastern and western glacial lineages. The cpDNA diversity and population structure of endangered mountain spruces such as *Picea mexicana* in the Sierra Madre of northern Mexico and *Picea rubens* in the Appalachian Mountains of eastern North America has also been investigated (Jaramillo-Correa et al. 2015). The genetic data revealed the signature of population declines for both species, with a quite ancient and severe bottleneck for *P. mexicana* dating back at least to the last interglacial, and a somewhat less severe and likely more recent bottleneck for *P. rubens*.

The study of the hybrid zones is also a common theme of our research program, not only for purposes related to the management of natural genetic resources, but also to better understand the evolutionary trajectory of these zones and their implications for differential adaptation and speciation in trees. A study has been completed in western Canada where *A. balsamea* and *Abies lasiocarpa* come into contact in western Alberta, by using species-specific paternally inherited cpDNA and maternally inherited mtDNA markers (Cinget et al. 2015b and PhD thesis of B. Cinget). The results indicated limited introgression outside the zone of contact and the stability of the zone for the

greater part of the Holocene since the first postglacial contact. This is in contrast to the zone of contact between *Pinus contorta* and *Pinus banksiana* currently in western Alberta, which was shown to have extended much further to the east during the middle Holocene using species-specific mtDNA and cpDNA markers (see our previous report and Godbout et al. 2014 for a review).

A study of the patterns of introgression in eastern North America between *P. mariana* and *P. rubens* using gene SNPs spread on the 12 spruce chromosomes has been completed. The results indicate that while most of the loci were selectively neutral, some permeable loci were more subject to introgression, indicative of ancestral polymorphism along with balancing selection. A smaller set of loci spread over 10 chromosomes was observed. They are reflecting impermeable regions, thus indicative of species boundaries and ongoing divergent selection and speciation. Together, these heterogeneous patterns indicate a young and incomplete speciation process between these progenitor-derivative species.

In the footsteps of our pioneering studies on spontaneous hybridization and introgression between exotic and native species in the genus *Populus* (see our previous report), we have pursued similar studies in the genus *Larix* with similar finding regarding variable spontaneous rate of hybridization and hybrid recruitment depending on the local abundance of the native species *L. laricina* (Meirmans et al. 2014). A multi-locus phylogeny of the genus *Fraxinus* has been reconstructed with all species worldwide with new insights on the long-term evolution of temperate tree species and interesting phylogeographical implications in various parts of the world (Hinsinger et al. 2013). In addition, ancient contact and reticulation during the milder climate period preceding the final elevation of the Himalayas has been observed between nowadays-disjunct Asian and European lineages (Hinsinger et al. 2014).

In collaboration with the Univ. Autonomous of Mexico (J.P. Jaramillo-Correa), a study comparing the variation of a STR-rich mitochondrial intron (nad5-4) with 11 mtDNA genes devoid of STRs within the genus *Abies* and in other Pinaceae has revealed that the regions flanking the STRs harbored mutation rates as low as those estimated in mitochondrial genes devoid of repeated stretches, and that repeated stretches are common in all sorts of plants including angiosperms, but their accumulation in STR hotspots appears to be taxon-specific (Jaramillo-Correa et al. 2013). Thus, the study suggests a new mutagenic role for repeated sequences in the plant mitochondrial genome.

These phylogeographical and genetic diversity studies have implicated various collaborators including the late Tom Ledig (Univ. of California at Davis and member of the North American Forest Commission), who unfortunately passed away in May 2015, and who has devoted much of his fruitful professional life to the conservation genetics of endangered species. They also included the collaboration of J.P. Jaramillo-Correa (Univ. Autonomous of Mexico), D. Hinsinger and N. Frascaria-Lacoste (Univ. of Paris at Orsay and AgroParisTech), P. Meirmans (Univ. of Amsterdam), M. Perron (MFFFP), and N. Isabel (NRCAN).

#### ASSOCIATION STUDIES, QTLs AND GENOMIC SELECTION (LEADER JEAN BOUSQUET)

Under the SMarTForests project, genomic selection (GS) approaches have been developed for *P. glauca* based on the discovery of high-confidence gene SNPs for most of the transcriptome (Pavy et al. 2013a) and the development of high-throughput genotyping chips containing large numbers of SNPs using the Illumina Infinium technology (Pavy et al. 2013b). GS approaches have been tested for both a large effective size population of half-sib families (Beaulieu et al. 2014a) and a small effective size population of full-sib families as part of the advanced breeding population of MFFPQ (Beaulieu et al. 2014b). On average, accuracies of around 0.40 and around 0.80 were respectively obtained for populations of half-sibs and full-sibs, and for characters such as mature growth, wood density and micro-fibril angle. Accuracies were marginally higher for wood density, reflecting the higher heritability for this trait. Much of the accuracy appeared based on relatedness and long-range linkage disequilibrium (LD) rather than short-range LD between quantitative trait loci (QTLs) and markers, and given that the accuracies obtained with the population of half-sibs were about half of those observed for the population of full-sibs, because of the large number of fathers siring half-sibs. The trend was also the same for conventional models using mature field data to estimate breeding values. There was little loss of accuracy when including different test sites into the same GS model or by applying a GS model developed for one site to another one, reflecting little *genotype x environment* interactions as usually seen in populations of eastern white spruce. GS models did very well by recovering 90% of the accuracy and genetic gains obtained by using conventional models at the mature age. So, even if they are

marginally less precise, much larger gains per unit of time could be achieved by using predictive GS models at an early age (Beaulieu et al. 2014b). The study on full-sibs was conducted in collaboration with MFFFP (A. Rainville).

Regarding *P. mariana*, another highly reforested species in eastern Canada, a QTL and an association genetics study for growth and phenology traits based on a candidate gene SNP approach has also been conducted (Prunier et al. 2013), in collaboration with scientists from NRCan (N. Isabel and J. Beaulieu) and MFFFP (M. Despons). While identifying small effect QTLs involved with these traits, the study indicated that the approach of including gene SNPs previously tested positive in environmental association studies or segregating in DNA pools made of extreme phenotypes for adaptive characters resulted in a much higher rate of positives than the traditional approach solely based on random SNPs from candidate genes. It also showed that cohorts of positive SNPs overlapped partially among the different years when the population was assessed, indicating partially changing sets of genes involved. A large SNP resource made of nearly a hundred thousand high-confidence gene SNPs obtained after exome capture has been constructed and validated by genotyping (Pavy et al. submitted). An Infinium genotyping chip has also been constructed and is being used to test GS in advanced-breeding populations of MFFFP (Lenz et al. in preparation with the collaboration of MFFFP, M. Despons). Similar gene SNP resources have also been developed for *P. abies* (e.g. Pavy et al. 2013b) and a genotyping chip has been developed and used collaboratively to build a gene map and identify QTLs related to *P. abies* resistance to *Heterobasidion* by a Swedish group (M. Lascoux, J. Stenlid, M. Lind, T. Kallman et al.) (Lind et al. 2014).

#### FUNCTIONAL GENOMICS OF WOOD TRAITS AND INSECT RESISTANCE (LEADER JOHN MACKAY)

The research under this theme spanned three areas: gene expression profiling, functional genomics of wood formation and secondary metabolism, functional genomics of insect resistance. It aims to enhance our understanding of the conifer transcriptome as it relates to diversity and genetic architecture of complex traits.

Our activities in the area of gene sequencing have led to the development of DNA chip for analyzing gene expression comprehensively; it is comprised of an oligonucleotide microarray (MA) representing 23,853 genes (Raherison et al. 2012). We have used this microarray to investigate various aspects of tree growth and development as well as spruce budworm resistance. We have carried out a first survey of gene expression variation across the major organs and tissues in white spruce (*Picea glauca* (Moench) Voss) (Raherison et al. 2015). This analysis highlighted the modular organization of the white spruce transcriptome and revealed its functional organization. We used these data to reconstruct gene expression networks particularly focusing on the contrast between earlywood and latewood and thus identified potential regulators of wood formation. One of these candidate regulators (the gene PgNAC-7) was specific to early wood and was independently shown in spruce to activate the expression of several secondary cell wall enzymes involved in lignin and cellulose synthesis, and MYB transcription factors (Duval et al. 2014). Over the years we have reported on several of these MYB transcription factors showing that they form a large gene family which includes members involved in wood formation and in defense responses. Recently, we showed that these to major classes of MYB transcription factors may be antagonists with some acting as activators and others acting as repressors of the same enzyme encoding genes (Bomal et al. 2014). Together, these studies provide us with a more complete picture of the genes that regulate wood formation, their activities and interactions.

Gene expression profiling also enabled the recent discovery of a gene with a major effect in natural resistance against the spruce budworm (Mageroy et al. 2015), with end goal to provide tree breeders with informative markers for this trait. We used microarray profiling to show that a sequence encoding a glycosyl hydrolase (PgbetaGlu-1) was very strongly expressed in trees that clearly resisted to spruce budworm defoliation, and had very weak expression in non-resistant trees. The variation in expression was genetically heritable and closely correlated with accumulation of phenolic compounds that are toxic to spruce budworm larvae. We are presently investigating the extent of natural variability in this gene's expression and in the accumulation of the phenolic compounds in breeding populations and in forests presently infested by spruce budworms.

Comparing groups of trees that are of different genetic backgrounds or have contrasted phenotypes is also producing promising results in other areas of investigation. These include the ability of vegetative buds to undergo a transition toward somatic embryogenesis (Rutledge et al. 2013). By comparing different genotypes that respond

different to somatic embryogenesis induction treatments it was shown that stress response genes may prevent the reversion to the embryogenic state.

We previously reported on the development of a novel experimental approach in which the haploid megagametophyte is used to characterize heritable variation in gene expression within individual trees (Verta et al. 2013). In a further study, we used both the haploid megagametophyte and the diploid embryo from the same seeds to investigate the genetic architecture of gene expression variation in white spruce (Verta et al. 2014). The study outlines the genetic landscape effects and the mode of action in enabling gene expression variation; it provides insights into the impact of evolutionary forces on gene expression.

#### GENOME SEQUENCING AND ANALYSIS (LEADER JOHN MACKAY)

As part of the SMarTForests project, we recently contributed to a report describing the sequence of the entire genome of white spruce (Birol et al 2013; De la Torre et al. 2014). We have made several updates and improvements to the genome assembly (Warren et al. 2015). Improvements have been achieved by sequencing a second white spruce genotype and using it to resolve the structure of the white spruce genome, and developing custom bioinformatics solutions; however, we have found that the overall assembly remains relatively fragmented, meaning that further improvements are needed. The *Picea glauca* genome sequencing and assembly data are available through NCBI (accession #: ALWZ0100000000 PID: PRJNA83435 at <http://www.ncbi.nlm.nih.gov/bioproject/83435>). We have also collaborated with Swedish scientist's sequencing the *P. abies* genome. Recently, our genome data were uploaded and made publicly available in the ConGenie database which integrates both the white spruce and the Norway spruce genome sequences. This integrate database is expected to facilitate comparisons between species.

We have also carried out a fine-scale analysis of the evolution of gene structure in *P. glauca* focusing on the structures of the 35 genes which were compared with *Populus trichocarpa*, *Arabidopsis thaliana* and *Zea mays*. The number and length of exons was well conserved but on average *P. glauca* genes had two to four times more intronic sequence than flowering plants, due to the presence of a few long introns. We found that longer introns do not seem to hinder or otherwise impact levels of gene expression despite the likely higher energetic cost for transcribing genes with long introns (Sena et al. 2014)

#### MOLECULAR ECOLOGY, ENVIRONMENTAL GENETICS AND MANAGEMENT - INTERNATIONAL LEVEL (LEADER DAMASE P. KHASA)

At the international level, the first comprehensive textbook on tropical agroforestry (Atangana et al. 2014) has been published by Springer. It includes all aspects of agroforestry in the tropics to date including emerging issues, such as agroforestry for integrated pest management, biofuel production, carbon sequestration, mitigation of climate change and REDD+ (reducing emissions from deforestation and forest degradation, including conservation and sustainable management of forests and the enhancement of forest carbon stocks) process. The textbook is intended for agroforestry students, teachers and practitioners. This work involved several collaborators including A. Atangana and S.X. Chang (Univ. of Alberta), and A. Degrande (World Agroforestry Centre). It was conducted within the framework of the Training Project in Natural Resources Management in the Congo Basin led by D.P. Khasa and supported by the Dept. of Foreign Affairs, Trade and Development Canada, DFAIT (2008-2013). The Agroforestry program at Univ. Laval is unique in Canada and is now expanding in tropical French speaking countries through international projects on training in management of natural resources, and with the use of digital learning tools to reach more students on-line (Khasa et al. 2015).

The study on provenance-progeny variation in survival rate and growth parameters of *Irvingia gabonensis* seedlings in native production zones of Cameroon is now completed (Onguene, Fouda, Atangana, Khasa, in preparation). This work showed that the survival rate of *I. gabonensis* seedlings was negatively correlated with altitude, and varied greatly with provenances and tree-within provenance. The best progeny was from a transitional locality, in spite of considerable dissimilarity in provenances and tree-within provenance in survival rate, leaf number, seedling volume and total biomass. The lowest progeny performance was recorded in southernmost provenances. Progeny

performance was positively and significantly correlated to provenance geographic localities. Moderate to high heritability values characterized leaf number, seedling volume and total biomass. This work involved the collaboration with N. A. Onguene (Institute of Agricultural Research for Development) and J.C. Fouda (Univ. of Yaoundé). Under the supervision of L. Bernier (Univ. Laval), PhD student J. Djeugap Fovo (now lecturer at the Univ. of Dschang, Cameroon) has recently completed his thesis on germination constraints and interspecific diversity of pathogenic fungi associated with *Ricinodendron heudelotii* in Cameroon, with the support from the Training Project in Natural Resources Management in the Congo Basin. A review paper from this thesis has highlighted the constraints and opportunities of tree domestication of this agroforestry species in Cameroon (Djeugap et al. 2013).

With the support of the Agence universitaire de la francophonie (AUF) and NSERC, we have completed the work on the impacts of monospecific exotic and native tree species plantations on the diversity and abundance of mycorrhizal communities and the physico-chemical soil properties in the Sahelian area (Sene et al., 2013a,b). The work provided information about the natural legume nodulating rhizobia and arbuscular mycorrhizal fungi communities associated with a range of old tree plantations and some aspects of their relationships to soil factors, which may facilitate the management of man-made forest systems that target ecosystem rehabilitation and preservation of soil biota. We have also completed a study on the impact of mycorrhizal inoculation on the development of *Intsia bijuga* grown on soil under exotic species *Casuarina equisetifolia* plantation in Madagascar (Ratahiriarisoa et al. 2014). The results showed that ectomycorrhizal inoculation caused a significant increase in phosphorus and nitrogen contents in plant leaves, and in soil assimilable phosphorus, total microbial activity and carbon. Consequently, an increase of the aboveground biomass was observed. Another study conducted in Madagascar showed that the propagation of an invasive species, *Grevillea banksii*, affects the dynamics of mycorrhizal fungi communities associated with native tree species (Andrianandrasana et al. 2014). In the latter study, it was found that the development of *G. banksii* was accompanied by a high formation of cluster roots and that soil occupied by *G. banksii* decreased the global microbial and phosphatase activities. Consequently, *G. banksii* disturbs the structure and the dynamics of symbiotic microflora such as arbuscular mycorrhizal fungi and rhizobia associated with two native tree species (*Intsia bijuga* and *Dalbergia trichocarpa*). The findings illustrate the negative impact of *G. banksii* propagation on the regeneration and the conservation of native tree species in Madagascan forest. In the context of community management of natural resources in Madagascar, strategies like more equitable distribution of wealth, environment improvement and evolution of control management responsibility for protected areas from the government of Madagascar to local communities, under the jurisdiction of community forest management committees, are crucial to achieve a more sustainable management plan based on community natural resources (Aymoz et al. 2013, Dhital et al. 2015).

With the support of NSERC and DFAIT through the Canadian Bureau for International Education (CBIE) under the framework of the Graduate Students' Exchange Program (GSEP), the work on the biology and biogeography of Eurasian invasive species is progressing well with important breakthroughs for *Conyza canadensis*. First, we have shown with well replicated transcontinental field studies, glasshouse experiments and individual-based models, that *Conyza canadensis* suppresses plant diversity in its nonnative ranges but not at home (Mnzaor et al. 2014). Second, we have demonstrated greater diversity of restriction groups (RGs) and sequence groups (SGs) of the PCR-RFLP of SSU rRNA gene in *Conyza* rhizosphere in native than in non-native sites (Mnzaor et al. 2015). In the introduced region, however, the arbuscular mycorrhizal fungi (AMF) diversity was more in uninvaded than in invaded sites. The species composition of AMF varied significantly between native and non-native regions and so also between invaded and uninvaded habitats. Though difference in AMF diversity between *Conyza* invaded and uninvaded sites may be attributed to invasion, the role of other evolutionary factors seems likely for differences between the native and non-native regions. We suggest that the ecological processes underlying these evolutionary differences in two biogeographic regions, besides the intensity of urbanization, might play some role in these differences. We have also used this simple and cost effective PCR-RFLP followed by sequencing to study the molecular diversity of ectomycorrhizal communities in *Eucalyptus urograndis* monocultural and silvopastoral plantation systems in Brazil (Pickford, Bousquet, Kasuya and Khasa, in prep.). The results showed significant differences in diversity indices between the agroforestry system and the other two systems. The phylogenetic analysis of the fungal ITS sequences revealed high diversity of Thelephoraceae in the three systems. Sclerodermataceae and Hydnangiaceae were not as diversified as Thelephoraceae, but amongst the most abundant.

For our work at the Canadian level, we have pursued the development and use of molecular markers in ecological studies of mycorrhizal fungi and plants. We are currently optimizing new microsatellite markers isolated from *Pisolithus tinctorius* and *Rhizophagus irregularis* (formerly *Glomus irregulare*), two model mycorrhizal fungal species. We also have developed novel CpDNA polymorphic microsatellite markers for phylogeographic implications in *Lemna minor*, another invasive aquatic species (Wani et al. 2014). These new chloroplast microsatellite markers for *L. minor* and haplotype distribution patterns indicate a complex phylogeographic history that merits further investigation.

Significant progress has also been made at the *in vitro*, *in vivo* and field levels for enhanced revegetation and reclamation of oil sands disturbed sites using actinorhizal and mycorrhizal biotechnology. Relationship between genetic variability in *Rhizophagus irregularis* and tolerance to saline conditions has been established and led to the selection of a salt-tolerant isolate that could, in the future, be used to pre-inoculate plants used in reclamation of saline substrates produced by oil sand industry (Campagnac et al. 2013). The growth and development of both green (*Alnus viridis* ssp. *crispa*) and speckled (*Alnus incana* ssp. *rugosa*) alders in varying proportions of oil sands process-affected materials (OSPMs) mixed with pristine sand (from 0 to 100 %) both in the presence and the absence of their symbiont, *Frankia* sp, showed that alder survival was not impaired by the presence of OSPMs, although symbiosis establishment was less efficient when the alders grew in pure OSPM residues (Bissonnette et al. 2014). Actinorhizal alders are very tolerant to OSPMs: when grown in pure OSPMs (100%), their dry biomass was 15 times greater than that of non-nodulated alders. Moreover, symbiotic alders allocated a higher proportion of their biomass to aerial parts, regardless of the OSPM percentage, whereas non-symbiotic alders showed a greater biomass investment in roots. When symbiosis was present, the alders thrived and produced a large amount of biomass that was highly correlated to the total nodule biomass. The pre-inoculation of alder seedlings in commercial greenhouses prior to their outplanting on OSPM-affected reclamation sites could significantly accelerate their growth and development. The effectiveness of ectomycorrhizal inoculation at the tree nursery seedling production stage on growth and survival was examined in *P. banksiana* and *P. glauca* trees planted in oil sands reclamation sites (Onwuchekw et al. 2014). The results suggest that the inoculation of conifer seedlings with ectomycorrhizal fungi could potentially be carried out on a large scale in tree nurseries to benefit postplanting performance in oil sands reclamation sites. However, these practices should take into consideration the differences in responses between the different plant species and fungal strains.

The BIOFILTREE project jointly funded by l'Agence nationale de la recherche of France (ANR) and the NSERC international strategy is not completed. This work involved the isolation, identification, and selection of *Frankia*, ectomycorrhizal and endophytic fungi for reclamation of gold mining sites. Several ectomycorrhizal and endophytic fungal isolates were isolated from the mining and natural sites and characterized using molecular approaches. Three field trials (2011, 2012, 2013) were established in gold mine tailings in Val d'Or in the Abitibi-Témiscamingue region of Québec, by exploiting actinorhizal and mycorrhizal symbioses and amendment treatments. Metagenomics of the microbial communities over time and space as well as the physiological and gene expression studies of the micro- and macrocosm experiments were conducted. Two MSc students enrolled at the Univ. of Sherbrooke, one MSc student at McGill Univ. and one MSc student and one postdoc at Univ. Laval were involved in these studies. Several publications arising from this project are now in preparation. The synopsis of the results was presented at two major Mine Closure conferences 2014 and 2015 (Nadeau et al. 2014, 2015). Our results suggest that inoculating seeds or seedlings with site-adapted microbial inoculants such as ectomycorrhizal (ECM) fungi, arbuscular mycorrhizal (AM) fungi, ericoid mycorrhizal fungi, nitrogen-fixing bacteria (e.g. *Rhizobium sensu lato*, *Frankia* spp.), and plant growth promoting rhizobacteria (PGPR), before outplanting, can tremendously increase plant health, growth, and establishment success on mining sites. The work aimed at assessing people's perception of and expectations for mine site restoration, particularly phytoremediation strategies, and analyses of their preferences for species that were used in phytoremediation, using multiple correspondence analysis as implemented in the software XLSTAT, showed that people's perception is influenced by the effects of mining activities on human health and region attractiveness (Vodouhe and Khasa 2015). Among restoration methods, phytoremediation was rated positively (65.2%) with regard to its environmental potential, aesthetic and consideration for future generation followed by fillings and excavating. According to them, mine sites need to be restored at any costs and shrubs are their preference as vegetation component for restoration because of their quick growth compared to trees and worries that trees can be harvested by logging industry before site rehabilitation is fully completed. In terms of weed management of future plantations, it has been shown that sheep vegetation management could be an ecologically effective method for controlling competing vegetation in young



conifer plantations in the central interior of British Columbia and thus, could increase annual tree growth (Serra et al. 2014).

QUANTITATIVE GENETICS AND CLIMATE CHANGE RESEARCH  
(LEADER JEAN BEAULIEU)

We compared height of 15-year-old white spruce full-sib families obtained by crossing trees from the same provenance and trees from different provenances (Doerksen et al. 2014). Height means differed between intra- and inter-provenance crosses, which we hypothesized was due partly to unidentified relatedness among intra-provenance base parents resulting in mild inbreeding and inbreeding depression among their offspring. A dense array of 5,844 single nucleotide polymorphisms was used to directly construct a genomic relationship matrix (G) that had four elements ranging from 0.17 to 0.24, between five intra-provenance base parents. Adjusting the numerator relationship matrix for this relatedness among base parents produced inbreeding coefficients of  $F_i \approx 0.1$  in their offspring, which displayed depressed height growth. Accounting for inbreeding level as a covariate in a mixed model decreased grossly overestimated (up to 2x) dominance variance in models without the covariate adjustment. Height growth decreased 39 cm (~ 6 %) for every 0.1 increase in  $F_i$ .

Quantitative genetics studies were also carried out in collaboration with Prof. Cuauhtémoc Sáenz Romero of the Univ. Michoacana de San Nicolás de Hidalgo, Mexico. Due to climatic change, it might be expected that in the next years, the habitat of *Pinus hartwegii* Lindl. could sharply decrease. It is then convenient to establish conservation strategies that diminish or counteract the effects of the climatic change. However, a first step in establishing conservation measures is to quantify the adaptive variation of the species and the evolutionary forces that may have shaped it. To understand the evolutionary forces acting on *P. hartwegii* populations, patterns of genetic variation, assessed through quantitative traits and isozymes, were compared in 12 natural populations along an altitudinal transect in Michoacán, Mexico, by estimation of  $Q_{ST}$  and  $F_{ST}$  statistics (Viveros-Viveros et al. 2014). Although significant genetic differentiation among populations was found for quantitative traits and isozymes, most of the quantitative traits showed a strong clinal pattern of variation associated with site elevation, but not for isozymes. Average  $Q_{ST}$  value (0.200) was higher than  $F_{ST}$  value (0.127), but was not significantly different. Results indicate that natural selection, imposed by the altitudinal environmental gradient, is acting on quantitative traits, while other evolutionary forces, like genetic drift and gene flow, are acting on isozymes.

In collaboration with H.A. Margolis (Univ. Laval) and M.S. Lamhamedi (MFFPQ), and with funding obtained from FRQNT, we carried out a study aiming to gain insight into the stability of *P. glauca* family genetic response to climate change (Carles et al. 2015). Seedlings from 60 full-sib families were subjected to a combination of two temperature regimes and two levels of CO<sub>2</sub> over two growing seasons. A series of morphological, phenological and physiological traits were assessed on the seedlings during their first three years of growth. We observed that warmer temperatures and CO<sub>2</sub> elevation had a positive effect on the height and diameter growth of 2- and 3-yr-old white spruce seedlings full-sib families. Instantaneous water use efficiency was the only physiological trait that was affected positively by the CO<sub>2</sub> treatment, showing a 51% increase that was consistent across families. The absence of significant family × treatment interactions suggests that performance of this set of families would not be decreased by the anticipated climate change under the conditions of high water availability used in this experiment. However, only traits at the juvenile stage were considered in the present study.

In collaboration with S. Rossi (Univ. du Québec at Chicoutimi), a study has been initiated to quantify genetic variation associated to budburst in boreal populations of *P. mariana*, which is a key determinant of their adaptation. While previous results by Rossi's group have shown extensive year-to-year variation and plasticity for this trait, the early results of a replicated experiment in greenhouse indicated that significant variation among boreal provenances exists, with earlier and quicker budburst for northern populations. Significant variation among families within provenances was also noted, as previously for budset in *P. mariana* and as previously observed for *P. glauca* and for other conifers. Notwithstanding gene flow and through recombination and natural selection, this variation should



contribute to local adaptation of the next generation in the context of naturally-regenerated forests facing climate change (Rossi and Bousquet 2014).

The rapid rate of current and expected climate change is expected to exceed the capacity of many tree species to adapt locally, putting them at risk of extirpation at a local or regional scale, and even over a large part of their current distribution range. Data from white spruce, black spruce and jack pine provenance tests established in Québec were used to develop transfer models with the aim to provide indirect estimations of the potential response of provenances to rapid climate change. These models integrate several climate scenarios to predict future height gains or losses for different time periods, or for different sites following a seed source transfer (Rainville et al. 2014). For *P. mariana* and *P. glauca*, the potential effects of climate change were estimated in terms of future yield in merchantable volume (m<sup>3</sup>/ha) rather than height growth. Simulations showed that in the short term (2046-2065), white spruce plantation yield from local seed sources is expected to increase in all three of Québec's bioclimatic domains because of a positive effect of climate change, especially in the north (balsam fir and black spruce domains). However, during the following period (2081-2099), productivity would slightly decrease in the sugar maple and balsam fir domains. The contribution of climate variables and climate models to total error of predicted yield is small for *P. glauca* plantations. For *P. mariana*, a slight yield increase is predicted in the short term in the balsam fir and spruce domains, but productivity could drop by one-third in the sugar maple domain. For the longer term (2081-2099), the error associated with *P. mariana* plantation yield predictions, mainly due to climatic factors, is too large to allow accurate predictions. For *P. banksiana*, the transfer model proved ineffective in predicting future plantation yield in Québec. These results allow the choice of the best adapted seed sources (local seed orchards) to plant in different regions of Québec, in order to counter the anticipated productivity losses due to climate change or to maximize future yields of black spruce and white spruce plantations. This strategy should help the Québec reforested forests to adapt to future climate and maintain their overall productivity. Even so, based on our predictions, an important loss of productivity is expected for *P. mariana* plantations in the sugar maple domain, no matter which seed source is used.

One-year-old *P. glauca* seedlings derived from somatic embryogenesis and produced by JDI were studied in microgravity conditions in the International Space Station (ISS) and compared with seedlings grown on Earth (Rioux et al. (2015). Leaf growth was clearly stimulated in space whereas data suggest a similar trend for the shoots. Needles on the current shoots of ground-based seedlings were more inclined towards the stem base than those of seedlings grown in the ISS. Amyloplasts sedimented in specialized cells of shoots and roots in seedlings grown on Earth while they were distributed at random in similar cells of seedlings tested in the ISS. In shoots, such amyloplasts were found in starch sheath cells located between leaf traces and cortical cells whereas in roots they were constituents of columella cells of the cap. Nuclei were regularly observed just above the sedimented amyloplasts in both organs. It was also frequent to detect vacuoles with phenolic compounds and endoplasmic reticulum (ER) close to the sedimented amyloplasts. The ER was mainly observed just under these amyloplasts. Thus, when amyloplasts sediment, the pressure exerted on the ER, the organelle that can for instance secrete proteins destined for the plasma membrane, might influence their functioning and play a role in signaling pathways involved in gravity-sensing white spruce cells.

#### RESEARCH ON WOOD PROPERTIES (LEADER JEAN BEAULIEU)

A series of additional studies were carried out on *P. glauca* wood properties. Tree growth, as measured via the width of annual rings, is used for environmental impact assessment and climate back-forecasting. We studied this natural process at various scales in white spruce stems (from cell and fiber within a growth ring, to ring and entire stem) in one, two, and three dimensions (Dutilleul et al. 2014). A new approach based on computed tomography scanning was developed to study tree growth and wood density (indirect measures) in 3D from stem sections, at a scale sufficiently small to allow the delineation of reliable limits for annual rings and large enough to capture directional variation in growth rates. Graphical and quantitative analyses were reported for white spruce trees with circular vs non-circular growth.

There is a growing interest in improving knowledge on wood quality of forest resources to allow for an end-product oriented supply for the wood-processing industry. We investigated the differences of *P. glauca* wood traits from 13 ecological regions of the province of Québec and identified climatic and geographic variables and tree

characteristics that may help predict wood traits (Lenz et al. 2014). One hundred and eleven wood disks were used to determine wood traits that are related to wood anatomy and to mechanical use of wood. The results indicate significant differences between ecological regions for most of the investigated wood traits. Samples from several coastal regions showed, for example, wood anatomy that is advantageous for some pulp and paper grades, but they offer comparatively weak estimates for mechanical applications. Ordination and projection to latent structures allowed us to identify maximum temperature, degree days, geographic location, tree height, and tree diameter as important predictor variables for wood traits. The quality of models was variable and  $R^2$  values ranged from 0.13 to 0.59. Prediction errors in an independent sample were mostly below 10%, and bias was below 5%. Although the prediction of wood mechanics was less precise, positive results are an encouraging step toward obtaining a wood trait signature for different environments (provenances).

The establishment of patterns of radial and longitudinal variations and the development of models to predict the wood anatomical properties, especially from juvenile wood, are of interest for both wood industry and researchers. Linear regressions were used to predict whole-tree, breast height and mature tracheid length and diameter in *P. glauca* and the West-Brown-Enquist (WBE) model was used to predict the variation of tracheid diameter (Mvolo et al. 2015a). Tracheid length and diameter increased from pith to bark. Tracheid length decreased, while tracheid diameter increased from apex to lower heights. Cambial age was the most important predictor of tracheid length. The final tracheid length models with either a log transformation or a third-order polynomial of cambial age explained 82% of the variation in the whole-tree tracheid length. At breast height, 83% of the variation in the whole tracheid length was explained using the juvenile value at a cambial age of 3 years. Up to 87% of the variation was explained by the model, including the average value of juvenile wood. However, mature wood tracheid length at breast height could not be predicted from juvenile wood. Distance from the apex predicted the tracheid widening in outer rings but failed to predict tracheid expansion of samples collected at fixed cambial ages. The WBE model explained 86% of conduit widening in the outer rings.

Estimations of transition age (TA) and juvenile wood proportion (JWP) are important for wood industries due to their impact on end-product quality. However, the relationships between analytical determination of TA based on tracheid length (TL) and recognized thresholds for adequate end products have not yet been established. We used three different statistical models to estimate TA in *P. glauca* based on TL radial variation (Mvolo et al. 2015b). We compared the results with technological maturity. A two-millimeter threshold, previously suggested for good paper tear strength, was used. Tracheid length increased from pith to bark and from breast height to upper height. Juvenile wood was conical with the three models. At breast height, TA ranged from 11 to 27 years and juvenile wood proportion ranged from 15.3% to 47.5% across the three models.

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## UPDATING ADVANCES IN RESEARCH AND DEVELOPMENT IN THE FIELDS OF PLANT PRODUCTION AND SOMATIC EMBRYOGENESIS IN QUEBEC

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**Key words :** forest nursery, seeding date, physico-chemical properties of growing media, short-day, climate change, assisted migration, somatic embryogenesis, clonal forestry, *Picea glauca*.

The use of forest plantations with high morpho-physiological quality plants produced from genetically improved seeds can increase the productivity of Quebec's forests. Each year, more than 120 million seedlings are produced in the 19 forest nurseries in Quebec (6 public and 13 private).

For environmental and financial purposes, Quebec's forest nurseries must constantly innovate to develop new techniques that will improve seedling quality and forest productivity. These techniques allow not only to meet the standards and criteria of morpho-physiological quality of seedlings applied in Quebec, but also to reduce the leaching of minerals, thereby helping to preserve groundwater quality and reduce costs related to the use of fertilizers.

To increase forest productivity, Québec promotes a gradual integration of somatic embryogenesis in clonal forestry, seed orchard management and cutting propagation programs. Continuous technology transfer to Quebec forest nurseries and technical support are among the priority activities of our team. Whether in technical committees or visits, this transfer allows forest nurseries to incorporate the latest technological innovations. These improvements are the result of a synergy with other researchers and practitioners from different organizations both national and international. The present report outlines the salient facts and results of the principal studies related to new developments on seedling production under forest nursery conditions, morpho-physiological variation of white spruce seedlings from various seed sources and implications for assisted migration and the integration of somatic embryogenesis in the reforestation chain.

## SEEDLING PRODUCTION

### Effects of seeding date on growth and mineral nutrition of white spruce seedlings (2+0) in relation to the root insufficiency

In the search for solutions to reduce the culling rates of large sized white spruce seedlings (*Picea glauca* [Moench] Voss) due to insufficient root development, containerized cultures sown on different dates were compared on an operational scale under forest nursery conditions. The main objective was to evaluate whether earlier sowing could improve seedling growth, especially root growth compared to sowing one month later. After two growing seasons, seedlings sown earlier were significantly superior to seedlings sown one month later, in terms of height (60% difference), diameter (45%), above-ground and total dry mass, root dry mass (108-110%) and root cohesion. For both treatments, foliar nitrogen concentration averaged 2.25%, which is well above the minimal foliar concentration requirement of 1.8% for forest seedlings shipped for reforestation in Quebec. Though foliar concentrations for other mineral elements differed slightly between treatments, no visible deficiency symptoms were observed. To improve root growth and maximize the performance of white spruce seedlings, nurserymen should favour an earlier sowing date. However, early sowing alone is not enough to reduce seedling culling rates due to insufficient root development. Attaining this objective also requires optimizing growing techniques at each step during production, and taking into account the specific needs of white spruce for water and mineral nutrients (see Lamhamedi *et al.* 2013a for more details).

### Root growth, plug cohesion, mineral nutrition, and carbohydrate content of 1+0 *Picea mariana* seedlings in response to a short-day treatment

A short-day (SD) treatment was applied to containerized 1+0 black spruce (*Picea mariana* (Mill.) B.S.P.) with the objective of increasing root mass and root-plug cohesion. The SD treatment resulted in the induction of bud formation, cessation of height growth, and significant increases in carbohydrate content (sucrose, pinitol, and starch), root nutrient contents, and root dry mass. Allometric models showed that given the same shoot mass, the average seedling grown under the SD treatment had 25 % more root mass than those in the control treatment, which led to a significant improvement in root-plug cohesion. Seedling quality evaluation before delivery to the planting site showed that 91 % of 1+0 black spruce seedlings subjected to SD treatments conformed to quality standards compared with 71 % for those subjected to the control treatment. These results indicate that the use of an SD treatment may improve the profitability of forest nurseries by increasing the quality and quantity of shippable seedlings (see Lamhamedi *et al.* 2013b for more details).

### Peat substrate aeration properties and growth of containerized white spruce: a case study in a forest nursery

Seedlings are rejected every year in forest nurseries because of insufficient root development and root plug cohesion. Adequate substrate bulk density and aeration properties are of critical importance for root growth and water and nutrients uptake. The quality norms for nursery substrates, based in part on the ratio of coarse to fine particle sizes, have recently been questioned with respect to substrate performances. Hence, experiments were conducted under commercial nursery conditions to determine the effects of substrate physicochemical properties on seedling growth and mineral nutrition over two growing seasons. Seedlings were grown in different substrates composed of coarse and fine (threshold particle size: 0.5 mm) peat particles, perlite and vermiculite, covering a broad range of physical properties with adequate available water and various coarse to fine particles ratio and aeration properties. Substrate properties had a significant effect on growth parameters but the effects differed between the two experiments. Seedling growth after one growing season was affected by water stress and low gas diffusivity. This study emphasizes the importance of management on substrate performances, indicating that short periods of moderate water stress (matric potential lower than -10 kPa) may hinder seedling growth. They also suggest that when irrigated substrates are maintained within the 0 to -5 kPa matric potential range, gas diffusivity values should not be lower than 0.003–0.005 cm<sup>2</sup> s cm<sup>-2</sup> s<sup>-1</sup>, in agreement with modeled anaerobic oxygen profile in root plugs. Hence, when designing and using substrates at the nursery scale, more attention should be paid to aeration properties found in the container after potting and to the management rather than to the ratio of coarse to fine particles (see Boudreau *et al.* 2013 for more details). This project was carried out in close collaboration with several researchers (Dr. Steeve Pépin and Dr. Jean Caron, Laval University).



### **Fine-scale geographic variation in photosynthetic-related traits of *Picea glauca* seedlings indicates local adaptation to climate**

Climate-related variations in functional traits of boreal tree species can result both from physiological acclimation and genetic adaptation of local populations to their biophysical environment. To improve our understanding and prediction of the physiological and growth responses of populations to climate change, we studied the role of climate of seed origin in determining variations in functional traits and its implications for tree improvement programs for a commonly reforested boreal conifer, white spruce (*Picea glauca* [Moench] Voss). We evaluated growth, root to shoot ratio, specific leaf area (SLA), needle nitrogen (Nmass), total non-structural carbohydrates (NSC) and photosynthetic traits of three-year old seedlings in a greenhouse experiment using seed from six seed orchards (SO) representing the different regions where white spruce is reforested in Québec. Height and total dry mass (TDM) were positively correlated with photosynthetic capacity (Amax), stomatal conductance (gs) and mesophyll conductance (gm). TDM, but not height growth, was strongly correlated with latitude of seed origin (SO) and associated climate variables. Amax, gs, gm, and more marginally, photosynthetic nitrogen use efficiency (PNUE) were positively associated with the mean July temperature (MJT) of the SO, while water use efficiency (WUE) was negatively associated. Maximum rates of carboxylation (Vcmax), maximum rates of electron transport (Jmax), SLA, Nmass, NSC, and root to shoot ratio showed no pattern. Our results did not demonstrate a higher Amax for northern seed orchards, although this has been previously hypothesized as an adaptation mechanism for maintaining carbon uptake in northern regions. We suggest that gs, gm, WUE and PNUE are the functional traits most associated with fine-scale geographic clines and with the degree of local adaptation of white spruce populations to their biophysical environments. These geographic patterns may reflect in situ adaptive genetic differences in photosynthetic efficiency along the cline (see Benomar *et al.* 2015 for more details). This project was carried out in close collaboration with several researchers (Dr. Hank Margolis, Dr. Lahcen Benomar, Dr. Jean Beaulieu and Dr. Jean Bousquet).

### **Morpho-physiological variation of white spruce seedlings from various seed sources and implications for assisted migration**

Due to climate change, genetically improved trees may no longer be adapted to the sites where they are intended to be planted. Assisted migration is a potential adaptation strategy for maintaining forest productivity and reducing vulnerability in the face of a changing climate.

Morpho-physiological responses of white spruce seedlings from eight seed orchards were evaluated in a nursery and at three planting sites. The modeling of growth curves showed that the height of both the southern orchards and second generation orchards was significantly higher than the other orchards. A multiple regression model showed that the final height of the plants was significantly correlated with climatic conditions of the orchards. The orchard and the planting site significantly affected height growth of seedlings. The results of this study and those obtained over the longer term should help to refine the operational rules of seed transfer for assisted migration (see Villeneuve *et al.* 2015 for more details). This project was carried out in close collaboration with several researchers (Dr. Hank Margolis, Dr. Lahcen Benomar, Josianne DeBlois, Marie-Claude Lambert, Dr. Jean Beaulieu and Dr. Jean Bousquet).

### **Adaptation to climate change: the case of extreme winter in forest nurseries in Quebec**

Climate change is likely to increase vulnerability of plants produced in forest nurseries. This could result in increased mortality of tree seedlings and potentially affect the profitability of some forest nurseries. To evaluate the tolerance of plants to frost in winter and test the use of adaptation measures, prevention and winter protection (geotextiles and artificial snowmaking), an experimental design simulating winter climate extremes, has been installed under forest nursery conditions.

The lack of snow in early winter increases, alone, the percentage mortality of plants from 5 to 23% according to species. This mortality can be further enhanced by the interaction of several factors, including the severity and long-term exposure plants to freezing temperatures.

If in mid-December snow accumulation is insufficient, the nursery could apply artificial snow to protect tree seedlings against winter frost. Our results show that the presence of a snow covering (5 - 10 cm) is an excellent insulator that protects tree seedlings against the low temperatures extremes (see Lamhamedi 2012 for more details).

## SOMATIC EMBRYOGENESIS

Somatic embryogenesis (SE) provides, from a single seed and asexually, an unlimited number of somatic embryos that later became plants whose genotype is identical to that of the original embryo from seed. These plants are therefore a clone or variety.

Priorities for research and development in the field of ES are focused on the morpho-physiological characterization of somatic clones (SC) in the nursery, the integration of stock somatic plants in the cuttings propagation program (CPP), the correlation between the performance of clones under nursery and site conditions.

### **Clonal variation of white spruce somatic clones and their use in the cutting propagation program**

Since 1997 we work on the morpho-physiological characterization of white spruce somatic clones (1+0 and 2+0), developing the catalog of nearly 1,500 clones, and their integration in the cuttings propagation program (CPP) in Quebec. Our initial work on integration of somatic clones in the CPP began in 2002 with somatic clones of Norway spruce. However, the emphasis is more on somatic embryogenesis of white spruce. Before SE can be introduced into CPP, we evaluated the physiological and morphological attributes of cuttings produced by somatic stock plants. Different rooting experiments were conducted under operational conditions.

Our results showed that clonal effects were consistently significant and several differences were also observed among clones for all rooting traits of cuttings and yield of cuttings produced by SC. These traits exhibit high clonal heritability values (Lamhamedi, 2015 pers. com., Lamhamedi and Gravel-Grenier 2012).

### **Genetic control and nursery-plantation genotypic correlations for growth characteristics of white spruce somatic clones**

Height and diameter of white spruce clones were measured over two growing seasons in a forest nursery and for 4 more years at two field sites. Clonal heritability was moderate and stable in the nursery, but decreased with age, reaching 0.26 and 0.11 for height and 0.14 and 0.04 for diameter, respectively, at the two field sites. Height exhibited weak to moderate clonal heritability and strong genotypic correlations with the different growth characteristics. The strong age-age genotypic correlations ( $\geq 0.809$ ) and nursery-plantation correlations ( $\geq 0.347$ ) for height indicate that selection for height can be conducted at a young age thus reducing the costs of clonal tests (See Wahid *et al.* 2013 for more details). This project was carried out in close collaboration with several researchers (Dr. Hank Margolis, Dr. Nadya Wahid, and Dr. Jean Beaulieu).

## CONCLUSION

The production of high quality seedlings is the result of the acquisition and development of new knowledge and its continuous transfer and implementation at an operational scale. In close collaboration with researchers and graduate students from different research centers, the seed and plant production research group has emphasized the transfer of expertise and knowledge and the integration of research results in Québec forest nurseries. This transfer has been realized through the scientific and technical publications, visits and conferences, as well as workshops. During these events, researchers, technicians, nurserymen and graduate students from different research centers in Québec or elsewhere are invited to present their research results. The content of these conferences is principally directed towards forest nurserymen in Québec, as well as managers who work in the field of forest regeneration.

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## ADVANCES IN FOREST GENOMICS AT THE CANADIAN FOREST SERVICE, QUEBEC REGION

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This report summarizes the progress of the Canadian Forest Service (CFS), Canadian Wood Fibre Centre (CWFC) and Laurentian Forestry Centre (LFC) research in forest and environmental genomics for the period 2013-2015. Our activities have mainly focussed on research on spruce genomics and the environmental genomics of poplars. This work was made possible through grants obtained from the Genomics R&D Initiative Funds and through our participation in the SmartForests Project (MacKay, Boehlman, Bousquet, et al.), a major initiative funded by Genome Canada, Genome Quebec, and Genome BC to develop molecular breeding in softwood trees. On another front, studies of gene-flow among poplar and larch species were made possible by grants from the Canadian Regulatory System for Biotechnology (N. Isabel). Parts of this project were done in collaboration with Université Laval (J. Bousquet), Forêt Québec (P. Périnet et al.), Agriculture and Agri-Food Canada Saskatchewan (B. Schroeder) and other members of the LFC (A. Séguin). A project was carried out on the development of a traceability system in white spruce thanks to funding from the Fonds québécois de recherche sur la nature et les technologies (J. MacKay, J. Bousquet, N. Isabel, L. Tremblay).

Research accomplishments were made possible with the generous contributions of dedicated staff and collaborators who deserve our thanks. They are: Sébastien Clément, Marie Deslauriers, Éric Dussault, Marie-Claude Gros-Louis, Philippe Labrie, Manuel Lamothe, Patricia Lavigne, Jean-François Légaré, Daniel Plourde, Esther Pouliot, and many summer students.

## TREE GENOMICS

### White Spruce (*Picea glauca*) and Black Spruce (*Picea mariana*)

During the last 2 years, grants were obtained from the GRDI to finish gene and QTL mapping and perform genomic selection of wood traits, both in white spruce. These grants were also used as co-funding for two of the research activities of the SMartTForests Project, a major initiative, funded by Genome Canada, Genome Quebec and Genome BC for developing molecular breeding in softwood trees and co-led by J. MacKay and J. Bousquet (U. Laval).

*Wood Traits.* In collaboration with Université Laval colleagues, wood traits of about 750 black spruces were assessed for wood density and microfibril angle. The same trees were also measured for total height and diameter at breast height. Genotyping of these trees had also been carried out in conjunction with our academic colleagues thanks to a grant from FQRNT. Close to 5000 high- quality single nucleotide polymorphism markers (SNP) were available for each tree. Genotypic data are stored in data files on NRCan servers. Genomic selection models were built and prediction accuracy for the traits of interest was calculated using various cross-validation schemes. Preliminary estimates of prediction accuracy for wood and growth traits were high, indicating that genomic selection would be a powerful tool for improving black spruce, especially for small effect size advanced breeding populations. Additionally, opportunities were found to reduce the number of genetic markers as well as the amount of phenotyping necessary for model construction. This will allow us to reduce costs in view of application to larger breeding populations. A scientific paper is currently in preparation and will be submitted before the end of 2015 (Lenz et al. in prep.).

Secondly, we have completed a study aiming to demonstrate that genomic selection (GS) for wood and growth traits can significantly help reduce breeding cycles for an essentially undomesticated species such as white spruce, and even for large populations of open-pollinated families (Beaulieu et al. 2014a). A second study on genomic selection of white spruce wood and growth traits initiated in 2012-2013 and using 1700 trees representing 49 full-sib families of two breeding groups of the Québec breeding population was completed. Data were analyzed and a scientific paper reporting the results obtained was recently published (Beaulieu et al. 2014b). Based on the results obtained with the large population of open-pollinated families and those of the population of full-sib families of limited effective size, we believe that marker-assisted early selection could be successfully implemented in Canadian white spruce breeding programs, especially when combined with reproduction techniques such as somatic embryogenesis (SE) and rooted cuttings. This research will be continued in the large-scale applied project FastTRAC (Fast Tests for Rating and Amelioration of Conifers) that aims to bring genomic selection to an operational scale in white spruce and Norway spruce breeding programs. The project is co-led by J. Bousquet (Université Laval) and G. Smith (CWFC, FPI) and was launched in spring 2015 under the Genomics Applied Partnership Program (GAPP) of Genome Canada, supported by Génome Québec, Forest Products Innovations (FPI), CWFC, the ministère des Forêts, de la Faune et des Parcs du Québec, J.D. Irving Ltd., NRCan, the New Brunswick Tree Improvement Council (NBTIC) and the New Brunswick Department of Economic Development.

Thirdly, we began to transfer the genomic selection technology to J.D. Irving Ltd. to be implemented in their SE pipeline. Genomic selection models were developed for 125 of their white spruce SE lines using markers that were identified as being associated with wood and growth traits in our previous studies. It was possible to demonstrate that the prediction accuracies obtained were even higher than those of pedigree-based prediction. These positive preliminary results with the transfer of genomic selection are very encouraging.

*Gene and QTL mapping* A new white spruce genetic map of 9000 genes was generated (Pavy et al. in preparation) and will serve as a basis of comparison among conifer species. During the last year, several experimental plantations, which include clonally replicated individuals (2000 seedlings genotyped for thousands of SNPs through

SMarTForests), were established on 4 sites along latitudinal and altitudinal gradients. This will allow us to evaluate new traits central to climate adaptation and to resistance mechanisms against forest pests, such as the accumulation of phenolic compounds. This project will lead to the development of new tools for large-scale characterization of traits useful in genomic selection testing.

*Traceability Method:* In collaboration with the Direction générale de la production de semences et des plants forestiers in Québec, we worked at developing a traceability system that will be applied to the production of white spruce elite varieties using somatic embryogenesis (SE) in provincial nurseries of the province. The controlled crosses and SE steps that lead to their production make them very prone to human and/or technical errors. The objective was to select a minimum number of informative SNPs (among genomic data made available from previous projects) and use them to distinguish between the different clonal elite varieties (using a unique genetic fingerprint) and to verify their family of origin.

A set of 37 SNPs were selected using *in silico* datasets representing the controlled crosses produced by the breeding program. They were used to verify the identity and the cross of origin for 1077 trees (2168 trees), produced by SE and planted in one field test. Using assignment analyses, we estimated that 10.67% of all individuals were illegitimate, *i.e.* that they were not the trees that they were supposed to be. We also determined that most of the errors probably occurred at the controlled crosses stage. The next step will be to translate the results of this study into recommendations to the tree nurseries and to verify the identity of varieties stocked in cryogenic banks. A manuscript presenting the development method and results is currently being written (Godbout et al. in preparation)

#### Eastern White Pine (*Pinus strobus*) and Western White Pine (*Pinus monticola*)

*Phylogeography.* We investigated the influence of two different physiographic landscapes on the population structure and postglacial colonization histories of two white pine species: *P. monticola*, which occurs in the highly mountainous region of western North America, and *P. strobus*, which occurs in a much less mountainous area in eastern North America. To characterize the patterns of genetic diversity across the ranges of both species, 158 and 153 SNPs derived from genes were genotyped on range-wide samples of 61 *P. monticola* and 133 *P. strobus* populations, respectively.

In *P. monticola*, a steep latitudinal decrease in genetic diversity likely resulted from postglacial colonization involving rare long-distance dispersal events. In contrast, no geographic patterns of diversity were detected in *P. strobus*, suggesting recolonization via a gradually advancing front. For each species, STRUCTURE analyses identified distinct southern and northern genetic groups that likely originated from different glacial lineages. At a finer scale, and for the two species, smaller subgroups were detected that could be remnants of cryptic refugia. We discuss the importance of our findings for conservation programs and predictions of species' response to climate change. The study was carried out by Simon Nadeau (M.Sc. student) in collaboration with Kermit Ritland (UBC) and is currently accepted for publication in the *American Journal of Botany* (Nadeau et al. in press).

## ENVIRONMENTAL GENOMICS

### Poplars and their Hybrids

Plants with novel traits (PNT) are those that are new to native populations and have the potential to impact the environment. We are exploring the risks PNTs pose to natural forest using poplar trees (*Populus* sp.) as a model system. Exotic poplar cultivars are used for a wide range of economic and horticultural applications and are prevalent on the landscape. Most exotic cultivars are complex crosses between native and exotic poplars and are capable of interbreeding with native species. Following Wilkinson et al. (2003), we examine the risk of exotic gene

movement into native poplar populations by quantifying spontaneous hybridization, assessing exotic gene introgression, and evaluating the fitness of exotic hybrids. This work was conducted in collaboration with researchers at Natural Resources Canada (CFS-LFC), Agriculture and Agri-Food Canada, and the ministère des Ressources naturelles du Québec.

**Marker Development.** To detect exotic poplar gene regions, we developed a diagnostic panel of 36 SNPs that could distinguish 8 poplar species (*Populus angustifolia*, *P. balsamifera*, *P. deltoides*, *P. fremontii*, *P. laurifolia*, *P. maximowiczii*, *P. nigra*, and *P. trichocarpa*) and their early generation hybrids (Isabel et al. 2013). We also developed panels of SNPs to evaluate long-distance gene flow and population structure within *P. balsamifera* and *P. deltoides* (Meirmans et al., Godbout et al., manuscripts in preparation).

**Evaluation of Spontaneous Hybridization.** We genotyped seeds from native poplar stands located adjacent to exotic poplar shelterbelts from central Canada and next to exotic poplar plantations in eastern Canada to detect spontaneous hybridization. Hybridization rates ranged from 0.1-2.3% in western Canada and were significantly lower than those observed in eastern Canada (Meirmans et al. 2010). Hybridization was dependent on the pollen donor and recipient genotype (Talbot et al. 2012).

**Native Hybrid Zones.** Hybrid genotypes that arise between plant species frequently have increased susceptibility to arthropod pests and fungal pathogens. This pattern has been attributed to the breakdown of plant defenses ('Hybrid susceptibility' hypothesis) and (or) to extended periods of susceptibility attributed to plant phenologies in zones of species overlap and (or) hybridization 'phenological sink' hypothesis). We examined these hypotheses by assessing the susceptibility of parental and hybrid *Populus* host genotypes to a leaf spot disease caused by the fungal pathogen *Septoria musiva*. Genotypes for 214 individuals, originating from the Red Deer River, AB, were obtained using 27 species-specific SNP markers. 27.7% of trees were classified as admixed individuals (*P. balsamifera* x *P. deltoides*). A subset of these trees was inoculated with four isolates of *S. musiva* and evaluated for their susceptibility. A further 53 trees were grown in a common garden to assess the effect of genotype on variation in leaf phenology. Leaf phenology was more variable within the category of admixed genotypes than within categories of either parental species. Leaf phenology was also more variable for the category of trees originating in the hybrid (*P. balsamifera* – *P. deltoides* [hybrid and parental genotypes combined]) zone than in adjacent pure zones of the parental species. The results from the common garden experiment support the 'phenological sink' hypothesis. This work was conducted by Jared LeBoldus from North Dakota State University (LeBoldus et al. 2013).

A tri-hybrid region represents an ideal playground for testing the Hybrid Bridge Hypothesis, which is an ecological hypothesis that argues that hybrids allow associated organisms (e.g., herbivores or pathogens) to encounter and potentially adapt to the genome of another species in a series of intermediate genotypes that effectively act as "stepping stones" that bridge two genomes. Such interactions have been tested by collecting *P. balsamifera*, *P. deltoides* and *P. angustifolia* and their natural hybrids in the drainage basin of the Oldman River in Alberta. We used molecular detection techniques to characterize the nature and extent of hybridization for 719 *Populus* trees. On the genotyped trees, we then surveyed ten species of gall-formers to examine how arthropod distributions are influenced by genetic similarity among host tree genotypes. We observed that host genotype and genetic similarity strongly affect the distributions of gall-forming species, individually and as a community. These effects were detected in the wild and experimentally in common gardens. Moreover, correlations between the diversity of host genotypes and their associated arthropods identify hybrid zones as centres of biodiversity and potential species interactions with important ecological and evolutionary consequences. This work was conducted by Kevin D. Floate from Agriculture and Agri-Food Canada (Floate et al. in press).

**Estimation of Rates of Introgression.** We screened adult trees from natural stands in western and eastern Canada for exotic gene regions and detected fully mature exotic hybrids (Talbot 2012, Thompson 2010, Roe et al. 2014a,b), providing evidence for exotic gene introgression in native stands. Exotic introgression was also found to be biased towards *P. balsamifera* (Roe et al. 2014).

**Realized Rate of Exotic Gene Introgression.** We assessed the relative fitness of *P. balsamifera*, *P. deltoides*, native hybrids, and exotic hybrids by comparing reproductive biomass, viability, and disease susceptibility among each tree class. Seedling recruitment was also evaluated for sexually produced progeny to further evaluate the competitive ability of each tree class. Exotic hybrids showed reproductive fitness traits and disease susceptibility intermediate to native species at the same locality (Roe et al. 2014b).

#### Larches and their Hybrids

**Evaluation of Spontaneous Hybridization.** Using a combination of two chloroplast and three mitochondrial DNA markers, we estimated the rate of spontaneous hybridization taking place between native (*Larix laricina*) and exotic (*L. decidua*, *L. kaempferi*, *L. sibirica*) species of larch in two arboreta. More than 5000 seeds and 1000 recruits from the two experimental sites were sampled and genetically identified. The occurrence of spontaneous hybridization between *L. laricina* and the exotics was found both in seeds and from recruits freely established near the plantations. A low hybridization rate (3% or less) was observed among the seeds of *L. laricina* maternal trees. Spontaneous native-exotic hybrids were also observed to establish and survive in the environment under the current climatic conditions at a similarly low rate. However, a much higher and variable rate of establishment was observed for recruits with exotic components (up to 34.6% at one site). These results indicate that the spontaneous hybridization and the recruitment of hybrid and exotic recruits do occur and should be taken into consideration in the management and establishment of plantations of fast-growing species with exotic components. This study was conducted by Patrick Meirmans (now at the University of Amsterdam, Meirmans et al. 2014).

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## **NORTHEAST SEED MANAGEMENT ASSOCIATION (NeSMA) SUMMARY OF 2013 and 2014 FIELD OPERATIONS**

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NeSMA is a partnership of 8 forest companies and the Ontario Ministry of Natural Resources and Forestry (MNR) operating in north-eastern Ontario. The company has tree improvement programs for black spruce (*Picea mariana* [Mill.] BSP), jack pine (*Pinus banksiana* Lamb.), and white pine (*Pinus strobus* L.).

### **FIRST-GENERATION PROGRAMS**

Cone collection was the primary activity in the seed orchards; crown management was completed to facilitate the cone collection.

The Edward Bonner black spruce seed orchard is comprised of 3 separate non-contiguous fields; since 2005 these fields have been managed as separate orchards for 3 different forest units managed by 2 NeSMA partners. In 2013, more than 8,300 trees were topped in the 3 fields.

The volume of black spruce cones collected from orchards in 2013 was 232 hecto-liters and 97 for jack pine. In 2014 the black spruce volume was 247HL and jack pine was 177HL. The majority of the black spruce cones in 2014 were collected at the Island Lake Tree Improvement Area where close to 6,000 trees were topped as part of the process. Cones were collected for the first time in a previously thinned genetic test associated with the Timiskaming program.

Forest Health Technicians with the MNR visited some genetic tests and seed orchards to determine whether any insect or disease threats were present and the status of any previously known pests.

The Ontario Stewardship Rangers completed an inventory/assessment of the trees in the Ingram Township white pine genetic test. In addition, the Ewanturel Township test white pine data previously analyzed was helpful in the 2014 field identification of selections for future work.

Competition was removed from the black spruce and jack pine realized gain trials in 2013 and these were measured and the data analyzed in 2014.

Data was analyzed for the Wendigo jack pine and the Algoma black spruce programs to support planning their future development.

## SECOND-GENERATION PROGRAMS

Hearst Forest Management completed a health and survival survey of the trees planted in 2012 to establish a jack pine clonal seed orchard in Casgrain Township near Hearst. The genetic tests and clonal archive were monitored to track their health and condition.

Analysis of the 2013 data collected at two Elite and two Infusion tests for the Timiskaming black spruce program provided information for planning; in addition, selections were identified in the field and individual cone collections were made from each selection. Data for the Saganash jack pine program was analyzed and more than 400 of the black spruce and jack pine selections preserved in the pollen bank/archive were tested to verify their viability.

## OTHER ITEMS

Work continues to plan for maintaining a sufficient adapted seed supply for the future. The changing climate will significantly affect this process.

Beginning in 2013 information and support have been provided for a project to revise and update the information in the forest genetics layers of the government Land Information Ontario system

**FOREST GENETICS AT THE ONTARIO FOREST RESEARCH INSTITUTE (OFRI) 2013 - 2015**

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**Keywords:** White spruce, provenance test, climate change adaptation, assisted migration, White pine blister rust, genetic resistance, introgressive hybridization, endophyte, Himalayan blue pine.

**FOREST ADAPTATION STRATEGY TO CLIMATE CHANGE**

**P. Lu, W. C. Parker, S. J. Colombo and R. Man**

The Ontario Ministry of Natural Resource and Forestry has recently completed an organizational transformation, which resulted in the realignment of science and research in a new branch (i.e., the Science and Research Branch, SRB) within the Provincial Service Division. The SRB subsequently launched its Integrated Science Action Plan (ISAP), which outlines OMNRF research priorities in the coming years. Among those, protecting bio- and genetic diversity, developing forest adaptation strategy to climate change and managing invasive species are highly consistent with OFRI's the ongoing forest genetic research projects.

To develop forest adaptation strategy to climate change, we made efforts to re-measure the 410-series of white spruce range-wide provenance tests located within Ontario. This series of provenance tests, which involves 245 range-wide provenances tested at 15 locations along climate gradient in Ontario, was established by the Canadian Forest Service in collaboration with Ontario Ministry of Natural Resources and forestry in the 1980s. The recent measurement data, together with those of earlier measurements, are expected to generate information for answering a number of resource management questions relating to climate change adaptation of the species.

A separate ongoing study is with eastern white pine, which involves a collaborative research with the USDA-FS in re-measuring eastern white pine long-term range-wide provenance tests located across Canada and the USA. In addition to measuring tree health status and size, we also took incremental wood core samples for tree-ring chronology and x-ray densitometry analyses. Data collection is now complete with data analysis ongoing.

In addition, we conducted controlled-environment studies to understand the responses of native Ontario forest species to simulated winter and spring warming. Poor synchronization between tree phenology (such as earlier than normal bud flush) and spring climate caused by irregular early spring warming has been the reasons large scale frost damage in Ontario over the past a few years. Differential phonological responses by tree species to early spring warming may provide a mechanism for mitigating spring frost damage through forest management options, such as species selection, during forest regeneration in hazard-pro areas.

## WHITE PINE BLISTER RUST RESISTANCE

P. Lu, R. Wilson and G. W. Adams

We continue to maintain and monitor a number of field research trials for white pine blister rust resistance aiming to evaluate the performances of selected backcrosses of hybrids between eastern white pine and Himalayan blue pine (*Pinus wallichiana*). The main objectives of these trials are to evaluate cold hardiness, growth rate, tree form and white pine weevil infection. Blister rust resistance is assessed more rigorously and precisely through artificial disease inoculation experiments with post-inoculation seedling survival as the sole criterion. Preliminary results have been promising as top hybrid-backcross families outperform pure eastern white pine controls, which validate Ontario's retrogressive hybridization breeding strategy for enhancing eastern white pine with blister rust

resistance. As new selections of seedlings from advanced generation hybrid backcrosses start to flower, we expect to produce next-generation backcross progeny containing a greater proportion of eastern white pine genome, while maintaining strong blister rust resistance.

In a parallel development, we are collaborating with the J.D. Irving Limited, NB, to assess the efficacy of applying endophytes at seedling stage in reducing white pine blister rust incidence in field plantings. In this study, newly germinated seedlings are sprayed with endophyte inoculum solution on the needles, which will result in the infection of seedlings with endophytes. Endophytes are beneficial fungi isolated from natural and native ecosystems, which do no harm on white pine seedlings after infection, but will secrete toxin to reduce spore germination and mycelium growth of other pathogenic fungus, such as white pine blister rust (*Cronartium ribicola*). In the ongoing experiment, we inoculated about 6,000 eastern white pine seedlings with two strains of endophytes selected by the J.D. Irving in the greenhouse. Seedlings are being monitored in the greenhouse for two growing seasons, and will be planted out in three field research trials, using uninfected eastern white pine as controls, for long-term monitoring.

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## PRESENTATION

“Performance of Eastern White Pine Hybrids in Field Trials”. At IUFRO conference “Challenges and Opportunities in (1) Genetics of Five Needle Pines and (2) Rust of Forest Tree Research: Conservation, Evolution and Sustainable Management in a Changing Climate”. 15-20 June, 2014. Ft. Collins, Colorado, USA.

## AAFC-STB AGROFORESTRY DEVELOPMENT CENTRE - TREE IMPROVEMENT SUMMARY

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**Keywords:** shelterbelt, agroforestry, tree improvement, *ex situ* conservation.

The Prairie Shelterbelt Program was initiated by the Government of Canada in 1901 to provide farmers with hardy trees and shrubs for shelterbelts to protect farm yards, reduce soil erosion, protect livestock, enhance biodiversity and improve air quality. From 1901 to 2013 over 600 million tree seedlings were provided to prairie farmers protecting more than 700,000 hectares of agricultural land. Since the commencement of the Prairie Shelterbelt program in 1901, significant advances have been made in the selection and improvement of tree and shrub species provided through the program. The shelterbelt tree improvement program at Indian Head, designed specifically to develop adapted, genetically superior tree and shrub species for prairie shelterbelts has been under way since the beginning of prairie settlement in the 1880s. Since then tree improvement and evaluation has been led by Angus McKay (1888-1903) Norman Ross (1904-1941), John Walker (1942-1946), Bill Cram (1947-1974), Gordon Howe (1975-1980) and William Schroeder (1981-2015). Over the years a number of people have contributed to the tree improvement program, most notable were Carl Lindquist (1953-1984), Dan Walker (1984-2011), Salim Silim (2002-2010), Ron Gares (2012-present) and Raju Soolanayakanahally (2005-present). Improvement efforts touched many genera and species but focused primarily on the genera *Populus*, *Pinus*, *Picea*, *Larix*, *Fraxinus*, *Quercus*, *Celtis* and *Salix*. During the 127 years the program encompassed species introductions and testing, plant exploration, classical breeding and selection, *ex situ* conservation and genomics. Since its inception, the breeding program developed 15 poplar clones and improved seed strains for over 20 species. The breeding efforts of the past 127 years have changed the landscape and quality of life for thousands of prairie people. In 2013, the last trees from the Prairie Shelterbelt Program were planted following over a century of federal government support for tree planting in western Canada. This report presents a summary of the tree breeding program.

## TREE IMPROVEMENT STRATEGY

With the commitment to establishing genetically diverse tree plantings the ADC improvement program concentrated on developing superior seed propagated populations. The steps followed to accomplish this are outlined below.

1. Sample the native range of the species, collect seed and assess the range of genetic variation in major traits using provenance trials.

2. Superior trees were selected from provenance trials, vegetatively propagated and planted in clonal banks.
3. Superior trees were crossed and their progeny evaluated under a variety of site conditions.
4. Based on the performance of progeny, trees in the clone bank were rogued leaving only trees that produce progeny with the desired traits. These rogued clone banks were transformed to seed production orchards.

#### *QUERCUS MACROCARPA* Michx.

*Quercus macrocarpa* (bur oak) improvement aimed to improve the adaptability of *Quercus* seed populations for prairie growing conditions, study genotype-environment interactions and develop improved seed strains for prairie agroforestry. Since 1992, the oak improvement program has assembled over 300 populations, evaluated the populations in seed source trials at locations in Manitoba, Saskatchewan and Alberta and developed an improved seed strain, for agroforestry planting in the Canadian Prairies. A clonal seed orchard for the production of the improved seed strain is located at Indian Head, SK. Living bur oak germplasm collections are retained at Indian Head and Birch Hills, SK and Edmonton, AB.

#### *LARIX SIBIRICA* Ledeb.

*Larix sibirica* (Siberian larch) seedlings originating from the Ural mountains in western Siberia were planted at the Indian Head Forest Nursery Station in 1908. In 1976, progeny from the 1908 plantation were planted in shelterbelts at the PFRA Shelterbelt Centre at Indian Head. In 1983, 29 superior trees were selected from these shelterbelts and planted in a clonal seed orchard. Seed from these trees were tested as accession E5100 at 12 test locations in Manitoba and Saskatchewan. Performance of the seed strain was superior to other seed strains and sources at all trial sites. Referred to as 'Lindquist' this seed strain was used in the Agriculture and Agri-Food Canada Prairie Shelterbelt Program. The *Larix* improvement program has assembled multiple seed sources of *Larix* species from their native ranges in Eurasia. These living collections are retained at Indian Head and Birch Hills in Saskatchewan.

#### *FRAXINUS PENNSYLVANICA* Marsh.

The *Fraxinus pennsylvanica* (green ash) improvement program was initiated in 1985 when superior trees were selected from native green ash stands in Saskatchewan and Manitoba. These trees were progeny tested and the top genotypes vegetatively propagated and planted in a seed orchard. The seed strain originating from this orchard was superior to other seed strains and sources at trial locations in Manitoba and Saskatchewan. The seed strain 'Plainsman' was used in the Agriculture and Agri-Food Canada Prairie Shelterbelt Program and is widely planted throughout the prairie provinces. In 1996, additional seed sources were obtained from the northern Great Plains states. This and other *Fraxinus* germplasm has been retained in living collections at Indian Head, Birch Hills, Outlook in Saskatchewan and Smokey Lake in Alberta.

*POPULUS* Since 1947, the poplar improvement program has been active testing populations from breeding programs, selecting and producing new hybrids and maintaining collections of poplar species and hybrids. From its beginning, the program has bred and evaluated over 40,000 *Populus* genotypes at 40 trial locations across the prairie provinces. This breeding program has released a total of 15 cultivars. Of these the most prominent are Walker, Assiniboine, Okanese, Katepwa, CanAm and Sundancer. These cultivars constitute the majority of poplars planted in the Canadian prairies for agroforestry and plantation forestry. Living collections of *Populus deltoides* (Indian Head), *P. balsamifera* L. (Indian Head, Prince Albert, Quebec City, Fairbanks) and *P. simonii* (Indian Head) are retained in common gardens.

#### *PINUS SYLVESTRIS* L.

Provenance tests of Russian seed sources of *Pinus sylvestris* (Scots pine) were established in 1962 at the



Agroforestry Development Centre, Indian Head, Saskatchewan. Data from these trials indicated that the 'balcania' ecotype from Voronezh, Province of Orel and Smolensk, as well as the 'eniseensis' ecotype from Central Siberia and the 'altaica' ecotype from southern Siberia were best adapted to the Canadian prairies. Plus trees from these populations were used in a breeding program to develop, hardy, fast growing trees with minimal winter browning and long needle retention. Full-sib progeny from crosses between selected trees were tested and the best performing parents vegetatively propagated and planted in a clonal seed orchard. Progeny from this seed orchard was extensively tested across the prairie provinces and proved to be superior to other seed strains. Referred to 'Prairie Green' Scots pine, this improved seed strain was used in the Agriculture and Agri-Food Canada Prairie Shelterbelt Program.

#### *PINUS PONDEROSA* Dougl.

Provenance tests of northern great plains seed sources of *Pinus ponderosa* (ponderosa pine) were planted at Indian Head, Saskatchewan in 1969, 1984 and 1988. Results from these trials indicated that trees originating from Valentine and Ainsworth, Nebraska and Rosebud, South Dakota were well adapted to the Canadian prairies. Progeny testing of selected trees from these specific sources was completed and outstanding individual trees with superior growth and minimal winter browning selected. These trees were planted in a seed orchard for the production of an improved seed strain for the Canadian prairies. Living collections of *Pinus ponderosa* seed sources are retained at Indian Head, Saskatchewan.

#### *PICEA PUNGENS* Engelm.

In 1959, vigorous *Picea pungens* (Colorado spruce) trees were selected from 1911 plantings on the Indian Head Forest Nursery Station. The selections were based on blue needle colour and overall vigour. These trees were crossed to identify the best parental combinations to generate a high percentage (>90%) of progeny with silver-blue needle colour. In addition to having silver-blue needle colour, progeny from these trees have proven to be well adapted to southern prairie conditions withstanding drought and exposed treeless environments. The best performing parents were vegetatively propagated and planted in a seed orchard. This improved seed strain has been widely planted throughout the prairie provinces.

#### *CELTIS OCCIDENTALIS* L.

In 1990 AAFC collected and propagated seed sources from native *Celtis occidentalis* (hackberry) stands in Manitoba, North Dakota and South Dakota. Seed source trials were established in Manitoba and Saskatchewan in 1993 and 1994. The trials are designed to determine which sources were best adapted to the prairies. Both trial sites were evaluated after 20 years and data showed large variability in adaptation of seed sources. One seed source originating from North Dakota produced high quality progeny. This source is a reliable seed stain for hackberry plantings in the Canadian prairies.

### GENETIC RESOURCE COLLECTIONS

A major component of AAFC tree improvement was conservation of woody genetic resources. This included sampling native populations and establishment of common gardens. These collections facilitate access to genetic resources for breeding and the study of genetic diversity. The collections provided for *ex situ* conservation of native woody plant populations which supported Agriculture and Agri-Food Canada's actions in biodiversity. The gene pool of collections was conserved in common gardens or gene banks established within the native range of the species. The vegetative collections include 756 populations and 24,500 individual genotypes of the following species: *Prunus virginiana* var. *melanocarpa*, *Crataegus rotundifolia*, *Shepherdia argentea*, *Hippophae rhamnoides*, *Symphoricarpos occidentalis*, *Quercus macrocarpa*, *Fraxinus pennsylvanica* var. *subintegerrima*, *Populus deltoides* var. *occidentalis*, *Populus balsamifera*, *Prunus nigra* and *Prunus americana*. The majority of these living collections have been retained, most are on private property and receive little to no maintenance so long term viability or access is uncertain. In 2015, seed representing the improved seed strains from the AAFC improvement program as well as seed collections for *Crataegus rotundifolia*, *Prunus pennsylvanica*, *Prunus virginiana*, *Rosa acicularis*, *Rosa woodsii*

and *Symphoricarpus occidentalis* were transferred to the National Tree Seed Centre at Fredericton New Brunswick.

#### LONG TERM GENETIC TESTS

The AAFC-STB Agroforestry Development Centre has established over 200 genetic trials in the prairie region. Genera included in the tests are *Pinus*, *Picea*, *Larix*, *Juniperous*, *Acer*, *Quercus*, *Populus*, *Salix*, *Prunus*, *Hippophae*, *Celtis*, *Symphoricarpus*, *Rosa*, *Shepherdia* and *Fraxinus*. These tests were evaluated every five years for survival, growth, adaptability and pest resistance. Information from the tests describe genetic variability within genera and is used to recommend new species and/or provide data to support release of new clones or seed strains. Some trials have been in place since the early 1960s. In 2014 all trials were surveyed and the majority were alive and in fair to good condition.

#### CONCLUSION

Tree improvement is an important activity and the sustainability of prairie tree plantings depend on our ability to maintain the biological and genetic diversity that is needed for healthy landscapes. It is important, especially with native plants, to work with the greatest degree of diversity possible allowing us to maintain healthy populations that have the genetics to withstand the movement of disease through a population of plants. This requires a continued commitment to the study of plant communities with less emphasis on individual specimens.

**FOREST GENETICS AND TREE IMPROVEMENT ACTIVITIES FOR ALBERTA TREE  
IMPROVEMENT AND SEED CENTRE  
2013 – 2015**

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**Keyword:** Climate change, seed germination, gene conservation, disease resistance, Dothistroma

This report summarises genetics, tree improvement, gene conservation and seed biology and technology activities the Alberta Tree Improvement and Seed Centre (ATISC) did since July 2013. These activities are either new or completion of those ATISC reported in 2013. Climate change adaptation, gene conservation and restoration for limber and whitebark pine, seed germination and long-term storage, spread of Dothistroma needle blight in lodgepole pine provenance and progeny trials, and DNA-based monitoring of white spruce seed orchard pollen contamination were some of the main activities conducted in this reporting period.

## SEED BIOLOGY AND TECHNOLOGY

### Alberta Seed Conservation Course

Alberta Tree Improvement and Seed Centre has offered this free 2-day course seven times since 2012 with 58 attendees from across British Columbia and Alberta and feedback has been extremely positive. Attendees have come from various backgrounds including academia, government, energy and forest companies, private restoration and seed collecting companies and the nursery industry. Topics covered include seed moisture relations, assessment & collection, seed handling, tetrazolium viability testing, seed longevity, storage behaviour, germination & viability, and seed dormancy plus a cut testing lab and a germination problem solving activity. ATISC will continue to offer this course in March each year.

### Water activity use

Alberta will be moving away from traditional moisture content standards for seed moisture testing. For the past 3 years, ATISC has been focusing more on using non-destructive water activity measurements to determine optimum storage conditions for tree seeds, shrubs and herbaceous seed that we are storing for reclamation use. Because the oil contents of limber and whitebark pines and non-tree species varies considerably, this switch will ensure optimum storage conditions for all seed, not just commercial conifers. Since 2012 we have completed desorption isotherms on five Alberta conifers, which together with international seed storage standards, are helping us set new moisture measurement standards in the 2015 revision of the Alberta Forest Genetic Resource Management and Conservation Standards.

### Seed management monitoring

In 2011, the conservation & research seed retesting program was put on hold until the data accumulated from 30+ years of retesting could be analysed and changes made to the program. Statistical analyses of 82 seed lots comprising 13 Alberta coniferous and deciduous species has been completed and the generated survival curves are providing a wealth of information to help best manage future seed lots for both short term operational and long term conservation use. ATISC restarted the program in 2014 with new seed lots, new longer retesting intervals and new species to fill knowledge gaps about non-timber and endangered species. We will actively monitor and adjust the program to include/exclude seed lots when required and to detect significant declines in viability to improve our knowledge of seed longevity in storage.

### Endangered Species

Whitebark pine seed has been collected routinely and stored for *ex situ* conservation at ATISC for the last 15 years and this species is now listed as federally endangered in Canada. Good germination protocols and estimates of seed cold storage longevity are necessary. Previous germination tests on Alberta seed have achieved only 25-55% germination of viable seeds spread over a long period, making propagation expensive, difficult and time consuming. Low and inconsistent results also meant that storage longevity monitoring was impossible and seedling production for white pine blister rust screening would be inefficient or incomplete due to the loss of genetics linked with deeply dormant seeds. In 2013, a large germination trial was conducted to find a method suitable for large-scale production of whitebark pine seedlings in Alberta, taking practicalities and economics into account while retaining the genetic integrity of the seedlot. A new germination protocol was developed and is currently in use. This new protocol achieves 95-100% germination of viable whitebark pine seeds found across Alberta. In this way, we can ensure that we are testing efficiently and preserving as much genetic material as possible, as well as lowering the cost of seedling production.

In addition, now that the necessary germination protocols exist for both limber and whitebark pine in Alberta, an artificial ageing longevity trial was set up in 2014 to develop comparative longevity estimates for these two species. From tests conducted on limber pine during the 2012 maturity trial, we suspected that limber pine was a short-lived species in cold storage (showing significant decline in less than 25 years) and preliminary results from the 2014/15 ageing test have confirmed this suspicion. Tests on whitebark pine are ongoing. We will compare artificial ageing test of limber and whitebark with that of lodgepole pine and black spruce to get more accurate estimates of cold storage life times for the two endangered species. We are currently investigating alternative storage methods for limber and may be extended to whitebark pine.

### DOTHISTROMA NEEDLE BLIGHT

Dothistroma needle blight outbreak occurred in 2012 in a pine clone bank located at ATICS as stated in the CFGA 2011-2013 report. Since then the disease has been found on lodgepole pine in several other locations in Alberta including a 30-year old provenance test, a 15-year old progeny test and a seed orchard. In 2014, the government initiated a project with Dr. Richard Hamlin at the University of British Columbia to identify the *Dothistroma* species causing the disease and to study genetic variation among fungal isolates using genomic-based methods. The methods were developed by the Tree Aggressors Identification using Genomic Approaches (TAIGA) project. Foliar samples from locations with suspected *Dothistroma* infections were collected in Alberta and British Columbia. The presence of the disease was confirmed in four out of five sampled locations in Alberta and in all six locations sampled in BC. The infected Alberta areas are all centrally located in the province while the location where the disease was not found is in the northwestern Alberta. The fungal species identified by sequence analysis is *Dothistroma septosporum* (also known as *Mycosphaerella pini*).

### RESTORATION OF ENDANGERED LIMBER AND WHITEBARK PINE

Both whitebark pine and limber pine are endangered species in Alberta. Both now have approved provincial recovery plans that represent the culmination of years of research and collaboration across agencies and jurisdictions<sup>2</sup>. Nearly all of more than 280 provincial long-term health transects established to monitor status and trends of these endangered species were re-measured in 2014 by staff from the province, Parks Canada, and regional staff. This data was used to target priority areas for cone collection and restoration activity in 2015 and subsequently.

These recovery plans provide a framework for action. Starting in 2015, cone collections will focus on supporting urgent recovery actions including identifying, documenting and protecting potentially blister rust-resistant parent trees, establishing blister rust screening capacity, and ultimately towards producing operational quantities of rust resistant seedlings which are the key to restoration. Other key activities will include developing more accurate range maps with supporting data including abundance and mortality to target recovery activities more effectively; continuing extension and outreach with tenure holders, recreational users, and landowners; establishing provenance trials to support modified seed zones for these species; and continuing to support other priority items in recovery plans.

For several years, Alberta has collaborated with Canadian Forest Service (CFS), U.S. Department of Agriculture Forest Service – Dorena Genetic Resource Center and Coeur D'Alene Nursery, the Province of British Columbia, and academic institutions such as University of Calgary and University of Alberta to advance research projects and applied studies on these species. Results on limber pine seeds from Alberta sources sent to the US for rust resistance screening and also assayed for key genetic resistance markers in Victoria BC by CFS have yielded some promising results, as have whitebark pine sources sent to Coeur D'Alene for rust resistance screening and phenotypic testing. We look forward to further results and to continuing these collaborations to support species recovery, and are working to develop capacity for in house testing.

## GENETIC CONSERVATION PLANNING

The 2009 Gene Conservation Plan for Native Trees of Alberta<sup>3</sup> is due for an update following the changes in species conservation status and changes in protected areas since it was first developed. The plan summarizes provincial priorities for *in situ* gene conservation, which is primarily served by protected areas such as parks and wilderness areas. Forest tenure holders engaged in tree improvement are also obligated to establish *in situ* conservation areas in representative habitats within each seed zone their tenures overlap. These populations must be large enough to sustain evolutionary processes. This is a requirement under the provincial Forest Management Planning Standard, which is structured to follow the Canadian Standards Association Standard CZ809-02 for sustainable forest management. The *in situ* gene conservation plan set out the framework for an *ex situ* provincial gene conservation plan, which has had a draft completed and under review during this reporting period. When finalized, this plan, which focuses on *ex situ* conservation of all indigenous provincial species, will be available on the ministry website.

## MOUNTAIN PINE BEETLE REHABILITATION CONE COLLECTIONS

To date, Alberta has 8,235 kg of lodgepole pine seed dedicated to rehabilitation of mountain pine beetle impacted areas. The provincial program is administered through Forest Resource Improvement Association of Alberta (FRIAA), which administers grant-based allocations on an annual basis. The beetle infestation is tracked by monitoring the most severely attacked areas, and areas where emergence rates indicate increasing populations, and a buffer surrounding those zones. Many areas affected are remote and may not be rehabilitated as part of post-harvest reforestation obligations. This program facilitates restoration and ecosystem recovery in those areas, and supports rehabilitation activities associated with salvaging beetle-killed timber or wood in imminent danger of mortality due to the beetle epidemic.

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<sup>2</sup> Alberta Whitebark Pine Recovery Plan 2013-2018, ISBN 978-1-4601-1100-0 (online) & Alberta Limber Pine Recovery Plan 2014-2019, ISBN 978-1-4601-1848-1 (online)

<sup>3</sup> Gene Conservation Plan for Native Trees of Alberta, ISBN 978-0-7785-6493-5 (online)

## CLIMATE CHANGE ADAPTATION

Since 2012, Alberta Tree Improvement and Seed Centre (ATISC) and private forest companies involved in genetic tree improvement have been implementing the Tree Species Adaptation Risk Management (TSARM) project. The Climate Change and Emission Management (CCEMC) Corporation initiated and funded the TSARM project with financial contribution from the Forest Management Branch. The project sought to, (i) evaluate the risks climate change poses to tree breeding programs that supplies reforestation seed and clones on public land; (ii) analyze provenance and progeny trial data across lodgepole pine and white spruce breeding programs to explore options for assisted population migration; (iii) develop cost-efficient vegetative propagation methods for aspen, which would enable the species to be artificially regenerated on a large scale to replace wild clones that may be thousands years old and thus lagging behind climate change; and (iv) establish new provenance trials in areas that were originally unimportant for field testing, but currently have dry climates to allow testing for drought tolerance.

Data analyses used height growth with ages of progeny and provenance trials ranged from 7 to 35 years. Results show that, (a) genetic differentiation among families and provenances is greatly related to regional variation in temperature than precipitation; (b) for most of the breeding regions, local seed appear to be the best choice for maintaining forest productivity; (c) opportunity exist for limited movement of seed from lower to higher elevations and from lower to higher latitude segments of neighbouring breeding regions to boost forest productivity. Because Alberta is predicted to be warmer and drier, adaptive traits such as survival during early stages of stand establishment may be more important in the future than height growth. Therefore, future assessments of young field trials will concentrate on early age performance to establish the cause of tree mortality at the stages when trees are highly vulnerable. Data from such assessments will enable the province to address regeneration-related challenges, especially in drought-prone areas. The TSARM has now ended and the government will review the results and how to integrate them into provincial seed transfer standards as part of the Alberta climate change adaptation strategy.

## WESTERN GALL RUST GENOMICS

Alberta Innovates Bio Solutions (AI Bio) and the Forest Management Branch are funding a genomics research at the University of Alberta. This project is part of the province's effort to address existing and emerging forest insect and disease problems that are expected to increase in a warmer and drier climate. The project is focused on the genetics of lodgepole pine, jack pine, the hybrids of the two species, and the fungus (*Endocronartium harknessii*). It involve inoculation of pine genotypes from different breeding programs using fungal spores from lodgepole pine natural range in Alberta and British Columbia, especially where the rust is prevalent, and in pure jack pine populations outside the hybrid zone. The research team is made up of scientist from the University of Alberta (Departments of Biological Sciences & Renewable Resources), Canadian Forest Services, Forest Management Branch, in collaboration with British Columbia Ministry of Forests, Lands and Natural Resource Operations.

## POLLEN CONTAMINATION IN SEED ORCHARD

Alberta Tree Improvement and Seed Centre is currently genotyping clones in white spruce seed orchards and background trees at its headquarters in Smoky Lake, Alberta. Orchards for most of the white and black spruce breeding regions, and research spruce plantations are located in this Smoky Lake complex. Genotyping of clones, background stands and seedlots from the seed orchards is aimed at establishing the "actual pollen contamination" (fertilization by contaminant pollen) and possibly replace the pollen capture and counting method of determining pollen contamination rate that ATISC currently uses. It is assumed that, because not all outside pollen entering the seed orchard end up fertilizing orchard clones, contamination rates determined by a pollen capture method may be higher than the actual contamination rates determined by DNA-based paternity analysis. In addition, results from this genotyping work may be used to assign/fix the "expected rates of pollen contamination" to each seed orchard and replace annual pollen contamination monitoring.

## STAFF CHANGES

In November 2014, Ms Jodie Krakowski joined the Alberta Tree Improvement and Seed Centre as a forest geneticist. Before moving to Alberta, Jodie was an employee of the British Columbia Ministry of Forests, Lands and Natural Resource Operations. She is a gene conservation enthusiast with an exceptional dedication to conservation

and restoration of limber and whitebark pine. Jodie is both a registered professional biologist and professional forester.

In January 2015, Mr. Leonard Barnhardt retired from the Alberta public service after more than 25 years of dedicated work with the Alberta Tree Improvement and Seed Centre. With the background in both forest ecology and genetics, Leonard was a strong supporter of genecology, provincial standards on seed transfer, gene conservation and management of forest genetic resources. He was a long-term member of the Alberta Forest Genetic Resources Council and the Alberta representative to CONFORGEN. Until his retirement, Leonard was the manager of the Alberta Tree Improvement and Seed Centre.

**In June 2015, Ms. Lee Charleson joined the Alberta public service as the manager of the Alberta Tree Improvement and Seed Centre. Ms. Lee has previously worked with forest companies in Alberta and prior to her current at the Alberta Tree Improvement and Seed Centres, she was a government staff with the British Columbia Ministry of Forests, Lands and Natural Resource Operations**

## WESTERN FOREST PRODUCTS –SAANICH FORESTRY CENTRE ORCHARDS

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**Keywords:** seed orchard, supplemental mass pollination, parent tree,

Western Forest Products (WFP) produces high quality seed for our forest regeneration programs in our operations in coastal British Columbia. Seed that is surplus to our needs is available to other licensees operating in the Maritime Seed Zone. At our Saanich Forestry Centre we manage seed orchards for four conifer species: coastal Douglas-fir (*P. menziesii* (Mirb.) Franco var. *menziesii*), western redcedar (*Thuja plicata* Donn ex D. Don), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and Sitka spruce (*Picea sitchensis* (Bong.) Carr.). We manage yellow-cedar (*Callitropsis nootkatensis* (D. Don) Oerst) hedges as well. Our annual planting programs average 7.5 million annually for our tenures on Vancouver Island and the mainland coast. Seed for more than 80% of our planting stock comes from orchard sources, delivering gain in volume or pest resistance.

We implement management techniques including orchard roguing, ramet replacement, to maintain leading-edge orchards. We manage for improved seed quantity by applying induction, supplemental mass pollination, insect control and management, and cultural management.



Western Forest Products supports research into genetics, tree seed production, and related studies conducted by researchers at the Ministry, Universities, Forestry Canada, and associated consultants and contractors. We are active in the BC Forest Genetics Council and its committees.

#### COASTAL DOUGLAS-FIR

Low and high elevation coastal Douglas-fir comprises about 35% of our annual planting program. We manage three orchards to meet our needs.

We have increased capacity and the genetic quality in our coastal Douglas-fir orchards in the past decade. Our newest low-elevation orchards include selections with average genetic worth for volume of 19. Wood density is a second trait of interest: included parent trees are projected to maintain wood density values to deliver a high-value product at rotation. These parent trees include third-generation parents identified in the province's coastal Douglas-fir breeding program. As the orchards are not at full capacity yet, WFP presently purchases and deploys seed from other producers, together with the seed from our orchards. Our 2014 crop produced seed for 2.5 million plantables.

We manage a high elevation Douglas-fir orchard at the Saanich Forestry Centre. The breeding value of the stock in this orchard is 13. We registered a 2014 crop for 100 k seedlings from this orchard.

We provide seed and pollen monitoring data for studies into parental contributions that UBC's Dr. El Kassaby and his research team are conducting. Estimates for pollen contamination are being examined; these have significant impact on the seed lot genetic quality scores.

#### WESTERN REDCEDAR

Western redcedar likewise comprises about 35% of our annual planting program. As the provincial western redcedar breeding program identifies additional selections for orchards, WFP incorporates those selections in our orchards. Roguing and ramet replacement balanced with maintaining production keep these orchards at the leading edge. Our western redcedar orchards delivered a seed crop in 2014 for more than 10 million seedlings with volume gain of 17%.

We also manage a western redcedar orchard comprised of selections from Haida Gwaii only. This orchard seed is preferred to wild seed by most licensees operating there. The 2014 crop produced seed for 385 k plantables.

Our Operations have established browse resistance operational trials, using the stock selected and further developed by breeding, as part of the BC Ministry breeder's western redcedar program.

#### WESTERN HEMLOCK

We manage two western hemlock orchards: one for each of the low elevation and high elevation seed planning units. In 2012 we rogued the low elevation orchard to raise the potential quality of seed crops to a genetic worth

of 17. The 2014 crop provides seed for 660 k seedlings and delivers a volume gain of 17% at rotation. The high elevation orchard produced a 2014 crop for 390 k seedlings with a genetic worth of 12. Western hemlock seedling requests average 10% of our annual planting program.

#### SITKA SPRUCE

The weevil-resistant Sitka spruce orchard at Saanich Forestry Centre has not produced a crop since 2010, but a reasonable crop is anticipated this year. This orchard includes selections identified by the Ministry's Sitka Spruce breeding program, and its seed delivers 85% weevil resistance to regeneration programs. WFP requests for Sitka spruce are between 2% and 5% of our annual planting program. Regeneration from this seed demonstrates very good growth when grown together with non-resistant stock on sites where weevil hazard is low or nil. Selection for volume as a secondary trait is anticipated from among the weevil-resistant parents.

We also manage a Sitka spruce orchard with selections only from Haida Gwaii, where the weevil is not present. In 2014, this orchard produced seed for an estimated 396 k seedlings with volume gain at rotation of 6%.

#### YELLOW-CEDAR

WFP continues to deliver high gain cuttings from our yellow cypress cuttings selections. We review the rooting success of our selected clones annually through testing and operational production. Our material delivers stock with a projected volume gain of 20% at rotation. Costs for rooted cuttings are significantly higher than that of seedlings, and nursery capacity is limited. Yellow cypress demand ranges from 5% to 10% of our annual planting program, and we meet 30% to 50% of that demand with high gain material.

Three decades ago, when nurseries were experiencing germination challenges with wild seed due to lost stratification, wild seed crops were sporadic and costly, and orchard production in our established orchards was not successful, cuttings were selected as a means of keeping yellow cypress in our planting programs. Today, we have significant improvement in nursery handling of yellow cypress seed and delivery of planting stock, and numerous pollen and seed production studies indicate that yellow cypress seed production is possible in cooler, more moist environments. WFP is initiating the development of a woods-orchard to deliver gain more broadly at lower cost through seed deployment.

**BRITISH COLUMBIA MINISTRY OF FORESTS, LANDS AND NATURAL RESOURCE  
OPERATIONS**

**FOREST GENETICS SECTION TREE IMPROVEMENT BRANCH**

**Charlie Cartwright, Barry Jaquish, Stephen Joyce, Dave Kolotelo, Greg O'Neill, John  
Russell, Michael Stoehr, Nick Ukrainetz, Chang-Yi Xie, Alvin Yanchuk**

**Ministry of Forests, Lands and Natural Resource Operations (FLNRO)**

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This report covers the breeding, testing and selection progress for the economically-important trees species of British Columbia for which a breeding program exists, and outlines some of the research carried out by our team.

**Interior Spruce**

Barry Jaquish

The BC interior-spruce tree-improvement program was initiated in the late 1960s and has progressed to the point where virtually all of the approximately 90 million interior spruce seedlings planted annually originate from improved class “A” seed orchard seed. The first-generation open-pollinated and polycross testing program includes 2533 families and 75 test sites, the oldest of which is 43 years old. To date, the second-generation testing program includes 753 full-sib families and seven test sites. Highlights of the 2012–14 reporting period include 1) the forward selection of 100 trees from full-sib second-generation progeny tests in the Bulkley Valley seed planning zone, 2) the establishment of two full-sib second-generation progeny tests for the Thompson Okanagan seed planning zone, and 3) the continuation of screening select seed orchard parents and second-generation selections for resistance to terminal weevil (*Pissodes strobi*). Screening of 58 parents from seed orchard 211 for the high-hazard Prince George seed planning zone was very successful and several resistant genotypes were identified. Overall, the probability of surviving severe weevil attack ranged from 81 percent (genotype PG29) to 25 percent. Results from this artificial weevil screening will assist in the development of molecular markers for weevil resistance by the Genome Canada SMartForest project.

**Western larch**

Barry Jaquish

Western larch (*Larix occidentalis*) seed orchards are in full production and provide improved seed for the annual planting of approximately seven million seedlings. Second-generation breeding is nearly complete for both western larch seed planning units, and full-sib second-generation progeny tests should be established in the near future. The western larch seed planning zones were realigned in 2010 to allow limited planting outside of, and primarily northwest of, its natural range. These changes were made to 1) account for expected climate change, 2) facilitate assisted migration of western larch, and 3) increase tree species diversity in the central interior.

**Interior Douglas-fir**

Barry Jaquish

The interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) program began in 1982 and has progressed to the point where first-generation progeny tests are 20–30 years old, seed orchards are in production, and second-generation

breeding is nearly complete in high priority zones. Grafting commenced to establish new seed orchards for the Thompson Okanagan seed planning zone. Open-pollinated families of all seed orchard parents (150 in total) were grown for screening for resistance to *Armillaria* root disease. This screening is a cooperative project between FLNRO and the Canadian Forest Service (Victoria).

## **Lodgepole Pine**

Nicholas Ukrainetz

The lodgepole pine (*Pinus contorta* var. *latifolia*) breeding program has progressed from open-pollinated (first generation) progeny testing to full seed production in several seed planning zones. Five zones were identified as high priority for advanced generation breeding. Information from open-pollinated testing was used to rank parents for growth and wood density. Top parents were selected for second-generation (F1) breeding and were crossed to produce a growth and a wood density breed. The second-generation progeny tests are now between 10 and 15 years from planting. Age 10 height data were used to make forward selections in two zones (153 and 199 selections), with the remaining three zones scheduled for assessment over the next two years. The highest priority trait continues to be volume at rotation; however, three pathogens have been identified for resistance breeding: western gall rust (*Endocronartium harknessii*), Comandra blister rust (*Cronartium comandrae*) and Dothistroma needle blight (*Dothistroma pini*). We are also continuing to survey attack by mountain pine beetle (*Dendroctonus ponderosae*) when progeny tests are attacked. Data from first and second-generation progeny tests were used to rank parents for resistance to mountain pine beetle, Dothistroma needle blight, and Comandra blister rust; small crossing programs were completed in order to provide material for future testing and resistance research. Crossing for resistance to western gall rust will commence in 2016. There are two series of realized gain trials that will be monitored to verify breeding values and gain estimates for parent trees and seed orchards.

## **Western White Pine**

Nicholas Ukrainetz

The western white pine (*Pinus monticola*) breeding program is split into two breeding populations servicing coastal and interior regions of the province. The primary focus of the breeding program is white pine blister rust (*Cronartium ribicola*) resistance; secondary priorities include maintaining adaptation to local biotic and abiotic factors, and enhancing growth. The program began with controlled inoculation trials of seedlings grown from seed collected from wild trees that had no active blister rust infection. Seedlings were monitored for infection for several years then selected for seed production in seed orchards. The coastal program began F1 crossing and deployed four series of progeny tests, which are now serving as a source of material for seed orchards. Infection data from these trials have helped to accurately rank parent trees for resistance to blister rust and growth. Approximately 120 forward selections have been made from progeny tests for future breeding and orchard production. The interior program acquired 50 trees from the Idaho breeding program that tested well and have formed a strong genetic base. Controlled crosses were created among the Idaho parents and the progeny screened in controlled inoculation trials. The focus of the interior program now is on F1 breeding among resistant parents and the establishment of progeny field trials to assess field level resistance. Mature realized-gain trials indicate strong resistance from orchard populations. A fifth series of progeny tests was deployed on four sites in maritime, subarctic, and interior continental regions with controlled-crosses among trees from the northwestern United States, coastal B.C. and Idaho. This series will provide information about genotype-by-environment interactions for growth and resistance, which will provide information for making recommendations about moving material between coastal and interior regions.

## **Interior Hardwoods**

Nicholas Ukrainetz

Testing and selection of hybrid poplar in northern environments is ongoing. A clonal test at Red Rock was used to characterize several controlled crosses among individuals of *Populus trichocarpa*, *P. deltoides*, *P. maximowiczii*, and *P. nigra*. Several clones were identified with superior growth characteristics and tolerance to northern climates and these have been selected to create a stool bed which will be available for operational use and further research. An extensive genecology and open-pollinated progeny testing program has been established for paper birch (*Betula papyrifera*). There was significant genetic variation for volume; several forward selections have been made and grafted into two seed orchards for operational seed production.

### **Ponderosa Pine**

Nicholas Ukrainetz

There is one active seed orchard for ponderosa pine (*Pinus ponderosa*), which was created based on information and selections from a provenance test including material from the species' range in B.C. and northwestern U.S. The orchard population reflects the species variation in B.C.; deployment of orchard seed will be restricted to an area that reflects the current realized niche of the species. The fundamental niche of the species is expected to increase with climate change. Individuals with superior growth characteristics in provenance tests will be selected and archived for a future breeding program.

### **Coastal Douglas-fir**

Michael Stoehr

Complimentary tests are used for the advanced generation testing and selection of coastal Douglas-fir (*Pseudotsuga menziesii*), with 4 GCA tests and 2 full-sib (fs) family-block tests in three series and a set of 4 full-sib tests for Series 4. Analysis for the complimentary tests follows a combined (GCA and fs tests) mixed linear model using ASReml, with the selection criterion being height at age 11, and forecast to volume at rotation age (60 years) with the use of an age-age correlation to account for uncertainty. Wood density, estimated for each family using the Resistograph, and stem form (ramicorm branching and sinuosity) are secondary traits in this selection scheme. As part of final selection, all forward selections from the fs tests are screened for the native needle disease, Swiss needle cast (*Phaeocryptopus gaeumannii*). In 2015, selections from Series 2 were distributed and planted in seed orchards. These selections have an average genetic gain of around 25%. In the fall of 2015, selections will be made in Series 3 (out of 4) and grafts will be planted in seed orchards in the spring of 2017. Trees in Series 4 will be selected in 2018. For the next generation of improvement, the breeding strategy is based on 4-parent diallels per subline with additional crossings across Series, and possible open-pollinated testing followed by molecular paternal analysis. It is anticipated that 128 parents form the breeding population (4 parents x 32 sublines) with an expected  $N_e$  of around 80 to 90. Conventional selection procedures will also be supplemented with genomic selection approaches for wood quality, by screening the genotypes of the forward selections for markers associated with high wood density in the parental (training) population.

### **Western Redcedar**

John Russell

Tree breeding for western redcedar (*Thuja plicata*) involves the development of three overlapping breeding populations from first-generation selections: 1) volume growth and cedar leaf blight (*Didymascella thujina*) resistance, 2) heartwood durability, and 3) deer resistance.

*Volume growth and cedar leaf blight resistance (CLB):* Breeding for first-generation polycross testing, involving approximately 1000 parents for the Maritime Low Seed Planning Unit (SPU), is complete. Seven annual series of tests have been established totalling 46 sites covering six seed planning units. Parental breeding values for volume at rotation and cedar leaf blight resistance are currently being estimated from all seven series based on 10-year data

(1000 parents). Rogued and new seed orchards are currently producing seedlots with a genetic worth up to 20% volume at rotation with partial resistance to CLB. Advanced generation breeding is currently ongoing using assortative mating with partial diallels.

*Heartwood durability:* The original 350 BC parent trees selected in the early 1970s (also represented in the first three series of polycross testing described above) were assessed for heartwood durable chemicals using wood cores from grafted ramets established in Cowichan Lake Research Station (CLRS) clonebanks and industry seed orchards. Fifty selections based on tropolone and lignan concentrations have been cloned and established in an advanced generation breeding orchard and advanced generation breeding is currently ongoing using assortative mating with partial diallels.

*Deer resistance:* Initial selections for a deer resistant population were made from both a family/population study (within-family forward selections) and the CLRS clonebank (parental backward selections) based on high needle monoterpene concentration and low deer browse. These selections were bred in an advanced generation population using assortative mating with partial diallels. Early selection for foliage monoterpenes has resulted in approximately 100 forward selections for high needle monoterpene concentration. Breeding F2 population is currently ongoing.

The development of a broad-spectrum, durable population for resistance to known and unknown pests under a changing climate is ongoing involving the above populations. A UPP grant from Genome BC in conjunction with Bohlmann lab at UBC (CEDaR) has been exploring the use of multi-trait genomic selection in this durable population. The main economic trait driving this project is heartwood durability, a trait that cannot be evaluated until after 30 years in progeny trials.

## **Yellow Cypress**

John Russell

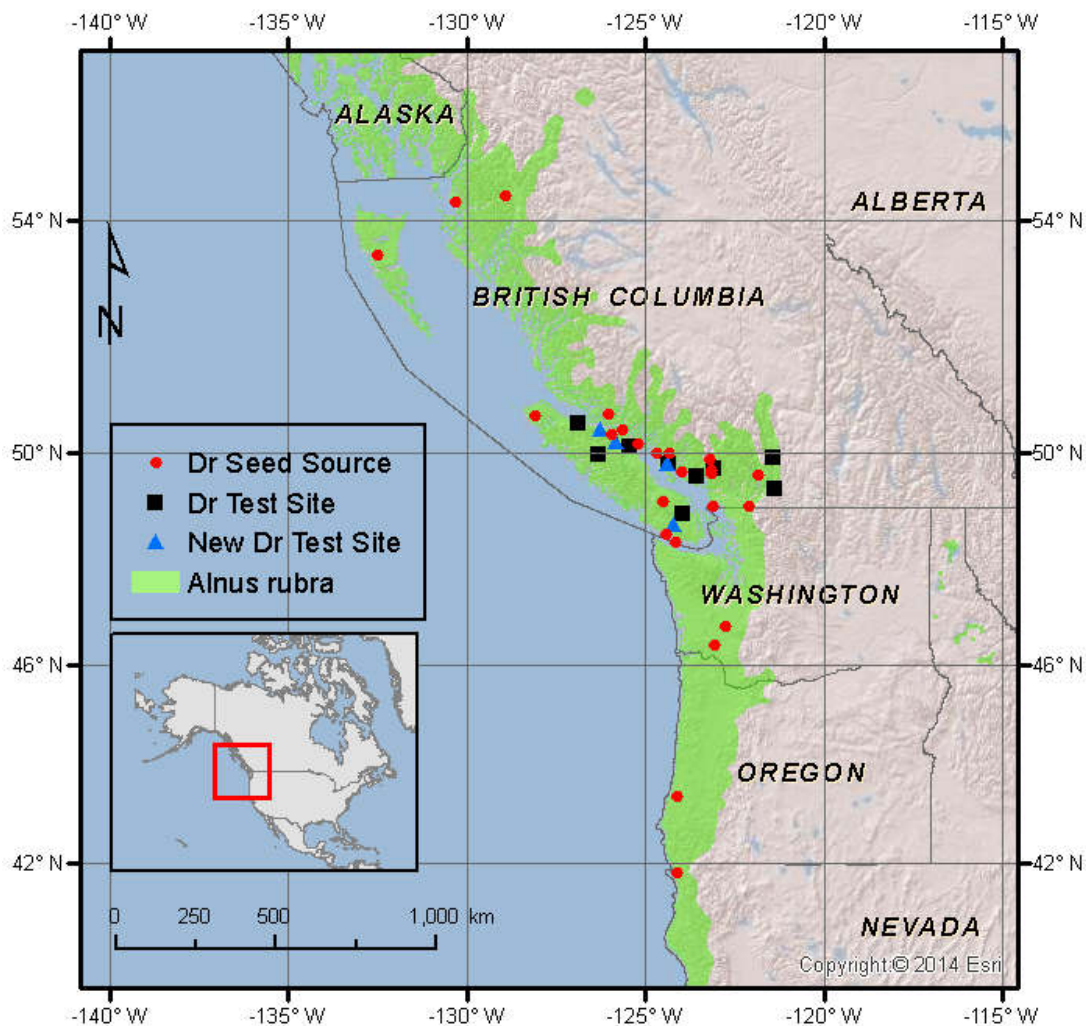
Yellow cypress (*Callitropsis nootkatensis*) is a major component of high-elevation coastal BC forests. The genetic management of this valuable species involves gene conservation, genecology, and tree breeding. Fifteen-year measurements have been completed for the yellow cypress genecology trials, which comprise approximately 50 populations established on 10 sites throughout BC. Analyses indicate the lack of a correlation between seed-origin climatic descriptors and mean provenance performance as seen in the 10-year data. Current liberal seed transfer guidelines reflect this random provenance genetic variation.

Yellow cypress propagates vegetatively in nature and cone crops are usually infrequent with historically poor seed viability and germination. Tree improvement has focussed on a clonal strategy. Twelve-year data from over 20 trials (Western Forest Products Inc. and FLNRO) have been collected and clonal genetic values from approximately 5000 clones have been estimated. Elite clones, based on volume and form, have been established in operational hedge orchards. The advanced generation is currently being bred using assortative mating. Research is ongoing to improve seed production efficiencies in clonal trials established in native yellow cypress ecosystems.

## **Red Alder**

Chang-Yi Xie

Thirteen provenance-progeny trials in two test series have been established. Test population includes about 10 families from each of 24 natural populations ranging from northern British Columbia to California and 32 control-pollinated families from a clone bank. The first test series was established at nine sites (black squares) with 5 replications at each site. The main objective of this series of tests is to collect genecology information for developing seed transfer guidelines and to select superior seed sources. The second test series was established at 4 sites with 15 replications at each site. The main objective of this test series is to estimate genetic parameters and make selections for future breeding and seed production.



## Climate change adaptation research

Greg O'Neill

BC's Climate-Based Seed Transfer technical working group completed the technical aspects of a proposed new seed transfer system for BC. A report describing the proposed system, including a comprehensive approach to assisted migration, went through external scientific review (April 2015) and is under stakeholder review (July 2015).

Age-10 height was collected in September 2014 on the interior spruce climate change/genecology provenance trial (128 populations at 17 test sites; a project done in collaboration with Barry Jaquish). Age-5 height and pest damage are being collected on the 15 species tested at the 48 sites in the Assisted Migration Adaptation Trial (AMAT) (<http://www.for.gov.bc.ca/hre/forgen/interior/AMAT.htm>). Both of these trials are long-term field projects intended to provide a better understanding of tree species' climate adaptation and guide seedlot selection in a changing climate, and will be used to help refine BC's new seed transfer system.

Age-6 data collected on the interior spruce climate change/genecology provenance trial, together with age-16 data from various Douglas-fir provenance trials were used in an analysis that examined transferability of orchard and

wildstand seedlots. Results confirmed the growth superiority of the orchard seed sources while demonstrating that selected seed sources were considerably taller than wildstand seed sources when transferred short or medium climate distances. See report by O'Neill, Stoehr and Jaquish (2014) below.

## Genetic Conservation

Charlie Cartwright

Whitebark pine (*Pinus albicaulis* Engelm.) is a high elevation species which has 47% of its natural range occurring in BC. It has undergone catastrophic population declines largely due to white pine blister rust (*Cronartium ribicola* J. C. Fisch.) but to a lesser extent by mountain pine beetle (*Dendroctonus ponderosae* Hopkins), wild fire and human activity. Further vulnerability results from slow growth and late onset of cone production plus almost exclusive reliance on Clark's nutcracker (*Nucifraga columbiana* Wilson) for distribution of seeds. As a result, whitebark pine is blue listed (at risk) in BC under the Endangered Species and Ecosystems Program in Ministry of Environment (MOE). More significantly, it was classified as endangered under the federal Species at Risk Act (SARA) in 2012. The repercussions are that Environment Canada (EC) must file a recovery plan within two years and status reports periodically thereafter. Though SARA applies directly to federal land, it is written such that it is incumbent on the provinces (clauses 5, 34[2], 39[1]1, and 60[1]1) to support federal action which can be demanded through orders to comply.

Gene conservation activities in Tree Improvement Branch commenced in 2006 with natural stand seed collections for rust resistance screening by then white pine breeder John King. Cone collections have continued to supply the FGC Gene Conservation TAC (GCTAC) *ex situ* reserve seed collection at the Tree Seed Centre led by Dave Kolotelo. Genetics trials are currently being established to 1) identify rust resistance populations and individual trees plus 2) develop climate-based seed transfer. This is led by Charlie Cartwright, Nick Ukrainetz (pine breeder) and Michael Murray (regional pathologist in Nelson) and will evaluate 500 parent trees.

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## **BRITISH COLUMBIA MINISTRY OF FORESTS,**

### **Cone and Seed Improvement**

Dave Kolotelo

The Tree Seed Working Group continues to provide extension on seed science and technology through its biannual Newsbulletin and its biennial workshop. The workshop in Fredericton had a theme of “Development and Application of Water Activity for Monitoring Seed Moisture Content” and also included virtual tours of several Tree Seed Centres from across Canada. Since the last CFGA meeting in Whistler (2013) we have produced Newsbulletin editions 58, 59, 60 and 61. Previous Newsbulletins can be found at this new web address <http://www2.gov.bc.ca/gov/content/industry/forestry/managing-our-forest-resources/tree-seed/tree-seed-centre/cone-seed-improvement-program/tree-seed-working-group>

Several areas were of key focus the past two years: our Kilning Strategy and seed extraction efficiency; genetic conservation and implementation of water activity. The kiln strategy revolves around ensuring structural integrity of our 27 year old kiln (done); obtaining a controlled Temperature\* Humidity chamber to test various kilning regimes (ongoing) and to eventually modify our kiln environmental controls to improve its efficiency. Trial work continues to focus on lodgepole pine in terms of both breaking cone serotiny and subsequent refection of cone scales to maximize seed extraction. Seed extraction efficiency is focused on refining Quality Assurance methods to help make the appropriate re-kilning decisions.

Genetic conservation has focused on expanding our seed bank with collections of non-commercial tree species. We obtained a water activity meter and we have been using this to non-destructively assess that the seed is dry enough for freezer storage. We hope to expand this technology to other uses at our facility. Time and effort has gone into specific genetic conservation extension and co-operation efforts with whitebark pine. During this time I have also reviewed the dormancy-breaking treatments applied to our most dormant commercial species – western white pine and yellow cypress.

I continue to participate in several committees: ISTA Forest Tree and Shrub Committee; Genetic Conservation Technical Advisory Committee (TAC); Coastal and Interior TAC. I helped organize the TSWG workshop on water activity and have made presentations and initiated discussions regarding tree seed on a multitude of topics. I provide facility tours, supervise our Testing group and continue to assist with succession strategies.

PROCEEDINGS  
OF THE  
THIRTY-FOURTH MEETING  
OF THE  
**CANADIAN FOREST GENETICS  
ASSOCIATION**

**PART 2  
Symposium**

Fredericton New Brunswick

Editors

Kathleen Forbes

COMPTES RENDUS

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TRENTE-QUATRE CONGRÈS

DE

**L' ASSOCIATION CANADIENNE  
DE GÉNÉTIQUE FORESTIÈRE**

**2<sup>e</sup> PARTIE**  
**Colloque**

Frédéricton Nouveau-Brunswick  
17 au 20 août 2015

Rédactrices/Rédacteur

Kathleen Forbes

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# Abstracts - Invited Papers Session

## **Plantation Silviculture in the 21st Century - Optimizing Stand Value by Integrating Site, Genetics, and Cultural Treatments (G x E x M)**

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Historically most forest landowners have focused on minimizing per hectare costs associated with plantation establishment and tending. This approach has resulted in millions of hectares of planted forests with growth rates that are substantially lower than what is biologically possible and financially optimum. Fortunately, during the past 25 years considerable advances have been made in our understandings of the genetic, ecological, and physiological basis for productivity. This knowledge has dramatically increased expectations concerning the productivity and value of plantations.

To realize these expectations, integrated silviculture regimes are needed where the crop trees, soil, and other vegetation are actively managed to optimize value. Implementation of these regimes requires ecophysiological knowledge concerning how a crop tree's genetic make-up interact with the environment to affect productivity, stem quality, wood quality, and susceptibility and resistance to insects and diseases. In addition, a site specific understanding of what resources limit production (temporally and spatially) and how cultural treatments can be used to ameliorate these limitations are required.

With tens of millions of hectares already planted with improved material and more than a million hectares being planted every year, many questions and concerns are being raised by forest managers. These include: what genotype(s) should be planted on what sites, what gains in volume and value will be realized at final harvest, what traits will lead to value gains at the end of the rotation, will the benefits of deploying clonal material offset the costs and risks, is the genetic material that is current being planted plastic enough to continue to thrive as climate changes, does the use of clonal material provide the opportunity to plant fewer trees per hectare, will thinning be needed, will other novel silviculture systems be possible, will landowners be able to realize gains in wood quality?

Applying our developing knowledge concerning G x E x M provides the basis for answering many of these questions. Intensive plantation silviculture is similar to modern agriculture but still firmly based on forestry's strong ecological foundations.

## **Adding value with tree improvement: a case study from British Columbia**

**Jack Woods**

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Forest genetics includes a broad range of activities. This presentation will focus only on tree improvement as an economic endeavor that is intended to add value to timber production. It is important to fully understand what constitutes value by examining the products derived from logs and the probable trends in product development. Logs from Canadian forests are utilized primarily for dimension lumber, pulp, and paper. There has been a strong trend in wood manufacturing to effectively utilize poorer-quality logs. Milling developments continue to change relatively quickly and will continue to find ways to profitably utilize poorer quality fibre. Timber production and silvicultural interventions take place at a much slower pace than milling upgrades and product development. Against this background of long growth rotations and shorter cycles of utilization and milling, opportunities to improve wood quality through tree improvement have limited value. The only trait that is consistently in demand is faster growth and higher log-volume production. This argues for selection for fast growth or selection for pest resistance pests are an issue.

In British Columbia, a system of quantifying growth gains from tree selection through to final rotation has been developed. The initial and most critical component of this quantification of gain is the development of a breeding value that reflects expected volume gain at an average stand-rotation age. This breeding value is then used in seed orchards to estimate a seedlot genetic worth for growth (GWg). All orchard seedlots produced in BC have a GWg associated with them that can then be used by operational foresters to evaluate the genetic quality of the seedlot. Growth models have a function that allows the input of a seedlot GWg to estimate stand growth and yield. These growth and yield data may then be utilized by timber supply models to estimate timber availability over time from a management unit or forest estate. Through these steps, determinations of annual harvest can incorporate genetic gains from tree improvement programs. The additional value prospects from this system will be discussed.

## Breeding and seed orchards in Sweden – how to get gain into the forest

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Forest tree breeding in Sweden is carried out by Skogforsk and is jointly financed by the forest industry and the Swedish government. Skogforsk also carries out mass propagation for establishing seed orchards, funded and operated by stakeholders (land owners, plant producers).

The general aims of Swedish forest tree breeding are: i) to increase value production in cultivated forests; ii) to prepare for climate change; and iii) to conserve and manage genetic diversity. Breeding for the four main species Scots pine, Norway spruce, silver birch, and lodgepole pine is carried out according to a Multiple-Population Breeding Strategy (MPBS) in separate unrelated subpopulations, each with 50 or more breeding parents. The various subpopulations are field tested and bred for adaptation to different combinations of latitude and temperature conditions, including combinations that lie outside what is normal under the present climate. This results in suitable material for the various geographical/climatic regions, in addition to a state-of-readiness for climate change. By selecting the genetically best trees from one or several breeding populations for mass propagation (seed orchards or clone mixtures), improved regeneration stock is created with the desired adaptation profile.

The establishment of a third round of seed orchards for Scots pine and Norway spruce, planned for 2002–2020, is soon to be completed. Orchard clones are selected based on genetic testing with an average genetic gain in full-rotation productivity of ca. 25 %. The program is nationwide, including almost 600 ha of orchard area at an annual budget exceeding 100 million SEK (ca 15 million CAD), and will fully supply future demand for seedling production and seed for direct seeding.

Economic calculations show convincingly the financial benefits of using improved reforestation material: the higher the site index the larger profit. In fact, it is difficult to find any investment in silviculture that beats genetically improved seed and seedlings. A genetic gain of 25% will double the land expectation value on a fertile soil at an interest rate 2.5%. The use of such seedlings creates room for additional investment corresponding to a plant price of four times more than current, making possible alternative propagation methods such as somatic embryogenesis. It is obviously preferable to allocate the genetically best material to the most valuable sites. For example, selective harvest from superior clones in current orchards should be used for seedling production, while the remainder can be used for direct seeding on less fertile land.

In parallel with the economic calculations, hands-on recommendations on suitable reforestation material for specific sites are needed to get the genetic gain into forest production. We have developed a web-based decision-making tool “Plantval” (<http://www.kunskapdirekt.se/sv/KunskapDirekt/Alla-Verktyg/Plantval/>) that is operating and free of charge. All available Swedish seed sources, improved and unimproved, are listed and given a production index (combination of survival and growth rate) depending on chosen planation site. Predicted survival and growth are based on transfer functions and genetic gains from orchards are added. By taking into account both the present climatic conditions, which are crucial for seedling survival and establishment, and future conditions, which

determine the growing conditions during the rotation, it is possible to optimize the deployment of reforestation stock currently available.

Skogforsk and Luke (The Natural Resources Institute Finland), with input from SMHI (Swedish Meteorological and Hydrological Institute) and FMI (Finnish Meteorological Institute) and funding from Föreningen Skogsträdsförädling (Swedish Tree Breeding Association) and the European Commission's Framework Programme 7, have developed new transfer functions valid for Scots pine both in Sweden and Finland north of latitude 60°N. These functions are based on new climatic indices used in scenario and ensemble analyses in climatic research. The indices are also corrected for bias towards observed climatic indices. Yearly mean temperature, temperature sum, and altitude of the site together with latitudinal transfer from provenance origin were the main explanatory variables for growth and survival. An updated decision-making tool, where the user specifies both reforestation sites and climatic scenarios, is now under construction. The tool will also make possible the use and trade of reforestation material over national borders. A similar project where Sweden, Finland, Norway and the Baltic States collaborate to develop response functions for Norway spruce has recently been started within phase two of the research programme Future Forest funded by Mistra (the Swedish Foundation for Strategic Environmental Research), SLU, Umeå University, Skogforsk, and the Swedish forest industry.

## **Incorporating genetic gain into growth and yield modelling**

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Intensification of silviculture to maintain softwood harvest levels on public and private lands has been a dominate theme in New Brunswick over the past 40 years. Planting with improved genetic stock of spruce (*Picea* spp.) and jack pine (*Pinus banksiana*) began operationally in 1992. As these plantations are still young (<25 years), and because genetic stock planted between 1992 and present has continually improved, long-term estimates of plantation growth and yield with gains are not available. Therefore, observed genetic gains from tests and consultation with experts are used to modify existing simulation models of individual tree growth in plantations (without gain) in order to estimate improvements in stand volume, time to harvest, and product distributions for long-term wood supply planning. Genetic gain studies and modeling approaches used by the New Brunswick Department of Natural Resources to incorporate genetic gain expectations in stand and forest forecasting are discussed.

## Overview of NBTIC- Forty Years of Cooperation

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The New Brunswick Tree Improvement Council (NBTIC) was established in 1976 to coordinate tree improvement efforts of government and industrial agencies and to facilitate the free exchange of genetic material and information. At the time, reforestation programs were expanding rapidly and all agencies recognized the impact tree improvement could have on wood supply.

Although NB is one of the smallest provinces in Canada, forestry is a major industry with approximately 50% of the land base managed for the province by four licensees. The annual planting level on Crown land is approximately 12,100 ha (22-25 million) most of which is full planting. The cooperative nature among the members of the Council has allowed NB to be a forest genetics leader in Canada.

At present, the Council is comprised four industrial companies, two universities, and provincial and federal governments. The provincial government is the lead agency for coordinating and conducting breeding programs and growing seedlings for genetic testing. Industrial members participate in selection and breeding and are responsible for planting, managing, and measuring the tests. Several industrial companies also produce seed and seedlings for their freehold reforestation programs.

Plus tree selection, orchard establishment and genetic testing have been carried out steadily over the last forty years. A total of 3119 first-generation, 1167 second-generation and 40 third-generation plus trees have been selected. Over 350 tests have been established on over 400 ha. First-generation programs for black spruce and jack pine consisted of seedling seed orchards and open-pollinated family tests, in contrast to those of white spruce and tamarack which were clonal seed orchards and control-pollinated progeny tests. Production from the seed orchards has been regular and heavy. By the early 1990's (in only 15 years), most reforestation stock was being grown from orchard seed. Seed orchard management including fertilizer and GA applications both in orchards and clone banks have been used routinely to increase cone production.

Seedling production in the province has remained steady over the last number of years with all of the seed (black spruce, white spruce and jack pine) coming from improved second-generation orchards. Other species such as Norway spruce and red spruce have been added to the program. Species priorities have changed over time with white spruce, black spruce and red spruce representing the majority of the species currently planted. To date, 304 ha of orchards have been established yielding 791,060 litres of cones, 5942 kg of seed and over 2.5 billion seeds!

Over the last 40 years NBTIC has made significant progress in improving the quality of trees planted in New Brunswick. The success of the program is directly related to the leadership and scientific direction from CFS, the support from all of the NBTIC members over the years and the commitment and leadership from DNR. Together we have made a positive impact on the reforestation program in NB.

## **Genomic selection – Potential and current limits of application in boreal conifer breeding programs**

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Genomic selection has been proposed as an alternative to conventional pedigree-based selection, where pedigree information is replaced with dense genetic marker information to estimate the genetic value of each individual. Genomic selection can theoretically capture the Mendelian sampling component in the absence of recorded phenotypes because at an appropriate marker density, all loci that explain some of the phenotypic variation are presumably tagged.

At the current stage of the development of the technology, genomic selection could not replace conventional breeding in conifers. But high prediction accuracies can be obtained with well-trained models applied to select tree from the same population. Breeding cycles could be hastened as well as the production of superior plantation stock in combination with somatic embryogenesis, which is being scaled up at the operational level in several jurisdictions producing improved spruce seedling stocks. Because boreal conifers have long breeding cycles in part due to long delays in evaluating traits at the mature stage, they likely represent the case where early forward selection by use of predictive genomics will generate the most important improvement of gain per unit of time and tangible economic benefits.

## **Integration of somatic embryogenesis and genomic selection with multi-varietal forestry**

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Multi-Varietal Forestry (MVF) is defined as the deployment of a range of genetically tested tree varieties in commercial plantation forestry. Somatic embryogenesis (SE) and cryopreservation are the enabling technologies for the implementation of MVF. Recently, it has been shown that genomic selection (GS) has a great potential to be incorporated with MVF.

MVF is well suited for intensively managed, high-productivity sites. MVF integrated with GS offers a drastically faster and much greater genetic gain than that from traditional tree breeding. In white spruce breeding in eastern Canada, for example, the gain is delivered 15 years sooner than the traditional seed orchard breeding. Moreover, GS will make the testing and selection efforts more efficient and streamlined through pre-screening.

Sufficiently refined and efficient SE protocols for commercial MVF are available for a number of conifers, primarily the spruces, some pines, and a few larches, but more refinements are required for several economically important conifers. The main challenge for implementing industrial MVF, however, is the high cost of SE seedling production due to manual handling of embryos, both pre- and post-germination. In order to be cost effective, it requires the development of a mechanized embryo handling system for transplanting into mini-plugs for greenhouse culture. With the lack of an automated transplanting system, complementary serial rooting of cuttings may be used as a mass propagation tool once the suitable varieties are developed from the SE-based system.

In addition to obtaining a large genetic gain at significantly reduced time, MVF offers flexibility to adapt to changing breeding goals, environment and disease and insect conditions, and this flexibility is provided by the up-to-date information obtained from varietal field testing. Furthermore, in MVF, plantation diversity is dynamically managed over time by carefully balancing genetic gain and diversity based on the best available varietal field test data.

**Key words:** Cryopreservation, deployment strategy, genomic selection (GS), multi-varietal forestry (MVF), somatic embryogenesis (SE), varietal field testing



## **Sustainable management of forest genetic resources under changing climate and environmental conditions**

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Global climate and environment changes are subjecting our forest ecosystems and forests, especially boreal and temperate forests, to significant stresses, which can negatively impact their adaptation, health, productivity and fitness; consequently affecting sustainability of forest genetic resources. For sustainable management of forest genetic resources under changing climate and environment conditions, it is critical to understand genetic/genomic basis of responses and adaptation of forest trees to changing conditions so that measures to mitigate the negative impacts of climate and environment changes could be developed by selecting, creating, and conserving genetically diverse and healthy forest resources. Genomics research and applications provide excellent opportunities to address these crucial research needs. I will discuss how structural, functional and population genomics and bioinformatics research and applications can contribute to decode responses of forest trees to climate and environment changes and understand the genetic basis of responses and adaptation of forest trees to climate change and air pollution by presenting highlights of our own research on these aspects. In black spruce (*Picea mariana*), we have identified and characterized genes expressed differentially in response to climate change conditions (elevated CO<sub>2</sub>, drought, and combined elevated CO<sub>2</sub>-drought co-stressed conditions), their metabolic pathways and relationships with relevant physiological traits, and mapped quantitative trait loci for phenotypic and ecophysiological traits related to growth and adaptation to climate change. We have also examined adaptive response of red spruce (*Picea rubens*) to sulfate air pollution and identified genes showing signatures of natural selection in response to air pollution. I will discuss the results of these studies and their implications and applications for sustainable management of forest genetic resources under changing climate and environment conditions.

# **Genomics of local adaptation in trees: Potential and constraint in the context of past and contemporary climate change**

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Forest tree populations are exquisitely adapted to their local environments, but climate change is substantially altering adaptive landscapes, which is expected to lead to widespread maladaptation of tree populations to their seasonal temperature regimes. Adapting management strategies to account for these changes depends crucially on an understanding of the genomic architecture of adaptive traits. Until recently, such studies have been constrained by relatively sparse sampling of the genome, but the advent of next generation sequencing allows for true genome-wide genotyping that enables a more comprehensive characterization of the evolutionary forces shaping patterns of variation. One promising approach to achieve this is exome capture, in which RNA baits are synthesized that target genomic intervals of interest. We used this technology to retrieve most of the ‘gene space’ in *Populus trichocarpa*, which has become a model species for understanding tree biology and evolution. Samples were collected from across the natural species range, from California to Alaska, and across two altitudinal transects in southwest British Columbia. This collection was planted and phenotyped at common garden sites in Virginia and British Columbia. The genotypes and phenotypes were coupled with climatic data to unravel the genomic basis for local adaptation at coarse (latitudinal) and fine (altitudinal) spatial scales through tests of local selection, genotype-phenotype and genotype-environment associations, and characterization of ‘deleterious’ alleles. We will discuss these results in the context of managing local adaptation under climate change.

# Abstracts - Volunteer Papers Session

## Climate and Economic-smart Forest Regeneration

Daniel W. McKenney<sup>1\*</sup>, John H. Pedlar<sup>1</sup>, Jing Yang<sup>2</sup>, Alfons Weersink<sup>2</sup> and Glenn Lawrence<sup>1</sup>

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Seed source selection is one of many challenges for forest managers under an evolving climate. If greenhouse gas emissions continue on current trajectories, climate model projections suggest radical changes in Canada's climate this century – well within standard rotation periods for most commercial species in Canada. The sobering implication is that many plant populations are likely to be growing outside of their comfort zone during the course of this century with survival, growth rates, disturbance regimes, and even silvicultural investments being affected.

We present a framework and case study that maps the growth expectations and net present value (NPV) associated with planting black spruce (*Picea mariana*) and white pine (*Pinus strobus*) seed sources across a study area centred on Ontario, Canada. The model accounts for climatic influences and change through the use of universal response functions (URF), which (in principle) predict the growth of any seed source under any climatic conditions. The use of the model will be demonstrated for two locations in northern Ontario; both species exhibited significant variation in NPV across the study area and significant gains associated with “climate-smart” seed movements. For example, the NPV associated with potential white pine seed sources varied by more than \$1500/ha for a planting site at North Bay, Ontario. We also compared the NPV maps to climate similarity maps to examine the degree to which simple climate matching can act as a rule-of-thumb or proxy for the detailed genecology relationships contained in the URF. Climate similarity was calculated using an existing tool called Seedwhere, which was developed under the premise that local seed (and hence local climate) is generally best. Under climate change, Seedwhere can assist forest managers in finding locations where climate change projections are reasonably similar to current maternal climates.

Overall, the Seedwhere-based climate similarity maps were well-correlated with the URF-based NPV maps; however, there was poor agreement regarding white pine seed deployment from North Bay, where the two approaches identified opposite seed transfer directions. We propose that this situation can arise when species show strong genetic adaptation to a central climatic optimum. Some implications and possible research directions are discussed. The climate similarity and URF models are now available as a web-based Decision Support Tool (<http://gmaps.nrcan.gc.ca/climwhere/>).

**Preliminary study of the effect of climatological factors on tree-ring widths of  
*Fagus orientalis* Lipsky in the Caspian Forest of Iran**

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**Keywords:** Caspian forests, crossdating, dendrochronology, *Fagus orientalis* L., ring width.

The effects of global climate change on forest growth and the formation of annual tree-rings have been observed in many parts of the world. This study aims to understand the impacts of climate factors on the annual growth variations of oriental beech (*Fagus orientalis* Lipsky) at the Kheyroud Forest Research Station located in the Caspian forests of Northern Iran. Twenty disc samples were randomly taken from altitudes of 1160 m above the Caspian Sea. Ring widths were measured in the laboratory using TSAP-win software and a LINTABII Machine. Since false and missing rings are typical in beech trees, skeleton plots were created to enhance crossdating accuracy. Chronologies were observed for a total of twelve samples. Meteorological data for rainfall and air temperature were recorded at the Nowshahr Meteorological Station, located near the study sites. Results showed a significant correlation ( $R=0.54$ ) between mean annual air temperature and radial growth as indicated by annual tree ring width. During the growing season, August temperatures reveal a significant correlation ( $R=0.41$ ) with tree ring widths while the highest association was observed in the previous December ( $R=0.44$ ) and the weakest association was found in February ( $R=0.31$ ). Excluding the month of March ( $R=0.33$ ), there appeared to be no significant correlation between precipitation accumulated during other months and tree ring widths. In addition to oriental beech, there are many other species important to this region that may be sensitive to climatological parameters. Thus, it is important to conduct more comprehensive research to expand our knowledge about the impacts of climate change on trees growing in the Caspian forests.

## Endophyte enhancement of conifer seedlings to improve tolerance to insects and disease

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Collaborative research between J.D. Irving, Limited and Carleton University began in 1998 to evaluate the potential of inoculating spruce seedlings with selected native strains of endophytic fungi (fungi living in conifer needles) to improve tolerance to eastern spruce budworm. Basic research conducted earlier at the University of New Brunswick during a serious spruce budworm epidemic had demonstrated that some endophyte strains isolated from relatively un-defoliated conifer trees in the region produced anti-insectan secondary metabolites. Methodologies have been developed to inoculate seedlings in the nursery with highly selected strains of endophytes producing different secondary metabolites to improve seedling tolerance to defoliators later in life. We have documented that the strains remain in the tree (up to age 12 tested so far), the anti-insectan metabolites continue to be produced and that feeding on endophyte treated trees reduces survival of spruce budworms through to adulthood. New research is progressing on developing the same approach to improve white pine blister rust tolerance in white pine. Endophytes have been isolated which have been demonstrated to be anti-fungal. We believe that this novel approach to improving the seedling's tolerance to insect and disease attack will be a useful tool in sustainable forest management.

## Reflections on Prairie tree breeding 1888 to 2015

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Since the commencement of the Prairie Shelterbelt program in 1901, significant advances have been made in the selection and improvement of tree and shrub species provided through the program. The shelterbelt tree improvement program at Indian Head, designed specifically to develop adapted, genetically superior tree and shrub species for prairie shelterbelts has been under way since the beginning of settlement in the 1880s. During this period, tree improvement and evaluation was led by Angus McKay (1888-1903), Norman Ross (1904-1941), John Walker (1942-1946), Bill Cram (1947-1974), Gordon Howe (1975-1980), and William Schroeder (1981-present). Improvement efforts touched many genera and species but focused primarily on *Populus*, *Pinus*, *Picea*, *Larix*, *Fraxinus*, *Quercus* and *Salix*. During the 127 years the program encompassed species introductions and testing, plant exploration, classical breeding and selection, *ex situ* conservation and genomics. Since its inception, the breeding program developed over 25 poplar clones and improved seed strains for over 20 species. The breeding efforts of the past 127 years have changed the landscape and quality of life for thousands of prairie people. In 2013 the last trees from the Prairie Shelterbelt Program were planted following over a century of federal government support for tree planting in western Canada. This paper presents a reflective view of the tree breeding program, particularly the people, its accomplishments and impacts.

## Genetic gain and gene diversity of seed orchard crops

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**Key words:** status number, effective population size, group coancestry, inbreeding, fertility variation, ramet variation, gene migration, sibling coefficient

Seed orchards are the major tool for deploying the improvement generated by breeding programs and assuring the consistent supply of genetically improved seed. Attainment of genetic gain and monitoring of gene diversity through selection and breeding were studied and reviewed considering the factors: selection intensity, genetic value, coancestry, fertility variation, and pollen contamination. The optimum goal of a seed orchard is achieved when the orchard population is under an idealized situation (e.g., panmixis, equal gamete contributions from all parental genotypes, non-relatedness and no pollen contamination). In practice, however, due to relatedness among parents, variation in clonal fertility and ramet number, and gene migration from outside, the realized genetic gain and gene diversity deviate from the expectation. In the present study, the genetic value of seed orchard crops (genetic gain) could be increased by selective harvest, genetic thinning and/or both. Status number was used to monitor the loss of gene diversity in the process of forest tree domestication, and calculated to be reasonably high in most seed orchards. Fertility of parents was estimated based on the assessment of flowering or seed production, which was shown to be under strong genetic control. Variation in fertility among orchard parents was a general feature and reduced the predicted gene diversity of the orchard crop. Fertility variation among parents could be described by the sibling coefficient. On average, the sibling coefficient was estimated to be 2 (i.e., the coefficient of variation of 100% for fertility variation among genotypes). Besides the fertility variation, it was possible to consider the phenotypic correlation between maternal and paternal fertilities, and the rate of pollen contamination. Status number was increased by controlling parental fertility, e.g., equal seed harvest, mixing seed in equal proportions and balancing parental contribution. By equalizing female fertility among over-represented parents, it was possible to affect a favorable tradeoff between gene diversity and seed production. If the status number of orchard crop is not large enough, loss of gene diversity, random drift in gene frequency and potential inbreeding problems could occur in subsequent generations. Genetic loss or erosion did not seem to be alarming during the domestication of forest trees because a large number of parents were commonly used in first-generation seed orchards. An understanding of reproductive processes and monitoring of the impacts of the management practices are essential to maximize genetic gain and to maintain sustainable gene diversity in seed orchard programs. Such factors are described, and general principles and practices of seed orchards are reviewed in the presentation.

## General over-view of Turkish seed orchards and its effect on plantation performance

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Seed orchards and its effect on **plantation** forestry are reviewed based on early published papers together with Turkish tree breeding history. The Turkish forest tree breeding was started from the research on seeds and the selection of seed stands at the beginning of 1960's. The first "National Tree Breeding and Seed Production Programme", which was a part of the Turkish–Finnish project, implemented in the cooperation between the Turkish Ministry of Forestry and Enso Forest Development Oy Ltd that was running between 1994 and 2003. Establishment of seed orchards was one of the most important of stages in tree breeding programme. Turkish seed orchards have been established generally with clones or seedlings propagated from plus trees selected phenotypically from seed stands. Turkey has a total of 179 seed orchards that was established by 6,476 clones at 1,314 ha with nine tree species mainly Turkish pines (*Pinus brutia*, *P. nigra* and *P. sylvestris*). For instance, the total of 72 orchard were established by 3,025 clones at 562 ha in *Pinus brutia*. There are large differences among orchards and within an orchard for the number of clones and the number of ramets per clone. It has been reported that the seed sources have the high level of genetic diversity and the variation in seed stands transfers successfully into seed orchards and plantations by 25-30 clones on Turkish red pine (*Pinus brutia*) and Turkish black pine (*Pinus nigra*) (Velioglu *et al.*, 2003a&b; Bilir *et al.*, 2004). Variance components due to families in the seed orchards were in general higher than those in the seed stands in seven year field test of Turkish red pine (Gulcu and Celik, 2009). Seedlings of seed stands showed higher growth performance than those of seed orchards at the nursery stage of Turkish red pine (Dilaver *et al.*, 2015). Other published materials are also reviewed and contributed for the establishment of future seed orchards and plantation forestry in Turkey.



## Mating system dynamic temporal variation in a coastal Douglas-fir seed orchard

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As a key component of the tree improvement delivery system, seed orchards play a significant role as the production populations responsible for packaging the genetic gain and diversity captured through breeding. To reach this goal, seed orchards are expected to function as a closed, perfectly random mating population, which, in reality, is rarely fulfilled due to parental fertility variation, reproductive phenology asynchrony, and gene flow (pollen contamination). The actual performance of the mating system within the orchard is thus of primary interest. We studied the mating system dynamic of a second generation, wind-pollinated, coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) seed orchard over a four-year period to obtain information under various combinations of seed crop management practices such as supplemental mass pollination (SMP) and/or bloom delay (overhead cooling) and different cone-crop sizes. This study utilized six polymorphic microsatellite (SSR) markers to generate DNA fingerprints of 58 and 489 individuals representing the seed orchard's parental and offspring (2008 seed crop) populations, respectively. The DNA fingerprinting data were used in a likelihood based pedigree reconstruction framework to assign the seed crop parentage using the CERVUS software (Version 3.0) (Kalinowski *et al.* Mol. Ecol. 2007). The parentage assignment permitted the determination of parental gametic contribution, selfing rate, and the extent of external gene flow. The results obtained from 2008 were compared to the seed orchard's previous performances.

Gametic contribution results indicated that 80% of the seed crop gametes were produced by 64% of the orchard's parents, an estimate that is higher than that observed from the other three studied years (range: 37 – 52%). A 16% selfing rate was estimated, value is similar to what was previously observed (range: 12 – 17%). Finally, external pollen contamination rate of 28% was determined from the total paternal gametes, indicating that the pollen contamination was quite serious in this particular year compared to previous findings (range: 10 – 18%). The observed high pollen contamination rate could result from the lack of crop management practice implementation in 2008 (i.e., no SMP or bloom delay), leading to an extended reproductive phenology developmental period (i.e., lack of enhanced reproductive phenology synchrony) and the absence of pollen augmentation through SMP. On the other hand, the considerable external pollen boosted the outcrossing rate, possibly explaining the observed relatively low selfing rate, specifically when excessive inbreeding was expected due to the extremely low cone-crop size.

This conducted temporal study enabled the comparison of the same seed orchard's performance under different management practices and aimed at providing a guideline for the various seed crop management options implemented and assisting in determining the genetic quality of the orchard's seed crops.

## Genomic selection in black spruce: towards its operational application

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**Background:** More than 100 million black spruce (*Picea mariana* (Mill.) B.S.P.) seedlings are planted every year in the Canadian boreal forest. Due to its economic and ecological importance, the species has been subject of several breeding initiatives aiming to improve growth and more recently wood properties. Genomic selection (GS) is thought to be a valuable approach to overcome the time-consuming selection for mature traits such as wood quality, which can take up to 30 years to assess through field tests in boreal conifers. GS should be particularly appropriate and economically feasible at an operational scale in advanced-breeding populations with small effective size. But until recently, genomic resources for black spruce were not available in order to test breeding approaches based on genomic selection.

**Methodology:** In a first step, we developed a large-scale SNP (single nucleotide polymorphism) marker registry representative of the black spruce transcriptome as well as a genotyping chip. Then, using an advanced-breeding population of the Quebec Ministry of Forests, Wildlife and Parks, we evaluated different scenarios of model construction and validation that are of importance for operational integration of genomic selection into breeding programs. The 734 trees analysed for this study belong to 34 full-sib families from a partial diallel mating scheme and were established in two ecologically contrasting environments in eastern Quebec. Height and diameter at breast height (DBH) were measured to characterize growth; wood density and cellulose microfibril angle (MFA) were also determined. The latter traits are important characteristics for mechanical wood quality and were obtained from wood cores sampled at DBH. All sample trees were genotyped for 4993 SNP markers using the developed Illumina Infinium iSelect array.

**Results:** The quality of genomic selection models was evaluated through the correlation between cross-validated breeding values and known breeding values for each trait. When all markers were considered, the accuracy of models was high and reached values around 0.8 for wood density and height growth, and somewhat lower values for the two remaining traits. These estimates were generally congruent with those obtained for *Picea glauca* bi-parental families for the same traits. The relative efficiency of genomic selection models versus conventional pedigree-based models was high, with values reaching 100%. Only for DBH, a slightly higher absolute gain was estimated with the pedigree-based approach. Similar patterns of model precision and estimated gains were observed when genomic selection models were trained and validated on different sites. This suggests low G-by-E interaction in this population, which should however be confirmed on a wider range of sites. In a further step, we tested subsets of markers and trees. Accuracy of genomic selection models based on 450 to 500 trees was comparable with accuracy of models including all available sample trees, although slight differences between traits were observed. Similarly, only 500 to 1000 markers were necessary to reach model accuracy estimates that

were comparable with accuracies obtained with the entire marker set. Both findings indicate that costs for phenotyping and genotyping could be further reduced, which would improve the cost-to-benefit ratio for application in the operational tree breeding program.

**Conclusions:** Overall, the present results are an encouraging step towards operational application of genomic selection in slow-growing boreal conifer species. Wood quality traits are expensive to evaluate and cannot be assessed until maturity is reached. Our results indicate that genomic selection for these traits is possible at the seedling stage, with genetic gain per year being two to three times higher than that obtained with the conventional selection approach.

**Local climatic adaptation not detected for white spruce (*Picea glauca* [Moench] Voss) populations from Ontario  
in reciprocal transplant experiments**

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Local climatic adaptation is a fundamental assumption for delineating seed zones and is commonly assumed to occur in forest tree species that show genetic differentiation among natural geographic populations, especially for those with widespread natural distribution with populations growing in distinct environments. However, due to a scarcity of suitable data, the inference of local adaptation has rarely been validated through reciprocally transplanted common garden experiments (RTCGEs). In this study, we restructured data from tree provenance tests to approximate RTCGEs for detecting local climatic adaptation among white spruce (*Picea glauca* (Moench) Voss) natural populations in Ontario. Although white spruce populations originated from ten climatically distinct locations in Ontario showed significant clinal variation and genotype-by-environment interaction in survival and tree height growth, local populations did not significantly outperform the non-local ones. Populations originated from south-central Ontario did not suffer higher mortality than local populations at more northerly planting sites resulting from causes such as frost damage, and they have shown superior growth across field tests. This suggests an absence of local adaptation for northern white spruce populations sampled from this part of the species range in Ontario. We discuss the implications of this result for the interpretation of tree provenance test results and the development of climate change adaptation strategies, such as assisted migration, for white spruce to enhance forest adaptation and productivity under current and future climate. We also discuss the importance of conserving white spruce genetic resources in south-central Ontario.

## **Evidence that allelic introgression from *Populus balsamifera* (balsam poplar) underlies local adaptation in *P. trichocarpa* (black cottonwood)**

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Understanding the genetic basis of local adaptation is a key goal in evolutionary biology, in particular the roles of introgression (i.e. gene flow between species) as a mechanism for generating allelic variants of adaptive value. Natural hybrid zones in forest trees provide novel systems to study the ecological significance of adaptive genetic variation arising from introgression. Previous landscape genomic studies in *Populus trichocarpa*, a keystone tree species with a widespread distribution in western North America, indicated genomic footprints of admixture with its sister species *P. balsamifera* near potential contact zones in British Columbia. These studies also identified candidate genes for local adaptation in *P. trichocarpa*. Here, we explored patterns of introgression and signals of local adaptation in pure *P. trichocarpa*, pure *P. balsamifera*, and admixed populations from wild populations in British Columbia and Alberta (Canada), focusing on previously identified genomic regions with signatures of local adaptation. Employing scans of single nucleotide variants and ancestry analysis, our results indicate that a telomeric region in chromosome 15, containing several candidate genes for local adaptation, shows strong evidence for introgression from *P. balsamifera* into *P. trichocarpa* in populations from the northern range of its distribution. Further genomic analyses revealed signals of selection in these genes, and functional analyses based on gene expression variation and correlation with adaptive phenotypes to higher latitudes (i.e. short growing seasons) suggest distinct functions of the introgressed alleles. Overall, our results support the hypothesis that this is a case of adaptive introgression in an ecologically important forest tree.

**Genetic consequences of anthropogenic disturbances and population fragmentation in *Acacia senegal* (L) Willd.: The case of Lake Baringo Forest Ecosystem, Kenya**

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**Keywords:** *Acacia senegal*, genetic structure, bottleneck, molecular markers, Bayesian methods, Lake Baringo

The acacia bushland and woodland ecosystem in Kenya has been seriously impacted by anthropogenic disturbances through over utilization and infrastructure developments. The pattern of genetic diversity and structure was analyzed on a tree species, *A. senegal*, which plays a significant role as a source of fodder but has high potential for economic returns through gum arabic production. Leaves from 330 individuals belonging to 11 populations within L. Baringo ecosystem affected by different levels of human disturbances were genotyped using nine polymorphic nuclear microsatellite markers. High genetic diversity was reported for all the populations despite human disturbances. The average number of alleles observed per locus per population was 7.434 ranging from 6.667 (Kampi ya moto, Lake Bogoria and Tangelbei) to 8.333 (Kampi ya samaki). Among the 11 populations the highest gene diversity ( $H_E = 0.632$ ) was recorded in Kokwa population with the lowest value of 0.570 observed in Rimoi; however, the largest value of  $H_O$  (0.570) was observed in Kampi ya moto population with the lowest value of 0.558 recorded in Lake Bogoria. No significant difference was reported in gene diversity among the different levels of disturbance however significance was reported on mean number of alleles and allelic richness with least-disturbed populations recording higher values. No recent population bottleneck footprints were detected for the species within the ecosystem. Overall population differentiation was moderate and significant ( $P < 0.001$ ;  $F_{ST} = 0.067$ ;  $R_{ST} = 0.189$ ). Furthermore no clear relationship was observed between genetic and geographic distance ( $P > 0.05$ ) suggesting lack of pattern of isolation by distance. The differentiation therefore may be due to effects of human disturbances. Analysis of population structure using Bayesian model suggested the presence of three gene pools as most probable, although most individuals showed mixed ancestry. Residual diversity and genetic structure of populations of *A. senegal* within L. Baringo forest ecosystem suggest a limited impact of human disturbance on molecular genetic parameters, however conservation strategies are required to safeguard the population from further degradation

## Abstracts - Posters

### **Fecundity variation, genetic relatedness and gene diversity in a 1.5 generation seed orchard of *Pinus koraiensis***

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**Keywords:** coancestry, fecundity, sibling coefficient, effective number, gene diversity, relatedness, correlation

Reforestation can be achieved naturally or artificially. For artificial regeneration, seeds are collected from natural stands or from seed orchards. Seeds are important in both natural and managed populations as a resource of reproductive material and also for their commodity value in the production of forest products. To meet immediate seed needs, one can collect seeds from individually good phenotypes, good stands, seed production areas (seed stands) and proven seed sources. Long-term seed needs are often met by establishing seed orchards; greatly improved seeds are obtained from seed orchards. Seed orchards are the most common and cost-effective means of making available a stable supply of genetically improved seed.

A seed orchard is defined as an area where seeds are mass-produced to obtain the greatest genetic gain, as quickly and inexpensively as possible. Also, it is defined as a plantation of selected clones or progenies which is isolated or managed to avoid or reduce pollination from outside sources, and managed to produce frequent, abundant, and easily harvested crops of seed. In advanced-generation orchards, breeding values of orchard parents will be known from genetic testing. With knowledge of breeding values and other genetic information, it would be possible to establish new seed orchards (e.g., 1.5 generation seed orchard).

Gene diversity of seed crop was estimated based on genetic relatedness (coancestry) and fecundity variation among clones for five consecutive years in the 1.5 generation clonal seed orchard of Korean pine (*Pinus koraiensis* E. et Z.). The seed orchard was initiated from ramets of plus trees that were genetically tested and selected based on the volume growth. Fecundity variation among clones was estimated as the sibling coefficient based on the clonal production of conelet divided by the total of conelet production. Region, sub-region and location from where plus trees were selected were geographically identified and grouped. Relatedness among region, among locations within regions, and among clones within location was assumed to vary for estimation of the gene diversity.

Gene diversity was calculated as the concept of status number ( $N_s$ ), which was half the inverse of group coancestry. Four scenarios of genetic relatedness among groups were imposed: 1) no related and non-inbred clones, 2) all half-sib clones, 3) all full-sib clones and 4) coancestry varied among clones, locations, sub-regions and regions.

The average of conelet production was peak in 2010 and 2011, moderate in 2013 and 2014, and poor in 2012. When pooled, each clone produced an average of 13.7 conelets. Except the poor conelet year, the correlation coefficients of Pearson's and Spearman's correlation were all positive and statistically significant. The good conelet producer gave good conelet production consistently. Cumulative distribution of projected total conelet contributions of clones showed that the observation of conelet production was deviated from the expectation of equal production among

clones. The sibling coefficient was highest at poor conelet year (2012) and lower at poor years.

Under the scenario 1), the  $N_s$  was ranged from 11.1 to 38.2 for five successive years. The scenario 3) showed much lower  $N_s$  compared to the other scenarios. The relatedness among regions, sub-regions, locations and/or clones decreased the  $N_s$  and the fecundity variation boosted the decrease. On average (pooled),  $N_s$  was calculated to be 38.2% of census number ( $N$ ) with the range from 8.9 to 40.6 under the scenarios.



**Estimates of genetic parameters for height and stem forking of black spruce in  
New Brunswick**

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Genetic variation for growth and stem forking were evaluated in black spruce [*Picea mariana* (Mill.) B.S.P.] first-generation progeny tests. Data available from 2 test series (1979 and 1980, with 157 and 128 half-sib families respectively, and 6 field trials per series across New Brunswick, Canada) were analyzed. Individual tree height (HT) and stem forking (SF) were periodically measured between ages 5 to 35, and 5 to 20 years, respectively. Results showed that HT was highly genetically controlled, with individual narrow-sense heritability estimates of 0.64 (range: 0.47-0.91), and family mean heritability estimate of 0.80 (range: 0.73-0.86). Individual narrow-sense heritability for HT increased from trial ages of 5 to 15 years and then declined, whereas family mean heritability remained stable. Estimates of type-B genetic correlations for HT were high and stable across trial ages and test series with the mean of 0.88 (range: 0.77-0.98). Individual narrow-sense heritability for SF was low (mean  $h_i^2=0.08$ ) and remained constant over time for both series. However, family mean heritability estimates for SF were high (mean  $h_f^2=0.56$ ). This result suggested that stem forking is partially under genetic control at family level but not at the individual tree level. The type-B genetic correlation estimates for SF were high (mean=0.74). Family-by-site interactions were weak for HT and SF over all trial ages.

## Genetic resistance to western gall rust in jack pine in New Brunswick

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**Keywords:** jack pine, western gall rust, genetic parameters, tree improvement

Developing resistance to western gall rust (WGR) is important for maintaining healthy and productive jack pine plantations. In this study, we estimated genetic parameters of resistance to WGR, based on data collected from three 2<sup>nd</sup> generation full sib progeny testing series of jack pine, planted in northeastern NB. Results indicated that (1) resistance to WGR in jack pine was controlled by both additive and dominance gene effects, with the latter playing a greater role; (2) narrow-sense heritability estimates for resistance to WGR were low (mean=0.05; series range=0.00~0.09), suggesting a polygenic gene reaction mode. Broad-sense heritability estimates were moderate on individual tree basis (mean=0.17) and considerably higher on the full-sib family mean basis (mean=0.87); and (3) mid-parental additive genetic and dominance effects on WGR were empirically correlated ( $>0.65$ ), indicating that incorporating breeding for WGR resistance into the current jack pine tree improvement programs with seed orchard approach could partly capture the benefit from dominance effects. While genetic gains in WGR resistance could be realized through various breeding and deployment schemes, it appeared that rapid improvement could be achieved through backward selection on full-sib family means.

## **Adjusting to water stress: dehydrins genes in white spruce**

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### **Background**

Forest trees must be to adjust to temperature and water availability changes throughout their life cycle. Trees have developed mechanisms to regulate water relations and prevent the loss of intracellular water and dehydration and, reduce risks associated with the formation of ice particles. Dehydrins are the best known dehydration protective proteins in plants [1] and appear to be particularly important in conifer trees. They usually accumulate in maturing seeds or are induced in vegetative tissues in response to increased salinity, dehydration, cold stress and frost.

A total of 53 dehydrin genes have been identified in the white spruce transcriptome database [2], which is many more than in herbaceous angiosperms studied to date [3,4,5], but only a few of them have been investigated in conifers [6,7,8]. As a basis to understand the role and evolution of dehydrin genes in conifer adaptive responses, we aimed to: (1) identify full length gene sequences of dehydrins in white spruce; (2) classify these genes based on conserved amino acids motifs (segments A, E, S, K) and (3) trace the evolutionary origin of dehydrins in white spruce studying phylogenetic relationships (4) analyze the expression profile of dehydrins under water stress.

### **Methods**

Dehydrins were identified in the gene catalog available for white spruce [2,9]. We analyzed sequences with completed Open Reading Frames (ORF) and compared their DNA sequences to other conifers, poplar, arabidopsis and grapevine. The identification of amino acid motifs was used to classify the sequences among the three possible groups in gymnosperms (SKn, Kn ou KnS).

We conducted a greenhouse experiment with three year-old seedlings of three white spruce clones to study the expression profile of dehydrins. The seedlings were exposed to one of two watering regimes: normal watering versus no watering during 29 days. Needles and roots were sampled in 6 different dates. At each sampling day the water potential of sampled plants was measured using a Scholander chamber. We extracted total RNA and we are analyzing expression by using reverse-transcriptase quantitative PCR (RT-qPCR).

### **Results and Discussion**

#### *Sequence analysis*

The 53 cDNA sequences of dehydrins were recovered in the white spruce gene catalog [2, 9]. We identified 31 sequences containing a complete ORF in white spruce. Seven amino-acid motifs were identified in the white spruce ORFs including two K-segments, a S-segment, a A-segment and E-segment as described by Perdiguer (2012) [6]

and two novel conserved motifs located at the beginning of sequences (B1 and B2).

The amino-acid motifs were used to classify the 31 sequences into six groups: three Kn groups that differ at the beginning of their sequences (presence or/and absence of B1 and B2 motifs), a SKn group which contains an A and E-segment (exclusive of conifers), a KnS group with short S-segments and a group that contains K-segments and A-segments that are less similar to the previously described segments from angiosperm plants. This classification showed that all dehydrins contain one or multiple K-segments.

In conifers, some variations in the K-segment have been reported and were observed in white spruce sequences. To date, the A and E –segments were identified only in the Pinaceae and the N- terminal region has been described as highly conserved in Pinaceae. We identified two novel motifs in this region (B1 and B2 segments) but they are not highly conserved in white spruce dehydrins. The different sub-groups within the dehydrins family were found to vary in diversity. The B1-Kn containing sequences were well conserved, whereas the B1/B2 – AESKn group was variable suggesting it may be more dynamic.

#### *Drought stress and gene expression*

The midday water potential measured in steams showed that from the seventh day without watering, the plants began experiencing a slight stress which increased progressively showing that the treatment had an impact on the plants. Gene expression analyses by RT-qPCR have been initiated and results will be presented.

#### **Conclusions and next steps**

The dehydrin gene family has expanded in white spruce and other conifers and, their coding sequences have evolved into many different classes according to their amino acid motifs.

The next steps are the construction of a phylogenetic tree to study the dehydrins gene family evolution and the completion of gene expression analysis in response to drought stress.

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## Identification and characterization of polymorphic microsatellite loci using next generation sequencing in *Quercus variabilis*

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Microsatellite markers are highly polymorphic and widely used in population and conservation genetic studies in forest tree species. Microsatellite markers can be directly developed by using genome sequences of the target species, and can be transferred from closely related species by the cross-species amplification. The latter method may result in low amplification rate and low level of polymorphisms, and also cause problems related to homoplasy and null alleles. Thus, microsatellite markers derived directly from genome sequences of the target species could be considered to be more useful for correct analysis and interpretation in the genetic studies. So far the identification of microsatellites for non-model species lacking genome sequences has been difficult, time- and labor-consuming. Recent advances in the next generation sequencing techniques give rapid and cost-effective methods to produce large amounts of genome sequences for discovery and development of microsatellite loci from non-model species. This study was conducted to develop microsatellite markers in *Quercus variabilis* Blume using next generation sequencing. *Q. variabilis* is a deciduous broad-leaved tree species occurring widely in Northeast Asia, including Korea, China, and Japan. In Korea, *Q. variabilis* is an ecologically and economically important tree species. A total of 305,771 reads (384bp on average) were generated on a Roche/454 GS-FLX system, yielding 117Mbp of sequences. The de novo assembly resulted in 7,346 contigs (total length: 3.78Mbp, mean depth: <10X). A total of 606 contigs (20.75%) including 911 microsatellite loci were derived from the 2,921 contigs longer than 500bp. The 911 microsatellite loci consisted mostly of mono-, di-, and tri-nucleotides repeats (65%, 16%, and 14%, respectively). A total of 180 primer sets were designed from the 911 microsatellite loci and screened in 54 *Q. variabilis* individual trees sampled from a natural stand to obtain polymorphic loci. As a result, a total of thirteen polymorphic microsatellite loci were selected and used for estimating population genetic parameters in the 54 individual trees. The mean number of effective alleles was 4.996 ranging from 2.439 to 7.515. The observed heterozygosity ranged between 0.731 and 1.000 with an average of 0.873. The expected heterozygosity ranged from 0.590 to 0.867 with an average of 0.766. The mean polymorphism information content was 0.732 with a range of 0.583 and 0.856. Null alleles were not detected in all loci. No significant linkage disequilibrium was detected after Bonferroni correction in all loci. In the near future, these novel polymorphic microsatellite markers will be used to study population and conservation genetics of *Q. variabilis* of Korea in more detail.

## **Foliar secondary metabolite levels of plantation lodgepole pine are strongly influenced by snowfall at the provenance origin**

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Pines, and conifers in general, produce a diverse array of volatile secondary metabolites in their foliage, including a wide variety of terpenoids. While some of these compounds have been shown to play a role in defense, others have little known about their biological function. Any investigation of the functional roles of these compounds is complicated by both the great variety of chemicals produced, and the complex nature of plant-environment interactions. As a first step in identifying why specific compounds are produced, foliar levels of individual compounds from lodgepole pine provenance trial trees (*Pinus contorta* var. *latifolia* Engelmann) were compared with historic climate data from parent tree locations to identify any potential relationships. Volatile foliar metabolites from trees representing 13 provenances were quantified by GC-FID, and interpolated historic climate data were generated from the ClimateBC model. While inter- and intra-tree variation was high, statistically significant correlations were seen between levels of certain compounds and specific climate variables. Historic precipitation as snow at provenance origin locations was found to be highly positively correlated with levels of a broad range of compounds in plantation tree foliage, chiefly monoterpenes, sesquiterpenes, and terpene alcohols. While snowfall has been linked to tree growth in other British Columbia tree species, evidence for a further relationship between growth and secondary metabolite levels in literature has been tenuous. The high correlation of a number of foliar secondary metabolites with provenance origin snowfall over geographically diverse sites indicates the presence of some physiologically important relationship that has not yet been well established.

## **Industrial implementation of multi-varietal forestry using somatic embryogenesis and genomic selection**

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The use of a range of genetically tested tree varieties in commercial planted forestry is termed as Multi-Varietal Forestry (MVF). Since early 1990s, industrial implementation of MVF using somatic embryogenesis has been carried out at JD Irving limited. The main advantage of MVF is to obtain much greater genetic gain than is provided by traditional seed orchard-based tree breeding while providing the flexibility needed to adapt to changing breeding goals or changing environments and climate. While the use of seed orchards remains the main method of obtaining improved seed, new biotechnologies such as somatic embryogenesis (SE) and genomics technology could provide more effective and alternative delivery of genetic improvement. SE is the most important biotechnology in conifers for the development and production of tree varieties with desirable traits and is sufficiently refined for several spruce and pine species for use in industrial application. Furthermore, the recent advancement in genotyping technology based on single nucleotide polymorphism (SNP) has led to the development of genomic selection (GS) that predicts ‘Genomic Estimated Breeding Values’, a technique that has already been successfully used in animal breeding. The current data indicated that a tree breeding strategy incorporating GS and vegetative deployment by SE (i.e., MVF) will result in a large genetic gain without waiting the time required to produce seeds. Consequently, the genetic gain is realized 15 years sooner than seed orchard-based tree breeding. At JD Irving Limited, MVF using GS will be implemented in the near future incorporating growth, wood quality and pest resistance traits.



## **Valuation of tree improvement in Alberta – applying the BC model to Alberta**

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Tree improvement is currently in flux in Alberta and discussions are ongoing between industry and the Alberta Government to redesign how programs and responsibilities will be managed going forward. Although tree improvement is, in general, conducted similarly throughout the world, each jurisdiction has its own history, species, conditions, relationships and biases. Using the program developed in BC called ‘Tree Improvement Investment Priorities and Value in British Columbia’ (TIIP) as a starting point, a comprehensive analysis will be undertaken within an Alberta context. This BC model was first developed in 1997 to assist the Forest Genetics Council of British Columbia with business and strategic planning (Woods, 2002). The primary goals of TIIP are: 1) To objectively rank seed planning units (unique combinations of tree species, geographic areas and elevation bands – equivalent to seed zones in Alberta) for breeding and seed orchard investments; 2) To generate discounted program values; and 3) To estimate timber supply gains over time (requires genetic analysis). Over the last decade (Woods, 2002) a full integration from basic science to operational production has developed in all tree improvement programs to ensure secure and highly productive seed sources for forest management areas throughout the province. In this study, we attempt to apply the BC model to Alberta while at the same time considering unique challenges present in Alberta, such as accounting for permanent land deletions (energy sector), or more globally, the influence of climate change. Furthermore, we will also consider social values as model parameters, for instance the importance of sustaining adjacent communities by providing and maintaining jobs. This new tree improvement model would be a significant change for Alberta and requires a scientific re-evaluation of how gain in tree improvement programs is valued. The initial parameterization of the model will be based on genetic analyses and associated data from two Controlled Parentage Programs (CPPs). These case studies will provide critical information to the Province in deciding whether or not to adopt a ‘BC’ type model for valuing tree improvement in Alberta.

The outcomes of this project will establish the value of tree improvement in Alberta through a combined analyses including increased fibre production along with links to economic and social values and adaptive capacity for continued sustainable forest management. The model will allow for well-informed valuation of current programs and the potential for future programs. As the burden of risk associated with continuing these programs is likely to move from a lift effect in annual allowable cuts, to a more social value on sustaining communities, maintaining current cut levels and ensuring forest health, a comprehensive assessment of value is warranted and timely. This project will be conducted in collaboration with industrial partners and the provincial government of Alberta in order to ensure that both the values and the parameters are pertinent.

# Abstracts Tree Seed Working Group

## **Seed viability prediction options**

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The need to be able to predict seed viability is important to collection and processing decisions. For collection decisions it is also necessary to have an understanding of the reproductive biology and anatomical changes taking place within the seed during development. The question of “when” and characteristics of immature and mature seed will be reviewed. Emphasis will be on Gymnosperm species, but some Angiosperm examples will be presented. The differences between germinability and viability will be discussed.

The second major question is ‘how’ and a review of available technique including tetrazolium, hydrogen peroxide, incubation, x-rays and cutting tests will be presented. Emphasis will be placed on the use of cutting tests and x-rays and a variety of examples will be provided discussing the advantages and shortcomings of each. Examples of seed deterioration and possible mechanisms will be discussed.

## **The practical applications of water activity to improve the efficiency of seed management systems**

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Water activity has great potential as a way to effectively manage seed moisture when storing seeds both short term and long term. It is low cost, easy to use, universally applicable to orthodox seeds, and rapid. In the practical application of water activity to seed management there are several considerations that will make it more understandable and workable for seed managers and their clients. The first point is the name. While water activity has meaning from a more pure scientific viewpoint, in practical application it is a foreign term. Relative humidity on the other hand is used in the daily conversation (e.g., weather report) and is what is actually measured in a water activity test. Therefore, equilibrium relative humidity is proposed as the more practical term. Bringing the seed into equilibrium with the air in the test chamber is critical and is influenced by seed size, seed coat, and rate of drying. Therefore, the effect of these factors is discussed. Adoption of a universal equilibrium relative humidity (ERH) is important both because time and resources are not sufficient to study each species in detail to find its optimum ERH for storage and the limits of the test equipment to measure the true ERH make a highly refined ERH recommendation meaningless.

## **Using water activity in tree seed banking and its implementation in Alberta**

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The three important environmental factors affecting the lifespan of orthodox (desiccation tolerant) seeds in storage are temperature, oxygen and moisture. The linear negative relationship between decreasing seed moisture and increasing seed longevity forms part of the basis of the seed viability equations developed in 1960-80, and these underpin all seed conservation practices today. Gradually in the past 30 years, there has been a move away from using the moisture content method to measure water in seeds and towards using water activity as a measurement and standard for seed storage. Water activity measures the potential energy of water in seeds and is usually a non-destructive process that takes approximately 5 minutes as opposed to the destructive moisture content method, which typically needs 17 hours. How water activity and the equilibrium relative humidity of seeds are used for drying and storage will be discussed, as well as the advantages of using this method to set standards. Changes to Alberta's provincial seed storage moisture limits in the upcoming version of the Forest Genetic Resource Management & Conservation Standards will also be discussed.

## **The application of water activity to the management of pollen and seeds in Quebec**

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Until now, moisture content (MC) measurements were used to determine the water status of forest reproductive material. This measure is destructive, time consuming, and provides only quantitative information. It does not describe the chemical availability of the water present in a compound, nor the resulting potential negative effects of water on the conservation of stored pollen and seedlots.

Measuring water activity ( $A_w$ ) allows this distinction. This tool was primarily developed for the agri-food industry (Scott 1953). It describes a product's water status qualitatively. This, in turn, provides information about the product's degree of stability and predicts its conservation potential cited as "shelf life". Indeed,  $A_w$  and not MC governs the development of microorganisms and the production of toxins, and thus, the reactions associated with food degradation (Scott 1953).

In a given system,  $A_w$  is the ratio between observed water vapour pressure and the one of pure water at the same temperature. In other words, it is the equilibrium relative humidity (eRH) of the air surrounding a system at the same temperature (Rahman and Labuza 2007).

$A_w$  describes the state of water in matrices, whereas eRH qualifies the surrounding environment. Measuring  $A_w$  also has the advantage of being rapid, easy, and most importantly, non-destructive. This allows a sample to be measured repeatedly during its conservation.

### **A Fruitful Collaboration**

Thanks to technology transfer and collaboration between France's Irstea (formerly called Cemagref) and Quebec's Direction de la recherche forestière (DRF) of the ministère des Forêts, de la Faune et des Parcs,  $A_w$  measurements have been used in Quebec since 2007 for the treatment of forest tree pollen and seeds.

The first step of this work involved the production of sorption isotherms (Brooker et al. 1992) to characterize water relations in seeds of the various tree species used in Quebec's provincial planting program. We characterized a total of 10 conifer and 5 hardwood species, with results similar to those obtained for French forest species. By analysing the isotherms, we were able to determine the optimal  $A_w$  for the conservation of each species,

and, for each, the resulting MC (Colas et al. 2010). We also determined the mean minimum MC which corresponds to combined water associated with cell membranes (monolayer water). Drying seeds below this minimum MC could impair their long-term conservation (Wang 1974, Sun 2002). The current target  $A_w$  for seed conservation in Quebec is 0.35 (Colas et al. 2010).

A water activity-regulated dryer was built from blueprints provided by Irstea (Baldet and Colas 2013). It enables the stabilization of seedlots at the targeted  $A_w$ . Its main feature is that it works without heat, on the basis of the 2-pressure principle developed by the National Institute of Standards and Technology (Hasegawa and Little 1977). It is simple and easy to set up and use, and allows the stabilisation of seed and pollen lots to a safe  $A_w$  level for their conservation in the best possible conditions.

### **Water Status During Conservation**

The water status of seeds or any other product is in balance with that of the environment in which it is conserved (Freire and Mumford 1986; McDonald 2007). For optimal conservation, each seed or pollen grain should contain only the water necessary for its survival, and no more.  $A_w$  is a reliable indicator of changes in the water balance between seeds and their conservation environment (Baldet et al. 2008). Yet, humidity is a risk factor which is incompatible with good-quality conservation. Seeds must be protected from ambient eRH using airtight containers (Wang 1974), or kept under controlled eRH conditions (Freire and Mumford 1986). Unfortunately, no container is completely impervious. Water vapour will enter or exit as long as equilibrium is not reached (Walters 2007).

A trial aimed at developing a frequency table for germination tests at the Berthier Tree Seed Center (BTSC) revealed that  $A_w$  increased significantly in preserved seedlots (Colas et al. 2012), thanks to permeability of the polymer and repetitive opening of the containers. The non-destructive nature of  $A_w$  measurements was a real advantage in this study. After measurement seeds were stabilized to the optimal  $A_w$  in the dryer controlled by water activity and returned to conservation. The same lots can be measured repeatedly.

We began work to develop an optimal storage container that would improve the conditions for long-term conservation of forest tree seed in Quebec. This involved developing a new polymer which was less permeable to water vapour. Results are expected by the end of 2015.

### **Conclusion**

$A_w$  measurements are now used operationally in Quebec for seed- and pollen lot treatment. This qualitative tool for monitoring the water status of stored lots was adopted because of its reliability and ease of operation. At the BTSC,  $A_w$  is now used to govern the final drying of seedlots during extraction and has been added to the list of quality control tests. Because of its non-destructive nature,  $A_w$  measurement will be an essential tool to verify the reliability of seedlot conservation in a conservation bank for forest genetic diversity. It is noteworthy that the

International Seed Testing Association has scheduled the implementation of a standard for water activity in its 2015 timetable.

### **Acknowledgement**

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## Abstracts – CONFORGEN FORUM

### **Forest genetic resources on the global stage – opportunities and challenges.**

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Tree genetic resources typically receive little attention on the global stage in spite of their value for improvement of tree-based products and for adaptation to change, most notably related to climate. The development and release of the first ever State of the World's Forest Genetic Resources report and associated Global Plan of Action by the FAO (<http://www.fao.org/forestry/fgr/64582/en/>) provides an opportunity to bring attention to the need for focused conservation efforts. Bioversity International (<http://www.bioversityinternational.org/>), a member of the CGIAR Consortium, is a research for development organisation which carries out research on agricultural and tree biodiversity, working closely with FAO and other international organisations. Bioversity's forest-related work aims to facilitate implementation of the Global Plan of Action by developing methods and tools for diversity characterisation and threat analysis, working with partners to prepare strategies and guidelines for conservation, supporting regional genetic resource conservation research networks and producing training materials. A project focused on a valuable but threatened African species, *Prunus africana*, exemplifies.

Bioversity's approach to prioritising populations through diversity and threat analyses. Recommendations to local managers include putting greater emphasis on planting the species in identified areas that are likely to continue to be suitable habitat as the climate changes. The species is one of thousands that are both useful to local people and threatened to some degree and while specific patterns of diversity and threats may be species-specific, methods to understand them can be more broadly applied. Approaches developed in Canada may be highly applicable to other regions. In fact a challenge for forest geneticists everywhere is how to monitor



changes in genetic diversity – identifying indicators that can be readily applied, easily understood and affordable.

## **The development of an access and benefit-sharing (ABS) regime for Canada**

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The presentation will provide an overview of the further development of domestic ABS policy in Canada, and the potential impact on the forest sector and forest-related activities

The domestic policy development process will help Canada to better understand the implications of the Nagoya Protocol for Canada, including helping to inform a decision on whether Canada should accede to (become legally-bound by) the Protocol.

### **International Protocol**

The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity was adopted in 2010 with the objective of achieving the fair and equitable sharing of the benefits arising out of the utilization of genetic resources. The Protocol came into effect in October 2014, and 62 Parties have now ratified. Canada has indicated that it wants to undertake additional analysis before determining its response.

Under the Protocol, genetic resources are defined “*any material of plant, animal, microbial or other origin of actual or potential value containing functional units of heredity*”. “Utilization of genetic resources” means to “*conduct research and development on the genetic and/or biochemical composition of genetic resources, including through the application of biotechnology*”<sup>4</sup>.

The utilization of genetic resources (e.g., researching plant extracts for active components that could be used as the basis for developing cancer drugs) may result in benefits that could be monetary, such as royalties or up-front payment, and/or non-monetary, such as collaboration in scientific research and institutional capacity-building. The

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*“Biotechnology”...means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.*

*“Derivative” means a naturally occurring biochemical compound resulting from the genetic expression or metabolism of biological or genetic resources, even if it does not contain functional units of heredity.*

Protocol also covers traditional knowledge (TK) associated with genetic resources from indigenous and local communities (e.g., knowledge about the healing properties of certain plants).

Under the Protocol:

- Access to genetic resources will be subject to the **prior informed consent (PIC)** of the Party providing the resource (unless otherwise determined by that Party); and
  - Genetic resources will be accessed and used according to **mutually agreed terms (MAT) negotiated between users and providers**. The terms may serve as a contract that can spell out how the resources are to be used, by whom, for what purpose, and how monetary and non-monetary benefits will be shared.

Parties are also required to have compliance measures in place to implement the Protocol, as well as monitoring. For further information: <http://www.cbd.int/abs/>

#### **The further development of ABS policy in Canada.**

Federal, provincial and territorial governments have discussed the further development of ABS policy in Canada. The federal government has also engaged extensively with stakeholders and Aboriginal communities on these issues. Environment Canada has produced a Discussion Document outlining a potential approach to ABS policy in Canada. The Discussion Document proposes a policy that would initially only apply to some genetic resources on federal lands or waters. Genetic resources which are currently in seed banks and other ex-situ collections would be excluded. Genetic resources entering collections accompanied by PIC and MAT agreements in future would need to track and honour the terms of those agreements, including with regard to third party transfer.

If Canada decides to implement a federal policy, it would likely be some years before it is in place. In the meantime, Canadians accessing genetic resources from countries which have ABS systems will need to abide by the requirements of those systems. The U.S. is not a Party to the Convention on Biological Diversity (CBD) under which the Protocol was negotiated and thus cannot become a Party to the Protocol, although it does have some domestic ABS rules, for example in national parks.

#### **Engagement of, and impacts on, the forest sector**

Forest sector stakeholders, especially the forest genetics community, have been engaged in this process to some extent, although more widespread engagement would be welcome as the policy development process continues. The general feeling from stakeholders is that a domestic policy will need to be as simple and efficient as possible to avoid slowing the transfer of genetic resources unnecessarily.

Effects in the forest sector could be felt in the academic, biotech, and non-forest products communities where there could be additional administrative requirements when exchanging forest genetic resources for research and development (e.g., having an agreement covering conditions for use and the sharing of benefits). It should be noted that forest genetic resources are found in trees, non-timber forest species (e.g., shrubs, fungi, forest herbs) and forest soil (e.g., fungi, bacteria).

**An overview of forest-tree genetic conservation work in British Columbia**

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British Columbia integrates genetic conservation efforts for forest-tree species with other forest genetics activities. Forty-two indigenous species are recognized. The core of this provincial program is a catalogue outlining the *ex-situ*, *in-situ*, and *inter-situ* genetic conservation status of each species. Some 9,000 *ex situ* conservation seedlots are maintained at the Provincial Tree Seed Center. New collections are added for priority needs when seed is available in wild stands. Projects to better understand genetic diversity and to meet specific species needs are supported.

## **Multi-species approach to recovery planning: potential application in New Brunswick forests**

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There is a growing emphasis on applying a multi-species approach to recovery planning in Canada, as the list of species at risk continues to lengthen. The forested landscape of New Brunswick is an appropriate candidate for this approach, as it is home to a number of species that are at risk across their Canadian range (e.g. Eastern Wood Pewee, Chimney Swift). The challenges, requirements and limits of multi-species planning will be explored through an overview of our forests at a broad scale (ecoregions, ownership) followed by consideration of habitat requirements at a finer scale (e.g. stand structure and composition) using forest birds as examples. This scenario will be presented within the broader context of recovery planning and the regulatory framework for species at risk.

## **Prioritizing forest tree species requiring ex situ conservation in Canada**

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Thirty five species were identified as requiring ex situ conservation measures based on a pan-Canadian CONFORGEN survey conducted in 2009 to identify native tree species which may be of concern and requiring conservation measures. This ex situ prioritization scheme was developed to assist in determining which of these species identified as requiring ex situ conservation by the survey may be in more urgent need of conservation. This scheme considers 3 indicators: 1: conservation ranking criteria, 2: eco-geographical ranking criteria and 3: feasibility of ex situ conservation. For each indicator a number of parameters are considered. The results show in assessing the 35 species identified as requiring ex situ conservation by the survey that there are three general groupings, those most in need, moderate need and low relative to the other species considered.



