PROCEEDINGS OF THE THIRTY-SECOND MEETING
OF THE
CANADIAN FOREST GENETICS ASSOCIATION

PART 1 Minutes and Members' Reports
PART 2 Symposium

Forest Genetics & Tree Improvement: New Knowledge, Challenges & Strategies

Génétique forestière & amélioration des arbres : nouvelles connaissances, défis & stratégies

COMPTES RENDUS DU TRENTE-DEUXIÈME CONGRÈS
DE
L'ASSOCIATION CANADIENNE DE GÉNÉTIQUE FORESTIÈRE

1ere PARTIE Procès-verbaux et rapports des membres
2e PARTIE Colloque

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Includes preliminary text and articles in French.
Contents : Part 1. Minutes and Member's Reports. Part 2. Symposium.

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PROCEEDINGS

OF THE

THIRTY-SECOND MEETING

OF THE

CANADIAN FOREST GENETICS ASSOCIATION

PART 1

Minutes and members' reports

Thunder Bay, Ontario
August 15–18, 2011

Editor
J.D. Simpson

Enquiries may be addressed to the authors or to Mr. Dale Simpson, Editor CFGA, Natural Resources Canada, Canadian Forest Service - Atlantic, P.O. Box 4000, Fredericton, New Brunswick, Canada, E3B 5P7. E-mail: Dale.Simpson@nrcan.gc.ca

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2012

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Forest Genetics Ontario
Lakehead University – Faculty of Natural Resource Management
SeedTek
AbitibiBowater

COMPTES RENDUS

DU

TRENTE-DEUXIÈME CONGRÈS

DE

L' ASSOCIATION CANADIENNE GÉNÉTIQUE FORESTIÈRE

1re PARTIE

Procès-verbaux et rapports des membres

Thunder Bay, Ontario
15–18 août 2011

Rédacteur
J.D. Simpson

Les demandes de renseignements peuvent être adressées aux auteurs ou à Dale Simpson, Rédacteur, ACGF, Ressources naturelles Canada, Service canadien des forêts - Atlantique, C.P. 4000, Fredericton, Nouveau-Brunswick, Canada, E3B 5P7. Courriel : Dale.Simpson@ rncan.gc.ca

Ces comptes rendus sont disponibles à : www.cfga-acgf.com

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pour
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2011

Sous le patronage de

Forest Genetics Ontario
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Ontario – Northeast Seed Management Association

Ontario – Superior-Woods Tree Improvement Association

Ontario – Ministry of Natural Resources

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BUSINESS MEETING MINUTES

CFGA/ACGF 32nd Business Meeting Minutes

Paul Charrette chaired the 32nd Business Meeting of the CFGA/ACGF held at Lakehead University, Thunder Bay, Ontario on Thursday August 18, 2011. Fourteen Active Members and 1 Honorary Member were present.

371 Minutes of the 31st Meeting

(as printed in the proceedings from the 31st meeting, Part I)

Motion: That the minutes of the 31st Business Meeting be approved as published.
Moved by: Dave Kolotelo
Seconded by: Michele Fullarton
Carried.

372 Membership

372.1 Honorary Membership

The names of three nominees were presented as follows:

Gaëtan Daoust
Narinder Dhir
John King

Motion: That the nominated Honorary Members be duly elected.
Moved by: Sally Aitken
Seconded by: Michael Stoehr
Carried

372.2 Active Membership

The names of six nominees were presented as follows:

Barb Boysen	Ontario Forest Gene Conservation Association Peterborough, ON
Lyn Dunford	Manitoba Forestry Branch Winnipeg, MB
Jodi Krakowski	Ministry of Forests, Lands and NR Operations Squamish, BC
Nicholas Ukrainetz	Ministry of Forests, Lands and NR Operations Vernon, BC
Tongli Wang	University of British Columbia Vancouver, BC
Chang-yi Xie	Ministry of Forests, Lands and NR Operations Victoria, BC

Motion: That the nominated Active Members be duly elected.
Moved by: Pengxin Lu
Seconded by: Leonard Barnhardt
Carried.

373 Chair's Report

The 32nd meeting of the Association was held for the first time in Thunder Bay, Ontario at Lakehead University. The meeting was hosted by Forest Genetics Ontario with support from the Faculty of Natural Resources Management at Lakehead University.

I would like to thank the members of the Organising Committee: Bill Parker, Bill Cole, and Pengxin Lu. I would also like to thank Dale Simpson for his overall guidance and support as well as for organising the Forum on the Conservation of Forest Genetic Resources and Dave Kolotelo for organising the Tree Seed Working Group meeting.

On behalf of the Organising Committee I would like to thank all those who contributed to making the meeting a success. I would especially like to thank the sponsors, AbitibiBowater and Seedtek, for their support.

The sponsored student program was a success as students from UBC, UNBC, U of A, U. Laval, and UNB attended the meeting. The winner of the Carl Heimberger award for the best oral presentation was Ashley Thomson and the winner of the Gene Namkoong award for the best poster presentation was Patrick Lenz. I would like to thank Michael Stoehr, Pengxin Lu, and Bill Parker for serving as judges for the awards.

Thanks also to everyone who participated in the meeting including the invited speakers, students, diehard CFGA goers, and new comers. I wish the organisers for the next meeting the best of luck and I look forward to seeing you in beautiful British Columbia in 2013.

Paul Charrette
Chair

374 Treasurer's Report

Tannis Beardmore, who had been Treasurer for 10 years, resigned in 2009 and Michele Fullarton agreed to assume the position. The financial statement for the period of August 2008 to June 2011 was presented by Michele and tabled for information and acceptance by the membership (see Attachment #1). The statement shows a balance of $22,374.87 in the Association's account.

Motion:	That the financial statement be accepted as presented.
Moved by:	John Mackay
Seconded by:	Bill Parker

Carried.

375 Financial Contributions

Forest Genetics Ontario
Lakehead University – Faculty of Natural Resource Management
SeedTek
AbitibiBowater

Motion:	That the CFGA/ACGF executive of the 32nd meeting express our sincere appreciation to these contributors.
Moved by:	Bill Parker
Seconded by:	Dave Kolotelo

Carried.

376 Editor's Report

The proceedings of the 31st meeting were uploaded to the web site in August 2009. An announcement was sent to all Active members, Honorary members, registrants, and International addresses that the proceedings were available on the web site. A copy of the proceedings was sent to Canadian universities and other libraries.

Membership consists of: 17 Honorary, 85 Active, and 60 Canadian libraries.

Dale Simpson

377 Education Committee

The Organizing Committee of the CFGA 2011 meeting sponsored students from five Canadian universities with an accredited undergraduate forestry program. This sponsorship is encouraged by the CFGA members and financially supported by conference registration fees and contributions provided by sponsors. Sponsorship covered costs of attendance including transportation, accommodation, and conference registration.

The five universities were asked to nominate a student to attend the meeting in Thunder Bay. They nominated the following students:

Danielle Bourassa	University of Northern British Columbia
Jin-hong Kim	University of New Brunswick
Blaise Ratcliffe	University of British Columbia
David Roberts	University of Alberta
Guillaume Tessier	Université Laval

The Gene Namkoong Award is presented to a student for the best poster. The winner was Patrick Lenz from Université Laval. The title of his poster was “Radial variation of wood traits – pathways for their genetic interpretation” and was co-authored with Claude Bomal, Sébastien Caron, Jean Beaulieu, and John Mackay.

The Carl Heimburger Award is presented to a student for the best oral presentation. Ashley Thomson from Concordia University received the award for a paper entitled “Comparative phylogeography of *Betula alleghaniensis*, *B. Lenta*, and *B. papyrifera* in eastern North America: influence of introgression, niche breadth, and glacial history”, co-authored with Selvaduri Dayanandan and Christopher W. Dick.

Pengxin Lu

378 Working Group Reports

378.1 Tree Seed Working Group

The Tree Seed Working Group (TSWG) continues to hold workshops at our National meetings and produce the TSWG Newsbulletin. Since our last meeting the following Newsbulletins have been produced:

# 48	December 2008	
# 49	July 2009	
# 50	December 2009	Seed Testing
# 51	July 2010	
# 52	December 2010	Genetic Conservation
# 53	June 2011	

All past Newsbulletins can be found at the following link http://www.for.gov.bc.ca/hti/treeseedcentre/tsc/tswg.htm. Anyone wishing to be on the electronic distribution list, please contact either Dale Simpson (dsimpson@nrcan.gc.ca) or myself (Dave.Kolotelo@gov.bc.ca).

The TSWG portion of the meeting (August 14, 2010) featured both a morning workshop and an afternoon tour of SeedTek (http://www.seedtek.ca/), their seed upgrading/ testing facility and the

AbiBow Kakabeka Tree Improvement property. The speakers for the morning session are indicated below:

Michele Fullarton	Tree Improvement and Seed Production by NB Department of Natural Resources
David Kolotelo	Challenges Processing Cones and Seed From Mountain Pine Beetle Killed Stands
Michael Stoehr	Inter *situ* Gene Conservation
Dale Simpson	*Ex situ* Conservation of Ash

In terms of the afternoon field trip I'd like to thank Paul Charrette and the organizing committee for incorporating this into the meeting. We had a very interesting visit to SeedTek to review their seed upgrading and testing facility. Thank you to Dave Haveman (owner) for hosting us and Daren Fellner, seed technician, who took us through various seed upgrading operations being used at the facility. At the AbiBow site, Megan Thompson guided us through the site to view jack pine clonal archives, progeny tests, and a seedling seed orchard. Of specific interest was the solar powered electrical fence used to protect the seed orchard from deer.

It was a good meeting in Thunder Bay and we look forward to hosting CFGA in BC in 2013. We are planning the TSWG workshop for July 22nd with a tour of our BC provincial Tree Seed Centre. Look forward to seeing you here.

Dave Kolotelo
Chair

378.2 Wood Quality Working Group

No activities to report since the last CFGA/ACGF conference in 2008.

379 Old Business

379.1 Printing of Proceedings (former business meeting item 367.1)

At the last Business Meeting it was moved that the Proceedings be provided by electronic means thus eliminating the cost of publishing them in paper format. The Proceedings of the 31st meeting were uploaded to the CFGA/ACGF web site in August 2009. All future Proceedings will be uploaded to the web site.

379.2 New Logo (former business meeting item 367.2)

Paul Charrette presented two versions for consideration. One consisted of the the Association's name in bilingual format inserted within the maple leaf of the current logo. The second version contained the Association's name in bilingual format in a circular format around the outside of the maple leaf. It was agreed that the Active Members be polled as to which version is preferred.

379.3 CFGA/ACGF Web Site (former business meeting item 367.3)

A web site was created by Gary Carleton, Carleton Communications, Duncan, BC with financial support provided by the BC Ministry of Forests, Lands and Natural Resource Operations. The site was up and running by the end of October 2008 (2 months following the CFGA meeting). The CFGA pays the annual fees associated with the domain names and web site hosting.

Several documents including history of the organization, a list of conference themes, student award program, and constitution were included. In addition, all past proceedings and documents back to 1937 were scanned under contract with the National Research Council Canada and placed on the web site. It was a stack of documents almost 1 m high.

Thanks to the BC Ministry of Forests, Lands and Natural Resource Operations for covering the costs of web site design and start-up. As well, thanks to Denise Tousignant, Quebec Ministry of Natural Resources and Wildlife for translating the documents.

380 New Business

none

381 Future Meetings

381.1 Location of the 2013 Meeting

Sally Aitken confirmed that the meeting will be in the Vancouver area. UBC and the BC Ministry of Forests, Lands and Natural Resource Operations will cooperate to organize it jointly with the Western Forest Genetics Association.

381.2 Location of the 2015 Meeting

Fredericton, NB.

381.3 Location of the 2017 Meeting

Alberta, possibly.

382 Election of New Executive

The following slate of officers will serve as the executive for the next CFGA/ACGF meeting:

Chairpersons:	Sally Aitken University of British Columbia Lee Charleson Ministry of Forests, Lands and Natural Resource Operations
Vice-Chairperson: Symposium	Sally Aitken University of British Columbia
Vice-Chairperson: Local arrangements	Diane Douglas Ministry of Forests, Lands and Natural Resource Operations
Treasurer:	Michele Fullarton NB Department of Natural Resources
Editor:	Dale Simpson Canadian Forest Service
Executive Secretary:	Dale Simpson Canadian Forest Service

383 Adjournment

Motion: That the 32nd business meeting of the CFGA/ACGF be adjourned.
Moved by: John Mackay

Attachment # 1

CFGA/ACGF
Financial Statement
August 2008 – June 2011

Holdings – August 18, 2008

Cash balance	$14,448.00
Total GICs	$16,000.00
Total Holdings	**$30,448.00**

Credits

Interest (on chequing account balance)	$ 7.15
Interest (from GICs)	$ 1,268.14
GIC (matured June 29/09 and rolled into chequing account)	$ 8,000.00
GIC (matured March 5/10 and rolled into chequing account)	$ 8,000.00
Total credits	**$17,275.29**

Debits

Student awards (2008 CFGA)	$ 800.00
Student Expenses (2008 CFGA)	$ 6,139.97
Proceedings (digitization and binding)	$ 1,582.00
Mailing (Proceedings and CFGA documents)	$ 589.26
Website (hosting and annual fees for domain)	$ 237.19
Total debits	**$ 9,348.42**

Guaranteed Investment Certificates

Total GIC holdings	**$ 0.00**

Holdings – June 17, 2011

Cash balance	$22,374.87
Total Holdings	**$22,374.87**

ACTIVITY REPORTS
FROM ACTIVE MEMBERS

TREE BREEDING AND CONSERVATION IN NOVA SCOTIA

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The Nova Scotia Forest Genetics Working Group (NSFGWG) is the coordinating body for tree breeding in Nova Scotia. The current objectives of the NSFGWG are to increase wood volume and quality and maintain broad genetic diversity in forest plantations through the production and utilization of locally adapted, genetically improved tree seed. Active members include the Nova Scotia Department of Natural Resources (NSDNR) which acts as coordinator, Abitibi-Bowater, Northern Pulp, New Page Corp, and J.D. Irving, Limited. The Canadian Forest Service has provided support services as well as technical and scientific expertise from the program's inception. Additionally, the NSDNR is responsible for developing and conducting research to improve forest management practices, developing strategies for meeting sustainable development objectives, maintaining the provincial forest inventory, and compiling and reporting on data relative to forest resources. These responsibilities have prompted the department to actively increase activities regarding the conservation of provincial forest genetic resources.

TREE BREEDING

Since the year 2000, industrial cooperative members have assumed the lead role in the day-to-day tree breeding activities formerly carried out by NSDNR. Indigenous species of interest include black spruce (*Picea mariana*), white spruce (*P. glauca*), and red spruce (*P. rubens*).

The first phase of the black spruce program involved collecting open-pollinated seed from plus trees and establishing seedling seed orchards and associated family tests of the half-sibs. For red and white spruce, a clonal approach was taken whereby cuttings from the plus trees were grafted into clone banks and seed orchards and clonal breeding values were predicted from polycross progeny tests. After the second round of selection in black spruce (i.e., second-generation), the clonal approach was applied. First-generation open-pollinated seed orchards supply the current seed requirements for the above mentioned reforestation species. The industrial cooperators have proceeded with second-generation black spruce, white spruce, and red spruce selection, breeding, and orchard establishment as information and support was provided by the NSDNR. General combining ability testing for juvenile height of the first-generation black spruce, white spruce, and red spruce populations is now complete.

Second-generation selections for black spruce were made in the first-generation family tests. By the year 2000, one tree from each of 363 of over 1050 families had been selected, on the basis of size and form characters, as ortets to form the second generation clonal breeding population. Eighty-eight of these clones form the seed production population in three clonal seed orchards. The remaining clones are represented in clone banks. Second-generation selection for white spruce has been completed. Based on parental breeding values (mid-parent values), derived from half-sib progeny tests, 103 of 459 white spruce specific crosses from a random pair-mate design were selected from which 410 trees (approximately four trees/cross), growing in selection plantations, were chosen on the basis of size and form characters to constitute the second-generation breeding population. Of these selections, 60 unrelated selections where chosen for deployment into second-generation clonal seed orchards. Second-generation selection in red spruce has been completed using a similar approach. As per white spruce, 122 of 167 red spruce specific crosses were selected from which 322 trees were chosen to constitute the second-generation breeding population. A total of 60 unrelated selections where chosen for deployment into second-generation clonal seed orchards. Data analyses of second-generation black spruce, white spruce, and red spruce progeny tests are ongoing.

Open-pollinated clonal seed orchard expansion into the second-generation continues with Northern Pulp establishing both a black spruce and a white spruce orchard at their East Mines facility. Abitibi-Bowater and NewPage Corp. have cooperated in establishing a white spruce orchard and a red spruce orchard at Bowater's Melvern Square property. J.D. Irving has also commenced the establishment of a second-generation red spruce orchard.

IN SITU CONSERVATION

Forest genetic resources in Nova Scotia are safeguarded through a network of *in situ* reserves composed of parks, wilderness areas, and other protected landscapes. However, the province has yet to evaluate how well existing protected areas meet the goal of safeguarding the genetic diversity of all indigenous tree species. The goal of such an assessment is to highlight the conservation gaps that are present within the *in situ* land network and address these gaps through conservation measures in order to mitigate genetic diversity loss due to climate change, other forest threats, and their interactions.

Preliminary results obtained from this gap analysis show that eastern white cedar (*Thuja occidentalis*) populations are not adequately represented in existing reserves. Based on this assessment, combined with the fact that eastern white cedar was listed as a vulnerable species in 2006, a management plan for native occurrences of eastern white cedar in Nova Scotia was prepared in July 2010 (http://www.gov.ns.ca/natr/wildlife/biodiversity/species-recovery.asp#cedar). The objective was to prioritize documented native cedar populations for *in situ* conservation to effectively address this species vulnerability through stewardship.

EX SITU CONSERVATION

The National Tree Seed Centre, Canadian Forest Service conserves seed from Nova Scotia tree provenances for *ex situ* gene conservation objectives. Increased collection efforts are expected for indigenous tree species lacking adequate *in situ* conservation coverage. Currently, white pine (*Pinus strobus*) is the only forest tree species conserved through additional *ex situ* activities. More specifically, a total of 55 clones were selected throughout the province and were established as a clonal seed orchard. In 2010, these clones were re-grafted and the orchard is in the process of re-establishment.

NEW BRUNSWICK TREE IMPROVEMENT UPDATE

Michele Fullarton and Yuhui Weng

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Keywords: Breeding, second-generation, $GA_{4/7}$, third-generation, seed orchard, black spruce, jack pine, white spruce, red spruce, Norway spruce, tamarack, NBTIC

The New Brunswick Tree Improvement Council (NBTIC), formed in 1976, is comprised of industrial, scientific, and government agencies. The Department of Natural Resources (DNR) coordinates the activities among members and is responsible for all breeding and growing of tests. NBTIC industrial members plant, maintain, and measure genetic tests. The tree improvement program in NB continues to focus on the main reforestation species: white spruce (*Picea glauca*) and black spruce (*Picea mariana*). Interest in jack pine (*Pinus banksiana*) has declined, but interest in planting red spruce (*Picea rubens*) continues to increase. Secondary species for tree improvement include balsam fir (*Abies balsamea*) for Christmas trees, Norway spruce (*Picea abies*), white pine (*Pinus strobus*), and tamarack (*Larix laricina*).

SEED ORCHARDS AND SEED PRODUCTION

Second-generation orchard establishment of black spruce and jack pine began in 1989. Orchard establishment is now complete for these species with a total of 34.3 ha. These orchards are meeting the reforestation demands for Crown land. The first planting of second-generation white spruce orchards was in 2000 and a total of 11.9 ha has been established. Some of these orchards produced their first crop in 2009. NBTIC members have also established 10 ha of red spruce and 12.4 ha of white pine clonal seed orchards in the past 3 years. Red spruce demand has increased to two million seedlings in 2011. White pine is deployed by mixing it in trays with white spruce and Norway spruce. A small third-generation jack pine orchard (1 ha) was established in 2011 by DNR.

Seed production in 2008 was modest, almost 24 million seeds, with the majority of that collected from second-generation black spruce orchards. In 2009, however, there was a bumper crop, with over 1 billion seeds collected. The majority of the seed collected was from white spruce first-generation orchards (~81%). In 2010, only a very small amount of black spruce was collected as cone production was virtually non-existent in agency orchards. In 2010, DNR injected over 6 000 first- and second-generation white spruce orchard trees with gibberelic acid ($GA_{4/7}$) to induce flower production. These cones will be harvested in 2011.

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. Planting has gone from a high of over 3 million to 325 000 in 2011. DNR did make 40 third-generation jack pine selections in order to establish a small orchard in 2011. 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 black spruce and 21 for jack pine. The full-sib testing is 83% complete for black spruce and 81% complete for jack pine. The black spruce breeding strategy is under review and will be modified for third-generation selection, breeding, and testing. For the past three years, second-generation white spruce breeding has focused on supplemental mass pollination of orchard clones. Pair-mating has not started but a preliminary, updated breeding strategy has been outlined and it will be used for future breeding and testing. In 2011, white spruce pollen was collected for use in pair-mates in 2012.

Testing continues to be an important component of the NBTIC program. Since 1976, 349 tests have been planted on over 390 ha. Since 2008, the ninth series of black spruce full-sib progeny tests and sixth series of jack pine full-sib series were planted. The material for these tests came from full-sib crossing done within sub-lines established from nursery progeny test information. These tests will be used for making third-generation selections. A white spruce polycross test of second-generation seed orchard clones was planted in 2011 for orchard roguing purposes.

Second-generation selections in white spruce began in the fall of 1998. The white spruce strategy is slightly different in that the second-generation selections originate from selection plantations laid out in 48-tree plots. These selections are based on results from 5- and 10-year-old polycross progeny test measurements. A total of 467 selections have been made for this population. Also, another 30 red spruce selections were made in 2008 and 2009, totaling 129 plus trees in the first-generation population. These have all been used for orchard deployment.

DATA ANALYSIS

The data analyst position has continued to be funded by the NBTIC. A total of 8 NBTIC test series were analyzed in 2009 and 2010 which included 2 progeny tests, 4 full-sib progeny tests, and 2 gain tests. In addition to that, a revised white spruce breeding strategy has been outlined, as well as a number of papers written and reviewed.

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NATIONAL TREE SEED CENTRE UPDATE

Dale Simpson and Bernard Daigle

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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 the period from 2008 to 2010.

Seed collection is a necessary activity to expand the geographic sampling for species and to add new species to the inventory. 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. Seed is stored at the Seed Centre in two collections: Seed Bank and Genetic Conservation. The Seed Bank collection is used to provide seed for research while the Genetic Conservation collection conserves natural genetic variation through collections from species or populations that are unique or threatened as well as samples from seedlots that are collected for the Seed Bank. During the three-year period over 330 collections were made from 27 species. An additional 110 seedlots from eight species were obtained via donation or purchase. The Seed Bank collection consists of over 6600 seedlots. The Genetic Conservation collection continued to grow. The quantities that are stored are variable depending on species and seed size. Over 4370 seedlots from 48 species are stored.

In response to the threat imposed by emerald ash borer (*Agrilus planipennis*) that is expanding its range in Canada a program for the conservation of ash seed has been led by the Seed Centre since 2004. 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. Seed collections have been hampered by irregular crops and the lack of resources to make collections. Nonetheless, over 530 seedlots from five species are in storage. Samples from these collections were sent to the National Centre for Genetic Resources Preservation in Fort Collins, Colorado, USA and the Millennium Seed Bank in England for back-up storage. Seed will continue to be collected as crops become available and resources permit.

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. Over 2700 tests were completed during the three-year period. The data not only provide an indication of the quality of each seedlot but when combined with previous test data 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. Some seedlots that have been in storage for over 50 years. When the current round of testing is completed the data will be evaluated.

Operational research continues to be an important activity. Evaluating germination test data, as described above, is one aspect. Another activity is developing or refining germination testing protocols particularly the type and duration of treatments applied to seed to promote germination. This is particularly important for species that produce dormant seed that generally involve varying durations of warm and cold treatments prior to germination testing. Exploratory trials using a single seedlot from each of pin cherry (*Prunus pennsylvanica*), choke cherry (*P. virginiana* var. *virginiana*), and black cherry (*P. serotina*) demonstrated that 6 weeks of warm treatment followed by 24 or 36 weeks of moist chilling resulted in the highest germination. Further trials will be conducted using more seedlots to fine-tune the warm and cold treatment durations.

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TREE IMPROVEMENT PROGRESS AT THE DIRECTION DE LA RECHERCHE FORESTIÈRE

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Keywords: white spruce, Norway spruce, black spruce, jack pine, hybrid larch, hybrid poplar, *Septoria* canker, controlled crosses, tree improvement, tree selection, provenance tests, progeny tests, molecular certification, hardwoods, forest yield, seed orchards

WHITE SPRUCE TREE IMPROVEMENT

André Rainville

In the past two years, we completed the selection of white spruce (*Picea glauca*) trees in first-generation progeny tests. They represent provenances covering the distribution of the species in Quebec. These trees will be integrated into the main breeding population. Efforts in clonal forestry, initiated in 2007, continued with the planting of 2 more clonal tests, each containing around 200 clones derived from somatic embryogenesis. Measurements at 4 years of age were collected in two clonal tests. Analysis revealed high clonal variability for growth and branching characters. Clone x site interaction was low, while site-site correlation was high. The relative performance of clones seems to be stable over time. Results were presented at a research symposium. We also investigated the possibility of early selection for wood characteristics and its impact on mechanical properties. Measurements taken on 30-year-old trees from a provenance-progeny trial show that selection concentrated on a few easily measurable traits, such as wood density and core length, holds promise of superior genetic gains for mechanical properties, but would have a negative impact on fibre anatomy related to pulp quality.

CLIMATE CHANGE ADAPTATION MEASURES

André Rainville

In 2010, in collaboration with the Canadian Forest Service (CFS), a special project was initiated to update the existing white spruce transfer model and to develop models for black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*). These models used data from 21- to 24-year-old trees in provenance tests established in Quebec. The R^2 of the models were 0.56, 0.71, and 0.34, respectively. For white spruce, the previous model developed by Andalou et al. (2004) had a R^2 of 0.34, which is much less than the new model's value of 0.56. This improvement is probably due to the inclusion of older climate data (1941–1970) in our new model, compared to the previous one (1971-2000). A possible explanation is that the climate had probably already begun to change in the 1971–2000 period. Future climate data were simulated by Ouranos, a consortium on climate change. Transfer models were then combined with biophysical site index models and integrated into Biosim, a simulation model developed by the CFS, to produce maps of future plantation yields. These tools are currently being used to redefine planting zones for seed orchards and to guide transfers for other seed sources. Results were presented at both a professional symposium and a forest research symposium.

In the spring of 2011, a project aimed at developing a conservation strategy for forest tree genetic resources was initiated as a joint effort consisting of a geneticist (André Rainville), a specialist on seed conservation (Fabienne Colas), and a researcher on the impacts of climate change on future forest tree species range and biodiversity (Catherine Périé). The different steps of the methodology and an overview of the preliminary results were presented.

LARCH TREE IMPROVEMENT

Martin Perron

In spring 2009, 80% of the intra and interspecific 10 x 10 factorial design between European larch (EL, *Larix decidua*) and Japanese larch (JL, *L. kaempferi*) was completed. Seedlings were grown in the nursery in order to establish two progeny trials in 2012. These progeny trials will contribute to understanding the genetic causes of heterosis in *Larix* x *marchlinsii* and will lead to recommendations of the best full-sib families to be used for cutting propagation.

Second-generation breeding populations (80 JL and 80 EL) should lead to merchantable volume genetic gains between 5.5% and 15% at year 30 (Perron 2010).

JACK PINE TREE IMPROVEMENT

Mireille Desponts

In 2009 and 2010, selections for two jack pine clonal seed orchards were completed for the eastern region of the province (Côte-Nord and Bas-Saint-Laurent – Gaspésie). In a few years, clonal seed orchards will be able to supply all breeding zones with highly improved seeds. In 2012, controlled crosses will be completed for second-generation jack pine for the southern zone and breeding for the northern zone will begin. For each breeding zone, polycrosses and controlled crosses (full-sib) will be carried out using about 300 selected trees.

BLACK SPRUCE TREE IMPROVEMENT

Mireille Desponts

In spring 2011, controlled crosses were completed for second-generation black spruce for the north-eastern region (Côte-Nord, Saguenay – Lac-Saint-Jean and Charlevoix). Also in 2011, a research project on the genetics of wood quality in progeny trials was completed, in which estimates of genetic parameters of wood traits were obtained. Results indicated moderate, unbiased individual-tree narrow-sense heritability (h_i^2) and unbiased half-sib narrow-sense heritability h_f^2 for wood density and stiffness (MOE). These traits will be integrated in future black spruce selections.

NORWAY SPRUCE TREE IMPROVEMENT

Marie-Josée Mottet

In 2009, progenies with superior growth and resistance to the white pine weevil (*Pissoides strobi*) were identified in three 10-year-old Norway spruce (*Picea abies*) full-sib progeny tests. Recommendations were made for the best crosses, in order to reproduce their progenies by cuttings for reforestation purposes. In collaboration with J.D. Irving Limited, New Brunswick, clones from resistant families and derived from somatic embryogenesis will be planted in two tests in 2012. In 2010, for the regions of Bas-Saint-Laurent and Gaspésie, a new seed orchard was established from selections made in 14-year-old open-pollinated progeny tests, based on height and weevil resistance. In 2009, in response to environmental certification concerns, the invasive potential of Norway spruce was assessed in 23 plantations. Results indicated that this species did not show any invasive pattern.

HYBRID POPLAR IMPROVEMENT PROGRAM

Pierre Périnet and Marie-Josée Mottet

The hybrid poplar improvement program produces new hybrid material on a regular basis for planting in Quebec. In addition to the selection of *Septoria*-resistant clones for southern Quebec, the program is mainly oriented towards the production of *Populus maximowiczii* hybrids that are well-adapted to forest sites located in bioclimatic zones 3, 4, and 5 (sugar maple–yellow birch, balsam fir–yellow birch, and balsam fir–paper birch zones).

Four *P. maximowiczii* x *P. nigra* (MN) clones were recently released for commercial planting in zones 1, 2, and 3 in southern Quebec and two *P.* x *rollandii* x *P. maximowiczii* (RM) clones for zone 5. From 2009 to 2011, ten clonal tests and one *P. balsamifera* full-sib progeny test were established in different locations in bioclimatic zones 1 to 5, and one experimental plantation was established at the Berthierville nursery. In 2011, pollen from *P. maximowiczii* and *P. trichocarpa* parental populations in Villeroy, Shipshaw, and Rigaud were collected and stored in our pollen bank.

In the global context of climate change, *Septoria musiva* monitoring is conducted in areas traditionally free of the canker (zones 3, 4, and 5). Unfortunately, some leaf spots or cankers have been found since 2001. Even though there are no indications of disease outbreaks in these zones, selection efforts are directed towards *Septoria* tolerant clones.

Several projects on poplar cultivation by steckling production (1-0 rooted cuttings) are continuing within the network "Réseau Ligniculture Québec", in collaboration with scientists, forest industry partners, and provincial nurseries. The poplar research team at the DRF contributes to different projects on *Septoria* canker, wood processing and utilization, and molecular genetics studies with scientists from Université Laval, University of Alberta, and CFS.

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RECENT ADVANCES IN TREE REPRODUCTION, SEED AND SEEDLING PRODUCTION IN QUEBEC

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In Quebec, the degree of integration between different steps in the reforestation process, from seeds to outplanting, is unparalleled. The Direction de la recherche forestière (DRF – the Research Directorate) of the Ministère des Ressources naturelles et de la Faune (MRNF – Ministry of Natural Resources and Wildlife) has a multidisciplinary research group which develops expertise in forest genetics, seed orchard management, seed technology, mass cutting propagation, somatic embryogenesis, seedling production, environmental monitoring, planting techniques, and forest plantation silviculture. In close collaboration with other research agencies (universities, research centres), this group is responsible for acquiring, integrating, and transferring research results related to seed and plant production to the 21 forest nurseries (6 government and 15 private), the provincial seed centre, and the cutting propagation and somatic embryogenesis centres. DRF projects must answer research and development needs and are subject to rigorous evaluations of both pertinence and scientific value by independent committees. The evaluation of these projects, as well as those submitted by other research institutions, is administered by an independent agency (Fonds québécois de la recherche sur la nature et les technologies). The present report outlines the salient facts and results of the principal studies related to new developments on water activity dedicated to seed quality evaluation, the genetics of seedling production mainly on root system development of white spruce (*Picea glauca*), and the integration of somatic embryogenesis in the reforestation chain.

SEEDS

Larix Indoor Seed Orchard

A new research project began in 2009 with the aim to better understand mineral nutrition for *Larix* grafts cultivated in the indoor seed orchard located at the Berthier Tree Seed Centre facilities. The objective is to increase and to obtain dependable seed production so that hybrid larch seedlings can be produced from seed and not solely from cuttings as is the present practice.

Water Activity

Since 2007, the DRF, in close collaboration with Cemagref (Nogent-sur-Vernisson station, France), has worked on the implementation of water activity (a_w) as a new quality assessment method for the management of orthodox tree seed. This simple and non-destructive technique permits quick, reliable, and economical control of the quality of pollen and seeds. A lot of work has been done mainly for the hydric characterization of the species used for Quebec reforestation program. This work, combined with results obtained previously in France and for two species from British Columbia, shows a tendency for a universal value of a_w that can be used to stabilize the moisture of seedlots in order to preserve them (Colas et al. 2008, 2010). Water activity is now routinely used at the Berthier Tree Seed Centre for seed moisture content tests and to monitor seed drying. Thanks to the collaboration with Cemagref, we built seed/pollen dryers controlled by a_w. This equipment is inexpensive, very simple to operate, and gives us a reliable method to better control pollen and seed drying.

For us, water activity is a very useful tool that can be used to monitor the quality of seed stored for conservation without destroying material (Baldet et al. 2009). The rapid acquisition of data and the fact that the assessment is non-destructive allow us to test our seedlots as frequently as is necessary. We noticed that repeated opening of the containers for sampling purposes resulted in an increase of both a_w and subsequent moisture content, and that the use of inadequate containers, permeable to humidity and fitted with a lid with an inadequate seal had the same consequences. This is a key problem for long-term conservation. A new project began at the DRF with one part dedicated to determining the best conditions for long-term tree seed conservation (Colas et al. 2010). We are collaborating with a plastics research centre (Centre de technologie minérale et de plasturgie inc., Thetford Mines) to develop a new container. The work will focus on a new polymer with the introduction of nanoparticles and the development of a new type of lid.

The first International Seed Testing Association (ISTA) workshop dedicated to a_w was organized by Cemagref in collaboration with DRF in October 2010 in France (Baldet and Colas 2010). Three ISTA technical committees FTS (Forest Tree and Shrub), STO (Storage), and MOI (Moisture) were involved with their respective chairs (Fabio Gorian, Hugh Pritchard, and Harry Nijenstein). This was the occasion for us to present different parts of our collaborative work on a_w. As a result, it was decided that a_w can be introduced into the ISTA rules as a new quality test. Further work on this topic will be undertaken by ISTA.

SEEDLING PRODUCTION

Growth and Mineral Nutrition of Seedlings Produced from Ten White Spruce Seed Orchards

To meet the needs for improved spruce seeds, 17 first-generation white spruce seed orchards (SO) were established. These SOs are located in different bioclimatic regions and contain seed trees originating from geographically and genetically distinct sources. To evaluate the influence of SO on seedling growth and morphology, seedlings produced with seeds originating from the ten most commonly used first-generation white spruce SOs were raised under similar nursery conditions. Tissue nutrient concentrations of seedlings were similar for all the different seed sources during the second growing season. At the end of the growing season, only shoot phosphorus concentrations were significantly different. When modeled with a logistic function, there was a significant difference between height and diameter growth curve parameters of seedlings from distinct SOs during the second growing season. These differences led to significant differences in height and shoot dry mass of the seedlings at the end of their second growing season, but not in their diameter or root dry mass. The ten SOs were clustered in two groups according to the above-ground characteristics of their progeny. This limited amount of morphological diversity suggests that expanding the size of the present seed zones may be an appropriate course of action for white spruce seed production (Carles et al. 2011).

Genetic Variation in Seed Size and Germination Pattern and its Effect on Early Seedling Growth of 75 Open-pollinated White Spruce Families

We determined the degree to which 75 open-pollinated white spruce families differ in seed and germination characteristics and examined the extent to which these characteristics influence their early growth. Seed characteristics (1000-seed weight, length, width, area, and volume) were measured on 400 seeds per family. Germination parameters (germination capacity, peak value, and germination value) were determined for each of the 75 families under controlled conditions and germination patterns were modelled using the Weibull function. Seedling characteristics (height, diameter, shoot and root dry weights) were measured at the end of the first and second growing seasons under standard nursery cultural practices. Statistically significant family variation (p 0.0001) was found for all seed characteristics and germination parameters measured. The between-family variance explained 23% to 98% of the total variance of morphological and physiological seed characteristics. Family differences at the seed stage explained up to 33% (root dry weight) and 12% (shoot dry weight) of the family differences observed at the 1+0 and 2+0 seedling stages, respectively. Because of the probable genetic component of family variation in seed size, we do not recommend adjustment for this characteristic when estimating family heritabilities of seedling characteristics. Moreover, since a maximum of only 12% of the family differences observed at the 2+0 seedling stage are explained by the effect of seed size, selection for families with better juvenile characteristics could be made without considering the maternal effect on seed size (Carles et al. 2009).

Genetic Variation of 75 White Spruce Families in Rooting Ability of Cuttings

As part of the ongoing improvement program for white spruce, capitalizing on family genetic variability may improve the rooting ability of cuttings. Cuttings produced from 75 half-sib white spruce families were sampled during the rooting phase (B+0) and the two subsequent growing seasons (B+1, B+2) to evaluate growth variables, root system architecture, gas exchange, and nutritional status.

The estimation of genetic parameters, notably high individual and family heritability values for root mass during the first two years of growth, showed that the rooting ability of cuttings is under strong genetic control. Genetic correlations demonstrated the relationship of root mass with diameter or height. Family genetic variability is high enough to permit stock plant selection for mass propagation and improved rooting of white spruce cuttings (Gravel-Grenier 2009, Gravel-Grenier et al. 2011).

SOMATIC EMBRYOGENESIS

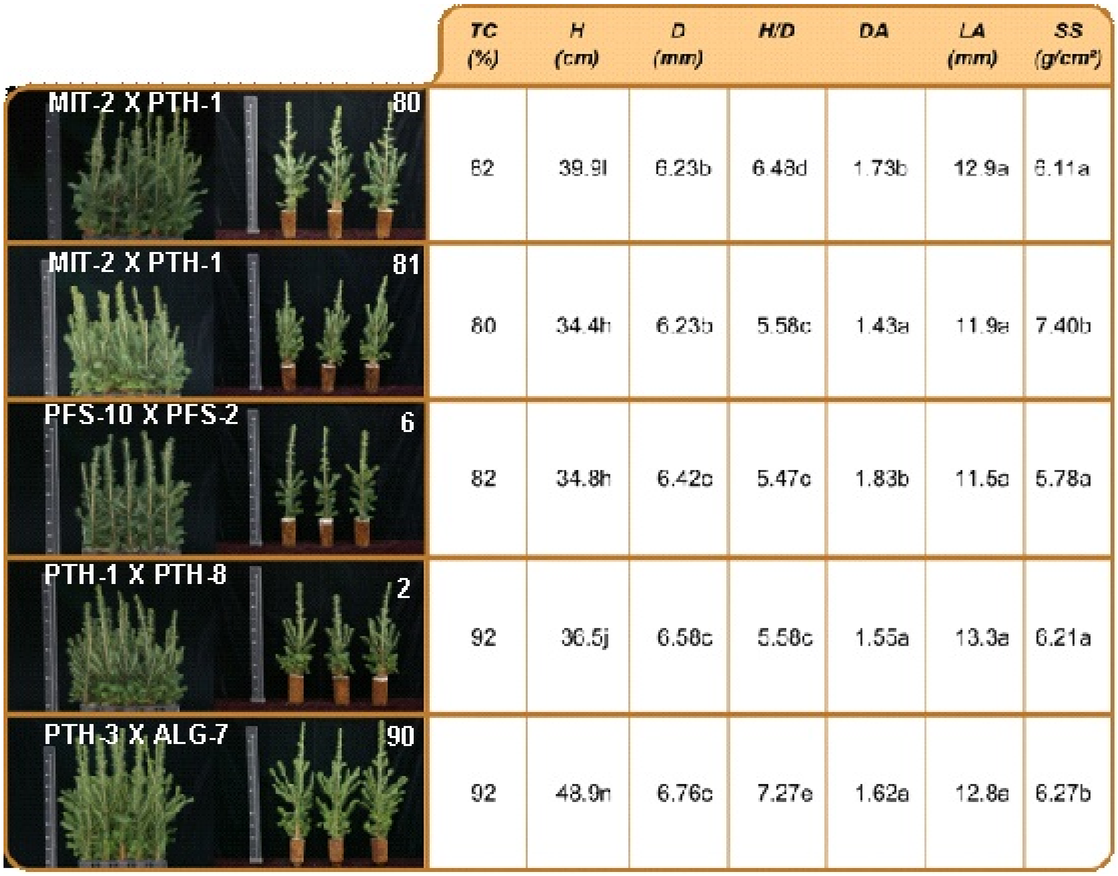
The Use of Somatic Clones for the Production of Seeds and Seedlings of High Quality

Quebec has some of the oldest clonal tests planted in Canada. Our results showed precocious male and female flowering of somatic white spruce and black spruce clones. Studies on flower development, pollen quality, and seed production by somatic clones revealed that these clones may be used as seed trees (Colas et Lamhamedi 2009, 2010, 2011). Controlled crossings between somatic clones were done and the seeds were characterized. After one growing season under forest nursery conditions, seedlings exhibited similar morpho-physiological variables compared to those produced from seed orchards.

Clonal Variation of White Spruce and its Use in the Cutting Propagation Program

Techniques for the operational production of seedlings by somatic embryogenesis (SE) are presently being developed. Each year, more than 30 000 seedlings are produced by SE and deployed for clonal field testing. The advantage of SE is the cryopreservation of juvenile tissue that can be multiplied in perpetuity, as many times as required. SE will help us to develop multi-varietal forestry. As part of a larger project, we are currently evaluating the feasibility of the operational integration of the clones produced by SE into the production chain of cuttings. We defined the pedigree of more than 800 clones and we characterized their morpho-physiological attributes in nursery and in field tests (see Fig. 1, Lamhamedi et al. 2000, Lamhamedi 2010). We subjected these 800 clones to the same morpho-physiological evaluation that is done for all seedlings produced in the nurseries (27 quality criteria, see Fig. 1).

The best clones are also evaluated with regard to their possible integration into the production chain of cuttings (Tremblay et al. 2008, Tousignant et al. 2008, 2009, Lamhamedi 2010). This evaluation is based on the clone characteristics exhibited during the successive steps of SE and their subsequent growth in the nursery. In this project, we retained the clone identity and were able to separate the genetic variance into its components (additive, dominance, and epistatic) to evaluate the broad-sense individual heritability for somatic clones. Our results also indicate that cuttings from white spruce stock plants produced by SE are suitable to produce high quality stecklings under nursery conditions.



	TC (%)	H (cm)	D (mm)	H/D	DA	LA (mm)	SS (g/cm²)
MIT-2 X PTH-1 80	82	39.9l	6.23b	6.48d	1.73b	12.9a	6.11a
MIT-2 X PTH-1 81	80	34.4h	6.23b	5.58c	1.43a	11.9a	7.40b
PFS-10 X PFS-2 6	82	34.8h	6.42c	5.47c	1.83b	11.5a	5.78a
PTH-1 X PTH-8 2	92	36.5j	6.58c	5.58c	1.55a	13.3a	6.21a
PTH-3 X ALG-7 90	92	48.9n	6.76c	7.27e	1.62a	12.8a	6.27b

Figure 1. Growth and shoot architecture of white spruce somatic clones (2+0) (Lamhamedi 2010). TC: rate of conformity which combines all 27 criteria; H: height, D: diameter, DA: needle density (number/ length), LA: needle length (mm), SS: specific leaf area (g/cm^2).

The evaluation of genetic parameters of clones at a juvenile stage is necessary to better understand the amplitude of clonal variability and the degree of genetic control. One hundred thirty-nine white spruce clones from 22 unrelated families were produced by SE. After one and two years of growth in a nursery, these clones were used for evaluation of growth characters and mineral nutrition (N, P, K, Ca, and Mg) of the shoot and root tissues. The analysis of variance showed a highly significant clonal effect for the majority of the growth characters at the end of both growing seasons and for the mineral status at the 2+0 stage. Our results reveal that height exhibits high clonal heritability which remained stable over the two growing seasons (H^2_c= 0.60). In addition, moderate genetic control was observed for most other growth characters and mineral nutrient contents (K, Ca, and Mg) at the end of the second growing season. Strong genotypic and phenotypic correlations were observed between height and diameter at the 1+0 stage and between height and the rest of the growth characters at the 2+0 stage. Strong positive genotypic and phenotypic correlations were also revealed between shoot mineral nutrient contents and total seedling mineral contents. We have to determine the impact of these results in terms of early selection to decrease the number of samples used in clonal tests and the costs associated with establishing high yield clonal plantations.

Growth and Performance of White Spruce Clones in Clonal Tests

After being characterized in the nursery, morpho-physiological characteristics of each clone are evaluated in clonal tests. Chosen sites are representative of the 2 white spruce bioclimatic regions (fir and maple breeding zones). In general, more than 200 somatic clones are tested each year.

In 2010, data collected in 4-year-old tests on shoot architecture, growth, and number of branches, revealed a low broad sense heritability for all morphological characteristics. However, height presented the highest clonal heritability (H^2_c= 0.137). High genetic correlations were observed between all the characters of growth and branch number. Genotype x site interaction is low and correlation between the sites is high. These results confirm the stability of morphological performance of several somatic clones and suggest that it is possible to integrate these criteria for early selection of somatic clones.

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 centres, the seed and plant production research group has emphasized the transfer of expertise and knowledge and the integration of research results in Quebec forest nurseries. This transfer has been realized through scientific and technical publications, visits and conferences, as well as workshops and training sessions on particular themes. During these events, researchers, technicians, nurserymen, and graduate students are invited to present their research results. The content of these conferences is principally directed towards forest nurserymen in Quebec, as well as managers who work in the field of artificial regeneration.

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FUNCTIONAL, ECOLOGICAL AND EVOLUTIONARY GENOMICS AT UNIVERSITÉ LAVAL

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Keywords: comparative genomics, chloroplast DNA, ectomycorrhiza, ESTs, environmental genomics, functional genomics, gene expression, genogeography, genome mapping, genome scans, marker-aided selection, microsatellites, mitochondrial DNA, molecular ecology, molecular breeding, phylogeography, poplars, population genomics, SNPs, spruces, structural genomics, tree improvement, wood formation, xylem

INTRODUCTION

This report summarizes the efforts and results obtained in forest and environmental genomics over the 2008–2011 period by our group at Laval University. The Canada Research Chair in Forest and Environmental Genomics (Tier 1, lead by J. Bousquet) was renewed and entered into a second cycle of seven years (2008 to 2015). The second phase of our major spruce genomics program *Arborea*, (lead by J. Mackay and J. Bousquet), was completed in 2011. The emphasis has been put on expanding spruce genomic resources and developping gene markers related to adaptation and wood characters of the two most widely reforested species in Canada, white spruce (*Picea glauca*) and black spruce (*Picea mariana*). The project has been supported by Genome Canada, Génome Québec, the Canadian Forest Service (CFS) Genomics R&D Initiative and NRCan-Forest Industry Strategy, and the Fonds québécois de la recherche en nature et technologie (FQRNT). It involved research scientists from Université Laval (N. Gélinas and P. Rigault), the University of Alberta (J. Cooke), the CFS (N. Isabel and A. Séguin), the Canadian Wood Fibre Centre (CWFC) (J. Beaulieu), the Québec Ministry of Natural Resources (MRNFQ) (M. Perron, A. Rainville, and M. Desponts), Agri-Food Canada (A. Bertrand), and FPInnovations (P. Bicho). *Arborea* has also produced an offshoot, project SMarTForests – Spruce Marker Technologies for Sustainable Forestry (lead by J. Mackay) scheduled to be launched in 2011, which is a large-scale collaborative effort implicating scientists of *Arborea* and those of *Treenomix* from the University of British Columbia. The project aims at sequencing the genome of white spruce and bringing spruce molecular breeding to an operational scale. It is supported by Genome Canada, Génome Québec, and Genome British Columbia with the implications of provincial and federal research agencies across Canada and Irving Forest Products.

In collaboration with a number of Canadian, European, and American partners and under the European Union Framework Program, we have also been involved with the set up of the FoResTTraC network, aimed at promoting and fostering collaborative forest genomics reseach across the Atlantic (lead by J. Bousquet and J. Mackay). In the footsteps of the joint meeting of the CFGA and IUFRO working parties on genomics, population genetics and conservation of genetic resources in Quebec City in 2008 (Bousquet et al. 2008), we have also contributed to the publication of a textbook presenting the state-of-the-art research currently conducted worldwide in conifer genomics research and related aspects (Plomion et al. 2011).

Work related to the Phylogeographical Atlas of Canadian Conifers (lead by J. Bousquet) has made significant progress with the support of the National Science and Engineering Research Council of

Canada (NSERC), Ministère du Développement économique et de l'innovation du Québec (MDEIE), and support from the Government of Mexico. Collaborators to this project are from CWFC (J. Beaulieu), the B.C. Ministry of Forests, Lands and Natural Resource Operations (B. Jaquish), the Univ. of Alberta (F.C. Yeh), the Nova Scotia Department of Natural Resources (M. Lemieux), and the Univ. Nacional Autonoma de Mexico (J.P. Jaramillo-Correa and L. Eguiarte).

During the last period, we hosted B. Boyle (now at the sequencing/bioinformatic platform of the Institute for Systems and Integrative Biology of Laval Univ.) and N. Pavy as senior research associates as well as several postdoctoral researchers, including C. Bomal, M.-C. Namroud, B. Pelgas (CFS), X.-X. Wei (now at the Institute of Botany of China), T. Doerksen (CWFC), C. Dhont, S. Sokolski, E. Campagnac, T.T. Hoa (now senior research scientist at the Institute of Agricultural Genetics, Hanoi, Vietnam), and A. Atangana (joint position with the Department of Renewable Resources, Univ. of Alberta). Many graduate students have been involved in diverse research projects, including, at the MSc level, J. Pickford, G. Sarasin, S. Gérardi (now at *Arborea*), I.M.J. Behavana, N. Onwuchekwa, N. Morisette, P. Talbot, M. Lemieux (from Nova Scotia Dept. of Natural Resources), E. Fortin, G. Tessier, C. Côté, and at the PhD level, M.A. Shah, S. Kone, N. Rasool, C. Rochon, P. Mutonkole, F. Kangueja, J. Umba, T. Lubalega, R. Boukadida, M. Da Silva Folli, F. Bedon, J. Godbout, D. Hinsinger (now at ENGREF-Paris), S. Legay, P. Lenz (CWFC), F. Pitre, J. Prunier, B. Cinget, J. Sena, J.P. Verta, and B. Dufils. Several of these students were or are currently co-supervised by adjunct professors J. Beaulieu, N. Isabel, and A. Séguin from the CWFC or the CFS.

PHYLOGEOGRAPHY AND PHYLOGENY

Jean Bousquet

We have determined the large-scale phylogeographic structure of a number of broadly distributed North American conifers at the range-wide level using cytoplasmic markers of the mtDNA and cpDNA genomes. Because they are maternally inherited, mtDNA markers are less dispersed than cpDNA or nuclear markers and their geographical patterns of genetic variation are more likely to reflect history, while cpDNA patterns would reflect more recent gene exchanges through pollen. Because these conifers were largely displaced during the Pleistocene and are now occupying once glaciated areas, these studies are useful to identify glacial vicariance and retrace the origin and colonization history of these species since the end of the last Pleistocene glaciation. New published contributions since 2008 include the work on lodgepole pine (*Pinus contorta*) in collaboration with A. Fazekas (Univ. of Guelph), F.C. Yeh (Univ. of Alberta), and C. Newton (Vizon Scitec); on Douglas-fir (*Pseudostuga menziesii*) in collaboration with J. Beaulieu (CWFC), J. Vargas-Hernández (Programa Forestal – Campus Montecillo, Mexico), and B. Jaquish (British Columbia Ministry of Forests, Lands and Natural Resource Operations); on eastern hemlock (*Tsuga canadensis*), black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*), in collaboration with M. Lemieux (Nova Scotia Department of Natural Resources), J.P. Jaramillo-Correa (Univ. of Mexico), and J. Beaulieu (CFWC).

For lodgepole pine, four to five genetically distinct glacial lineages with refugia on the coast, in the Beringia region and south of the ice sheets could be identified with evidence for higher diversity and a large zone of contact in central British Columbia (Godbout et al. 2008). While var. *contorta* matched the coastal lineage around Queen Charlotte Islands, much diversity was seen south on the coast, reflecting the location of a genetically distinct and highly diverse refugial zone. The geographic structure of var. *latifolia* was complex due to several lineages, and the Cascades and Rocky Mountains ranges appeared as potent vicariance factors.

For Douglas-fir, the geographical structure in the north was reminescent of that of lodgpole pine with the coastal and interior varieties matching distinct mtDNA and cpDNA lineages which existence was dated back to before the Pleistocene (Wei et al. 2011). A large zone of contact and higher genetic diversity were observed in central British Columbia, together with inter-mixed cytoplasmic genome assemblages arising from Holocene contact between the coastal and interior lineages. Mexican Douglas-fir appeared to be derived from the Rocky Mountains lineage but was genetically distinct, genetically less diverse, and geographically more differentiated, a likely consequence of the fragmented nature of the species at high altitude in Mexico. The study of the Mexican part of the distribution was a mandate from the North American Forest Commission.

For black spruce, evidence for an Alaskian refuge was seen and multiple genetically distinct lineages were observed with both cpDNA and mtDNA, again underlining the key vicariance roles played in the western part of the continent by the Rockies and in the eastern part by the Appalachian mountains (Gérardi et al. 2010). However, the geographical structure seen with mtDNA was shifted eastward for cpDNA at several places, indicating the capture of ancient mtDNA lineages by western cpDNA lineages. New mixed cytoplasmic assemblages arising from inter-lineage contacts during the Holocene were seen in such regions as in Alberta. The implications of this inter-mixing for adaptation remains to be investigated. Evidence for more extensive gene flow through pollen was widespread across the entire natural range, given the much lower population differentiation values for cpDNA than for mtDNA.

For eastern hemlock, no clear evidence of multiple glacial lineages was found, reflecting in part the smaller distribution of this species in eastern North America (Lemieux et al. 2011). However, a weak cpDNA signal was observed for the possible existence of a genetically distinct lineage west of the Appalachians, though it would not have expanded during the Holocene. The center of diversity was seen at the southern edge of the range where population differentiation and genetic diversity were maximum but highly distinct from the low level of genetic diversity seen in the sister and sympatric species *Tsuga caroliniana*.

For jack pine, evidence from mtDNA indicated that a large part of the Maritimes were colonized early in the Holocene by a distinct lineage that would have survived on emerged banks on the northeast coast during LGM, in addition to the existence of at least two genetically glacial lineages east and west of the Appalachians (Godbout et al. 2010). A large zone of high genetic diversity and Holocene contact was observed in Central Québec, reminescent of those seen in Central British Columbia for lodgepole pine and Douglas fir, or in Alberta for black spruce. Similarly as for eastern hemlock, many private haplotypes and higher population differentiation was seen at the southern edge of the species in the U.S., reflecting presumable remnants of a main glacial refugium. Little of this structuring was seen with cpDNA, indicating the homogenizing effect of gene flow by pollen.

Altogether, these studies suggest that similar vicariance processes involving mountain ranges and coastal areas took place during glacial times for western and eastern species, and that similar large zones of contact have formed during the Holocene, where different glacial lineages met on what once were glaciated lands (Jaramillo-Correa et al. 2009), even generating new inter-lineage genomic combinations (Gérardi et al. 2010, Wei et al. 2011). This trend is contrary to traditional expectations where diversity usually decreases as the distance from refugial zones increases. The results also show that the effects of Quaternary climatic oscillations on the population dynamics and genetic diversity vary considerably according to latitude for a given species, with genetic diversity patterns reflecting different processes taking place in the north and in the south, where conifer species have more restricted and fragmented populations at higher elevation (Jaramillo-Correa et al. 2009; Lemieux et al. 2011; Wei et al. 2011). The general consensus on the efficacity of pollen flow to homogenize genetic diversity in conifers was confirmed in all studies analyzing the two cytoplasmic genomes where generally much less population differentiation was observed for cpDNA than for mtDNA, in spite of usually higher genetic diversity detected for cpDNA (Jaramillo-Correa et al. 2009, Gérardi et al. 2010, Godbout et al. 2010, Wei et al. 2011).

Range-wide phylogeographical studies are progressing for *Abies balsamea*, *Larix laricina*, *Pinus strobus* , and *Taxus canadensis*, as part of the Phylogeographical Atlas of Canadian Conifers. A study involving cpDNA and mtDNA markers regarding the hybrid zone in Alberta between *Pinus contorta* and *P. banksiana* has also been completed in collaboration with F.C. Yeh (Univ. of Alberta). One of the results indicates the early postglacial colonization of eastern Alberta and western Saskatchewan by *P. contorta*, followed by range displacement by *P. banksiana* (Godbout et al. submitted).

The phylogeographic study of Mexican and Central American firs and the phylogenetic study of the entire genus *Abies* have been completed in collaboration with researchers from the Univ. of Mexico (Jaramillo-Correa et al. 2008, 2009; Aguirre-Planter et al. submitted). Subtropical firs harbored genetic signatures typical of relictual populations that were once much larger. The genogeographic structures were often complex with lack of congruence between maternal and paternal lineages. Future research on subtropical conifers will focus on variation related to demography and variation in candidate genes related to climate adaptation and how this variation is distributed among relic conifer populations.

The mtDNA and cpDNA phylogenies of the entire genus *Picea* have also been investigated (Bouillé et al. 2011). They were discordant in several repects. The mtDNA phylogeny, which is representative of the maternal lineages and of smaller historical gene dispersal by seeds, was geographically more structured than the cpDNA phylogenies, which are representative of the paternal lineages and of larger historical gene dispersal by pollen. These results indicate that geographic speciation (instead of ecological speciation) has been a main trend in the genus *Picea* and that ancient and more recent reticulation has been occurring frequently on a geological time scale paralleling the weak reproductive isolation often seen nowadays among spruce taxa. While mtDNA appeared to best track the speciation history of the genus (vertical evolution), the cpDNA phylogenies are likely to best represent the overall similarity between species for biosystematic purposes because they take into account reticulate evolution.

MOLECULAR ECOLOGY AND ENVIRONMENTAL GENETICS

Damase P. Khasa

The work on spontaneous introgression from exotic poplars into native congeners in eastern Canada has made significant progress, in collaboration with N. Isabel and co-workers (CFS), P. Périnet (MRNFQ), and with the support of the Canadian Regulatory System for Biotechnology Strategy (CRSBS), Ligniculture Québec, and Natural Science and Engineering Research Council (NSERC). Simulation work showed that introgression rates vary extensively and depend on a number of critical factors including the population structure of the native species and the selective advantage of exotic genes (Meirmans et al. 2009). Complex patterns of spontaneous hybridization between poplars exotic to eastern North America (*Populus nigra*, *Populus trichocarpa*, and *Populus maximowiczii* and poplars native to this region (*Populus deltoides* and *Populus balsamifera*) were observed using a large array of gene SNPs specific to one or the other species (Meirmans et al. 2010). Contributions from all three exotics were found in the offspring, confirming low reproductive barriers among poplar species in these sections. The frequency of hybrid offspring varied among pollen donors, recipient populations, and years. A remarkably high rate of sponteaneous hybridization was found in a small peripheral population of *P. balsamifera* at the southern edge of its distribution. This indicates that peripheral populations carry a higher risk of introgression as opposed to central populations where the rate of spontaneous hybridization was low. This trend should be attributable to the dilution effect of the local indigenous pollen cloud. Under the leadership of N. Isabel (CFS), these studies are currently being expanded to other regions of Canada.

At the international level and with the funding of Department of Foreign Affairs and International Trade of Canada (DFAIT) through the Canadian Bureau for International Education (CBIE) and under the framework of a Graduate Student Exchange Program (GSEP) and NSERC, the group has contributed to the characterization of microsatellite markers from *Acacia mellifera*, *A. brevispica*, and *A. senegal* (Omondi et al. 2010a). The genetic diversity and population structure of *A. senegal* in Kenya was investigated by using seven polymorphic nuclear microsatellite loci and two chloroplast microsatellite loci (Omondi et al. 2010b). In both chloroplast and nuclear datasets, high levels of genetic diversity were found within all populations and genetic differentiation among populations was low, indicating high gene flow. The analysis of population structure provided support for the presence of two groups of populations, with one representing the Rift Valley region while the other, Eastern Kenya. The spatial genetic structure and implications for conservation were also investigated for the threatened African species *Milicia excelsa* (Ouinsavi et al. 2009). Considerable genetic variability was detected and statistically significant genetic differentiation was found among populations. Genetic and geographic distances were positively correlated, indicating isolation by distance. Bayesian analysis showed partition of the genetic variation into four population clusters. Information gained from this study emphasized the need for the *in situ* conservation of the relict populations and the establishment of gene flow corridors through agroforestry systems by interconnecting these remnant populations. Using nuclear microsatellites (Anderson et al. 2010), the study on *Allanblackia floribunda* or tallow tree, a species distributed across rainforests of Central Africa (Atangana et al. 2010, 2011) failed to show geographic structure (Atangana et al. 2010). The observed lack of temporal stability in effective size of *Allanblackia* populations from sites postulated as rainforest refuges questioned their proposed location in Cameroon. Other molecular data (e.g., mtDNA markers) are needed to provide more insights into the debate.

We pursued the development and use of molecular markers in ecological studies of mycorrhizal fungi. PCR-RFLP, cloning, and DNA sequence analyses (e.g., of SSU rRNA genes) have been used to study the effects of soybean intercropping on the arbuscular mycorrhizal diversity of poplar (Chifflot et al. 2009). A total of 13 different phylotypes were identified. Phylogenetic analyses showed that these belonged to the genera *Glomus* (11), *Acaulospora* (1), and *Scutellospora* (1). The Shannon-Wiener diversity indices were 0.82 for soybean and 0.70 for poplar under the tree-based intercropping system, and 0.53 for poplar under the forest system. New markers based on protein-coding genes for the identification of arbuscular mycorrhizal fungi have also been developed (Sokolski et al. 2010).

Our work on root symbionts as biotools for enhanced reclamation of reconstructed soils of the oil sands industry has expanded during the last period, including research on ericoid, ecto- and arbuscular mycorrhizae as well as on actinorrhizae. After completing the work on *in vitro* and *in vivo* selection of ectomycorrhizal fungi species (i.e., *Hebeloma crustiniliforme*, *Suillus tomentosus*, and *Laccaria bicolor*) for use in the reclamation of saline-alkaline habitats, salt-tolerant strains on ericoid and arbuscular mycorrhizal fungi have been selected *in vitro* (Campagnac et al. in preparation). The evaluation of the field performance of alder-*Frankia* symbiosis (*Frankia* is a N-fixing actinomycete) for the reclamation of oil sands sites has also been conducted (Lefrançois *et al.* 2010). After two growth seasons, *Frankia*-inoculated and non-inoculated alders yielded comparable amounts of plant biomass but there was an increase in hydrocarbon (hexadecane, naphthalene, and phenanthrene) mineralization where the reclamation site had been planted with alder-*Frankia*. The alder rhizosphere samples all had comparable hydrocarbon mineralization rates. Profiles from denaturing gradient gel electrophoresis (DGGE) indicated a change in the microbial communities of the bulk soil between unplanted and alder-*Frankia* plots. Soil tests showed that alder-*Frankia* decreased soil pH (from 7.5 to 6.6 in first year and from 8.2 to 7.2 in second year) and plant-available sodium content (70% reduction), and had a positive impact on soil organic matter content (increase of up to 6 times in alder-*Frankia* plots). The field results confirmed that the use of alder-*Frankia* symbionts improved remediation capabilities and enhanced soil quality, supporting the potential of the alder-*Frankia* symbiosis as part of a reclamation strategy for the reforestation of these sites and the re-establishment of a balanced ecosystem.

With the financial support of Syncrude Canada, the CONRAD consortium, and NSERC a research project involving the isolation, identification, and selection of arbuscular and ericoid mycorrhizal fungi for reclamation of oil sand tailings has been completed. Our expertise in the use of microbial inoculants as biotools to enhance plant establishment of disturbed soils has been recognized internationally with the establishment of the BIOFILTREE Consortium. This joint research project ($ 1.2 million) is part of the NSERC international strategy to increase scientific collaboration with l'Agence nationale de la recherche of France (ANR) within the framework of the memorendum of understanding signed between Canada and France to establish mechanisms for joint funding of collaborative research and innovation projects. This project will strengthen the collaboration between the research and innovation communities of the two countries to achieve more rapid scientific and technical results, leading towards new innovative green technologies in the area of environmental and ecosystem health. Integrated bioremediation that combines fast growing and trace elements-tolerant species inter-cropped with the nitrogen-fixing alder together with *Frankia* and mycorrhizal symbionts is proposed as an alternative environmentally-acceptable and compatible technology for reclaiming disturbed and/or contaminated lands both in France and Canada.

Other studies have been conducted on mycorrhizas to look at the role of ectomycorrhizal fungi in biobeneficiation development for the reduction of potassium and phosphorus from Sishen iron ore in South Africa (Adeleke et al. 2010a, b), and to investigate the mycorrhizal status of exotic and native plants in relation to the problem of alien plant invasions in the Kashmir Himalayan (Shah et al. 2009a, b). This work was supported by NSERC and DFAIT through CBIE under the framework of GSEP. Other work on ectomycorrhizal fungi have focused on the ecology and management of the lobster mushroom (*Hypomyces lactifluorum* and *Russula brevipes*) in a jack pine stand from eastern Canada (Rochon et al. 2009).

FUNCTIONAL GENOMICS AND QUANTITATIVE GENETICS OF WOOD TRAITS

John Mackay

Our functional genomics and quantitative genetics research have continued to emphasize wood formation and properties in spruce trees. We utilised different approaches to investigate the genetic architecture of wood traits, including their heritability and the genes that control secondary vascular growth and development.

Large-scale complementary DNA (cDNA) sequencing has been applied to several conifer species as a means to discover genes (see the review of MacKay and Dean 2011). We used this approach and sequenced cDNA in 14 different white spruce libraries and obtained 146 616 white spruce high quality expressed sequence tags (ESTs). This work involved the collaboration of J. Cooke (Univ. of Alberta), J. Beaulieu (CWFC), and N. Isabel (CFS). These ESTs were analyzed together with 125 556 ESTs from previous *Arborea* and *Treenomix* (Univ. of British Columbia) research activities.

Iterative large-scale EST and cDNA clone analyses, and targeted FL-cDNA sequencing produced a gene catalogue of 27 720 unique cDNA clusters, 23 589 of which are represented by full length insert cDNAs (Rigault et al. submitted). We were able to estimate that the white spruce nuclear genome contains up to

32 520 expressed genes (i.e., 4800 missing from the dataset owing to incomplete, partially sequenced, and unsampled transcripts), representing up to 47 Mb of unique coding sequence. These estimates are in the same range as the genomes of model plants such as *Arabidopsis* and rice. Next-Generation sequencing methods were utilized to help confirm and enhance the gene catalogue by providing deeper coverage for rare RNA transcripts, by extending incomplete clusters and by yielding unsampled sequence. With the set of expressed genes from a conifer in full view, functional and protein domain annotations highlighted divergences and conservation between conifers and angiosperms. All of our sequence data have been made available through public databases such as Genbank.

Our functional genomics research program focuses primarily on wood formation and the genetic basis of wood properties. A major aim have been to identify gene sequences to be incorporated into association studies related to wood properties (Beaulieu et al. 2011a), which in turn should lead to practical tools and methods to enable the selection and breeding of trees based on DNA markers. This research is part of the *Arborea* genomics initiative (*Arborea* II) funded by Genome Canada, Génome Québec, and several other partners from the public sector. A particular emphasis has been placed on putative regulatory genes of secondary xylem (wood) formation conifers and poplars. The genes investigated include MYBs (Bomal et al. 2008, Bedon et al. 2010, Legay et al. 2010) and homoe domain leucine zipper proteins as potential regulators of vascular development (Côté et al. 2010). This work was carried out in collaboration with A. Séguin (CFS) and J. Grima-Pettenati (CNRS, France).

We have refined quantitative PCR (qPCR) as applied to the study of gene expression in conifers (Boyle et al. 2009) as well as developed and applied large-scale microarrays from cDNA amplicons (11 000 genes) for RNA profiling in trees (Pavy et al. 2008a). We identified a core set of xylem genes conserved in sequence and expression among angiosperm and gymnosperm plants, and to characterize the dynamic wood transcriptome. Microarrays have helped to link *MYB* transcription factors to phenylpropanoid metabolisms and secondary cell wall thickening in secondary xylem (Bomal et al. 2008, Bedon et al. 2010, Legay et al. 2010), *HD-zipIII* transcription factors (from poplar) to vascular development genes (Côté et al. 2010) and *WRKY* transcriptional regulator with defence response genes (Levée et al. 2009). More recently, an oligonucleotide microarray was developped (25 000 genes) in collaboration with the *Treenomix* program and has been used to validate the expression of candidate genes for wood formation (Beaulieu et al. 2011a).

We use quantitative genetics methods to investigate the genetic basis of complex traits such as wood density and cellulose microfibril angle, aiming to produce applied outcomes for tree breeding. A ring-by-ring analysis of wood anatomy, physical, and strength property traits in white spruce revealed that heritability varies with cambial age (Lenz et al. 2010, 2011). Trait-to-trait correlations showed that selection trade-offs may be expected when breeding for wood quality traits. We are now linking genomics and quantitative genetics to investigate the relationship between gene diversity and wood structure variation (Beaulieu et al. 2011a). A review of the quantitative genetics aspects of growth and wood formation has been completed (Beaulieu and Bousquet 2010).

STRUCTURAL, ASSOCIATION AND POPULATION GENOMICS

Jean Bousquet

As part of the *Arborea* collaborative project, we have completed the testing of a number of high-throughput single-nucleotide polymorphism (SNP) genotyping platforms where thousands of SNPs identified from resequencing expressed genes or from *in silico* identification in EST gene assemblies were genotyped for hundreds of trees. One of the genotyping platforms relies on the Illumina bead-array technology and the highly multiplexed GoldenGate allele-specific extension assay, where up to 1500 SNPs can be genotyped at a time (Pavy et al. 2008b). A success rate in excess of 70% of the SNPs with more than 95% of the trees successfully genotyped for each valid SNP was obtained for both white spruce and black spruce, which compares well with success rates reported in the the literature for human and mouse. The success rate reached 90% for SNPs previously genotyped, without reaching 100%, which is a normal limitation given the level of multiplexing of the assay. In collaboration with other groups (K. Ritland, Univ. of British Columbia; S. A'Hara and J. Cottrell, British Forestry Commission; M. Lascoux, Univ. of Upsala; P. Ingvarsson, Univ. of Umea; J.P. Jaramillo-Correa, Univ. of Mexico), the white spruce SNPs have been tested with success for a number of other spruce species (*Picea engelmannii*, *P. sitchensis*, *P. mexicana*, *P. abies*, and *P. mariana*), the transfer rate being inversely correlated with phylogenetic distance from white spruce. In collaboration with N. Isabel and J. Beaulieu (CFS) and under the collaborative project *Arborea*, these SNPs have been applied in a number of studies aimed at mapping genes and QTLs (quantitative trait loci) as well as identifying adaptive or functional polymorphisms from large numbers of candidate genes using association genetics and outlier identification approaches (see below). A laboratory integrated management system and database (LIMS-DB) has also been developed to retrieve and manage SNP information in collaboration with Jean Beaulieu's team at CWFC (Clément et al. 2010).

In collaboration with the team of N. Isabel (CFS), gene mapping in white spruce and black spruce has made significant progress with linkage maps attaining more than 300 genes (Pavy *et al.* 2008b) and recently, more than 800 genes, the most dense gene maps for any conifer (Pelgas et al. 2011). Next generation maps are in progress and will contain in excess of 9000 genes (Pelgas et al. in preparation). For a review on linkage mapping in conifers, see Ritland et al. (2011). Synteny and macro co-linearity was high between white spruce and black spruce genomes (Pavy et al. 2008b) and a large number of quantitative trait loci (QTL) for growth, budset, and budflush could be mapped onto the genome of white spruce in one of the most comprehensive QTL mapping study in any plant (Pelgas et al. 2011). The results show that many QTLs were replicated between pedigree populations, years, and environments and that a number of QTLs had pleitropic effects, which reconciles well with the observed genetic correlations between these adaptive characters. Percents of character variance explained by individual QTLs were generally below 10%, indicating that these characters are well under multi-gene control with small effects. Many QTLs are co-located with genes previously identified as differentially expressed or harbouring adaptive SNPs identified from outlier studies (below), though these potentially causal relationships should be considered temptative.

In collaboration with J. Laroche (Institute for Systems and Integrative Biology, Univ. Laval) and J. Beaulieu (CWFC), genetic differentiation among white spruce natural populations in Quebec was estimated for more than 500 gene SNPs. A few dozen outlier SNPs could be identified as candidates for local adaptation. Post-hoc relationships were found between SNP differentiation, gene functions, population phenotypic attributes, and bioclimatic parameters of population sources (Namroud et al. 2008). The study, one of the first in non model organisms, has been the subject of an editorial by Molecular Ecology. A similar study was also undertaken in black spruce, where out of nearly 600 gene SNPs tested, two dozen markers were found to be significantly related to temperature and precipitation variation of population origins (Prunier et al. 2011). A significant overlap in gene families was observed between these SNPs and those previously identified as candidates for local adaptation in white spruce. Relationships with average values of population phenotypic attributes could also be observed in several cases. The intensity of natural selection related to temperature adaptation since postglacial colonization could be further estimated, with selection values 10 times less than that reported in retrospective studies of domestication effets in agronomic plants, but 50 times higher than that under drift/selection equilibrium. These are among the first long-term estimates of natural selection related to climate adaptation in the natural environment.

In collaboration with the team of J. Beaulieu (CWFC), a pilot association study for wood characters in white spruce was completed with nearly 1000 SNPs from more than 500 candidate genes assayed on a population of nearly 500 (Beaulieu et al. 2011a). After FDR correction, more than a dozen SNPs could be identified with individual effects between 3% and 5%. For one of the first times in plant association genomics research, it could also be demonstrated in several instances that the genes carrying these SNPs showed significant expression differences among genotypic classes. The percent of trait variance explained reached up to 10% when considering combinations of a few SNPs. These results are encouraging and genotyping has recently been completed for a an expanded set of trees (3 times) and for many thousand gene SNPs (Doerksen et al. in preparation).

In collaboration with N. Isabel (CFS), studies of nucleotide diversity, linkage disequilibrium (LD) as well as demography and selection signatures of full-length sequences of regulatory genes were also conducted for boreal *Picea glauca*, *P. mariana*, and *P. abies* (Namroud et al. 2010). The results showed that LD is very low in spruce genes across species (half-decay of LD of less than 100 bp), and that large numbers of SNPs per gene will be necessary in association studies if one needs to capture all potentially explicative variation of candidate genes. There was significant heterogeneity of LD and selection signatures among genes and among species, but most genes in all tested species carried the demographic signature of a bottleneck followed by population expansion, which was found to be linked to LGM and ensuing Holocene post-glacial recolonization. These analyses of sequence signatures and LD have been expanded to encompass more than a hundred expressed genes from various functional classes in an effort to better orientate association studies (Pavy et al. submitted).

Overall, these different studies and progress indicate that spruces, in spite of their exceptionnally large genome size (in the scale of 10 times that of the human genome), have really entered the genomic era. Progress should be rapid on a wide spectrum of subjects and applications, such as expanding our comprehension of conifer genome structure and evolution, molecular breeding, as well as monitoring and management of functional genetic diversity in domesticated and natural populations. For instance, in collaboration with J. Beaulieu (CWFC), we have completed a pilot study in white spruce comparing SNP diversity at many hundreds of expressed gene loci between first-generation selected families at different selection intensities and unselected ones from the same provenances. The results show no loss of SNP alleles though allele frequencies were significantly changed at a handful of loci (Namroud et al. submitted). Not only should these studies be of assistance to manage breeding populations, but they could serve as benchmarks to validate the genetic diversity of improved varieties planted under the framework of forest certification.

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ADVANCES IN SPRUCE GENOMICS, QUANTITATIVE GENETICS AND POPULATION GENETICS AT THE CANADIAN FOREST SERVICE, CANADIAN WOOD FIBRE CENTRE, QUEBEC REGION

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This report summarizes the progress made by the Canadian Forest Service (CFS) - Canadian Wood Fibre Centre (CWFC) in forest genetics, genomics, molecular ecology, and biodiversity for the 2008–2011 period. Our research activities were mainly focused on spruce. This work was made possible through grants obtained from the NRCan–Forest Industry Long-Term Competitiveness Strategy; the CFS–Genomics R&D Initiative; and through our participation in the *Arborea* project (J. Mackay and J. Bousquet, Univ. Laval), a major initiative funded by Genome Canada and Genome Quebec to develop molecular breeding in softwood trees. We also continued investing considerable time and effort in population genetics studies of forest tree species as part of our participation in the research activities of the Canada Research Chair in Forest and Environmental Genomics (Tier I), via a Natural Sciences and Engineering Research Council (NSERC) strategic grant for the development of a Phylogeographic Atlas of Canadian Conifer Tree Species (J. Bousquet and J. Beaulieu), as well as a grant from the Ministère du Développement économique, de l'Innovation et de l'Exportation du Québec (MDEIE) to foster collaboration with Mexico (D. Khasa, J. Bousquet, and J. Beaulieu). Other research activities on the genetics of the white spruce root system were also conducted in collaboration with Hank Margolis of Univ. Laval and Mohammed Lamhamedi of the Ministère des ressources naturelles et de la faune (MRNF), with funding received from the Fonds québécois de la recherche sur la nature et les technologies (FQRNT).

Research accomplishments were made possible thanks to the generous contribution of dedicated staff and collaborators who deserve our recognition. They are: Michèle Bernier-Cardou, Pamela Cheers, Éric Dussault, Philippe Labrie, Audrey Lachance, Isabelle Lamarre, Patrick Laplante, Daniel Plourde, Esther Pouliot, and several summer students.

SPRUCE GENOMICS

During the last three years, grants were obtained from the CFS–Genomics Research & Development Initiative (GRDI) and the NRCan–Forest Industry Long-Term Competitiveness Strategy to carry out association studies of wood traits, both in white spruce and black spruce. These grants were also used as co-funding for research activities of the *Arborea* project, a major initiative funded by Genome Canada and Genome Quebec to develop molecular breeding in softwood trees and co-led by J. Mackay and J. Bousquet (Univ. Laval).

A first discovery population of 495 white spruce (*Picea glauca*) was assembled along with a validation population consisting of 200 trees. Increment cores were collected from these trees and phenotypic values for a variety of wood traits and properties were assessed at FPInnovations–Paprican's facilities in Vancouver, British Columbia. Hence, radial profiles of over 25 wood physical properties were obtained for each of the 695 trees. An Oligo Pool All array including 1536 single nucleotide polymorphisms (SNPs) harboured by 625 candidate genes for wood formation was built and the discovery population was genotyped using the Illumina GoldenGate genotyping assay. Candidate genes were either selected based on expression profiling studies and literature reports or were detected *in silico* from the gene sequences. Association studies are underway and the first gene variants associated with wood traits in white spruce have been identified. Data analysis made it possible to find between 8 and 21 SNPs that were significantly associated ($P \leq 0.01$) with each of the earlywood, latewood, and total wood traits. After controlling for multiple testing ($Q \leq 0.10$), 13 SNPs were still significant across as many genes belonging to different families, each accounting for 3% to 5% of the phenotypic variance in 10 wood characters (Beaulieu et al. 2011). When analyzed simultaneously, the cumulative effect of multiple significant SNPs for the same trait could explain a higher proportion of the total phenotypic variation (e.g., 9.7%, 8.7%, and 11.1% for average ring width in latewood, cell wall thickness in early wood, and percentage of earlywood, respectively). The SNPs that were found significant in the first association study are being tested in collaboration with Y.S. Park (CFS) using a 14-year-old clonal test established in New Brunswick.

The white spruce discovery population was expanded from 495 to 1700 trees and wood physical traits of the additional trees were also assessed by FPInnovations using the SilviScan technology. Thanks to our collaboration with *Arborea* and funding from Genome Canada and Genome Québec, the large discovery population could be genotyped using the Illumina Infinium iSelect genotyping bead chip. Hence, over 6700 high-quality SNPs were available for new association studies. Preliminary results of single-locus analysis show that a total of over 100 SNPs were found associated with wood traits, each explaining between 1% and 5% of the phenotypic variation in these wood traits. Analyses were also carried out with the subset of SNPs found significantly associated at $P \leq 0.05$ with a given wood trait using the Bayesian hierarchical model (BAMD). Hence, for wood density, a subset of 47 SNPs with effects significantly different from zero was delineated, which could explain close to 40% of the variation. These results show that Bayesian multilocus approaches are promising for the identification of groups of markers that could be used for the selection of trees with desirable wood attributes. A manuscript is in preparation to report the results of these new analyses, which were presented at the "Genomics-based Breeding in Forest Trees Symposium" held in Davis, California, in June 2011.

Efforts were also made to develop molecular breeding for wood traits in black spruce (*Picea mariana*). Wood physical properties of a discovery population of 400 samples were assessed by FPInnovations using the SilviScan technology. The population was assembled from a progeny test in New Brunswick with the collaboration of the New Brunswick Tree Improvement Council (Kathy Tosh). The search for SNPs in gene sequences amplified using primer pairs developed for white spruce was achieved for over 1500 contigs using white spruce cDNA sequences available through our partnership with *Arborea* and funding previously obtained from FQRNT with J. Bousquet for black spruce marker development. The development of an Oligo Pool All (OPA) array to genotype the discovery population was completed and association studies were carried out using multi-SNP Bayesian hierarchical models to identify gene variants that have the potential to be used as selection tools. We have begun testing markers to rank a Newfoundland and Labrador black spruce breeding population for wood traits.

In collaboration with *Arborea* (J. Bousquet) and the financial support of FQRNT, outlier detection methods were used to scan the black spruce genome for gene SNPs involved in adaptation to variation in temperature and precipitation. This scan involved close to 600 SNPs from over 300 genes. Using two detection approaches, a total of 26 SNPs from 25 genes distributed among 11 of the 12 linkage groups were detected as outliers with F_{ST} values as high as 0.078. Several genes carrying outlier SNPs belonged to gene families previously found to harbour outlier SNPs in white spruce. The functional annotations of these genes and regression of SNP frequencies on climatic variables supported their involvement in adaptive processes. Results were published in Molecular Ecology (Prunier et al. 2011).

A Laboratory Information Management System (LIMS), supported by a relational database (TreeSNPs), was developed for SNP discovery in our genomics projects in collaboration with *Arborea* researchers (Clément et al. 2010). Its functions include bulk data submission, reporting of candidate gene selection, cDNA and PCR primer sequences, amplification success and resequencing results, and export of tables (CSV format) for downstream analysis. The system, source code, and documentation are available for download at http://treesnpspub.arborea.ulaval.ca:3000/download . A second database (PhenoTree) was developed and it characterizes each tree in our discovery and validation populations by its pedigree, growth environment, and phenotype. Phenotypic data include tree morphological data (dbh, height, crown dimensions, branching), wood chemical determinations, and physical properties.

QUANTITATIVE GENETICS

Quantitative Genetics of Wood Properties

In collaboration with J. Mackay (*Arborea*), we investigated the genetic control of wood physical properties in white spruce as a function of cambial age (up to 16 years at DBH) to improve juvenile wood attributes of this species. Increment cores were taken from 375 trees randomly selected from 25 open-pollinated families in a provenance-progeny test established on three sites. High-resolution pith-to-bark profiles were obtained for microfibril angle (MFA), wood stiffness, wood density, tracheid diameter, cell wall thickness, fibre coarseness, and specific fibre surface using the SilviScan technology. The heritability estimates obtained indicate that genetic control of cell anatomy traits and wood density increases with cambial age, whereas the genetic control of MFA and wood stiffness remains relatively low across growth rings. Significant genetic gains could be expected in tree improvement programs for wood density, radial tracheid diameter, cell wall thickness, and specific fibre surface. Genetic and phenotypic correlations between wood traits along the cores from pith to bark were also estimated. Results show that most correlations become stronger in magnitude in rings closer to the bark. An exception to this rule was found between MFA and wood stiffness, where correlations were strongly negative from the pith to the bark. Age-age correlations for different wood traits were found to be high and possible gains from early selection were estimated to be good in ring 8 and older for most traits. MFA was the trait with the strongest potential for selection as early as ring 4, but a detrimental correlation with wood density may represent a drawback of this juvenile selection approach. Results were published in two scientific papers (Lenz et al. 2010, 2011).

Other Quantitative Genetics Studies

In collaboration with H.A. Margolis (Univ. Laval) and with funding from FQRNT, the genetic variation and control of seed and germination characteristics were investigated, as well as the extent to which they influence the early growth of 75 open-pollinated white spruce families. Seed characteristics studies included 1000-seed weight, length, width, area, and volume whereas germination ones included germination capacity, peak value, and germination value. Seedlings were also produced and measured at the end of the first and second growing seasons. Significant variation due to families was found for all the traits, with the between-family variation explaining 23% to 98% of total variation in the various seed traits. Family differences at the seed stage could explain up to 33% of root dry weight at the first-year seedling stage and 12% of shoot dry weight at the second-year seedling stage (Carles et al. 2009).

Genetic variation in rooting characteristics of white spruce cuttings harvested from 2-year-old stock plants of the same 75 open-pollinated families was also explored. Growth, root system architecture, and gas exchange of the cuttings during the rooting phase and the two subsequent growing seasons were evaluated. Root initiation as well as root development during the two following years were found to be under strong genetic control. Strong positive correlations between plant root and aboveground traits at the end of years 2 and 3 suggest that an indirect selection for families producing cuttings with heavier root dry masses could be based on aboveground traits. Results were published in the Canadian Journal of Forest Research (Gravel-Grenier et al. 2011).

Quantitative genetics studies were also carried out with collaborators on one Mexican and one African tree species. For the first study, 13 *Pinus patula* populations were sampled in the state of Oaxaca, Mexico, along an altitudinal gradient (2400 m to 4000 m). Seedlings were grown in tree pots in two environments: in a shade house located in Ixtlán de Juárez and in a greenhouse followed by a shade house in Quebec City. Total seedling height was measured at 6 months of age in both locations. Results indicate that the populations differed significantly and that there was no significant genotype x environment interaction. The population found at 2650 m was the tallest. These results suggest the presence of a weak altitudinal pattern of variation in seedling height. This research was carried out by Professor Cuauhtémoc Sáenz-Romero during his sabbatical stay in Quebec City (Sáenz-Romero et al. 2011). We also determined whether *Allanblackia floribunda*, a forest tree species growing in Africa and valued for the fat extracted from its seeds, could be genetically improved for fruit/seed production. Seventeen to forty fruits from each of 70 trees distributed among four sites in Cameroon were sampled. Fat was extracted from the seeds and the stearic and oleic acid contents of the fat were estimated. Highly significant ($P<0.0001$) between- and within-tree variation characterized the fruit and seed traits. Stearic and oleic percentages in seed fat ranged from 44% to 66% and from 25 and 48% per tree, respectively. Moderate repeatabilities were estimated for fruit traits and mean seed mass. Moderate positive relationships were also found between stearic and oleic acid percentages. Results of the study were published (Atangana et al. 2011) and show that there is a potential for breeding *Allanblackia floribunda* for stearic and oleic acid contents in seed fat.

POPULATION GENETICS

Phylogeography

During the 2008–2011 period, we continued investing time and energy in the development of knowledge in phylogeography of North American conifer species. Studies were carried out in collaboration with Jean Bousquet's (Univ. Laval) team and support from NSERC, and results were published in various scientific journals. Besides completing an invited review on the phylogeography of North American trees (Jaramillo-Correa et al. 2009), the first study aimed to determine whether the phylogeographic structure of jack pine (*Pinus banksiana*) populations in eastern Canada was supporting the existence of a coastal glacial refugium in mid-latitudes. One maternally inherited mitochondrial DNA (mtDNA) minisatellite marker and four paternally inherited chloroplast DNA (cpDNA) microsatellite markers were used to assess the range-wide geographical structure of jack pine populations. A total of 1240 trees was sampled from 83 populations. Fifteen mitotypes and fifty chlorotypes were found. Results show that populations from the Maritimes present a unique mtDNA background characterized by very low diversity and the preponderance of a distinctive mitotype. The distribution of cpDNA diversity was not spatially structured, although three chlorotypes were restricted to the east. Based on the patterns observed, we concluded that populations from the Maritimes region likely derive from a genetically depauperated north-coastal refugium (Godbout et al. 2010).

In a second study, we also focused our interest on eastern North America. Our objective was to determine whether genetic diversity in eastern hemlock (*Tsuga canadensis*) is uniformly distributed or structured; this information is relevant to help guide conservation efforts. A total of 892 individuals from 60 populations representative of the entire natural range was sampled and genotyped at seven polymorphic cpDNA markers. Sixteen chlorotypes were identified. Among-population differentiation was low (G_{ST} = 0.02). Moreover, the distribution of chlorotypes did not show a strong geographical pattern. A spatial Bayesian approach made it possible to reveal two distinct groups of populations, with the southern Appalachian populations harbouring greater population differentiation and conserving relatively high allelic richness. In an article published in the Canadian Journal of Forest Research (Lemieux et al. 2011), we concluded, based on the patterns observed, that the southern part of the eastern hemlock range should be considered high priority for *ex situ* conservation.

In a range-wide population genetics study of black spruce (Gérardi et al. 2010), we compared transcontinental chloroplast and mitochondrial DNA population structures in order to identify putative ancestral and new cytoplasmic genome assemblages. A total of 597 trees was sampled in 32 populations, three of them being from Alaska. Trees were genotyped for four mtDNA polymorphic markers resulting in 10 mitotypes and five cpDNA regions generating 93 different chlorotypes. Mean within-population diversity and allelic richness for cpDNA markers were 0.80 and 4.21, respectively, and diminished westward. Population differentiation estimates were G_{ST} = 0.104 and G_{ST} = 0.645 for cpDNA and mtDNA, respectively. At least three genetically distinct cpDNA lineages partially congruent with those inferred from mtDNA data could be delineated. Patterns observed in Alaska suggest that black spruce could have survived the last glacial maximum there. Results also suggest the occurrence of extensive pollen gene flow between ancestral lineages that preferentially occurred west to east, while seed-mediated gene flow was geographically restricted.

With the financial support from MDEIE and in collaboration with J. Bousquet and D.P. Khasa (Univ. Laval), Douglas-fir (*Pseudotsuga menziesii*) was also the subject of a range-wide phylogeography study and results were published in Tree Genetics & Genomes (Wei et al. 2011). This study was also a mandate from the North American Forest Commission of the FAO. Two organelle DNA markers with contrasting modes of inheritance were genotyped for 613 trees from 44 populations covering all of the species' natural range, from Mexico to Canada. Two mitotypes and 42 chlorotypes were recovered in this survey. Significant subdivision was observed in both genomes, which is an indication that seed and pollen gene flows were restricted. Three distinct cpDNA lineages were delineated, corresponding to the Pacific Coast, the Rocky Mountains, and Mexico. The northern populations were characterized by a high level of genetic diversity, indicating a large zone of contact between coastal and Rocky Mountains lineages. A possible northern refugium was also inferred from the data collected. The Mexican lineage, which appeared to originate from the Rockies lineage, was characterized by the lowest genetic diversity but the highest population differentiation. Our results emphasize the pressing need for conservation of Mexican Douglas-fir.

Mating Patterns in a Breeding Orchard

A study was carried out to test the predictive value of biological and ecological variables on male fertility patterns in a white spruce breeding orchard using genetic markers and a full-likelihood Bayesian paternity analysis. The orchard located at the Cap Tourmente Wildlife Reserve contains 104 clones from 36 provenances, with up to 10 ramets per clone. Each individual ramet was assessed visually for the number of male strobili produced over a 2-year period. Moreover, five seedlots were collected from each of two designated mother clones in the orchard. A random sample of seed from lots collected during both years was sown and grown in a greenhouse. A total of 522 surviving offspring were genotyped along with the 104 clones from the orchard at nine sequence tagged sites (STS) and two microsatellite loci. Results of the analyses (Doerksen et al. 2011) showed that distance alone was predictive of siring success, whereas fecundity and a provenance indicator variable captured additional – but not all – remaining variation. Using additional non-genetic measures to predict siring success increased individual probabilities of paternity over a genetic-only model. Not all successful males were consistently successful over the years. Overall rate of selfing was 14% in the surviving (56% – 63%) seedlings. For 6% of the sampled progeny, breeding orchard fathers could not be assigned. These results highlight the need to be cautious when open-pollinated seedlots are collected in breeding orchards for reforestation needs.

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NORTHEAST SEED MANAGEMENT ASSOCIATION (NeSMA) SUMMARY OF FIELD OPERATIONS

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NeSMA is a partnership of nine forest companies and the Ontario Ministry of Natural Resources (MNR) operating in north-eastern Ontario. The company has tree improvement programs for black spruce (*Picea mariana*), jack pine (*Pinus banksiana*), and white pine (*Pinus strobus*) This report summarizes the main operational activities of the company during the 2008, 2009, and 2010 field seasons. Due to the downturn in the forestry sector the field operations have been considerably reduced compared to previous years. The majority of the work was in the 13 first-generation seed orchards. The advanced generation work concentrated on maintaining existing sites.

FIRST-GENERATION PROGRAMS

The majority of the work consisted of crown management and cone collection in 9 black spruce and 4 jack pine seed orchards. The total volume of cones collected was 310 hL with a 50:50 split between jack pine and black spruce. The MNR paid for the collection of 124 hL of these cones in orchards NeSMA partners did not plan to collect from. The federal government Community Adjustment Fund (CAF) paid for the thinning and cone collection at 2 jack pine seed orchards and the roguing of one black spruce seed orchard. The CAF also covered the cost of cone crop assessments for 2 years in 13 seed orchards. The Curtis and Lastheels partner-managed orchards had brushing, topping, and cone collection work completed. A multi-year project to manage all of the tree crowns in the Lastheels black spruce orchard was completed.

Forest Health Technicians visited several genetic tests and seed orchards. Insecticide spraying took place in 2 consecutive years at the white pine genetic test at the Gurd Tree Improvement Area to control the false pine webworm (*Acantholyda erythrocephala*). Clipping of leaders in the same test was done to control the white pine weevil (*Pissodes strobi*). White pine blister rust (*Cronartium ribicola*) control measures were undertaken in 2 of the white pine genetic tests planted in 2006. The lower branches on each tree were removed and infected trees were cut and taken off the site. The trees at these tests were also measured. Competing vegetation was removed from a third test also established in 2006.

Each year Northern College students, Ontario Rangers, and Ontario Stewardship Rangers completed maintenance work at a seed orchard, white pine genetic test, and the black spruce clonal archive. The company continued its community education work through a 6 week co-op placement with a student in the High Skills Forestry Major Program at Englehart High School as well as lectures and field outings with students from Northern College.

Tree improvement sites on the Spanish and Northshore Forests were visited with industry partners to focus on assessing their potential to provide improved seed if active management was resumed. A tentative strategy was written. The partners of the Island Lake jack pine seed orchards visited the orchards to consider their long term viability and management options. Thinning of these orchards is planned for 2011.

SECOND-GENERATION PROGRAMS

The black spruce clonal archive was inventoried and tagged. The genetic tests and clonal archive were monitored to track their health and condition. A total of 317 additional plus tree selections were made for the Ivanhoe, Timiskaming, Saganash, and Superior programs with supporting funds from MNR/Forest Genetics Ontario (FGO). The information for the additional plus tree selections made in 2009 was compiled. The genetic tests for the Ivanhoe Program were assessed and measured. A plan was developed to preserve the elite selections in the Stoddart Township black spruce family tests which are threatened by the potential construction of a phosphoric acid plant. Support from FGO allowed investment protection projects in 8 black spruce tests.

Pollen collection and controlled crosses were completed in the Jack Pine Pilot Program tests in 2 consecutive years followed by collection of the cones resulting from the first year of this work. The Pilot Program elite tests were thinned to their final configuration and the material chipped.

SUPERIOR-WOODS TREE IMPROVEMENT ASSOCIATION

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The Association, a partner in Forest Genetics Ontario (FGO), has made good progress over the past three years in its cooperative tree improvement programs in jack pine (*Pinus banksiana*), black spruce (*Picea mariana*), and white spruce (*P. glauca*). The members have actively managed first-generation programs and are completing the initiation of second-generation black spruce and jack pine programs.

Highlights from the second-generation programs include the production of grafted stock and the establishment of new clonal seed orchards, as well as the establishment of the final elite population tests, with assistance from FGO. To prevent damage from deer browsing the members have established and maintained electric fences around two sites. In addition Tiller netting (i.e., plastic mesh sleeves) has been placed over the leader of trees during the fall and winter to deter deer browsing. Members were able to collect two cone crops from the young clonal orchards.

The first-generation programs provide improved seed for operational tree planting. Nearly all black spruce seedlings are now grown with improved seed and the number of improved jack pine seedlings planted annually is increasing. Two seed orchards were rogued to their final level and there are plans to complete final roguing in a third orchard.

The Association also continued to assist with a number of research initiatives and to support students studying forest genetics at Lakehead University. The members assisted Dr. Pengxin Lu, Ontario Forest Research Institute, with his jack pine rooted cutting trial. The Association and FGO also provided assistance to Dr. Bill Parker, Lakehead University, for his projects on seed source portfolio selections in black spruce and jack pine to compensate for predicted climate change in Ontario.

FOREST GENETICS AT THE ONTARIO FOREST RESEARCH INSTITUTE (OFRI) 2008 – 2011

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Keywords: White pine blister rust, invasive species, genetic resistance, introgressive hybridization, climate change, adaptation strategy, assisted migration, provenance, bud flush, white spruce, hybrid poplar, somatic embryogenesis

WHITE PINE BLISTER RUST RESISTANCE

P. Lu and D. Derbowka

Enhancing genetic resistance in eastern white pine (*Pinus strobus*) to white pine blister rust (caused by *Cronartium ribicola*) continues to be a research focus at OFRI. Initiated by Dr. C. Heimburger in the 1940s, this long-term R & D project attempts to breed major resistance genes to *C. ribicola* into *P. strobus* from Eurasian white pines through introgressive hybridization. While being conceptually straightforward, this breeding strategy took several decades to be implemented. Progress is being made in producing the third-generation hybrid backcross between *P. strobus* and *P. wallichiana*, which is 15/16 *P. strobus* by pedigree.

Testing and characterizing the backcross progeny for disease resistance, growth, and adaptation are among the major research components. Disease resistance is primarily assessed through artificial inoculation of pathogen basidiospores onto white pine seedlings at 1 – 2 years of age in controlled environments, while adaptation and growth performances are evaluated through field trials established under varying climatic conditions. Results to date are encouraging as the hybrid backcrosses have shown much higher post-inoculation survival than progenies of *P. strobus* (>60% vs. 0%) and they demonstrated competitive growth performances and wood quality in south-central Ontario. Research is continuing in understanding the inheritance of blister rust resistance; resistance mechanisms; and characteristics of the backcross progenies in morphology, physiology, and reproductive biology. Vegetative propagation using somatic embryogenesis in conjunction with a varietal deployment strategy is also ongoing.

SOMATIC EMBRYOGENESIS

P. Lu and D. Derbowka

OFRI has established its somatic embryogenesis (SE) applications laboratory to support Ontario's tree improvement programs and endangered species conservation effort. With the generous technology transfer by Drs. Y.S. Park and K. Klimaszewska of the Canadian Forest Service, OFRI staff has learned to use SE technology to propagate white spruce (*Picea glauca*) and white pine operationally. OFRI's SE lab has an immediate application in a pilot white spruce tree improvement research project that studies a multi-varietal tree improvement strategy using clonally replicated progeny tests. Another major SE application is the propagation and cryo-preservation of elite eastern white pine hybrid backcrosses selected for blister rust resistance. In the coming years, SE procedures will be developed for propagating butternut (*Juglans cinerea*) for endangered species conservation and restoration in Ontario.

HYBRID POPLAR GENETIC RESOURCES

P. Lu, S. Blake and D. Derbowka

Ontario invested heavily in the past (1950s–1980s) in breeding hybrid poplars for biomass and bio-energy production, with hundreds of clones selected. These genetic resources are now at risk due to tree mortality in old field clone banks and tests, resulting from short life span of trees, pests, and land use changes. To conserve hybrid poplar genetic resources, we recently surveyed old field achives and trials, collected cuttings from the available clones, and started to propagate them through rooting cuttings. The objectives are to plant these existing clones into new gene conservation archives and update their pedigree database for potential use for Ontario's bio-economy.

FOREST ADAPTATION STRATEGY TO CLIMATE CHANGE

W.C. Parker, S.J. Colombo, P. Lu and N. Eskelin

A research project was initiated in OFRI to assess assisted migration as an adaptation strategy to climate change for Ontario's forests. This research attempts to fill information gaps about population-climate interactions for Ontario's forest tree species through analyses of data from long-term provenance tests and supplemental seedling experiments in controlled environment chambers. The approaches of assisted migration and its effects on forest adaptation will be assessed under projected climatic scenarios of the future. Ongoing work includes tree measurements and wood core analyses with a series of white pine range-wide provenance tests across Canada and the USA in collaboration with USDA, Forest Service partners; and conducting a short-term genecology study under controlled environments in growth chambers.

WHITE SPRUCE BUD FLUSH STUDY

P. Lu and R. Man

We investigated the cumulative thermal requirements and thermal models in predicting the time of seedling bud flush among white spruce provenances in Ontario. The objectives were to assess the potential effects of assisted migration on bud flushing time, a delay of which may reduce the risk of late spring frost damage. Bud flush data were collected from seedlings growing in growth chambers under simulated climatic patterns. The results indicated that although there was considerable variation among provenances, the geographic trend in bud flushing time was not consistent with that shown in a range-wide provenance study. Thus a simple northward movement of seeds over a relatively short distance was unlikely to result in an appreciable benefit on this fitness trait but artificial selection through tree improvement could be effective.

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MANITOBA'S TREE IMPROVEMENT PROGRAM

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Keywords: jack pine, black spruce, white spruce, hybrid poplar

Manitoba Conservation – Forestry has established tree improvement programs in breeding zones across the province (Fig. 1) for the three main coniferous reforestation species in Manitoba: jack pine (*Pinus banksianna*), white spruce (*Picea glauca*), and black spruce (*Picea mariana*). Tree improvement work in many of these zones is accomplished through cost-sharing agreements with the three forest companies responsible for forest renewal in Manitoba: Tolko Manitoba, Tembec Forest Resources, and Mountain Forest Section Renewal Company (MFSRC). This report will highlight the major activities completed from the summer of 2008 to the winter of 2010.

TOLKO MANITOBA – MANITOBA CONSERVATION CO-OPERATIVE PROGRAMS

Due to the economic conditions in the forest industry, only the highest priority work to maintain the programs for the Tolko Tree Improvement Co-operative was completed during this three year period.

The first roguing of the black spruce Simonhouse seedling seed orchard in the Highrock Breeding Zone took place in the fall of 2010. The bottom 25% ranked families, 5131 trees, based on 10-year family test measurements were removed. Post roguing, the orchard has 15 529 trees remaining with a composition of 33.4% top ranked, 33.4% second quartile, and 33.2% third quartile families.

Twenty-year measurements of the second family test for the Nelson River Black Spruce Breeding Zone is being considered with the possibility of moving forward with a second-generation program based on analysis of the 20-year data.

In the Saskatchewan River White Spruce Breeding Zone, 23.1 hl of cones were collected from the Reader Lake seed orchard in the fall of 2009.

TEMBEC FOREST RESOURCES – MANITOBA CONSERVATION CO-OPERATIVE PROGRAMS

In the Lake Winnipeg East Black Spruce Breeding Zone, selective roguing of 245 trees was completed in the Brightstone orchard in the fall of 2008 in conjunction with crown management and cone collection. Cone induction treatments using gibberellin injections had been conducted in half of the orchard in the spring of 2007. Spring flower development surveys in 2008 indicated a promising cone crop; however, a good cone crop did not occur in part due to squirrel caching activities in the orchard. The orchard is now composed of 3521 trees with 56% from top ranked families, 37% from second quartile families, and 7% from the third quartile families.

MFSRC – MANITOBA CONSERVATION CO-OPERATIVE PROGRAMS

Maintenance (mowing) continued on the black spruce family tests and seedling seed orchard that were established in the Mountain Breeding Zone in 2001. The 10-year family test measurements for this zone are scheduled for 2011.

In the Mountain White Spruce Breeding Zone, 2.85 hl of cones were collected from the Birds Hill white spruce clonal seed orchard in the fall of 2008. Cones collected from the top 25% clones were kept separate forming an elite seedlot. Early samples made during cone collection identified poor yields and the presence of a high spruce seed moth population. Cone collection was discontinued at this point. A later cone sanitation collection was made to remove the overwintering spruce seed moth larvae and reduce the population on site. In 2009, 18.8 hl of cones were collected from this orchard. The previous fall's spruce seed moth sanitation program was successful in removing the population within the orchard and yields were good.

MANITOBA CONSERVATION PROGRAMS

The Mantagao black spruce mass selection seed orchard (MSSO) in the Interlake Breeding Zone was selectively rogued in the fall of 2008 to improve spacing and reduce crowding. This was performed in conjunction with a cone collection. Topping was also performed during cone collection on selected trees having a good cone crop. A total of 2.9 hl of cones was collected. The roguing removed 386 trees leaving 3920 trees in the orchard with 53% from top ranked families, 38% from second quartile families, and 9% from the third quartile families.

A western gall rust incident survey was performed at the jack pine MSSO in the Interlake Breeding Zone, the Cranberry Portage MSSO in the Northern Jack Pine Bbreeding Zone, and the Hillside Pedigreed seed orchard in the Eastern Jack Pine Bbreeding Zone in the spring of 2008. A western gall rust incident survey was performed at the Root Lake MSSO in the Northern Breeding Zone in the spring of 2009. Surveys were timed for rust sporulation to aide visibility when surveying the upper crowns. Information on exhibited family and individual-tree susceptibility to the rust will be used in future roguing decisions.

Jack pine cone collections were conducted at the Cranberry Portage MSSO in the Northern Breeding Zone, and the Hillside Pedigreed seed orchard in the Eastern Breeding Zone.

The province cooperated with the Canadian Forest Service in the measurement of Forest 20/20 hybrid poplar trial plantations in the fall of 2008 and again in 2010 to determine clone performance over a number of sites.

The province, in partnership with the Manitoba Forestry Association, is responsible for delivering the Trees for Tomorrow program. This program is part of the Manitoba government's action plan to reduce greenhouse gas emissions by 2012. The Trees for Tomorrow program is committed to planting over one million trees a year in Manitoba over the next five years – for a total of five million. In 2008, a total of 260 110 trees (91 230 hybrid poplar and 168 880 conifer species) were provided to landowners for use in afforestation efforts. A total of 816 162 trees (362 536 hybrid poplar and 453 626 conifer species) were provided to landowners in 2009. A total of 1 392 315 trees (466 365 hybrid poplar and 925 950 conifer species) were provided to landowners in 2010.

The province is also cooperating with CON**FOR**GEN and CAFGRIS. Fifty litres of red ash (*Fraxinus pennsylvanica*) seed was collected from the northwest region of the province (The Pas area) and sent to the National Tree Seed Centre.

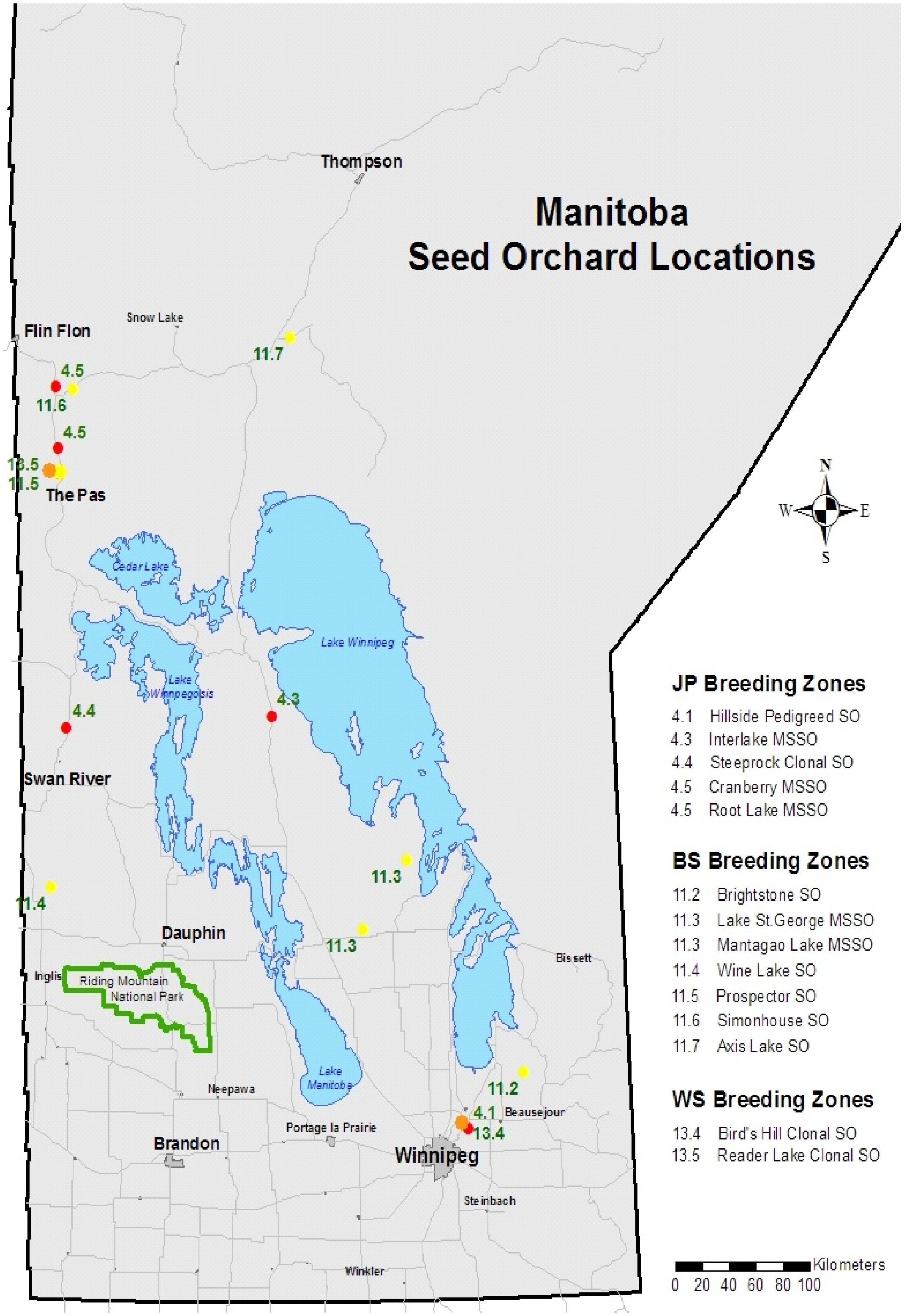

Figure 1. Map of seed orchard locations for the Manitoba tree improvement program.

GENETICS AND TREE IMPROVEMENT PROGRAM, 2008–2011 ALBERTA SUSTAINABLE RESOURCE DEVELOPMENT

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Keywords: Provenance studies, climate change, mountain pine beetle, conservation

This report summarizes the activities of the Forestry Division's genetics and tree improvement program for the period 2008–2011. A major focus of the program in this period has been on provenance studies of white spruce (*Picea glauca*); studies of variation in shoot phenology, water use efficiency, and genotype X environment interaction are expected to help guide deployment of this species on public land in the face of climate change. The eastward spread of the mountain pine beetle (MPB) has also had a large impact on the program in recent years; an extensive conservation effort has been underway since 2008 to collect seed and scion material from at-risk populations of lodgepole pine (*Pinus contorta* var. *latifolia*), jack pine (*P. banksiana*), whitebark pine (*P. albicaulis*), and limber pine (*P. flexilis*). The Alberta Forest Genetic Resource Management and Conservation Standards (FGRMS), developed primarily for commercial tree species, are now being applied to all woody species used in the reclamation of public land. This reflects the ongoing activity in the oilsands and the concomitant increased need for diverse types of reclamation material. The Alberta Tree Improvement and Seed Centre (ATISC) has initiated a floral induction study using gibberellin $A_{4/7}$ and girdling in white spruce. The aim is to increase seed production for oilsands reclamation in northeastern Alberta. Seed deployment on public land has been facilitated with the recent development of two map products: one facilitates wild seed and propagule deployment in the appropriate seed zone and the other, deployment of seed orchard seed in the approved controlled parentage program region (breeding region). Two long-term members of the Alberta genetics and tree improvement program have retired: Dr. Narinder Dhir, the founder of the program, retired in 2008 and Corrine Andriuk, the seed specialist, retired in 2010. Alberta Sustainable Resource Development and ATISC are grateful for their dedicated service.

PROVENANCE AND CLIMATE CHANGE RESEARCH

Shoot Phenology in the White Spruce Complex

We assessed variation for the dates of bud flushing and bud-set among 90 provenances of white spruce, Engelmann spruce (*Picea engelmannii*), and interior spruce from across western North America planted at Hay River near High Level, Alberta (59°08'N; 117°34'W; 370 m). This trial is part of the British Columbia-led genecology and climate change adaptation study of the white spruce complex. Bud flushing was scored as the number of days from May 1st to the date when the bud scale ruptured to expose green needles. Bud-set was assessed on a five point categorical score ranging from zero (no visible bud) to four (fully formed bud). Geographic and climatic trends were apparent only when data were analyzed by province to account for the complex topographic landscape of western North America. For example, within Alberta where geographic and climatic trends were the strongest, bud flushing on terminal and lateral shoots was negatively correlated with latitude of seed origin: r = -0.72 and r = -0.65, respectively. This indicates a tendency for provenances from high latitudes to open buds earlier than provenances from low latitudes. Bud flushing on terminal and lateral buds was positively correlated with elevation of seed origin: r = 0.64 and r = 0.60, respectively. This indicates a tendency for high elevation provenances to open buds later than low elevation provenances. The latitudinal and elevational effects may be confounded because, for Alberta, high elevation provenances are also southern provenances and vice versa. In terms of the Alberta climate, bud flushing on lateral shoots was positively correlated with growing degrees days above 5°C ($r = 0.54$). This shows that, provenances from warmer regions at mid-latitudes and mid-elevations opened buds later than provenances from cooler regions.

Within Alberta, bud-set on terminal and lateral shoots was negatively correlated with growing degree days (GDD): $r = -0.90$ and $r = -0.91$, respectively. This indicates a strong tendency for provenances from cooler environments to set buds earlier than provenances from warmer environments. Elevation and GDD of seed origin were also factors that explained variation in bud flushing and bud-set among provenances from British Columbia and Idaho.

Water Use Efficiency in White Spruce

We conducted a pilot study on water use efficiency (WUE) in white spruce provenances at two sites. Ten provenances were selected from locations with varying moisture stress based on the ratio of GDD and mean annual precipitation (MAP) to represent moist and dry seed sources. Likewise, the two test sites were selected to represent moist (Fort McMurray) and dry (High Level) environments. WUE was determined by analysing the ratio of stable carbon isotopes (^{13}C vs. ^{12}C) in two types of wood samples: 1) overall carbon isotopic content of a tree for the entire period of 27 years of field growth and 2) carbon isotopic content during the driest year identified by the size of growth rings. A larger content of the heavier ^{13}C relative to the lighter ^{12}C carbon isotope in the sample results in a high WUE indicating that the tree is able to undertake photosynthesis at low moisture levels. At both the dry and moist sites, and for both types of samples, provenances from drier environments had higher ^{13}C content than provenances from moister environments. Provenance variation for WUE was greater at the drier site than at the moister site. At the drier site, the correlations between 27-year diameter at breast height (D27) and ^{13}C were 0.84 and 0.66 for the type 1 samples and type 2 samples, respectively; the correlations between ^{13}C and 27-year height (H27) were 0.25 and 0.46 for type 1 samples and type 2 samples, respectively. This shows that, in a dry environment, provenances with high WUE had better growth than provenances with low WUE. On the moister site, H27 and D27 were poorly correlated with ^{13}C: $r < 0.15$ and $r = 0.24$, respectively. The correlation between ^{13}C and MAP of seed origin was moderately negative, $r = -0.30$ to -0.47, for both types of samples on both sites. ^{13}C was correlated with GDD, $r = 0.59$, only for the type 2 samples on the drier site. These correlations show a tendency for provenances from dry environments to exhibit greater WUE than provenances from moist environments. They also show that provenance variation in WUE is best detected in the driest period of the tree’s life.

Variation and Genotype X Environment Interaction

We studied variation among nineteen white spruce provenances in Alberta for growth potential (height and DBH) and optimum climate. Growth potential was assessed using time series data from eight provenance trial sites measured at three-year intervals beginning at age twelve and ending at age twenty-seven years. Optimum climates for individual provenances were derived from a second-degree polynomial regression of 27-year height on a test site climatic index developed from eleven climatic variables by principal component analysis. Rweyongeza et al. (2010a) published the results. Briefly, the results showed similar patterns of population differentiation between growth potential and optimum climate. In Alberta, white spruce populations appear to be differentiated into three main groups: 1) populations at high latitudes in the north, 2) populations in the Rocky Mountains in the southwest, and 3) populations in mid-latitudes and mid-elevations in the boreal forest. Rweyongeza (2011) used the climatic index developed earlier to analyze genotype X environment interaction (GE) for white spruce in Alberta using the same series of provenance trials. The additive main effect and multiplicative interaction (AMMI) analysis revealed a close association between provenances and sites with similar climates. Results on provenance variation and GE are being integrated into the Alberta guidelines for seed transfer across seed zones taking into consideration both present and anticipated future climates.

ALBERTA PINE SEED CONSERVATION PROGRAM IN RESPONSE TO MOUNTAIN PINE BEETLE

Alberta Sustainable Resource Development (ASRD) has been monitoring mountain pine beetle (MPB) in Alberta since 1977. According to ASRD's 2007 Mountain Pine Beetle Management Strategy, since the 1990s MPB has spread to west-central and north-western Alberta as its range expands due to a warming climate. Catastrophic long distance dispersal events took place in the summers of 2006 and 2009 resulting in an epidemic infestation of lodgepole pine, hybrid, and jack pine stands in north-western Alberta. ASRD declared MPB a pest emergency on April 11, 2007 and, with industry and other stakeholders, developed and implemented an aggressive control program to reduce infestation, contain spread, and salvage heavily infested stands.

The Alberta Mountain Pine Beetle Management Strategy prescribes the required activities and management responsibilities of ASRD, industry, federal agencies, municipalities, and landowners. However, because forest genetic resource management of MPB susceptible pines required specific consideration ATISC developed an action plan for MPB management in 2007. A main objective of this plan was to carry out conservation seed and scion collections for lodgepole pine, jack pine, whitebark pine, and limber pine.

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

For lodgepole pine, jack pine, and their hybrids, conservation seed collections were made based on regional geographic sampling and available genetic information. Selection of limber and whitebark pine populations for seed archiving and scion collections for grafting was based on regional representation and ecological classification because knowledge of adaptive genetic variation for these species in Alberta is limited.

To date, ATISC has completed 53 bulk and 67 single-tree lodgepole pine cone collections representing 35 seed zones. Whitebark and limber pine collections were done in collaboration with the Alberta Forest Health Section; ASRD Forest Areas; Alberta Tourism, Parks and Recreation; the Canadian Forest Service; and Parks Canada. In total, 190 single tree and 11 bulk whitebark pine collections and 265 single tree and18 bulk limber pine collections have been completed. Whitebark and limber pine collections are particularly costly as many of the stands can only be accessed by helicopter and cone crops must be caged prior to harvest to prevent predation by Clark's Nutcracker (*Nucifraga columbiana*). This necessitates visiting each collection site at least twice in a crop season.

In addition to the cone and scion collections, there are currently 2400 limber pine and 400 whitebark pine seedlings growing at ATISC which have been saved from seed germination tests. These seedlings were grown to test ATISC's propagation capability and are to be used for outplanting in select locations and for a pilot grafting program.

ATISC also played a role in ASRD's efforts to increase the operational pine seed inventory to support future renewal strategies in Alberta's MPB affected forests. Forest industry companies collected cones in conjunction with their harvest activities in priority seed zones where pine seed was needed as identified by ASRD. Forest industry proponents wishing to collect cones submitted proposals and, once approvals were issued, carried out collections for ASRD. Reimbursement costs for collections were set. ATISC staff conducted field checks, monitored cone quality, and received and registered the seed. Over 1800 kg of seed were collected.

Additional pine seed collections were carried out for ASRD in priority seed zones where harvest activities were not occurring or where forest companies were harvesting but not collecting cones. These collections were done through seed collection contracts administered by ATISC. Three ground collection contracts and 15 aerial collection contracts were awarded and completed resulting in over 960 kg of seed registered for use on public lands.

SHRUB SPECIES SEED COLLECTIONS

ASRD's Alberta Forest Genetic Resource Management and Conservation Standards (FGRMS) set the policy framework for reforestation and reclamation of public lands. While the standards were implemented primarily for trees, they were also developed with the broad understanding that reclamation of public land by non-timber industries would utilize plant materials other than seedlings of reforestation tree species. The FGRMS are currently being used to guide the collection and deployment of materials of all woody species used in reclamation to ensure that local adaptation and minimum genetic diversity requirements are met. To date, 100 seedlots representing 11 new shrub species have been collected and registered for public land use.

SEED DEPLOYMENT TOOLS

ATISC created two Provincial seed deployment tools in 2010 and 2011. The first is a map product that facilitates wild seed (Stream 1) deployment. The maps were created by intersecting seed zone line work with the line work from 1:250 000 scale digital National Topographic System (NTS) grids. Attributes such as the Alberta Township Survey (ATS), major roads, and communities were added as geographic references to assist the user with determining the correct seed zone deployment area for reforestation and reclamation purposes. The map products are available in 50 individual Portable Document Format (PDF) files based on NTS grid identification number for total provincial coverage.

The second deployment tool facilitates deployment of seed orchard seed (Stream 2) and consists of digital line work (shapefiles) for all 21 approved Controlled Parentage Program (CPP) Regions (breeding regions). Shapefiles are available for a total of 21 regions: nine white spruce, three black spruce, six lodgepole pine, one jack pine, one Douglas-fir (*Pseudotsuga menziesii*), and one western larch (*Larix occidentalis*). These regions were delineated based on a combination of genetic and ecological information and forest management agreement boundaries.

More detailed information describing seed zone and CPP Regions is available in the FGRMS manual. The manual and digital line work for Stream 2 material is accessible online on the ASRD website or can be obtained free of charge by contacting ATISC at phone number (780) 656-5072. The stream 1 map products are completed but are not available online at this time.

SEED ORCHARD RESEARCH

A flower induction trial using three dosages of gibberellin $A_{4/7}$ applied alone or in combination with non-destructive girdling is being implemented in the Region E white spruce clonal seed orchard in the spring of 2011. The Region E and E1 orchards both provide white spruce seed for the northeast boreal lowlands and, in previous years, naturally occurring seed production in these orchards has been sufficient to meet demand. However, Region E1 is located in the heart of oilsands activity and recently several oilsands companies have become cooperators in the project. Consequently, demand for seed has increased. To meet this need, the E1 orchard is being expanded and the flower induction trial is being undertaken to develop a protocol for enhancing flower production in both of these orchards.

PLANT PROPAGATION

In 2009 and 2010, ATISC produced almost 60 000 seedlings for research and conservation purposes. About 11 000 seedlings were grown for two jack pine progeny trials to be established in 2011 and almost 5000 lodgepole pine, whitebark pine, and limber pine seedlings were grown for rootstock for grafting as part of MPB and white pine blister rust conservation programs. Seedlings were also grown for Arbor Day activities and for the Alberta Junior Forest Wardens program.

Due to the presence of *Pythium* spp. in the greenhouse water supply and *Fusarium* spp. in the soil, controlling root disease has become a major concern particularly in the greenhouse-grown two-year- old seedling stock. To curtail root diseases, greenhouse trials are being conducted with different soil mixes, container types, and rearing regimes.

About 1200 grafts were produced over the report period. The majority were grafts of lodgepole pine selections made as part of the conservation program funded by the MPB initiative.

RETIREMENTS

Dr. Narinder Dhir retired in 2008 after more than 30 years of guiding the progress of tree improvement in Alberta. Narinder was hired by the Alberta Forest Service, Timber Management Branch in 1975 to develop a genetics and tree improvement program for the province. At the program's inception, he was the only staff member and had no facilities. Under his direction, Alberta's tree improvement program has become very well established. Both government and industry led programs are making good progress: a network of field test sites, both government and co-operative, has been established across the province; and several seed orchards are now producing genetically improved seed for reforestation. Currently, most of the program delivery is done from the Alberta Tree Improvement and Seed Centre at Smoky Lake, a complex of greenhouses and laboratories with 50 ha of irrigated field space dedicated to clone banks, field trials, and seed orchards. It is also home to the reforestation seed bunker, which houses Alberta's supply of reforestation seed. During his tenure, the Alberta Forest Genetics Resource Council was established to promote dialogue between academics, government, industry, and other stakeholders; the Forest Genetic Resource Management and Conservation Standards, which regulate management of tree gene resources on provincial Crown lands, came into effect; the Genetics and Tree Improvement Unit took over management of reforestation seed storage and registration; and the Forest Genetics Association of Alberta was inaugurated to address the needs of smaller forest companies carrying out tree improvement and research work. Through all the administrative and scientific challenges over the years, Narinder was always the strongest and most determined advocate for maintaining a viable tree improvement program within the Alberta provincial government. We wish Narinder the very best and trust his retirement will be as productive as his career was with Alberta Sustainable Resource Development.

Corrine Andriuk, the Tree Improvement and Seed Centre's seed and wood lab scientist, retired in 2010. Corrine's career at the Centre began in 1978 when the facility was known as Pine Ridge Forest Nursery. From 1991 until her retirement she was a mainstay of the Centre's seed and wood program providing the support and data necessary for the various tree improvement programs to progress. She shepherded countless research seedlots through the registration, testing, and storage process and was much practiced in counting tree rings, measuring wood fibre lengths, and assessing wood density on superior tree wood samples. Her days of counting (i.e., cones, germinants, tree rings…) at the Tree Improvement Centre may be over but local community organizations are now benefitting from her attention to detail. We wish Corrine a happy and healthy retirement.

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WESTERN FOREST PRODUCTS – SAANICH FORESTRY CENTRE ORCHARDS

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Keywords: seed orchard, supplemental mass pollination, parent tree

Western Forest Products Inc. (WFP) delivers high quality seed to our forest regeneration programs and makes surplus seed available to other licensees operating in the Maritime Seed Zone of British Columbia. Seed orchards for four conifer species are managed at the Saanich Forestry Centre: western redcedar (*Thuja plicata*), coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*), western hemlock (*Tsuga heterophylla*), and Sitka spruce (*Picea sitchensis*). Yellow-cedar (*Chamaecyparis nootkatensis*) hedges are managed as well. The annual planting program averages 7.5 million seedlings to tenures all across Vancouver Island, the Queen Charlotte Islands, and the mainland coast from Powell River to the North Coast.

Orchard management techniques that are employed include orchard roguing, ramet replacement, and supplemental mass pollination to produce seed of greater genetic quality. Techniques for promoting increased seed quantity include 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 of Forests and Natural Resource Operations, the Universities of British Columbia and Victoria, Canadian Forest Service, and associated consultants and contractors. Input to gene resource management policy development in British Columbia is provided.

WESTERN REDCEDAR

The western redcedar orchard delivered a seed crop in 2009 for more than 1.7 million seedlings with volume gain of 16%. Roguing, based on new scores received from the breeder, moved the genetic worth of this orchard forward rapidly. Replacement of the rogued ramets is underway. The average breeding value of the orchard is currently 18. Western redcedar comprises about 35% of the Company's annual planting program.

COASTAL DOUGLAS-FIR

The 2009 Douglas-fir cone crop was phenomenal, given that the fully productive ramets number about 247 in this low-elevation orchard. Seed for 2.6 million seedlings with a genetic worth of 11 was harvested. Upgrading and replacement of the retiring Sechelt orchard, established at the Saanich Forestry Centre, includes the addition of 1322 ramets with a breeding value of 19. This capacity should meet the needs for low-elevation Douglas-fir when this orchard comes into full production. Third-generation selections are now available from the coastal Douglas-fir breeding program and are being incorporated into the orchards. With these selections, deployed seed crops will deliver more than 20% volume gain at rotation. In the interim, WFP purchases and deploys seed from other producers.

A second-generation high-elevation Douglas-fir orchard was established which replaces the mature first-generation orchard at the Sechelt orchard site at the Saanich Forestry Centre. The breeding value of the clones in this orchard is 13. Low- and high-elevation coastal Douglas-fir comprise about 35% of the annual planting program

WESTERN HEMLOCK

The low-elevation second-generation western hemlock orchard at Saanich Forestry Centre produces seed for 14% volume gain at rotation. With seed use for this species significantly reduced in recent years, the backlog of banked orchard seed is being deployed. This orchard will be upgraded by roguing. When banked seed supplies of reasonable quality run low cone harvests will resume.

A high-elevation second-generation western hemlock orchard is under development at this time. The breeding value of the clones in this orchard is 11.

SITKA SPRUCE

The weevil-resistant Sitka spruce orchard at Saanich Forestry Centre produced a good crop in 2010, with seed for 3.8 million seedlings. This orchard seed delivers 85% weevil resistance to regeneration programs. Though volume gain scores have not been developed for this orchard, seedlings from this seed demonstrate very good growth when grown together with non-resistant stock on sites where weevil hazard is low or nil.

YELLOW-CEDAR

Western Forest Products initiated clonal evaluation of yellow-cedar cuttings more than two decades ago. At present, the Saanich Forestry Centre is delivering yellow-cedar cuttings to reforestation programs with a projected volume gain at rotation of 20%. The selected clones were assessed for rooting and the better rooters were included in the deployment strategy. Because of the rooting challenge and the higher nursery cost to produce a steckling using this strategy, a significant portion of the reforestation program has been served by wild seed in recent years.

Cuttings were selected for reforestation programs when it was realized that seed orchard production in yellow-cedar was not reliable in low-elevation, warm, dry environments, wild seed crops were costly and sporadic, and seed stratification and germination was challenging. Significant progress has been made in seed stratification techniques in recent years and new information regarding pollen viability and management techniques is becoming available, indicating the possibility of orchard seed production in other settings. Western Forest Products will participate in projects to deliver seed from woods-orchards, using selections from both Ministry and company clonal programs as parent trees.

BC MINISTRY OF FORESTS, LANDS AND NATURAL RESOURCE OPERATIONS (MFLNRO) TREE IMPROVEMENT BRANCH

Brian Barber, Mike Carlson, Charlie Cartwright, Lee Charleson, Diane Douglas, Barry Jaquish, John King, Dave Kolotelo, Matt LeRoy, Sylvia L'Hirondelle, Leslie McAuley, Greg O'Neill, David Reid, John Russell, Michael Stoehr, Ward Strong, Chang-Yi Xie, Alvin Yanchuk and Susan Zedel

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BC's FOREST GENETICS CONSERVATION AND MANAGEMENT PROGRAM

Brian Barber

In 2010, following several government reorganizations, staff working in tree breeding and forest genetics research were assigned to Tree Improvement Branch (TIB), which has responsibility for tree seed policy, planning, extension, information systems, seed orchards, and the provincial tree seed centre. This amalgamation was relatively seamless, as our respective groups have worked closely together for decades. TIB now consists of 55 full-time staff and dozens of seasonal auxiliary staff with an annual budget of ~$ 9 million.

Strategic direction and recommendations for public investments in forest genetics research and operations continue to be provided by the Forest Genetics Council of BC (FGC). The FGC is a multi-stakeholder advisory body reporting to the Chief Forester, with representatives from the Ministry of Forests, Lands and Natural Resource Operations (MFLNRO), forest licensees, universities, and Natural Resources Canada. The goals and objectives of FGC aim to enhance the conservation, resilience, and value of BC's forest genetic resources.

FGC's objectives for increasing the amount and genetic worth of select (orchard) seed used for reforestation were recently incorporated into MFLNRO's Annual Service Plan performance measure for maximizing productivity of our forest resources. Currently over 50% of the 200 million trees planted annually in BC are grown from select seed with an average genetic gain of 15%. The use of select seed in 2011–12 will produce an additional 4 million m^3 of wood at 65 years, which represents over half the timber volume gain attributed to silviculture investments made through the Land-based Investment Program.

The economy, diminishing royalties from the forest sector, and increasing demands for other public services continue to put pressure on MFLNR's budget. However, funding and support for forest genetics research and operations within the Ministry and the private sector remain in relatively good shape. We are therefore still on target to increase the amount of select seed used to 75%, with an average genetic worth of 20%, by 2020, while also advancing climate-based seed transfer.

POLICY AND DECISION SUPPORT UNIT

Lee Charleson, Diane Douglas, Matt LeRoy, Leslie McAuley and Susan Zedel

A number of initiatives were reviewed and supported by policy and decision support staff. A review of the draft BC Forest Offset protocol document was undertaken to ensure forest offset protocols related to carbon sequestration and tree improvement (use of select seed) made appropriate references to seed use, including linkages to Forest and Range Practices Act legal requirements.

In timber supply, decision support services for the verification of genetic gain assumptions in timber supply data packages, analyses, and/or rationale reports were undertaken for several management units. Support was also provided to Forest Analysis and Inventory Branch in the form of rationale writing for the Kalum Timber Supply Area.

In climate change adaptation projects, extensive support was given to the MFLNRO Climate Change group – Forest and Range Practices Act policy assessment project.

Ongoing work in information system and extension activities included Seed Planning and Registry (SPAR) support to clients is ongoing throughout the year, with the busiest time between September and February. Eight SPAR training sessions on specific functions were conducted via Live Meeting from September to November 2010. Other extension projects include Extension Technical Advisory Committee and TIB projects.

New initiatives led by policy and decision support included Mountain Pine Beetle seed planning impact and opportunity mapping and new a policy for western larch seed use.

The TIB recognizes the need for spatially-enabled decision support tools to support seed planning activities such as cone collection and seed inventory management (http://www.for.gov.bc.ca/hti/seedplanning/MPB_Impact_Opportunity.htm). The results of the decision support project undertaken in 2010–2011 are intended to support both strategic and operational level seed planning activities. New decision support tools include: 1) an integrated MPBBIO geodatabase with seed planning spatial data sets, 2) a set of 'MPB Seed Planning' interactive PDF maps, and 3) Cone Collection Opportunity maps for two management units (Williams Lake and Quesnel Timber Supply Areas).

In June 2010, amendments to the *Chief Forester's Standards for Seed Use* were made to provide resource managers and forest practitioners the flexibility, innovation, and adaptive capacity to expand western larch through limited seed use beyond its contemporary range across B C. See link http://www.for.gov.bc.ca/code/cfstandards/amendmentJun10.htm.

These amendments, which came into effect on October 3, 2010, are based on recent scientific research (Rehfeldt and Jaquish 2010) that uses a bioclimate approach to match seed sources with areas projected to be climatically suitable in the future. A policy approach and risk assessment framework, which was further modified based on stakeholder review and input, was led by TIB staff and developed by a multi-disciplinary team.

The intended outcome of the policy initiative is to maintain or enhance ecosystem resilience and forest productivity, reduce tree species vulnerability (through increased tree species diversity), and to improve the chances that tomorrow's plantations are well adapted to the future climate.

Extension work about the new policy was made at field meetings, on-line training sessions, poster sessions at meetings, and in a "Forestry Team in Action" report in the B.C. Forest Professional magazine. For the sowing year ending in 2011, 330 thousand western larch were requested for the LW1 zone and nine thousand western larch were requested for the LW2 zone.

SEED PRODUCTION

David Reid

TIB operates six seed production sites around the province and has managed seed orchards since 1963. These sites produce approximately 40% to 50% of the orchard seed in BC and contain 40 seed orchards of nine species with approximately 50 000 ramets. A major expansion in the TIB orchard program was completed in 2010. This involved the purchase of 15 ha of new orchard land adjacent to the Skimikin Seed Orchard, the first land purchase for a TIB seed orchard in 30 years. Ten hectares of forested land was also cleared at this site for another seed orchard. The overall expansion amounted to an additional 16 000 ramets added primarily to the three orchard sites at Vernon (Kalamalka), Bailey Road, and Skimikin. Most of these additions were lodgepole pine (*Pinus contorta*), however, the first third-generation Douglas-fir (*Pseudotsuga menziesii*) seed orchard was established at the Saanich Seed Orchard site on Vancouver Island. One white spruce (*Picea glauca*) orchard was removed at Kalamalka as it had consistently produced such large volumes that a 25-year inventory of seed exists for that particular seed planning unit. A second-generation replacement orchard will be established there in a few years once breeding and testing is completed.

Seed production over the last three years was 865 kg in 2008, 718 kg in 2009, and a record crop of 1612 kg in 2010 from seven species in 24 TIB orchards. The 2010 crop is sufficient seed to produce over 233 million seedlings. To date, TIB orchards have produced well over 13 000 kg of seed since production started in 1974.

The TIB seed production group is operated on a cost recovery basis which means that seed sales to clients are used to pay for salaries and operating costs of the group. In 2010–11, TIB conducted a major cost exercise to update the Chief Forester's Surplus Seed Price Schedule. This schedule was last revised in 2003, so seven years of data were analyzed in the review. As a result, prices for five species stayed the same, four species were increased, and one species was reduced. The price schedule can be found on our Branch web site (www.for.gov.bc.ca/hti).

TREE SEED CENTRE

David Kolotelo

Since the last members report, Dale Simpson and I have produced six editions of the Tree Seed Working Group News Bulletin, numbers 48, 49, 50 (Seed Testing), 51, 52 (Genetic Conservation), and 53. The Newsbulletin reaches over 250 people worldwide and we are always looking for contributions relevant to tree seed science and technology. In celebration of our 50th anniversary of the Tree Seed Centre I edited the publication "Excellence in Cone and Seed Services" that can be found on this webpage http://www.for.gov.bc.ca/hti/publications/tsc/TreeSeedReport_web_August.pdf .

I continue to chair our Genetic Conservation Technical Advisory Committee. Personal efforts in this area involve reporting and planning our *ex situ* collections, investigating the seed storage behaviour of bigleaf maple (*Acer macrophyllum*), and attempting to improve germination in whitebark pine (*Pinus albicaulis*). I participate in other *ad hoc* committees or meetings as requested.

In 2009 I took over supervision of our testing area at the Tree Seed Centre. We completed a Testing Operating Manual and are currently reviewing all of our Quality Assurance programs. Program summaries have been completed for our fungal assay program, moisture content retesting, pellet assessment, and unkilned seed moisture content testing. Based on stratification trials performed in our lab we are changing our methods for yellow cypress (*Callitropis nootkatensis*) to improve germination. Ongoing activities include being a provincial resource for cone and seed related activities and continuous improvement activities at the Tree Seed Centre.

TREE BREEDING

Program Changes in Tree Breeding and Forest Genetics (Alvin Yanchuk)

After an 80-year presence in forestry in BC, the Research Branch of the BC Forest Service (BCFS) was terminated in November 2010. Many factors led to the unfortunate decision, ranging from large budget pressures in the Provincial budget, large reduction in forestry revenue, and many years of down-sizing (including a more recent one in 2009–10) in the Branch. Fortunately, the remaining components of the Forest Genetics Section were moved into TIB, which now brings all components of the tree breeding, policy, and delivery of improved seed into one Branch. This, in the long run, should put the breeding program back on a better foundation, rather than the trajectory it was on, and hopefully this will enable us to again develop into a world-class tree breeding agency.

The program lost some substantial intellectual capacity over the last few years, to the downsizing mentioned above. Drs. Mike Carlson and John King 'decided' to retire in 2010, and Jodie Krakowski was moved to another position in government. Several technical staff also retired, or had their positions made redundant; Doug Ashbee, Dave Ponsford, Helga Mehl, and Ian Cairns were all key members of the forest genetics and tree breeding group, and their experience and dedication will be missed. Alvin Yanchuk stepped down as Manager of the Forest Genetics Section in early 2010, and Barrie Phillips took over as Section Manager for Forest Genetics, in the TIB. Alvin also took a one-year leave of absence from the BCFS (Aug 2010 – July 2011) and worked in the genetics and tree improvement group at Scion (Forestry Research Institute), New Zealand.

Coastal Douglas-fir (Michael Stoehr)

In the spring of 2010, the fourth and last series of advanced generation tests was planted. This concludes the establishment of this phase of genetic testing and selection for coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). In each of the first three series, 4 GCA (polymix) tests and two complimentary full-sib family tests were carried out, testing a total of 271 parents and 305 full-sib families, respectively. In Series 4, 160 full-sib families were planted on four sites. Selections were made in the full-sib blocks for Series 1 and 2 with genetic gains averaging 25% for tree volume at rotation age. In a separate experiment, realized genetic gain tests were analyzed based on age 12 data. Expected levels of gains were observed for the mid-gain genetic groups (10% gain) and the top-cross entries (18% gain) planted at four spacings across five sites in coastal BC. In stem volume per tree, gains observed were greater than anticipated due to the added effect of increased diameter growth. No spacing x genetic entry interactions were found.

Western Redcedar (John Russell)

Management of the western redcedar (*Thuja plicata*) gene resource, a major component of BC's coastal mixed-coniferous forests, is in its infancy. The program had its beginnings in 1990 focussing on gene conservation and genecology. A tree breeding program was initiated in 1996. Tree breeding for western redcedar 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 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 have been estimated from all seven series based on 7- to 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 selfing.

Heartwood durability The original 350 BC parent trees selected in the early 1970's (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 CLRS clonebanks and industrial seed orchards. Fifty selections based on tropolone and lignan concentrations have been cloned and established in an advanced generation breeding orchard.

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 selfing. Early selection for foliage monoterpenes has resulted in approximately 100 forward selections for high needle monoterpene concentration.

The development of a durable population for resistance to known and unknown pests under a changing climate is ongoing involving the above populations. Genecological data and climatic modelling will assist in population structure and deployment.

Yellow Cypress (John Russell)

Yellow cypress 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-cedar propagates vegetatively in nature and, in addition, cone crops are usually infrequent and seed viability and germination, historically poor. Tree improvement has focussed on a clonal strategy. Twelve-year data from over 20 trials (Western Forest Products Ltd. and BCFS) have been collected and clonal genetic values from approximately 5000 clones have been estimated and 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 on improving seed production efficiencies in clonal trials established in native yellow cypress ecosystems is ongoing.

Western Hemlock and True Firs (Charlie Cartwright)

Though western hemlock (*Tsuga heterophylla*) is still a mainstay of the coastal forest industry harvest, most areas are left for natural regeneration. For this reason the forest genetics program has been de-emphasized. Current efforts are focused on deriving value from field trials already established. Breeding values from progeny test series established 10 years ago are being calculated and reports based on genecology installations and realized-gain trials will be forth coming over the next several years.

Since true firs are not easily managed in seed orchards, the genetics programs for them have looked at establishing seed transfer guidelines and identification of superior provenances for seed collection. With grand fir (*Abies grandis*) and noble fir (*A. procera*) recent efforts have involved grafting most productive, best form, range-wide materials from older test series for conservation. For sub-alpine fir (*A. lasiocarpa*) and Pacific silver fir (*A. amabilis*) provenance test series have been recently established and preliminary data are being collected.

Broadleaves (Chang-Yi Xie)

Red alder Commercial planting of red alder (*Alnus rubra*) is increasing, seed orchard establishment is being planned, and a moderate breeding program is under consideration. To assist seed orchard management and tree breeding, a red alder reproductive biology study has been initiated to provide information on male and female flower production, and timing of pollen shed and female receptivity for each clone in the clone bank and to develop pollen collection, processing, storage, and viability testing methodologies. Polycrossing of high breeding value clones will be completed in 2012 and second-generation progeny tests will be established in 2013–2014.

Black cottonwood Growth and health condition of 488 black cottonwood (*Populus trichocarpa*) clones from 30 populations (drainages) across the entire range in BC were examined in a provenance-clonal trial on the northern coast near Terrace. Three-year test results indicated that the southern populations were 87% taller than and twice as healthy as their northern counterparts. Regional differences accounted for most of the detected non-random variation with 55% in height and 70% in health condition, and demonstrated an ecotypic mode of differentiation between the two regions as previously observed in a common-garden test in the south at Surrey, near Vancouver. Of the 30 fastest growing clones free of any damage, 22 were from the southern region while only three were from the northern region. Performance was very consistent between the two sites at both population and clonal levels. The four tallest clones at the Terrace site, two from the Bell-Irving drainage in the north and two from the Nechako River drainage in the interior, were also the tallest at Surrey. Their 3-year heights are 2.5 to 3.5 and 5 to 12 times greater than their provenance means, respectively. Findings from this study provide further evidence for restrictedgene flow between the regions. The practical implications of those findings were also significant.

Black cottonwood in BC is much more abundant and extensive along the river drainages north of the no-cottonwood belt. It is unlikely that black cottonwood can be managed as a viable source of wood fibre in the province without expanding the land base to the northern coast. It is clear that the southern sources have superior photoperiodic response and using them for reforestation can greatly improve both the adaptedness and productivity of the plantations on the northern coast. The consistency of population performance between the two sites at Terrace and Surrey suggests that the entire coastal region can be treated as one seed planning/clonal deployment zone and the same seed sources or clones can be used for plantations across the entire region. Lack of genotype x environment interaction should also lower the cost of genetic improvement of the species.

Big leaf maple First assessment of height and diameter growth as well as health conditions was completed and data analysis will be done over the winter (2011–2012). There are four test sites, two on Vancouver Island (Skutz Falls and Sayward) and two on the mainland (Powell River and Carey Island). About 4500 trees of 400 families from 43 provenances, distributed across the entire natural range of the species, were planted at each site.

Interior Spruce (Barry Jaquish)

The BC interior spruce (*Picea glauca* x *engelmannii*) 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 40 years old. To date, the second-generation testing program includes 753 full-sib families and seven test sites. Through the 2008–10 reporting period, 140 trees from the East Kootenay and Prince George second-generation tests were selected, grafted, and established in holding beds in the Vernon area.

Western larch (Barry Jaquish)

Western larch (*Larix occidentalis*) seed orchards are in full production and provide improved seed for the annual planting of approximately six million seedlings. Second-generation breeding is nearly complete for each of the two western larch seed planning units. The highlight of the western larch program over the last two years is the realignment of the two seed planning zones to allow limited planting outside, and primarily northwest, of its natural range to account for expected climate change, to facilitate assisted migration, and to increase tree species diversity in the central Interior. Zone realignment was based on results from research that predicted the contemporary distribution from climate variables, genetic variation from provenance tests, and projections of future presence and absence and genetic variation provided by three global circulation models and two scenarios (Rehfeldt and Jaquish 2010).

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 20–25 years-old, seed orchards are in production, and second-generation breeding is nearly complete in high priority zones. In 2010, 35-year height and diameter were measured in the Trinity Valley range-wide provenance test. Progeny tests in three breeding zones (Shuswap Adams, Mt. Robson, and Prince George) were maintained and measured.

Lodgepole Pine (Nick Ukrainetz)

The lodgepole pine breeding program continues to focus on volume as the primary trait of interest for second generation breeding. Second-generation (F1) progeny tests have been established for five seed planning zones and we continue to gather information about secondary traits for insect tolerance and resistance, disease resistance, and wood quality. Results from surveys of open-pollinated progeny tests reveal patterns of heritable genetic variation for resistance to mountain pine beetle (*Dendroctonous ponderosae*), western gall rust (*Endocronartium harknessii*), and comandra blister rust (*Cronartium* *comandrae*) and data for these and other secondary traits will be incorporated into third-generation breeding to create more resilient breeding and production populations.

Interior Western White Pine (Nick Ukrainetz)

The western white pine (*Pinus monticola*) breeding program has two breeding zones: coast and interior. Information from blister rust screening was used to select parents to create an F1 population for field testing in the coastal breeding zone. Three series of F1 progeny tests have been established and will be used to generate information about disease resistance and growth under natural inoculation conditions. Plans are underway to establish a set of F1 progeny tests for the interior breeding zone.

Interior Hardwoods (Nick 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*). Results from genecology testing suggest that three breeding zones are appropriate for British Columbia east of the Coastal Mountains. There were significant patterns of heritable genetic variation for volume and several forward selections have been made and grafted into a seed orchard for operational seed production.

FOREST GENETICS, SEED TRANSFER AND PHYSIOLOGY

Climate Change (Greg O'Neill)

The Assisted Migration Adaptation Trial (AMAT http://www.for.gov.bc.ca/hre/forgen/interior/AMAT.htm) is a long-term multi-species field trial intended to provide a better understanding of tree species' climate adaptation. The trial involves 48 seed sources (mostly orchard seedlots) from 15 species that will be grown at 48 field sites in BC, Yukon, and neighbouring US states. Twelve sites are being established each year for four years. The field set of 12 sites will be planted in spring 2012. Their growth and health will be monitored every five years, beginning in 2013, and related to the climate of the plantations, enabling researchers to identify the seed sources most likely to be best adapted to current and future climates. The information will be used to revise BC's species and seed source selection guidelines, helping to ensure maximum health and productivity of BC's planted forests well into the future.

Adaptive Physiology (Sylvia L'Hirondelle)

The Adaptive Physiology research program helps forest geneticists and forest nurseries produce stress-resistant trees with high growth potential by testing genetic variation in frost hardiness, photosynthetic potential, and phenology, as well as heat, drought, and other stress tolerances. Recent work has included: 1) development of physiology tools to estimate population responses to a variety of climates, to assist in seed transfer under potential climate change conditions, 2) studying the relationship between patterns of genetic variation in adaptive traits and site ecology in populations of conifers such as *Picea glauca* x *engelmannii* and *Abies lasiocarpa*, and 3) improving the speed and accuracy of tests to predict overwinter storability of seedlings, field performance potential, and balanced seedling growth of major conifer species used in reforestation.

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FOREST GENETICS AT THE FACULTY OF FORESTRY, THE UNIVERSITY OF BRITISH COLUMBIA, 2008–10

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GENETIC CONSERVATION, POPULATION GENOMICS AND CLIMATE CHANGE

S.N. Aitken

In the Centre for Forest Conservation Genetics, we continue to explore the genetic structure, diversity, and capacity for adaptation to new climates of indigenous tree species using a wide range of methods. We continue to explore the genetic basis of local adaptation to climate using genomic tools. Jason Holliday used a population genomics approach to identify 35 SNPs associated with bud set timing and cold hardiness in Sitka spruce (*Picea sitchensis)* (Holliday and Aitken 2010)(Treenomix Project, Genome Canada and Genome BC). He also used re-sequencing data to detect the genetic signature of a series of successive bottlenecks resulting from serial postglacial recolonization (Holliday et al. 2010). Formerly a PhD candidate in our group, Jason is now an Assistant Professor at Virginia Tech. About half of the adaptive SNPs associated with climate-related phenotypic variation showed clinal variation across the species range in small samples of seedlings. Nina Lobo (MSc student) validated many of these clines in large samples of mature trees from across the species range. PhD candidate Jill Hamilton is using candidate SNPs from Sitka and white spruce (*Picea glauca*), microsatellite markers, and phenotypic variation to characterize the *P. sitchensis x P. glauca* introgression zone. In a similar project, PhD candidate Amanda de la Torre Cuba is characterizing the genetic structure and degree of introgression in the elevation-dependent hybrid zone between *P. glauca* and Engelmann spruce (*P. engelmannii*). She will also be evaluating the extent to which selection in the breeding program for the interior spruce species complex alters hybrid index. This research is funded primarily by Natural Sciences and Engineering Research Council (NSERC) and Genome BC.

We have completed studies of phenotypic and genotypic variation within less well studied indigenous tree species, funded primarily by the Forest Genetics Council of BC. Sierra McLane (PhD) completed her assisted migration experiment with the threatened species whitebark pine (*Pinus albicaulis*), establishing seed-based common gardens north of the current species range in subalpine areas predicted to be climatically appropriate with quite good early success (McLane and Aitken, in press). In her MSc research, Karolyn Keir found Pacific dogwood (*Cornus nuttallii*) to be relatively genetically depauperate based on nuclear microsatellite markers and chloroplast DNA sequence diversity (Keir et al. 2011). Despite this lack of genetic diversity, Jordan Bemmels found evidence for weak to moderate local adaptation in this species in a common garden experiment (BSc thesis; Keir et al. 2011). Colin Huebert (MSc student) found moderate local adaptation in a provenance experiment of British Columbia's only native oak species, Garry oak (*Quercus garryana*).

We are increasingly focused on predicting the effects of climate change on native tree species and developing strategies to mitigate these effects. Dr. Tongli Wang has been modeling current and future distributions of native species and ecosystems, as well as developing new analytical methods for using provenance trial data to predict the effects of climate change on populations (Wang et al. 2010). He has also expanded the ClimateBC software to western North America (ClimateWNA). Sierra McLane used these tools combined with dendrochronology to dissect genetic and climatic components of responses in growth rings of lodgepole pine (*Pinus contorta*) in the Illingworth provenance trial (McLane et al. 2011a, b). Dr. Pia Smets has been testing the physiological and growth responses of populations of several conifer species to temperature and drought. This research is funded primarily through the Forest Genetics Council of BC.

Many of the diverse objectives and approaches we have been using will be used in a large-scale genomics project entitled “AdapTree: Assessing the adaptive portfolio of reforestation stocks for future climates”, a new $4.7 million project funded by Genome Canada, Genome BC, and co-funding organizations (Project Leaders S. Aitken and A. Hamann (U of Alberta); Co-applicants J. Holliday, L. Rieseberg, R. Kozak, M. Whitlock, T. Wang, and D. Davidson). This project will integrate transcriptome sequencing, population genomic approaches, common gardens, and geospatial and climatic modeling approaches to address issues of genetic diversity and climate-based seed transfer in lodgepole pine and interior spruce in BC and Alberta, and will be the central focus of our research program for the next three years. Ian MacLachlan and Susannah Tysor will have PhD research projects associated with the AdapTree project and several new postdoctoral fellows and technicians will be joining our group.

Sally Aitken received the Canadian Institute of Forestry’s Scientific Achievement Award in 2009 and the UBC Killam Teaching Prize in 2010.

APPLIED FOREST GENETICS AND BIOTECHNOLOGY

Y.A. El-Kassaby

During the 3-year period, six graduate students successfully defended their dissertations. These include four Masters: 1 - Genealogical relationship among members of selection and production populations of yellow-cedar (*Callitropsis nootkatensis*) in the absence of parental information (Nasim Massah), 2 - Metabolite variation in ecologically diverse black cottonwood, *Populus trichocarpa* Torr. & A. Gray (Manal A. Fayed), 3 - Evaluating interior spruce genetic resources management practices through GIS-based tracking of seed deployment over time in British Columbia (Chen Ding Klaus), and 4 - Pollination dynamics in an advanced generation Douglas-fir seed orchard (Ben S.-K. Lai) and two Doctorates: 1- Molecular genetic diversity among natural populations of *Populus* (Mohamed Ismail) and 2 - Forest biomonitoring, biosecurity and DNA barcoding (Jeremy R. deWaard). At present, two graduate students are in the final stages of completing their dissertations (Irena Fundova’s Masters deals with *in situ* wood quality assessment in interior spruce and Tomas Funda’s Doctorate investigates the genetics of advanced generation seed orchards). Joseph Kess is well underway on his MSc study on gene flow in a Douglas-fir (*Pseudotsuga menziesii*) seed orchard. New PhD student Yang Liu is also joining the program. He completed his MSc with the Chinese Academy of Sciences and will be studying the molecular aspects of seed pretreatments. Two undergraduate students joined our group as summer students working on wood quality of western larch and comparative assessment of reforestation stocks.

Work on the Genome BC biofuel grant is progressing well and Dr. Hao Boa (PDF) has completed a study on genome-wide analysis of alternative splicing in *Populus* populations and Dr. Jaroslav Klapste (PDF) is analyzing the poplar feedstock project’s association genetics. This project has been extended to include balsam poplar and is shifting from xylem transcriptome to genome-wide analysis. The project is successfully funded by Genome Canada (Douglas, Mansfield et al.).

My independent research (funded by NSERC Discovery grant) focuses on the “Breeding without Breeding” concept with collaborative work with Drs. C. Liewlaksaneeyanawin (Thailand), M. Lstibůrek (Czech Republic), and E. Cappa (Argentina). Additional accomplishments include the publication of “Forests and Genetically Modified Trees” by the Food and Agriculture Organization of the United Nations, development of a user friendly seed orchard design computer program (in progress), and meeting my research obligation to my NSERC-Industry Research Chair.

Finally, this term is crowned by being the recipient of the IUFRO 2010 “Scientific Achievement Award” and being approved by the IUFRO Board to serve as the Co-coordinator of Division 2 (Tree Physiology and Genetics).

FORESTRY GENOMICS AND PLANT POPULATION GENETICS

Kermit Ritland

With Joerg Bohlmann (Micheal Smith Laboratory, UBC), I hold a Genome BC/Canada genomics project involving spruce. This involves several collaborations with Sweden, Scotland, and California, as well aswith colleagues at UBC (Sally Aitken, Shawn Mansfield, and Paul Wood). Sally Aitken and her student, Jason Holliday, have documented nucleotide variation at many genes putatively involved with adaptation.

Shawn Mansfield's post-doc, Rebbeca Dauwe, has mapped 150 metabolite QTLs and reconstructed pathways based upon these patterns in a 2x3 factorial design. Paul Wood is involved with GELS activities that research public perceptions about forest genomics. Post-doc Ilga Porth is currently finishing a study of 22 800 expression QTLs in the same experiment. In Scotland, our collaborators are developing SNPs for molecular breeding of Sitka spruce and have relied upon our EST-SSRs for initial work. Currently we are co-ordinating with the Quebec project to find additional SNPs. At Umëa, Sweden, Rosario Garcia-Gil has been working at developing a molecular marker map for Scots pine (*Pinus sylvestris*) and visited us for three months in early 2008 for this purpose. Further work involving Illumina assays is ongoing. In California, David Neale has collaborated with us in supplying candidate genes for resistance to the white pine weevil (*Pissodes strobi*) in spruce, as well as markers that can provide a syntenic map of the pine family. Recently, I was awarded a grant to study genome structure in spruce and pine, through comparisons of spruce and pine. This involves collaborations with both *Arborea* in Quebec (John Mackay) and the Mississippi Genome Exploration Laboratory (Daniel Peterson). Our main effort was to sequence 96 BACs in spruce and 96 BACs in pine, using BAC pools and 454 sequencing. The data are almost analyzed and from this, one can estimate gene number and trace patterns of transposon evolution over the past 100 million years in each species.

Through my NSERC grant, I have been involved with several projects in plant population genomics. In Ramsay et al. (2009), a MSc student shared with Loren Rieseberg, we identified 45 genes in the terpenoid biosynthetic pathway and inferred patterns of selective constraints in lineages involving *Arabidopsis*, *Populus*, *Ricinus*, and *Vitis*. Upstream genes had a greater constraint and a novel "pathway pleiotropy index" better explained patterns. Albouyeh and Ritland (2008) presented and evaluated an experimental design to estimate heritability of gene expression using parent-offspring regression with two-channel microarrays. Thompson et al. (2008) described estimators for three-gene identity coefficients and used these to demonstrate that clonal reproduction promotes inbreeding and spatial relatedness in yellow-cedar (*Callitropsis nootkatensis*). Ally et al. (2008, 2010) used aspen clones to study the nature of mutations in long-lived organisms and the 2010 paper received several commentaries in leading journals.

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P. Lu, P. Charrette and J.D. Simpson

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FOREST GENETICS AND TREE IMPROVEMENT: CHALLENGES AND OPPORTUNITIES

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INTRODUCTION

It is an exciting time to be a tree breeder. Worldwide, the markets for wood-based energy sources and biofuels, for cellulose and paper products, and for solid and reconstituted wood products for furniture and construction are strong, and will become stronger as economies in Latin America, Asia, and Africa grow. Concerns over climate change will make plantation forests, a renewable and sustainable resource, more attractive to investors and consumers. The need to make our plantation forests more productive, both to maximize economic return and to minimize pressure on native forests, is greater than ever. Tree breeders have great success stories to tell, and many of the traditional technologies and breeding strategies used in the past will continue to be effective in the future. But the world is constantly changing, and so this paper will briefly discuss some of the new opportunities and challenges associated with five areas of interest to tree breeders: wood properties, pests and pathogens, hybridization, pedigree reconstruction, and genomic selection.

WOOD PROPERTIES

Tree breeders have traditionally focused on growth, form, and adaptive traits (e.g., cold tolerance or pest resistance). Increasingly, wood properties are of interest to CEOs and mill managers, and therefore to breeders as well. For almost all species and products, it is possible to identify some wood characteristic that would be of value to improve, whether it be density, pulp yield, wood stiffness, higher uniformity, earlier transition from low-quality juvenile wood to high-quality mature wood, etc. For over 40 years, tree breeders have known that many wood properties are under a relatively high degree of genetic control and exhibit relatively low levels of genotype x environment interaction (e.g., Zobel 1961, van Buijtenen 1962). Wood density has received some emphasis in a number of programs; it can be measured accurately and easily with simple laboratory equipment, and wood density is an important trait both for pulp production and sawtimber quality, however, other traits, like fiber characteristics, chemical properties, wood stiffness, uniformity, etc. have not been widely emphasized in breeding programs. This was partially because techniques to assess samples for these properties were tedious and slow, and partially because the economic benefit of improving wood properties is less than the benefit from improving volume growth.

Today, advances in technology make indirect assessment of many wood properties relatively fast, inexpensive, and precise. For example, NIR spectroscopy can be used in a wide variety of ways in the forest industry – one recent issue of the Journal of Near Infrared Spectroscopy was entirely devoted to wood and wood products (JNIRS 2010, Vol.18, Issue 6). NIR spectroscopy is based on exposing a sample of interest to many thousands of discrete wavelengths of near infrared light, and measuring the reflectance or transmission. A training population of samples with a known property of interest (e.g., pulp yield, which is slow and expensive to measure) is used to build a multiple regression prediction model. The pulp yield of future samples can then be predicted based only on their NIR spectra. NIR was demonstrated to have utility to predict cellulose content of wood samples for breeding purposes as early as 1990 (Wright et al. 1990).

As long as the training data set is diverse enough to reflect the inherent variation of the future inference population, very good NIR models can be built for quite diverse traits. In fact, it is possible to develop models for a particular trait that fit a wide array of species from very different geographic regions, so-called “global models”, such as for pulp yield in eucalypts (Downes et al. 2010) or cellulose and lignin content in pines (Hodge and Woodbridge 2010).

Different NIR wavelengths are absorbed differently by chemical bonds in the sample, primarily the bonds between carbon, hydrogen, and oxygen atoms. As a result, NIR spectroscopy has normally been used to assay differences in chemical contents of samples in the pharmaceutical and agricultural industries. However, very good results have been obtained using NIR to assess a physical property of wood samples, the microfibril angle (MFA) of the cellulose microfibers within the tracheid cells of pine wood (Schimleck et al. 2002, 2005). This is done by scanning intact slices of wood from a normal increment core. Good global models for MFA for pine species can also be developed (Schimleck et al. 2010). Wood physical properties such as modulus of elasticity (MOE) can also be assessed quickly and cheaply in standing trees with acoustic devices (Grabianowski et al. 2006, Wang et al. 2007, Cherry et al. 2008, Mora et al. 2009). For temperate conifer species, which are characterized by a juvenile wood core with low stiffness wood (i.e., low strength wood, with low density and high MFA), using acoustic technology at a young age would probably be very valuable.

Fiber properties can still be a challenge to assess, but computer imaging systems can rapidly collect data on cell wall thickness, cell diameter, lumen size, etc. There are many new fiber length analyzers now available (Guay et al. 2005), and these could conceivably be fast enough for use in breeding programs. Some fiber dimension traits have been shown to be under genetic control, for example, Stanger (2003) demonstrated provenance and genetic variation within *Pinus patula* provenances for tracheid diameter and other fiber traits. Such traits could potentially be manipulated in a breeding program.

Certainly, breeders now have the opportunity to more fully incorporate important wood properties into their selection programs. More in-depth studies of large populations will provide good information on genetic control and genetic variance available for these traits. But for a trait to be important in a tree breeding program, in addition to being under genetic control, it must also be of high economic value relative to other traits of interest. For programs with pulp and paper as the product objective, there have been some very good studies estimating the dollar value of a marginal gain in final pulp production resulting from increases in volume growth (of most interest to foresters) and wood density and pulp yield (of most interest to mill managers) (e.g., Borralho et al. 1993, Wei and Borralho 2001, Lopez et al. 2009). These dollar values could be used by breeders to properly weight these traits in a selection index. For sawtimber producers, this information might be harder to come by, as sawmills, growth rates and log diameters, and market economics all vary substantially. Regardless of country and market, boards must meet some minimum strength criterion, however, boards are often graded visually, according to the number of rings/cm, or the numbers and size of knots. Genetic differences in density and MFA cannot be seen visually, so the only way for producers to recoup additional value for improvements in these traits would be if boards are machine stress graded. One comprehensive study on sawtimber economic weights was done for the *Pinus radiata* sawlog industry in New Zealand (Ivkovic et al. 2006). This study indicated that for an integrated forestry and sawmill operation, gains in volume (MAI) and wood stiffness (MOE) were almost equally important. The study assumed a profit function that incorporated increased value for higher stiffness logs, such as would apply if some type of machine stress grading system for logs and/or boards was in place. If the market is indeed moving toward machine stress grading in the future, breeders could begin to select today for wood strength properties.

PESTS AND PATHOGENS

Commercial forest plantation programs in the northern hemisphere have traditionally been based on native species, so northern foresters have always dealt with the native pests found in their region. In the southern hemisphere, forest plantations are based on exotic species, primarily *Pinus* and *Eucalyptus*. Often newly introduced exotic species have a period where there are few pest problems (Wingfield 2003), but eventually pests and pathogens make an appearance. Foresters and tree breeders in both hemispheres will face diverse and increasing threats from diseases and insect pests in the future. The world today is interconnected, with thousands of commercial flights and transoceanic ships traveling every day. This makes the accidental dispersal of pathogens highly likely, even with stringent phytosanitary protection systems in place. It seems reasonable to anticipate that a number of our commercial forest species will be severely challenged by something to which they have little tolerance. Most North Americanforesters are familiar with cases of chestnut blight *(Cryphonectria parasitica)*, Dutch elm disease *(Ophiostoma ulmii)*, and white pine blister rust *(Cronartium ribicola)*. A few more recent examples of this in the worldwide forest industry are listed below:

- *Dothistroma septosporum*, is a significant problem for the *P. radiata* industry in Australia, New Zealand, and Chile (Ivkovic et al. 2010, Watt et al. 2011).
- *Sphaeropsis sapinea, is a problem on P. radiata and P. patula* in South Africa (Wingfield et al. 2001).

- *Eucalyptus* rust, caused by *Puccinia psida*, a pathogen native to Central America and Mexico which attacks native Myrtaceae. The pathogen infected exotic eucalypt plantations and is now a worldwide concern (Coutinho et al. 1998).
- *Cryphonectria cubensis* which causes canker of *Eucalyptus*, first described in Cuba, now found in eucalypt growing regions throughout the southern hemisphere (Wingfield 2003).
- The hemlock woolly adelgid (*Adelges tsugae*), introduced into eastern North America from Asia in the early 1950s. The adelgid has now caused tremendous mortality throughout stands of eastern hemlock (*Tsuga canadensis*) in well over half the species range (Jetton et al. 2009, Krapfl et al. 2011).

Unfortunately, these types of situations will become more and more common (Wingfield et al. 2001, Wingfield 2003, Burdon et al. 2006). This potential increase in the number of pathogens to which a plantation species will be exposed makes it essential that breeders have access to a large array of genetic diversity. Species with resistance to a newly problematic pathogen can be deployed either as pure species (assuming acceptable adaptability and growth), or as tolerant hybrid partners which can be used to infuse genes into the primary commercial species. As an example, consider the case of *Fusarium circinatum,* which causes pitch canker in a number of pine species. The pathogen is probably native to either the southeastern United States, where it was first described in 1946, or Mexico, where it is widely distributed throughout the range of many native pines. In recent years it has been introduced into California where it caused substantial mortality on *P. radiata*, and around the world, with confirmed reports in South Africa, Chile, Japan, Spain, Portugal, and Italy (Carlucci et al. 2007, Bragança et al. 2009, Ganley et al. 2009). While a number of important plantation species are highly susceptible to the pathogen (e.g., *P. radiata, P. patula*), other species are extremely tolerant or resistant (e.g., *P. oocarpa, P. tecunumanii, P. maximinoi, P. jaliscana*) (Hodge and Dvorak 2000, Mitchell 2011). In southern and eastern Africa, one of the primary commercial pine species is *P. patula*, with around 1 million ha in plantation, and another species of importance is *P. radiata. Fusarium circinatum* is a serious problem in both seedling and rooted cutting nurseries, and is now present in older plantations. Despite sanitation efforts, in many cases, first-year field survival is often too low to support commercial forestry operations. Fortunately for the South African forest industry, good populations of resistant species already exist in the country, and the more resistant pure species like *P. tecunumanii* and *P. maximinoi*, and moderately resistant *P. patula x P. tecunumanii* hybrids are being increasingly deployed (Mitchell 2011).

Genetic Diversity and Risk Mitigation

Crop scientists have demonstrated that the use of varietal mixtures (e.g., multiple lines) of a single species, or species mixtures, e.g., barley and wheat, are effective in decreasing the incidence and spread of disease (Burdon et al. 2006). Similarly, genetic diversity within a single plantation tree species would probably be beneficial. Organizations considering or utilizing operational clonal forestry must answer questions about the appropriate number of clones to deploy in order to minimize risk from pests and other unpredictable climatic events. Burdon and Halliday (2006) suggested that once the effective number of unrelated clones (N_e) is above 20, a further increase does little to further reduce risk. Roberds and Bishir (1997) were more conservative, suggesting that 30 to 40 unrelated clones in plantations is roughly equivalent to very large numbers of unrelated clones in terms of risk of catastrophic failure. Many large forestry companies working with clonal eucalypts use 8 to 10 commercial clones at any one time, but some use as few as 4 or 5. The companies typically replace those clones and/or introduce some new clones every five to ten years. Commercial clones are usually deployed in a mosaic pattern across the landscape, so depending on rotation age, at any one time there may be 10 to 20 clones that make up the bulk of a plantation estate. The moderately low effective population sizes mentioned above (N_e from 20 to 40) suggest that a typical seed orchard with 30 to 40 clones would produce enough genetic diversity to substantially mitigate a fair amount of risk. Some modification might be necessary to minimize risk in the typical single species/variety plantation and breeding program (e.g., find a few more operational clones tobe deployed at any one time). But perhaps a better approach to further reduce risk might be through the development and use of alternate species/hybrids.

Phytosanitary Issues

Phytosanitary requirements for importation of plant material (seed and pollen) into many countries are growing more stringent in an effort to exclude pathogens. Australia and New Zealand are probably in the best position in this regard, as they have good scientists and very strong quarantine regulations, but also are both island nations that control all of their ports of entry. Most other countries in the world, particularly, in Africa, Asia, Europe, and to a lesser extent North America, share borders with many countries. If one neighbor has ineffective quarantines, this will likely affect other neighbors in short order (Wingfield et al. 2001).

Some countries ask for certification that seedlots are free from particular pathogens. It is difficult (if not impossible) to ensure that a seedlot is not carrying a pathogen, but tests can be conducted on seedlots, and negative results for the presence of a particular pathogen can be documented. Other countries, like Mexico, are becoming more and more restrictive on the export of any native genetic material. These developments are of interest to tree breeders because they have the potential to make our task more difficult. In the case of insect pests, with sufficient time and research investment, it would be possible in many cases to develop biological control programs that are effective. However, for a serious disease problem, the only cost-effective solution is likely the development of genetic resistance in the host tree population. It is important that breeders communicate with our colleagues in the phytosanitary arena to ensure that research quantities of forest genetic material can be exchanged safely and freely.

HYBRIDS

More than 95% of the world's forest plantations are planted with pure species (White et al. 2007). However, hybrids will become more important as plantation varieties in the future. Hybrids are already very important in *Populus* (e.g., *P. nigra* x *P. deltoides, P. trichocarpa* x *P. deltoides*, and many others*),* *Larix* (e.g., *L. decidua* x *L. laricina, L. decidua* x *L. kaempferi,* and many others) and *Eucalyptus* (e.g., *E. grandis* x *E. urophylla, E. grandis* x *E. camaldulensis,* and others). There have been success stories for pine hybrids as well (e.g., *P. rigida* x *P. taeda,* and *P. elliottii* x *P. caribaea)*. Pine and Eucalyptus hybrids will become increasingly important in the future.

There are many factors that will contribute to the increasing importance of hybrids. While it is difficult to project exactly what will happen in a specific region in terms of climate change, it seems safe to say that most regions will experience changes: warmer, drier or wetter, and with more hot and cold extremes. This will mean that species that had previously been well adapted to a particular region may become sub-optimal. The use of hybrids involving one parent what was formerly the primary commercial species will be a way to preserve the genetic gains made in growth, form, wood properties, etc., while infusing some additional adaptability, for example with increased drought or frost tolerance. Similarly, increased pest and pathogen problems will require resistance genes to be introduced into commercial varieties. These genes may not be found within the current commercial species and will need to be found in a hybrid partner. In addition, many hybrids show some kind of hybrid vigor or heterosis for growth traits, even though exactly why this occurs is still not well understood (Bradshaw and Grattapaglia 1994). Finally, breeders will want to combine the best traits possible into one "package", regardless of what species might currently carry those traits. For example, eucalypt breeders all over the world have an interest in hybridizing their primary commercial eucalypt species with *E. globulus*, in order to capture the outstanding wood properties of that species, regardless of whether *E. globulus* is at all well suited to their environment and climate.

For eucalypt breeders, hybrids offer a way to combine the superior traits of very different species. In general, hybrids are of more interest to *Eucalyptus* breeders and *Populus* breeders than pine breeders, for the simple reason that any outstanding hybrid clone can probably be vegetatively multiplied (e.g., through rooted cuttings), and thus used operationally. This is not true for most conifers, including almost all pines. Thus, to use pine hybrids commercially, there must be ways to mass produce F_1 seed, or to propagate hybrid families by rooted cuttings. Despite the challenges associated with pine hybrids, there are substantial possible benefits, and new technologies will make operational use of pine hybrids more feasible. One example is the use of mini-hedges or micro-hedges for rooted cuttings, which have led to vast improvements in cutting production per square meter of nursery bed, in rooting percentage, and in plant quality.

There are many "lesser known" or "obscure" species among *Pinus, Eucalyptus,* and other genera. As we learn more about these species, we will find that some of them have interesting characteristics that could be valuable. As one example, the wood properties of tropical pines like *P. tecunumanii*, *P. oocarpa,* and *P. maximinoi* are rather different than temperate pines like *P. taeda* and *P. radiata,* particularly in the juvenile core of the tree. The tropical pines have a more uniform density profile and much lower microfibril angles, producing higher strength wood (Camcore Annual Report 2008). Hybrids of tropical pines with temperate pines may thus result in faster growing trees which have a higher percentage of high quality wood. Another example, from the eucalypts, is the relatively unfamiliar species *E. benthamii,* which has a very restricted range in New South Wales, Australia. *E. benthamii* has shown great growth potential in temperate regions of southern Latin America and southern Africa. A significant disadvantage is that it does not root well, which is the case with a number of other cold tolerant species like *E. globulus*, *E. nitens*, and *E. smithii.* This will limit commercial deployment of these species to seedlings, but perhaps hybrids of these cold tolerant species with a more tropical species such as *E. grandis*, *E. urophylla*, or *E. pellita* might yield clones that both root well and display good cold tolerance.

Barriers to Hybridization

Production of almost any hybrid is more difficult than breeding with pure species. Compared to pure species, flower or cone abortion is higher and seed set for flowers or cones that do survive is typically lower. For example, in hybrids of *Picea rubens* and *Picea mariana,* pure species crosses had 6.6 times more filled seeds than hybrid crosses (Major et al. 2005). But some desirable hybrid combinations prove very difficult, if not impossible to make. Better understanding of the reproductive biology of *Pinus, Picea, Larix, Eucalyptus,* etc., and a better understanding of the nature of pre-zygotic and post-zygotic barriers may help breeders to overcome these barriers. Some very good work in this area has been done for the eucalypts (Potts and Dungey 2004). For the white pines, hybridization work has been conducted for many years, primarily in attempts to increase resistance to blister rust (caused by *Cronartium ribicola*) among susceptible species. There have been many white pine hybrid combinations attempted, and for those that have been unsuccessful, there appears to be variation in the nature of the barriers. For example, in a study of *P. cembra, P. koraiensis, P. flexilis,* and *P. strobus,* Kriebel (1972) found that the barriers to hybridization were mostly embryo inviability post-fertilization) rather than gametic incompatibility (pre-fertilization). In contrast, the incompatibility barriers between *P. lambertiana* and *P. monticola* appear to occur during the second year of the reproductive process but prior to fertilization, although the specific mechanisms differ for *P. lambertiana x P. monticola* and the reciprocal cross (Fernando et al. 2005).

One possible way to overcome hybridization barriers may be through protoplast fusion (Yanchuk et al. 2001). Protoplast fusion has been used to create somatic hybrids in citrus (Grosser and Chandler 2000, 2002), and has been investigated for species of *Ulmus* with limited success (Redenbaugh et al. 1981). More success was found with protoplast fusion of tamarack (*Larix laricina*) and two larch hybrids (*L.* x *leptoeuropa* and *L.* x *eurolepis*). Post-fusion products of both of the new hybrids (i.e., tamarack x hybrid larch) produced cell cultures and eventually somatic embryos (Pattanavibool 1998).

Hybrid Breeding Strategies

There are still many questions about long-term breeding strategies for hybrids. In some situations, one species may be nearly ideal in terms of growth, adaptability, wood quality, etc., but needs a disease resistance trait from a related, but less desirable species. This is the case with American chestnut (*Castanea dentata*) and Chinese chestnut (*C. mollissima*) which carries resistance to the chestnut blight caused by *Cryphonectria parasitica.* In this situation, the strategy has been to backcross hybrids to *C. dentata,* as much as possible keeping only the resistance genes from *C. mollissima.*

For many other hybrids, such as *P. elliottii* x *P. caribaea, P. wallichiana* x *P. strobus,* or *E. urophylla* x *E. grandis,* each of the pure parental species may bring more than one desirable trait to the hybrid. For example, in the case of *P. elliottii* x *P. caribaea, P. elliottii* brings adaptability to wet sites, good stem form,and higher wood density, while *P. caribaea* brings fast growth and responsiveness to vegetative propagation.

An often recommended strategy for hybrid breeding is Reciprocal Recurrent Selection (RRS). This strategy breeds both parental species as pure species, selecting new parents which are then used to create new hybrids. The new hybrids could be field tested prior to commercial deployment, or could perhaps be deployed without testing. The latter option would require that the hybrid performance be strongly related to pure species performance. Specifically, this would be feasible if General Combining Ability (GCA) within pure species was correlated with General Hybridizing Ability (GHA) in the hybrid. It would also be beneficial if there was little dominance variance, or Specific Hybridizing Ability (SHA), among the full-sib hybrid families. It is somewhat difficult to find general patterns in the literature, as the situation varies substantially depending on the species and the trait. For example, in a study of hybrids of *P. caribaea* x *P. oocarpa* and *P. caribaea* x *P. tecunumanii,* Brawner et al. (2005) found that the GCA of pure *P. caribaea* and the GHAs of *P. caribaea* in hybrid crosses with *P. oocarpa* and *P. tecunumanii* were strongly correlated. However, in a study of *P. elliottii* x *P. caribaea* hybrids (in similar environments), Brawner et al. (2003) found no correlation between GHA and GCA for 56 *P. elliottii* parents for growth traits. In a study of *E. nitens* x *E. globulus* hybrids, Volker et al. (2008) found that the correlation of GCA and GHA (for growth traits) for *E. globulus* parents was near zero, while for *E. nitens* parents was approximately 0.60. Dominance (SHA) variance was high up until age 6, but zero at age 10 years. In a study of *E. urophylla* x *E. grandis* populations, Bouvet and Vigneron (1996) reported that additive variance made up more than 70% of the total genetic variance in the hybrid, but that the *E. urophylla* (female) parents displayed substantially more GHA variance for volume growth than the *E. grandis* (male) parents. These kinds of differences may mean that very different breeding strategies would be optimal for different hybrids, which is probably to be expected.

A reciprocal recurrent selection scheme, by definition, will produce hybrids which are made up of 50% of each of the pure species genomes. The rationale behind using hybrids of native species as commercial deployment varieties is that the available native species are not perfectly optimal for plantation forestry. But in that case, there is not any reason to expect that the optimal "commercial variety" will be a 50:50 mixture of the available native species. For example, *P. strobus* x *P. wallichiana* hybrids have been made to introduce blister rust resistance from the *P. wallichiana* into *P. strobus.* The *P. wallichiana* brings resistance to blister rust, and higher wood density to the hybrid, while the *P. strobus* brings cold hardiness (Lu and Sinclair 2006). A breeder wanting to develop a hybrid performing well for all three traits (growth, cold hardiness, and wood density) would presumably need different percentages for genetic background from the two pure species, something a synthetic variety could possibly achieve, while a traditional RRS program likely could not.

This leads to consideration of a Synthetic Species hybrid breeding strategy. Essentially, the idea is to use F_1 hybrids as a base population, and begin breeding and selecting with the hybrid as though it were a pure species, for example, using normal recurrent selection. In theory, any genes of value, regardless of where they are in the two pure species, will eventually recombine and be selected for in some optimum proportion. This may work fine, especially with relatively closely related species. But as species become more distantly related, meiotic abnormalities may cause problems, particularly in early generations. Deng et al. (2008) examined meiosis in *P. wallichiana* x *P. strobus* and found a higher frequency of aberrant events such as chromosomal bridges, fragments, or micronuclei, in pollen cells of hybrids relative to the pure species. Myburg et al. (2004) studied F_1 hybrids of *E. grandis* x *E. globulus*, and found that the fertilization ability of F_1 gametes and viability of F_2 zygotes was affected by genic incompatibilities between the genomes of *E. grandis* and *E. globulus,* and by chromosomal rearrangements that result in abnormal meiotic products in the F_1 hybrid. If these types of phenomena are common for many hybrids, it may limit the applicability of synthetic species breeding.

To date, one of the best indications that synthetic breeding might be viable in some cases is the comparison of F_1 versus F_2 hybrids of the *P. elliottii* x *P. caribaea* hybrid in plantations in Queensland, Australia. Although specific F_1 families could be multiplied by rooted cuttings, use of the hybrid over large areas might require large numbers of plants, so the availability of F_2 seed, say from an F_1 seed orchard, might be very valuable. Across 3 field trials measured at ages 10 to 15 years, there was no significantdifference in volume growth between F_1 and F_2 seedlots, and both maintained the same superiority over the parental species (Dieters and Brawner 2007). This suggests that the observed heterosis in this hybrid is largely additive and is a good indication that a synthetic species strategy with this hybrid would be biologically feasible. Kerr et al. (2004a,b) describe the use of computer simulation to compare RRS (under a number of different parental and progeny selection strategies) with synthetic species breeding. They concluded that a synthetic species strategy would be more cost effective in most situations.

SOMATIC EMBRYOGENESIS

Clonal forestry has long been a reality for breeders working with *Eucalyptus*, *Populus*, and *Larix.* In these genera, plus others, vegetative propagation is relatively easy, making the step to operational commercial clonal forestry a short one. In the case of many conifers, particularly the pines, clonal forestry has been practically out of reach. Somatic embryogenesis (SE), along with cryopreservation, offers the exciting opportunity to practice clonal forestry with the Pinaceae. With SE, the breeder can theoretically preserve embryogenic tissue without loss of juvenility. This allows the breeder to test numerous clones in field conditions, theoretically for many years, until a final selection decision is made. The superior clones can then be extracted from cryopreservation, multiplied, and operationally deployed (Park et al. 1998). SE is now being used on a fairly wide scale in *Picea* and it is just beginning to be used on a commercial basis in *Pinus*, particulary *P. taeda* and *P. radiata.* The use of SE would allow pine breeders to utilize all of the genetic variation (additive, dominance, and epistatic) when selecting clones for commercial deployment, thus increasing the potential for genetic gains. This advantage becomes more significant as breeders seek to improve many traits simultaneously.

Currently, SE with conifers has been limited to juvenile explant material, e.g., immature embyros. While initiation rates for some species are very high (e.g., 65% for some *Larix* hybrids), often for pines, the rates are quite low (say from 1% to 10%). Full-sib families often show large differences in their amenability to the SE process. This appears to be under additive genetic control, suggesting that this trait could be improved (Park 2002). Simulation studies have revealed that variation in response to SE does not necessarily limit the genetic gain possible from selection of outstanding clones (Lstiburek et al. 2006). A logistical challenge is that there is often a very narrow window of time for collection of immature cones at the optimum stage for SE. For example, in *P. patula* in South Africa, the optimum time for immature cone collection is typically during the last two weeks of December (Glen Mitchell, York Timbers, personal communication). There has been some success in initiating somatic embryos starting with mature zygotic embryos as the explant tissue (e.g., Tang et al. 2001). If the initiation rates are high enough, this would be a tremendous logistical advantage in a breeding program.

For conifers, regeneration from tissue older than zygotic embyros is often very difficult (Bonga et al. 2010). Root and stump sprouts often exhibit a higher degree of totipotency than other tissue. Of the gymnosperms, it appears that only in *Sequoia sempervirens* have mature plants been derived from *in vitro* culture of root sprouts (Boulay 1987, Bonga et al. 2010). Very few pine species will produce root sprouts once a tree is past the sapling stage, but some exceptions are *P. oocarpa, P. canariensis, P. rigida, P. leiophylla,* and *P. teocote.* Are root sprouts in these species morphogenetically competent? What genes are active in these species that are not in others? Are these genes present, but “turned off” through some kind of epigenetic effects, and could they be “turned on” again through some sort of rejuvenation process?

Related to the topic of re-juvenation and epigenetics is the question of how SE plants will grow relative to normal seedlings. A possible concern with SE is the accumulation of epigenetic effects associated with aging and loss of juvenility during the SE and/or cryopreservation process. With rooted cuttings, it is clear for the pines that there is some kind of physiological maturation of hedges, and that after some period of time growth rates and/or rooting percentage of cuttings will decrease as hedge plants age. This might be 4 to 8 years for species like *P. radiata* or *P. elliottii* x *P. caribaea* hybrids, or as little as 3 years for a harder-to-root species like *P. taeda.* There appears to be relatively little data in the literature on the question of comparing overall growth of SE plants and normal seedlings from similar genetic backgrounds. In one report, somatic seedlings of *P. taeda* averaged 5.2 feet after two years growth in the field, compared to an average height of 8.6 feet for nearby normal seedlings (Pullman et al. 2003), but in a different study with 2-year-old *P. taeda* across 6 sites there were not large differences between seedlings and clones (Baltunis et al. 2007). In another study, SE clones of coastal Douglas-fir *(Pseudotsuga menziesii var. menziesii)* were planted with zygotic seedlings (normal seedlings) from the same full-sib families to compare growth. Results after 7½ years showed that the SE clones grew more slowly than the zygotic seedlings across five tests, 5.5 m versus 6.6 m in height, and volume of 10.2 dm^3 versus 15.1 dm^3 (Dean et al 2009). Normally, one would expect that 50% of the clones would be superior to the zygoticseedling population mean, but in this case only about 20% of the clones exceeded the zygotic seedling mean, and most of those came from a single fast-growing full-sib family. Similarly, O’Neill et al. (2005) examined 313 somatic clones from 11 full-sib families of *Picea glauca*, and reported slower growth of somatic seedlings compared to zygotics after 7½ years. Are these results typical of other conifer species? Will the size of the growth loss vary among species? If there is some epigenetic loss of growth potential in SE seedlings, this does not necessarily mean that we will not be able to identify very good SE clones that will exceed the genetic gain possible from using the best full-sib families (“family forestry”). However, they do suggest that higher selection intensities will be needed, that is, we will need to screen many clones per family in order to make up “lost ground” and make genetic gain. Breeders must take these effects into account when evaluating potential gain versus cost for an SE program.

PEDIGREE RECONSTRUCTION

To date, molecular markers have been used in tree improvement programs primarily for “finger-printing” and pedigree verification, which has significant value. In operational breeding programs, these tools have been used to confirm identity of clonal ramets in seed orchards, or to track identification errors made in progeny tests, etc. They have also been used to estimate the amount of pollen contamination coming from outside of production seed orchards (e.g., El-Kassaby et al. 1989, Adams et al. 1997). However, there are potentially other important roles for marker-based tools. One of those is Pedigree Reconstruction, the use of molecular to markers to ferret out the levels of relatedness among open-pollinated or polymix breeding populations.

The use of pedigree reconstruction in forestry was first suggested by Lambeth et al. (2001) as a way to recover paternal identity of progeny in pollen-mix families. The idea was that breeders could use pollen-mixes to efficiently create maternal family seedlots for progeny testing, but still recover paternal identity at some time in the future to make progeny selections with full pedigree control. The system was called Polymix Breeding with Parental Analysis (PMX/WPA), and it had the potential to be cheaper and easier than a full-sib mating and testing strategy, while yielding very comparable genetic gains. The suggestion was that parental identification could be done just on a top tier of selection candidates (to control relatedness of the progeny selections), or on the full population (to replicate a full-sib progeny test). El-

Kassaby and Lstiburek (2009) expanded this idea, and suggested the use of bulk orchard open-pollinated seed plus pedigree reconstruction of maternal and paternal identity in order to replicate a full-sib progeny test data set. They named this approach “Breeding Without Breeding” (BWB), since the breeder does not actually make any crosses, but still ends up with a full-sib progeny test data set. Assuming that parents of progeny can be accurately identified, BWB has the potential to capture a substantial proportion of the genetic gain possible from a complex mating scheme at a very low cost. In both the PMX/WPA and BWB approach, there will be unbalance in the resulting data sets in terms of differential contributions to the progeny population in terms of male and/or female parents, but this presents no computational difficulties, and will likely have only a small effect on the precision of genetic parameter estimates resulting from such a data set (e.g., Doerksen and Herbinger 2010).

Reconstructing a pedigree from very large marker data sets is challenging, and there has been much discussion about the optimal algorithms to accomplish this (e.g., Butler et al. 2004, Windig and Meuwissen 2004, Bink et al. 2008). Some methods seek to specifically identify parents, and these methods require that all parents are known. When parents cannot be assigned with confidence, some algorithms assign a subset of the progeny with “unknown” parents, decreasing the efficiency of the approach. A natural mating population (like a seed orchard) will likely produce an unbalanced population structure in the progeny, i.e., very different frequencies of male and female contributions. The presence of pollen contamination in seed orchards ensures that not all of the male parents will be known, with the result that there will be a large number of males each contributing a very low frequency of progeny in the population. With this kind of population structure, a molecular relatedness estimator will probably give better results than a parental allocation method (Blonk et al. 2010). Essentially, the difference between the approaches is that a parental allocation algorithm will classify every pair of observations as unrelated, half-sib, or full-sib, while a molecular relatedness estimator will use the similarity of markers to classify relatedness on a continuous scale. This allows the use of pedigree reconstruction without any knowledge of parental genotypes whatsoever. A study on a natural mating fish population (Blonk et al. 2010) indicated that this approach could result in an accuracy of prediction (the correlation between the true and predicted breeding of individuals) of 0.60, quite impressive for an approach with no controlled mating or testing.

The number of markers needed for such an approach is moderately high, but probably already within the range of operational tree breeding programs. For the case of the fish population mentioned above *(Solea solea),* 10 SSR markers with an average of about 14 alleles per marker were used. Hayes (2011) states that, for dairy cattle, pedigree reconstruction can be done with very accurate parental assignment using as few as 3000 SNP markers. Presumably, molecular relatedness algorithms would also work well with that number of markers.

In my opinion, a very exciting possibility is the use of pedigree reconstruction to “jump start” a tree improvement program. Often tree improvement programs begin by making phenotypic selections from commercial plantations, and then taking those selections into progeny testing. It seems likely that pedigree reconstruction (either with parental allocation or molecular relatedness) could be used to extract something like full-sib progeny test data from un-pedigreed plantation material (improved or unimproved). This would essentially advance a breeding program by almost a generation in one quick step.

GENOMIC SELECTION

Genomic selection (GS) is another potential use of molecular markers in tree breeding programs. Since at least the early 1990s, breeders have been intrigued by the opportunity to utilize marker aided selection (MAS), where presence or absence of some molecular marker (or many molecular markers) is associated with phenotypic superiority in some trait of interest. Molecular markers such as allozymes, restriction fragment length polymorphisms (RFLPs), or randomly amplified polymorphic DNAs (RAPDs) could all, in theory, be used in a MAS scheme. A classic paper by Straus et al. (1992) outlined a number of limitations of MAS, at least with the current types of molecular markers then available. Many of the limitations derived from an insufficient amount of linkage disequilibrium (LD) which is typically found in forest tree populations. Briefly, a molecular marker must be in LD with a quantitative trait locus (QTL) in order to be useful in any system of MAS. This means that when you see the marker, you have confidence that the tree also has the favorable allele for the QTL. Forest trees are predominantly outcrossing organisms, which leads to low levels of LD. In other words, most markers will not be associated with a “good” allele for any particular trait. High heritability traits are presumably controlled by fewer loci with large effects. Thus, only a small number of markers need to be in LD with the QTL important for a particular trait. However, with high heritability traits, only small numbers of related individuals are sufficient to predict breeding values of the parents of any particular individual, and for the individual the phenotype is a relatively accurate predictor of the genotype. In other words, with high heritability traits, it is easy to produce high family and within-family heritability. The result is that MAS would generally be most effective with traits with high heritability, but it is those traits where the benefit of MAS is relatively small.

Furthermore, even if some useful markers were found, there was a question of the consistency of LD associations between the markers and the QTLs. Not all markers will be in the same phase in all genetic backgrounds (e.g., in all full-sib families in which a breeder wants to use MAS). In addition, since not all alleles (either markers or QTLs) are present in all pedigrees, not all of the markers would be relevant in all situations. This means that correlations among markers and QTLs must be determined for all pedigrees of interest. And then there is the question of consistency of marker-QTL associations across generations. Selection will alter frequencies of both marker alleles and QTL alleles. How long will the association between marker and QTL last when the genetic backgrounds change?

To make MAS useful for tree breeders, very large numbers of markers are needed. Strauss et al. (1992) concluded that the use of MAS in forest tree breeding would be limited, but noted that MAS might potentially have more application in hybrid breeding, where hybrid crossing could generate relatively large, and perhaps relatively stable, amounts of LD.

Today, there are molecular markers available which may overcome some of the limitations, and make MAS possible. Single-nucleotide polymorphisms (SNPs) are mutations at a single nucleotide position, and each possible nucleotide represents a possible allele. SNPs are a very powerful marker system, with SNPs being abundant, typically bi-allelic in nature, and relatively inexpensive. Another marker system, Diverse Arrays Technology (DArT) may be even more powerful, at least in terms of being able to develop many markers at low costs.

Genomic selection can be thought of as a “new and improved” marker-aided selection. The distinguishing feature of GS is the use of fully saturated molecular maps of the genome and population-wide marker-phenotype associations. Essentially, by screening many thousands of low cost markers (e.g., SNPs orDArTs), and using a large population of accurately measured genotypes to look for associations with favorable phenotypes, we hope to find a sufficient number markers that are in LD with important QTLs even for low heritability traits (Grattapaglia et al. 2009). With enough marker candidates, it might be possible to find markers that are consistent across the entire population of interest, that is, that are in the same phase for all genotypes and pedigrees. In this case, GS would theoretically allow very accurate genotypic evaluation, increasing accuracy of selection, and thus genetic gain.

GS has been studied and increasingly used in animal breeding programs (e.g., see Goddard and Hayes 2007, Muir 2007, Hayes et al. 2008), and crop plants such as maize (Bernardo and Yu 2007) and barley (Heffner et al 2009). The two main advantages are the ability of GS to increase precision of breeding value prediction on progeny and to do that at a very early age. The comparison of interest is the genetic gain that can be realized based on selection on the expected breeding value (EBV) predicted from progeny testing, and the genomic expected breeding value (GEBV) predicted from the markers. This can be estimated from the accuracy of prediction of those breeding values, which is essentially the correlation between the true and predicted breeding value, $\mathrm{Corr}(g,\hat{g})$ (White and Hodge 1989). In dairy cattle, $\mathrm{Corr}(g,\hat{g})$ from a typical animal progeny testing scheme is around 0.75, and it has been demonstrated that GS can achieve this level of accuracy. As a result, there are already bull breeding companies in the dairy cattle industry using GS operationally (Hayes et al. 2008). Two strategies are in use: 1) to screen large numbers of bull calves to reduce the number that will be further progeny tested and 2) to select bull calves based on GEBV alone, that is, to eliminate progeny testing altogether. Similarly, in crop plants, simulations indicate that $\mathrm{Corr}(g,\hat{g}) = 0.85$ from GS are possible (Heffner et al. 2009), and one possible strategy would be to select only on GEBV every other breeding cycle, or in other words, to eliminate progeny testing every other breeding cycle. Clearly, these approaches offer the potential to make genetic gain more efficient per unit cost or per unit time.

Grattapaglia and Resende (2011) have outlined factors which will determine the utility of GS for forest tree breeding, which include: 1) the effective population size (N_e) and the genotyping density and 2) the size of the training population.

N_e and marker density The larger the size of the population of interest (N_e), the more markers that will be needed to ensure that useful markers will be found. GS depends on the markers being in LD in the same phase with the QTLs across the entire population. With N_e below 60, they suggest that 2 to 3 markers per centimorgan would be sufficient, perhaps around 5000 markers total. For larger populations, 20 to 30 markers per centimorgan would be needed, perhaps as many as 50 000 markers.

Training population size GS works in a similar way to indirect selection for wood quality using NIR spectroscopy. A training population must be measured for the traits of interest and all of the possible markers. This training population is analyzed to develop the marker relationships (i.e., identify which markers are useful), and then those markers are used to predict the GEBV for future selection populations. Training populations on the order of 1000 to 2000 genotypes are needed to accurately identify marker-QTL relationships.

Some recent results from a study on *P. taeda* shed some light on the potential of GS for tree breeding (Zapata-Valenzuela 2011). A group of 149 clones derived from 13 full-sib families (18 total parents) was tested across a number of field sites (approximately 3 to 6 sites per clone), and characterized for 3406 SNPs. Using half of the clones for the training set, GEBVs were then predicted for the remaining half of the clonal population. Accuracy of prediction was good for lignin and cellulose content (Corr(g,ĝ) ≈ 0.75), but only moderate for five-year height and volume growth (Corr(g,ĝ) ≈ 0.55 and 0.40, respectively). With more markers, accuracy would presumably increase, and one possible use of these markers would be to conduct GS on future offspring from this same set of full-sib families to preselect good clones. If GS was to be applied for multiple generations, it is likely that selection response will degrade over time (Jannink 2010). The higher the initial marker density, the slower the rate of degrade in response to GS (Solberg et al. 2008), however, at some point, a "re-calibration" of the markers would need to be done, that is, new markers identified using a new training population.

There are many technical questions associated the optimum statistical approaches to analyze the marker-phenotype data sets. But once a GS system is in place, GS could theoretically be beneficial to breeders by: 1) increasing accuracy of prediction (Corr(g,ĝ)), thus making more genetic gain per breeding cycle or 2) eliminating the need for progeny testing, thus reducing the breeding cycle and increasing gain per unit time. How valuable would these benefits be to forest breeders?

In my view, increasing the accuracy of prediction of breeding values is probably a minor benefit to forest tree breeders. A typical progeny testing scheme will very accurately predict parental breeding values (in other words, family heritabilities will be quite high). The advantage of GS would be in increasing the gain from within-family selection, that is, the identification of the best offspring in particular families. With low heritability traits, this gain could be significant. However, tree breeders have an advantage (one of few) over animal breeders in that trees can quite easily be cloned. Even for the difficult- to-work-with pines, rooted cuttings can be used quite easily to clonally test individual trees to increase the heritability of within-family selection (Shaw and Hood 1985). Combined selection with high family heritability plus high within-family heritability will result in high Corr(g, ĝ). Isik et al. (2004) examined a strategy of progeny testing with clonal replication for *P. taeda* for six-year-old volume growth. Assuming 25 clones/full-sib family, 4 test sites, and 4 ramets/clone/site, they estimated heritability of full-sib family means of 0.62, and a heritability for clones within family of 0.70. In another study for a similar trait (four-year-old volume), Isik et al. (2005) reported a within-family clonal repeatability of 0.60 using a design with only 2 sites and 9 ramets/site. Since heritability is directly related to selection accuracy (the square root of heritability = Corr(g, ĝ), see Hodge and White (1992), these values indicate that it would be fairly easy to obtain Corr(g, ĝ) of around 0.80 for clones. A complementary testing design with full-sib or half-sib families across a number of sites, and cloned full-sib family selection blocks on a few sites could also generate quite precise breeding value predictions.

This means that, for tree breeders, the main advantage of GS would probably come in reducing the length of the breeding cycle by eliminating the need for progeny testing. Assume that one could use GS at the seedling stage to make selections for the next cycle of breeding. Those selected trees must reach physiological maturity in order to produce sufficient flowers for breeding, and in this case, tree breeders are now at a disadvantage compared to animal breeders and most crop breeders. For long-lived trees, reaching reproductive maturity can take quite some time, 2–3 years for fast growing tropical eucalypts, or perhaps 3 to 6 years for more temperate conifer species. Tree breeders have some techniques to promote precocious flowering, e.g., topgrafting seedlings into the crowns of physiologically mature trees (Perez et al. 2007), or treatment with growth hormones like paclobutrazol (Moncur 1998), but even with these tricks, the need for time for trees to grow cannot be avoided.

For most forest tree species, breeders have found that juvenile-mature growth correlations are high enough to allow selection at $^{1}/_{3}$ to ½ rotation age, which could be as early as 3 to 4 years for a species grown on an 8-year rotation, and at age 5 for species on rotations of 15 to 20 years. Thus, for many species, it might be realistic to expect that GS would result in a reduction of the breeding cycle by only a few years, reducing the potential benefit. However, for long-rotation species, GS could conceivably reduce breeding cycle length substantially, perhaps by 50% or more. In my view, this suggests that tree breeders working with long-rotation species like *Pseudotsuga menziesii, Picea abies, Picea glauca*, *Pinus banksiana, Pinus sylvestris, Pinus lambertiana*, and *Pinus monticola* might reap the most benefit from GS in forestry.

Would tree breeders (or research managers or forest operations) be comfortable planting genotypes selected by GS? There will almost certainly be a need and desire to field test commercial varieties or clones prior to deployment (Heffner et al. 2009). Also there is still the issue of cost. Assuming a cost of US$ 0.01 / SNP / tree, and a relatively small breeding application (training population = 1000 trees, inference population = 10 000 trees, 5000 markers), the total cost would be $500,000. If a large number of markers tested on the training population are uninformative, perhaps the number of markers used on the inference population could be reduced, thus reducing the overall cost. But breeders must consider the alternatives: dollars invested in GS could go toward traditional progeny testing, and long after the data has been collected, the planted trees will eventually be harvested to generate income. Although some questions remain, the potential of genomic selection is exciting. It seems likely that GS will have real applications in some tree breeding programs over the next two decades.

SUMMARY – OUTLOOK FOR THE FUTURE

Many of these areas of opportunities and challenges overlap and interact. For example,

Somatic Embryogenesis and Hybrid Pines: Full-sib family forestry can work well with commercial pine hybrid production, but the ability to select and propagate clones would almost certainly provide the opportunity to make a significant increase in genetic gain. This might be particularly true for some hard-to-make hybrids, perhaps involving somewhat distantly related species, few plants might be produced, butthose could be outstanding phenotypes.

Genomic Selection and Hybrid Breeding: Hybrid populations will almost certainly have higher amounts of linkage disequilibrium than pure species populations, thus GS might be more effective (genetically) and more cost effective (requiring fewer markers). Many eucalypt breeders are already making three-way and four-way hybrids, for example, *(E. grandis* x *E. urophylla)* x *E. globulus*. Resende and Assis (2008) have proposed reciprocal recurrent selection between synthetic multi-species populations (RRS-SMSP) for eucalypts, with the idea of using GS to accelerate the breeding.

Disease Threat and Pedigree Reconstruction: The threat of new diseases may move many organizations to expand their species portfolio by developing plantation bases of new species. An organization might choose to purchase bulk orchard seedlots (i.e., genetically improved seed) from other geographic regions. Pedigree reconstruction could be used to “progeny test” those species, quickly providing improved material of a resistant species.

Tree breeders have always had an array of options for every phase of activity in the breeding cycle – selection, mating and testing designs, breeding strategies, propagation, and deployment. With new tools and technologies added to the already existing array, the development of an optimal strategy given a specific set of financial and logistical constraints will remain the breeder’s primary challenge.

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INVITED SYMPOSIA PAPERS

BREEDING FROM LODGEPOLE AND RADIATA PINE TO SCOTS PINE AND NORWAY SPRUCE – STRATEGIC DIRECTIONS OF TREE BREEDING IN SWEDEN

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Strategic directions for future Swedish tree breeding research are outlined and discussed by combining experiences of genetic improvement for lodgepole pine (*Pinus contorta* var. *latifolia*) and radiata pine (*P. radiata*) and application of the recent development of tree genomics.

The main focus of lodgepole pine genetic improvement in British Columbia was traditionally on delineation of seed transfer (planning) zones and units using large scale provenance trials, and the development of response surface and reaction norms of provenance populations. With the past decade of decimation of lodgepole pine forests by the mountain pine beetle (*Dendroctonus ponderosae*) and coping with realized and future climate change, an assisted migration program was initiated by redefining the seed transfer limits. Seed planning units form the basis for tree breeding and seed production planning. However only about 20% of lodgepole pine seedlings planted were of seed orchard origin with the remainder from superior-provenance seed collections and from wild stand collections.

In contrast, in Australia, almost 100% of planted seedlings were from radiata pine seed orchards. Radiata pine has been bred for three generations since the 1950s with realized genetic gain up to 33% from the first-generation and more than 10% gain from the second-generation for harvested volume. The focus of advanced generation breeding has shifted to optimization of production profits and optimization of deployment gain including:

1) development of economic breeding objectives,

2) application of Best Linear Unbiased Prediction using TreePlan and a Web-based interactive database for customized delivery of breeding values, tracking of pedigree, trials, mating, and selections,

3) infusion of new genetic material from the range-wide collections into existing breeding population,

4) increase of recombination rate and selection intensity by moving from discreet generation to a rolling–front breeding strategy,

5) implementation of MatePlan and purging of inbreeding depression to deal with inbreeding depression,

6) development of strategies dealing with adverse genetic correlation between wood volume and quality traits,

7) development of optimal deployment strategy - SeedPlan for maximizing deployment genetic gains, and

8) integration of genomics into selective breeding procedures to increase selection intensity.

The current Swedish tree breeding program and breeding strategy for Scots pine (*P. sylvestris*) and Norway spruce (*Picea abies*) were established towards the end of the 1980s and possess mixed features of lodgepole and radiata pine. Twenty-four breeding populations for Scots pine and 22 for Norway spruce were organized according to photoperiod (latitude) and growing season (temperature gradient). The breeding strategy mainly involves within-family selection with progeny testing to verify elite selections, uses double-pair mating for the generation of the same number of families as the previous generation, and adopts a simple experimental design for progeny testing.

Recently, the methodology for estimating breeding values has been refined and TreePlan has been introduced for the prediction of individual breeding values. The third round of seed orchards has been established for the deployment of seed from elite selections. A significant issue in deployment is high pollen contamination in seed orchards.

Using tree improvement experiences in lodgepole pine and radiata pine, and by absorbing recent rapid developments in genomics, three main research areas for accelerating Swedish Scots pine and Norway spruce breeding programs were identified to improve genetic gain: 1) sustaining genetic gain by redefining breeding zones and breeding populations for optimal adaptation of breeding and deployment populations, 2) increasing genetic gain by developing and adopting an optimal breeding and deployment strategy, and 3) realizing genetic gain earlier by integrating genomics into operational breeding selection.

Optimal breeding zones (number and size) will be redefined by matching genotypes with current and predicted environments of future plantations (under climate change model). This will be accomplished by meta-data analyses of G x E patterns for Norway spruce and Scots pine and by biogeoclimatic mapping of plantations.

Breeding objectives for solid wood production and for pulp and paper production will be developed as part of the overall breeding strategy to increase genetic gain for final products and production enterprises. This will be accomplished by estimating economic weights for solid wood, and pulp and paper production systems and for future carbon/bioenergy production systems. A large scale quantitative genetic survey is planned to estimate genetic variance and covariance matrices from early selection to harvest traits for Norway spruce, and Scots and lodgepole pine.

A flexible mating strategy through the control of co-ancestry and planned inbreeding for purging inbreeding depression is planned. Optimal selection and mating strategies and inbreeding effect are simulated using an allele model and controlled experiments including inbreeding are planned for the management of long-term genetic gain and adverse genetic correlation between wood quality traits and volume. Whether the current discreet generation strategy should be retained or migration to a rolling-front strategy is being studied using simulation. All individual tree selections are based on breeding values estimated from multi-traits, multi-sites, and multi-generations for the entire Sweden breeding program. Furthermore, a new deployment strategy is planned for optimizing genetic gain and production costs.

Novel genes for adaptation, fecundity, and wood quality traits are being explored using next generation sequencing technology including RNA sequencing. To realize genetic gain earlier and increase selection efficiency, population-based association of alleles with early selection and breeding objective traits are being implemented through candidate gene and genome-wide approaches for marker-aided and genome-wide selection. Advanced methodology of genome-wide association and selection using genomic estimated breeding value are being developed and tested for very early selection to increase selection intensity and genetic gain.

DEVELOPMENT OF DNA BASED TOOLS FOR MARKER ASSISTED SELECTION BY GENE MAPPING IN WHITE SPRUCE (*P. glauca*)

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Connecting phenotype to genotype is the basis for developing forest genetic applications such as marker assisted selection (MAS). Quantitative Trait Locus (QTL) mapping and genetic association mapping (or linkage disequilibrium (LD)) are two major approaches to find genes and DNA markers that control phenotypes of interest in forest trees. We have used these approaches to identify genes linked to or associated with growth, adaptation, and wood property traits in white spruce (*Picea glauca*).

GROWTH AND PHENOLOGY

The genetic architecture of bud phenology and height growth was investigated by assessing QTLs across pedigrees, years, and environments. A total of 11 distinct QTLs for bud flush, 13 for bud set, and 10 for height growth were localized on a linkage map. Nearly 50% of the QTLs were stable across environments and/or years and 20% were replicated between populations. The proportion of phenotypic variance explained by QTLs ranged from 3 to 22.2%, and QTLs accounted for up to 70% of trait variance. A genome-wide scan of 534 SNPs localized in 345 expressed genes identified 50 genes as candidates for local adaptation which included sequences which co-localized with the QTLs for bud phenology. Similarly we identified genes only or preferentially expressed in developing buds, which we hypothesize to play a more prominent role in bud formation, several of which co-localized with QTLs for the time of bud set.

WOOD PROPERTIES

Pilot association studies tested 469 trees and genotyped 944 SNPs in 549 genes related to wood development to identify diagnostic SNPs for wood traits. The SNPs were identified by association studies using a mixed-linear model for 25 wood physical traits, testing for additive and dominance effects. Thirteen SNPs had a significant effect ($Q<0.10$) in as many genes belonging to different families. Individual SNPs accounted for 3 to 5% of the phenotypic variance in 10 wood traits. The phenotypic variance explained by the SNPs reached up to 11% with approaches combining several SNPs. Significantly different transcript levels ($P<0.05$) were also found among the SNP genotypes for some of the genes.

Larger-scale association studies with 40 wood traits used an expanded population of 1700 trees and 6700 valid SNPs from 2660 genes for an average of 2.5 SNPs/gene (range 1 to 12). Single gene locus analyses identified a total of 73 different SNPs associated with up to 27 wood traits, each accounting for between 0.7 to 5.9% of the phenotypic variance. Bayesian multi-locus analyses showed that up to 60% of the variance could be explained by all the SNPs assayed for a trait such as average wood density, for instance. A subset of 47 SNPs with effects significantly different from zero could explain close to 40% of the variation in wood density.

CONCLUSIONS

QTL mapping and genetic association mapping results were reported. Our findings indicated that DNA markers may explain a proportion of the phenotypic variation that is sufficiently large to assist in tree selection and breeding programs. The use of markers holds the potential to shorten the breeding cycle and to facilitate selection of traits like wood properties which are expensive to measure.

GENOMICS OF FOREST TREES' RESPONSE TO CLIMATE CHANGE CONDITIONS AND ADAPTIVE TRAITS

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Rapid global climate change has become a prominent driving force of evolution in forest ecosystems, subjecting our planet's ecosystems to significant abiotic stresses. This can affect forest trees' fitness, adaptation, productivity, and evolutionary potential. Northern boreal and temperate forest trees/ecosystems are considered to be the most vulnerable to global climate change. There is an urgent need to understand the genomic basis of responses and adaptation of forest trees to climate change in order to develop and use genetically improved plant varieties and to conserve and sustainably manage well-adapted, genetically diverse and healthy forest genetic resources. We are using structural, functional, and population genomics approaches to address this very important aspect in economically and ecologically important North American boreal and temperate spruce (*Picea*) species.

We sequenced the whole transcriptomes (expressed genes) of two spruce species and identified and annotated genes expressed under normal, elevated CO_2, drought, and their combined conditions. Thousands of transcripts (genes) involved in several metabolic pathways showed differential expression (no expression, up-regulation or down-regulation) in response to elevated CO_2, drought, and/or their combined conditions. Responses to each treatment at the gene expression and physiological levels were correlated well among different genotypes. We identified and mapped quantitative trait loci (QTLs) for growth, biomass allocation, and water-use-efficiency traits in black spruce grown under normal and drought conditions. We are identifying and mapping genes and QTLs for traits related to growth and adaptation to climate change in spruce grown under ambient and elevated CO_2 conditions.

AN EPIGENETIC MEMORY IN NORWAY SPRUCE AFFECTS ADAPTIVE TRAITS

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In Norway spruce (*Picea abies*) an important adaptive mechanism termed an “epigenetic memory” has been identified. The memory affects the growth to dormancy cycle, which so importantly should be synchronized with seasonal change in temperature and day length. The temperature during post-meiotic embryogenesis and seed maturation shifts the growth cycle program of the embryos, resulting in significant and long lasting phenotypic change in the progeny, e.g., vital phenological processes with high adaptive values such as bud break and bud set. This phenomenon is not only evolutionarily interesting, but has clear practical implications for breeding and forest genetic conservation.

The underlying molecular mechanism that causes the ‘memory’ is still unknown, but transcriptional changes are clearly involved. Epigenetic regulation may be realized through several mechanisms, including DNA methylation, histone modification, chromatin remodeling, small non-coding RNAs, and transposable element regulation, of which non-coding RNAs might be one of the most important determinants.

It was somewhat unexpected and surprising to find that an epigenetic memory influences adaptive traits in common garden tests. These traits normally follow clear variation patterns among natural Norway spruce populations that parallel geographical or climatic clines at the origins of the populations. Such a clinal variation pattern normally suggests that strong natural selection is the causal factor shaping such a variation pattern.

The discovery of the epigenetic memory phenomenon arose from studying seedlings from seeds produced in Norway spruce seed orchards. Breeders planted parental grafts of northern Norwegian ecotypes in a southern seed orchard, the grafts produced seeds there, and the seedlings expressed a phenology surprisingly similar to that of native southern ecotypes. This was later confirmed by the observation that seedlings from central European trees, growing and producing seeds in central Norway, performed similarly to local Norwegian families, and very differently from seedlings from seeds produced at their central European origin. Differences in day length and temperature applied during male meiosis and microsporogenesis (pollen formation) did not affect progeny performance. However, differences in the maternal environment did so. The temperature during post-meiotic megagametogenesis (zygotic embryogenesis) and seed maturation shifted the developmental program of the embryos, resulting in significant long-lasting phenotypic changes affecting the growth cycle of the progeny. No effects of temperature treatments given during prezygotic stages where shown to influence the seedling performance.

The traits that are affected include the timing of dehardening and bud burst in the spring, cessation of leader shoot growth in the summer, as well as bud set and cold acclimation in the autumn. All of these traits occur earlier or later as an after-effect of the temperature applied during female reproduction and tested in full-sib progeny with identical genetic backgrounds. A colder environment during seed development epigenetically advances bud set and cold acclimation during autumn and dehardening and bud burst during spring in the progeny. The photoperiod during seed production also interacts with temperature in influencing these traits in the progeny. Progeny resulting from seed production at different temperatures during zygotic embryogenesis, followed by propagation of genetically identical clones at different temperatures during somatic embryogenesis, expressed a difference in timing of terminal bud formation equivalent to a 4–6° latitudinal ecotypic difference. The memory effects acting on the phenological traits last for more than 20 years after germination, and accordingly have implications for long-term growth under field conditions. Thus, this memory, affecting the climatic adaptation in Norway spruce, must be of an epigenetic nature and permanently fixed by the time the seed is mature.

This phenomenon is not only of great evolutionary significance but has clear practical implications for seed production. The memory may act as a mechanism to aid trees to cope with the rapid change in temperature due to global warming. It will also be of importance for the deployment of seedlings from seeds produced in seed orchards under warmer or colder conditions relative to sites where the plants are to be planted. The memory may also protect individuals within populations from mortality and thereby maintain greater genetic diversity in the populations. The presence of the epigenetic memory confirms that an exchange of breeding material of Norway spruce among countries can work nicely, in order to increase effective population size and counteract inbreeding in advanced generations. It may also be possible to exploit the phenomenon in producing seedlings by somatic embryogenesis with distinct phenotypes (epitypes), and thus specific adaptive properties. Although the epigenetic effect provides a highly important additional layer of adaptive flexibility, the genetic diversity and potential of the species are still of basic importance for the influence of adaptive traits.

Analysis of molecular components of epigenetic regulation raises one major question; which of the epigenetic molecular components regulates the memory? The epigenetic state (epitype) should be mitotically heritable and not involve alterations in the primary DNA sequence. The examples of transfer of the epitype to the next generation(s) imply that the epigenetic changes are somehow maintained or transferred after each cell division. DNA and histone methylation are obvious modifications which are epigenetically regulated, e.g., by RNA-directed DNA methylation. Making, maintaining, and erasing methylation are dynamic and complex processes involving a large number of genes. DNA methylation can be transmitted mitotically and meiotically. However, since DNA methylation levels change between seasons, it is not clear if DNA-methylation is the cause or the effect of the epigenetic memory regulation, and the methylation level is likely dependent on other factors. Increasing evidence is indicating that most epigenetic mechanisms of gene expression control include regulation by non-coding RNAs. It is becoming clear that these molecules play very important roles in various epigenetic modification mechanisms and plant development.

Our group (collaboration between the Norwegian Forest and Landscape Institute and The Norwegian University of Life Science) made a search of candidate genes that could regulate the memory, using specialized library sequencing approaches. Two subtracted cDNA libraries, representing genes that are mainly expressed in plants from a cold (CE) or a warm (WE) embryogenesis, revealed considerable differences in the transcriptomes. Many contigs were unknown. We used qRT–PCR to demonstrate that three genes with no matching in the database and 2 transposon-related genes were differentially expressed in close correlation with the memory-induced differences in bud set. We also prepared concatemerized small RNA libraries from seedlings of two full-sib families that originated from seeds developed in a cold and warm environment. One family expressed distinct epigenetic effects, while the other family did not. We used available plant miRNA query sequences to search for conserved miRNAs and from the sequencing we found novel ones; the miRNAs were monitored using relative real time-PCR. Sequencing identified 24 novel and four conserved miRNAs. Further screening of the conserved miRNAs confirmed the presence of 16 additional miRNAs. Most of the miRNAs were targeted to unknown genes. The expression of seven conserved and nine novel miRNAs showed significant differences in transcript levels in the full-sib family showing distinct epigenetic difference in bud set but not in the non-responding full-sib family. Putative miRNA targets were studied. Norway spruce contains a set of conserved miRNAs as well as a large proportion of novel non-conserved miRNAs. The differential expression of specific miRNAs indicates their putative participation in the epigenetic regulation.

The present knowledge of epigenetic inheritance is far from complete. In addition, information about possible epigenetic inheritance in mitochondria and chloroplasts is lacking. Furthermore, epigenetic inheritance systems may well vary between different taxa. The best-studied, short-lived *Arabidopsis* is not necessarily representative for the less studied and extremely long-lived woody species. It will be a challenge to disclose the epigenetic mechanism and to understand how the observed genetic variation in the epigenetic memory can help us unveil the molecular components, by using modern biotechnological and bioinformatic tools together with proper experimental material and designs.

THE BREEDING PROGRAM OF THE AMERICAN CHESTNUT FOUNDATION AT MEADOWVIEW

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Charles Burnham hypothesized in 1981 that the blight resistance of Chinese chestnut (*Castanea mollissima*) could be backcrossed into American chestnut (*C. dentata*), producing a tree that would perform in the forest similarly to the formerly dominant American chestnut. The American Chestnut Foundation was formed in 1983 to test this hypothesis, using a traditional plant breeding program. The program has been guided by full-time professionals since 1989. It was jump-started by using two B1 chestnut trees produced by the old breeding programs of the U.S. Department of Agriculture and the Connecticut Agricultural Experiment Station, known as 'Clapper' and 'Graves.'

The old breeding programs also largely developed the methods used in the current program and identified Chinese chestnut as the most promising source of blight resistance.

'Clapper' and 'Graves' were each crossed into approximately 30 American chestnut from the vicinity of Sugar Grove, Virginia, backcrossed again then intercrossed twice, with selection for blight resistance and recurrent morphological type prior to each cross. B3-F3 seedlings were planted back into the forest for testing beginning in 2009. The forest performance and blight resistance of those seedlings should start becoming evident by 2015 or 2020. Tests of their parents suggest that their blight resistance will fall somewhere between an intermediate and a high level. The first formal orchard test of the blight resistance of B3-F3 seedlings will be inoculated in 2011, which should clarify this somewhat. The ultimate blight resistance of these trees in the forest will be influenced by their adaptation and may also be affected by hypoviruses and other factors. It may take decades for a clear picture to emerge.

Other sources of blight resistance include 'Nanking' Chinese chestnut, which has been backcrossed into 20 American backgrounds. These will begin producing B3-F2 progeny in a few years. More than 20 other sources of blight resistance from Chinese chestnut have been backcrossed to varying degrees into American chestnut but into only a few backgrounds, so they would not by themselves constitute a viable breeding population. Other sources of blight resistance, such as *C. crenata*, have been used to lesser extents, and *C. henryi* and *C. sequinii* will be used in the near future. Using another type of blight resistance, American chestnut trees with low-levels of blight resistance have been increased into generic American chestnut and these crossed within and between sources of resistance. The best of these have intermediate levels of blight resistance.

Where possible, selection for American type was made using molecular markers, but this effort has been hampered by a lack of suitable markers, throughput, and funds. The fruits of the Fagaceae Genomics Project and the Forest Health Initiative may result in more routine use of markers in the project in the near future. It might be especially interesting to try to dissect out some of the components of forest performance, which may differ between lines within sources of blight resistance.

Strains of the blight fungus isolated from various hosts have shown no differences between host in general virulence. They have differed in general virulence among themselves. Isolates from the extremes of pathogenicity for virulent isolates are used to screen for resistance. The genome of two isolates has been sequenced and progeny of their cross evaluated for pathogenicity. We will look for QTLs for pathogenicity in their genetic maps.

REGENERATION DECISIONS UNDER AN EVOLVING AND UNCERTAIN FUTURE CLIMATE

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Climate is one of the major influences on forests and much effort is being expended to consider the possible consequences of rapid climate change on forests. In some parts of Canada forest managers are beginning to develop policies and practices that encourage the use of more southern seed sources, opening the specter for (relatively) widespread assisted migration of species and populations. A number of models/tools have also been developed that help to quantify plant response to climate and hence possible climate change.

In this talk I reviewed trends and prospects for climate in Canada including a brief discussion on the issue of the quality of Global Circulation Models (GCMs), the primary tool of the science world to identify the possible consequences of higher concentrations of greenhouse gases in the planet's atmosphere.

I will also briefly touched on the consequences of a rapidly changing climate on the distribution and performance of plant species. This is likely somewhat familiar territory for many. Research on the topic has burgeoned in recent years. But what faith can we put in such models? They range from simple climate envelops, more complicated statistical analyses to much more sophisticated process-based models that attempt to represent, for example reproductive processes and forest community outcomes into the future. And, how good are the GCMs models to support "place-specific" decisions like matching genotypes to future climates?

Part of this discussion touched on some early perspectives arising from the compilation and analysis of provenance trial data from across North America. The forest genetics community has a long history of moving plant material in a structured fashion. In the quest to gain more of an empirical insight on the subject of plant performance and climate relations we have compiled and collated published results from some 30+ provenance studies for a range of both hardwood and softwood species in both Canada and the United States. We have examined the correlations between a climate similarity index (between the trial location and the provenance source) and growth rates. We believe these results will help provide insights into the impacts of climate change and also represent a useful investment – making use of old trials. The presentation finished with some questions and suggestions for future work on the topic of regeneration decisions under an evolving and uncertain future climate.

GEOSPATIAL CLIMATIC ANALYSIS OF ADAPTIVE VARIATION IN BOREAL FOREST TREES: A 24 YEAR RETROSPECTIVE

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In 1992 the first version of ‘focal point seed zone’ was developed and published. This spatially explicit approach, based on the results of short term growth trials, made the site to be reforested into a ‘focal point’ and a unique seed zone was established for that site as needed. In 1996 the approach was modified to use climate-based regression models of provenance growth. In 2000 a spatially explicit differential systematic coefficient, again based upon the results of short term field trials, was adapted to indicate the rate of change of clinally expressed adaptive variation over distance and serve as an indicator of breeding zone boundaries. Beginning in 2001 range-wide provenance trials of white spruce (*Picea glauca*), black spruce (*P. mariana*), and jack pine (*Pinus banksiana*) were remeasured to develop response functions and transfer functions expressed as contour lines in present-day and future climates. In 2005, a maximal covering model was developed based on adaptive variation that selected a subset of breeding zones that maximally covered an area given a limit on the number of zones and the adaptive dissimilarity allowed within zones. In 2008, based on short term growth trials, modern portfolio theory was used to select a ‘seed source portfolio’, i.e., an optimal set of seed sources to be used in regenerating forests in an environment of multiple, equally plausible future climates. In 2011 a methodology was developed to develop robust reserve networks to conserve tree species across multiple climatic futures.

ADAPTING TREES TO A CHANGING CLIMATE: PHENOTYPIC AND GENOMIC APPROACHES

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The single largest threat to most native populations of trees in Canada is likely climate change. To quantify this threat, we need to better understand the adaptation of populations to climate. The analysis of phenotypic data on growth and survival from field provenance trials, and on growth, cold hardiness, drought tolerance, and phenology from nursery and growth chamber common garden experiments has provided an extensive literature on local adaptation and phenotypic plasticity in populations of widespread tree species. Re-analyses of these data from a climate-change perspective have provided valuable information on levels of plasticity and the predicted magnitude of adaptive lag under different climate change scenarios. Despite this rich knowledge of provenance effects for our most common species, numerous questions remain about the future of our native forests.

How can genomic knowledge inform our efforts to predict the condition of our future forests and their capacity to adapt to or tolerate new conditions over the next century? First, by using association mapping approaches, we can identify the genes and single nucleotide polymorphisms (SNPs) underlying phenotypic variation for key adaptive traits. Secondly, by applying population and landscape genomic approaches, we can characterize the adaptive surface of tree populations across the highly varied landscapes they occupy. We can also dissect the interacting effects of gene flow and natural selection on population differences, and identify those loci and SNPs that have significant clines along climatic gradients. One advantage of characterizing clines in adaptive SNPs over phenotypic clines is their independence from experimental environmental effects.

Once patterns of adaptive genomic variation are quantified, genomic knowledge can then be translated into changes in seed transfer and breeding programs for future climates. In the Genome Canada and Genome BC-funded AdapTree project, we are taking a population genomic approach to evaluate alternative climate-based seed transfer models. We will assess potential seed transfer systems for both lodgepole pine (*Pinus contorta*) and interior spruce (*Picea glauca, P. engelmannii*, and their hybrids) based on their capacity for matching genotypes to appropriate environments, and for managing among- and within-population genetic variation for key adaptive traits. We will also assess the impact of tree breeding on levels of adaptive genomic variation within reforestation seedlots, and on adaptive traits related to temperature and moisture stresses. Finally, we can assess the capacity for adaptation within populations based on levels of standing adaptive variation at the genome-wide rather than the phenotypic level.

VOLUNTEER PAPER ABSTRACTS

COMPLEMENTARY MULTI-VARIETAL FORESTRY FOR MEETING FUTURE CHALLENGES OF SEED ORCHARDS

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In the past 30 years, tree improvement programs around the world have contributed greatly to forest productivity and wood quality attributes of plantation forestry. The delivery of such improvement has been through seed orchards and this will continue to be the primary means of providing genetic improvement. However, seed orchards are often associated with several inefficiencies such as pollen contamination and unequal parental contributions. In addition, projected climate change further complicates efficiency of conventional seed orchards. With recent developments and refinements in somatic embryogenesis (SE) technology, complementary and/or alternative breeding, testing, and deployment strategies to meet these challenges have become available for several conifer species. In particular, SE enables Multi-Varietal Forestry (MVF), i.e., the use of tested tree varieties in high-value plantation forestry, offering: 1) much greater genetic gain than seeds orchards, 2) flexibility to adapt to changing climate and product goals, and 3) ability to manage genetic gain and plantation diversity. SE also simplifies breeding and selection schemes, offers an opportunity to develop pest-resistant tree varieties, and provides a new dimension for species conservation and restoration.

GENOTYPE X SPACING INTERACTIONS IN REALIZED GAIL TRIALS OF DOUGLAS-FIR

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Estimated genetic gains were verified for age 12 trees on five test sites across four spacings for second-generation improved coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Three genetic levels were tested in large area-blocks (12x12 trees) in each of the following spacings: 1.6 x 1.6, 2.3 x 2.3, 2.9 x 2.9, and 4 x 4 m. The genetic levels were top cross (TC), mid-gain (MG), and wild-stand controls with estimated genetic gains for TC of 18% and 10% for MG for tree volume at rotation age 60 years. Gains in expected tree height were close to predicted, but exceeded expectations in tree volume partially due to the additional effect of increased DBH growth. No significant interactions between genetic levels and spacing were observed. However, when the genetic merit of parents was calculated separately in each genetic level, significant spacing x genotype interactions were observed for both TC and MG parents. These interactions were due to rank changes in both height and DBH. Rank correlations ranged from 0.6 to 0.8 and from 0.5 to 0.7 for height and DBH, respectively. Practical implications of these findings were discussed.

THE ROLE OF MASS SELECTION IN ENHANCING GENETIC GAIN IN SECOND-GENERATION BREEDING POPULATIONS IN NORTHEAST ONTARIO

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An ideal second-generation breeding program would have at least two attributes: 1) a large unrelated breeding population and 2) high selection intensity based on breeding values. To optimize genetic gains for traits with modest heritabilities such as height and volume growth, selection intensity would preferentially be directed toward parental (backwards) selection. Unfortunately, because of the limited number of plus-tree selections, Ontario’s first-generation tree improvement programs were not designed to take full advantage of backwards selection. Simulation work in single-tree-plot progeny tests supported the hypothesis that using a comparison-tree approach to phenotypic selection in young plantations could exert a strong genetic selection pressure. A program of comparison-tree mass selection in operational plantations was implemented for all breeding programs in northeast Ontario. The resulting selections have expected breeding values comparable to the predicted breeding values of the selected elite breeding populations from first-generation breeding programs. Incorporating these new selections into the second-generation breeding program has the potential to address the twin goals of exerting high selection intensity while maintaining a large breeding population.

A PRACTICAL APPLICATION OF AN AGE-AGE CORRELATION TO EARLY SELECTION IN ALBERTA

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Many tree improvement programs rank parents and predict genetic gain at ages much lower than the half-rotation age recommended by Zobel and Talbert (1984) and other prominent tree breeders. In Alberta, where the rotation age for conifers ranges from 80 to 110 years, delaying selection and prediction of genetic gain until progeny trials are 40 to 55 years old is impractical and uneconomical. Therefore, data from progeny trials usually less than 25 years old are used. Although progeny trials on warmer, moister, and more fertile sites may have reached crown closure at this stage, a potential for large changes in family ranks still exists. To provide some protection against overestimation of genetic gain, the Government of Alberta uses the age-age correlation from the Lambeth (1980) equation to convert expected genetic gain at a juvenile age to expected genetic gain at a rotation age. Industry members of the Alberta tree improvement programs dislike the age-age correlation derived from Lambeth's (1980) equation because the equation was developed using data from non-boreal conifers, it is not related to the data from which genetic gain is predicted, and there is a general perception that the actual genetic correlation may be higher than the phenotypic correlation predicted by the equation. Therefore, the industry prefers stand dynamic-based biometric models (to be developed) to age-age correlation for predicting genetic gain at rotation age.

To address some of the criticisms of the Lambeth (1980)-based age-age correlation, the Alberta Tree Improvement and Seed Centre (ATISC) is developing an alternative correlation prediction equation using government and industry progeny and provenance trial data. In the Lambeth equation, the input variable is the ratio between selection and rotation ages, which allows the predicted correlation to be the same when the selection age is 25%, 50%, and 75% of the rotation age irrespective of the actual age interval between selection and rotation age (e.g., age 2 and 4 years has the same correlation as age 50 and 100 years). To prevent this, ATISC is using an age difference as an input variable into the regression. The advantage of this approach is that, for trials where serial measurements are available, the correlation and rotation age gain are both predicted from the same data. For trials where serial measurements are not available, the correlation is predicted from closely related trials in the same species and climate. A generic equation involving all species and trials is also being developed to be applied to species of lesser importance for which serial measurements are not available. Preliminary results indicate that: 1) results are similar across trials and species, 2) family and provenance trial data are equally good, and 3) correlations predicted from equations developed from breeding values and those developed from Pearson's correlations are similar.

WHOLE TRANSCRIPTOME SEQUENCING AND GENE EXPRESSION PROFILING IN BLACK SPRUCE (*Picea mariana*)

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Whole transciptome sequencing followed by bioinformatic analyses of sequences provides powerful approaches to identify and annotate expressed genes in a variety of organisms. We have undertaken whole transcriptome sequencing in black spruce (*Picea mariana*) using two next-generation sequencing platforms to identify and annotate differentially regulated transcripts in response to the main cause and effect of global climate change, which are elevated CO_2, drought, and the combination of both elevated CO_2 and drought. We used black spruce as a model species because it is one of the transcontinental conifers in the Boreal forest which are more vulnerable to global climate change. Moreover, black spruce is an ecologically and economically important species in Canada.

Needle tissue was collected at regular intervals from clones of four-year-old black spruce genotypes that were treated with ambient CO_2 (380ppm), elevated CO_2 (760ppm), watered/drought, and the combined elevated CO_2 and drought conditions. Total RNA samples were isolated from the needle tissues and then pooled according to the tissue collection intervals. The cDNA libraries constructed from the total RNA pools were sequenced by using long-read 454 pyrosequencer and short-read Illumina's sequencing by synthesis (SBS) systems. Quantitative RT-PCR (qRT-PCR) systems were also employed to quantify the amount of selected differentially regulated transcripts.

We obtained over 0.3 giga-base pairs (Gbp) transcriptome sequences of black spruce from four libraries sequenced using the 454 pyrosequencer. The transcriptome sequences were *de novo* assembled into over 30 000 contigs (sequence groups). Then, the assembled sequence groups were aligned with non-redundant protein sequences resulting in over 15 000 assembled sequence groups functionally annotated. The functionally annotated sequence groups were categorized by the gene ontology IDs and classified according to their molecular and cellular functions and cellular components. We found about 5000 full-length transcripts including open-reading frame (ORFs) from the functionally annotated sequence groups. We obtained over 50 Gbp transcriptome sequences of black spruce from sequencing of eight black spruce cDNA libraries using Illumina's SBS systems. The sequences were aligned with the 15 000 functionally annotated sequence groups in order to profile the transcripts differentially regulated under each of the treatment conditions. Consequently, we identified about 600 transcripts that were more than 10 times up- or down-regulated under different conditions. Furthermore, we discovered over 100 000 SNPs through the sequence alignment analysis. We are performing qRT-PCR analysis to verify the results obtained from whole transcriptome sequencing for a number of differentially regulated genes under different treatment conditions.

Overall, in black spruce, we have identified and functionally annotated genes expressed under ambient and climate change conditions as well as identified differentially regulated genes in response to climate change conditions. The results will help to deepen our understanding of tree's responses and adaptive mechanisms to global climate change at the molecular level. Also, the SNPs identified will yield molecular markers for various genomics, tree breeding, genetic resource conservation, and management projects.

GENETIC EVALUATION IN SPRUCE: CAN WE PREDICT RATHER THAN DESCRIBE?

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Large-scale candidate gene discovery for wood quality traits has taken place within the *Arborea* project at Laval University in collaboration with the Canadian Forest Service (Quebec). As polymorphic SNP loci were discovered within structural genes (cDNA), it was expected that some of them would have a moderate to large effect on the phenotype. However, unless locus effects and population samples are large, power to detect genetic variation between SNPs and quantitative traits is low. This hinders SNP use in genetic evaluations as SNPs are usually selected via truncation if deemed 'significant' and all variation due to small(er) effect loci is lost. As marker densities continue to grow, new statistical methods have been developed to capture variation from small effect loci. We investigated several of these multiple-SNP genetic models in a small black spruce (*Picea mariana*) and larger white spruce (*P. glauca*) population and discussed the challenges of using the information in a breeding program.

DO SELECTED FAMILIES OF WHITE SPRUCE PARTITION BIOMASS DIFFERENTLY FROM UNIMPROVED LOCAL SOURCES?

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White spruce (*Picea glauca*) is a valuable species for the paper industry in Minnesota. Through selection and progeny testing, genotypes with improved growth have been identified and placed into seed orchards to provide seed for commercial reforestation. Tree volumes of seedlings from these orchards exceed local unselected sources by as much as 30%. The mechanisms by which improved families achieve this growth remain elusive. Few studies have attempted to assess traits other than volume following selection. The objective of this study is to compare families with improved growth to bulked, woods-run sources with respect to biomass allocation to roots, shoots, and needles. Seed from four open-pollinated families from a local seed orchard and two woods-run sources was shipped to a nursery, germinated, and grown for one year prior to planting into individual 13-gallon pots. Sixty seedlings were destructively sampled and dried after each of three growth intervals: upon removal from the nursery styroblocks, following the first growing season outside, and following the second growing season. After the first growing season, seedlings did not differ significantly with respect to tree height, but the root systems of improved families were significantly larger than woods-run sources. A multivariate analysis on second-year growth confirmed that improved seedlings were taller and had higher needle weights than local sources. These findings were discussed and interpreted in the context of reforestation in both current and future climates.

BEST MATE INDICES TO PROMOTE EFFICIENCY IN MULTIPLE GENERATION BREEDING

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Intensive tree improvement strategies frequently include elegant multiple-cross mating designs such as partial diallels. With multiple-crossing schemes, random assignment of genotypes to crosses has been considered adequate. But, in today's economic climate the importance of efficiency in breeding strategies cannot be over emphasized. Single-pair mating has been shown to be very efficient at producing additional increments of genetic improvement through forward selection. However, the use of single-pair mating schemes triggers some concerns as to how to choose pairings. The 'z' distribution was used to consider the implications of assortative, complimentary, and random mating on expected attributes of the breeding population.

OPTIMAL BREEDING ZONES FOR WHITE SPRUCE IN ONTARIO UNDER CURRENT AND FUTURE CLIMATES

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Optimal breeding zones were developed for white spruce (*Picea glauca*) in Ontario under present and future climate conditions to examine potential shifts due to climate change. Sets of candidate (potential) breeding zones were determined based on the relationship between measured performance variables and climate. Candidate breeding zones were determined for current climate based on 1961–1990 climate normals and for future climate based on three different general circulation model predictions of 2041–2070 climate (CGCM2, HADCM3, and CSIRO). A decision-support model was used to determine the optimal subset of candidate zones which should form the operational zones for each climate scenario in order to maximize geographic coverage while limiting the total number of breeding zones used. Based on a maximum adaptive distance of 2.0 least significant difference (LSD) values between sites within zones, 14 breeding zones were required to cover the Ontario range of white spruce for the 1961–1990 data. Compared with breeding zones of other boreal conifers, current optimal breeding zones for white spruce were quite large, spanning up to 3° latitude and 10°–12° longitude and indicating large distances of effective seed transfer. Current breeding zones constructed based on a maximum adaptive value of 1.0 LSD value between sites within zones were comparatively more restricted and probably more accurately reflect true patterns of variation. Of the three general circulation models used to simulate future climate, HADCM3 B2 and CGCM2 B2 predicted 2041–2070 breeding zones that largely coincide with 1961–1990 zones while CSIRO B2 predicted much narrower future zones.

CHANGES TO BC'S SEED TRANSFER STANDARDS IN RESPONSE TO CLIMATE CHANGE

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British Columbia's Climate Action Secretariat envisions that "British Columbia is prepared for and resilient to the impacts of climate change." The BC Ministry of Forests, Lands and Natural Resource Operations has therefore, recently, begun to look at its policies and practices through a climate change adaptation lens. Changes to seed transfer standards and the introduction of climate-based tree species range and population expansion support these goals.

Recent changes to BC seed transfer standards in response to climate change include: 1) in 2008, an increase in elevation upper limits of 100 m or 200 m to many BC tree species for natural stand seedlots and seed orchard seedlots, and 2) in 2010, expansion of seed transfer limits of western larch (*Larix occidentalis*) beyond the contemporary range in areas projected to be climatically suitable in the year 2030.

Future work requires that a climate based seed transfer system be developed and BC's seed transfer will transition to the new system rather than continuing to amend and refine the existing standards.

The purpose of the recent and future seed transfer policy amendments is to address potential forest health and productivity impacts associated with a changing climate. The consideration and use of climate change adaptation strategies such as assisted range and population expansion are important tools for increasing ecosystem resilience and reducing tree species vulnerability in a changing climate.

IS INTRODUCED GENETIC RESISTANCE ACCEPTABLE IN MANAGING WHITE PINE BLISTER RUST IN *Pinus strobus*?

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White pine blister rust (caused by *Cronartium ribicola*) continues to be a major challenge for eastern white pine (*Pinus strobus*) regeneration in eastern Canada. There is little evidence to suggest that this forest health issue has been significantly alleviated by the changing climate or by improved silvicultural practices. Biodiversity and economic benefits created by the highly valuable forest species cannot be sustained when white pine disappears or declines in areas where blister rust hazard is high.

The lethality of *C. ribicola* is manifested by weak genetic resistance in eastern white pine to the introduced alien pathogen. The effectiveness of tree breeding within eastern white pine in an effort to mitigate the damage of *C. ribicola* has been largely unimpressive, despite considerable investment over several decades, which resulted in no operationally deployable resistant trees.

An alternative breeding strategy that breeds major resistance genes into *P. strobus* from Eurasian white pines through introgressive hybridization has proved to be more fruitful. Data from our resistance screening experiments with artificial disease inoculation suggest that strong resistance to *C. ribicola* has been passed onto progenies over multiple generations, including the third-generation hybrid backcross between *P. strobus* and *P. wallichiana*, which is 15/16 *P. strobus* by pedigree.

Field tests of eastern white pine hybrid backcrosses have indicated good growth rates and comparable wood attributes in areas where they have adapted well, particularly in Southern Ontario. The question now is: do strong blister rust resistance and good growth make eastern white pine hybrid backcrosses acceptable for operational deployment in high hazard areas?

SEED SOURCE PORTFOLIO SELECTIONS OF JACK PINE AT THREE ONTARIO LOCATIONS TO MINIMIZE RISK IN AN UNCERTAIN FUTURE CLIMATE

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Until recently no method or decision support model existed that could determine the most suitable seed source for artificial regeneration of a given site under the looming shadow of an uncertain changing climate. A decision support model capable of determining a mixture of seed sources that minimizes the risk of maladaptation under multiple climate change scenarios was used to select appropriate seed sources for 3 northwestern Ontario locations following an approximate latitudinal transect: Red Lake (north), Dryden (mid), and Fort Frances (south). Three models (general circulation models, the focal point seed zone model, and a decision support model) together with data collected from regional provenance trials were used to determine site-specific seed source selections (i.e., a seed source portfolio) at each site. Each site had 9 blocks with 9 plots within each block for a total of 81 plots/site. Each test had 8 seed sources planted including the local source. Each plot consisted of a 5 by 5 square layout of seedlings from a single source, with the exception of a single plot containing the mix of seed sources. All sites were planted in late summer of 2006. Extensive deer browsing severely damaged the seedlings at the two southern locations and chain link fences were established at these two tests in summer 2010. The Red Lake test was measured in the summers of 2009 and 2011. In 2009 the most southerly sources had both the lowest height growth (67%) and survival (62%). The local source had the highest survival (90%) and the second highest height growth.

BIO-CLIMATIC AND ECOLOGICAL GENETIC MODELS OF EASTERN WHITE PINE (*Pinus strobus*) AND PREDICTED RESPONSE TO CLIMATE CHANGE

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Approximately 120 000 forest inventory and ecological ground plots representing eastern North America were used to develop a climate model predicting the current distribution of eastern white pine (*Pinus strobus*) across its natural range. The resulting 8-variable random forest algorithm had an 8% overall error rate. This error was primarily a function of errors of commission, i.e., predicting presence of white pine for plots in which it was absent (error = 13%). In contrast, error of omission, predicting white pine absence when it was present, was 1%. Farm-field test and greenhouse data for 112 provenances representing the range of white pine in Ontario east of Lake Superior were analyzed using both nonparametric and parametric procedures to model the pattern of adaptive variation in eastern white pine. The resulting model accounted for 68% of the observed variation among seed sources for growth, phenology, and cold hardiness variables. Height data from two older range-wide provenance tests in Quebec and Maryland were consistent with the Ontario genetic model. Consequently, the Ontario genetic model was used to predict a range-wide pattern of adaptive variation. Climatic surfaces for three General Circulation Models (Canadian, Princeton, and Hadley GCMs), two scenarios, and three time steps (2030, 2060, and 2090) projected substantial geographic shifts in the realized climatic niche and adaptive structure of eastern white pine. The resulting models directly support the development of climate change response monitoring and management strategies.

REFUGIAL HISTORIES OF WESTERN NORTH AMERICAN TREE SPECIES: TAKING SPECIES DISTRIBUTION MODELS FURTHER INTO PHYLOGEOGRAPHY

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The cold temperatures and extensive continental ice sheets that dominated North America through the Pleistocene produced not only the diversity of the physical landscape but much of the genetic diversity of the modern biotic landscape as well. In recent years, species distribution models (or ecological niche models) have emerged as an effective tool for investigating the past and potential future distribution of species. Using these models in combination with palaeoclimate reconstructions from general circulation models, we assess the relationship between the extent and distribution of species glacial refugia and their modern genetic diversity. Results indicate that species ranges at the last glacial maximum, in addition to present-day ranges and species life history traits, account for much of the modern genetic structure. Furthermore, in addition to simple geographic space, we demonstrate that climate conditions at the last glacial maximum were limited in their diversity relative to the present day and thus represented an environmental bottleneck.

COMPARATIVE PHYLOGEOGRAPHY OF *Betula alleghaniensis*, *B. lenta* and *B. papyrifera* IN EASTERN NORTH AMERICA: INFLUENCE OF INTROGRESSION, NICHE BREADTH AND GLACIAL HISTORY

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This study investigated the chloroplast DNA (cpDNA) -based phylogeographic structure of *Betula alleghaniensis*, *B. lenta*, and *B. papyrifera* in eastern North America. Universal chloroplast microsatellite markers were used to assess cpDNA diversity and to determine the geographic distribution of haplotypes. Levels of polymorphism were low, with 7, 3, and 4 haplotypes recovered for *B. alleghaniensis* (n = 84), *B. lenta* (n = 28), and *B. papyrifera* (n = 28-84), respectively, based on 3 polymorphic markers from 61 sites across the geographic range. Extensive haplotype sharing within geographic regions was observed between *B. alleghaniensis* and *B. papyrifera* providing strong evidence of introgression. In contrast, *B. lenta* generally did not share haplotypes with other species. The phylogeographic pattern for *B. alleghaniensis* and *B. papyrifera* is congruent throughout areas of sympatry with distinct eastern and western populations, and a distinct Atlantic coastal haplotype which may have originated from a northeastern coastal refugium. No clear phylogeographic pattern was observed for *B. lenta*, as one haplotype dominated throughout its range. Further investigation of patterns of genetic variation and hybridization/introgression within these species is currently underway utilizing 10 polymorphic nuclear microsatellite markers.

GENETIC STRUCTURE AND VARIABILITY THROUGH TIME IN FOUR DISJUNCT PONDEROSA PINE POPULATIONS

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Climate change can influence vegetation in a variety of ways, including increases and decreases in established populations, extirpation of entire populations, and establishment of populations in new locations. Models of vegetation response to changing climate predict shifts in communities with resulting landscapes radically altered from their present states. Colonization of new sites is predicted to be widespread for many tree species, but the mechanisms by which this happens are poorly understood because establishment and expansion of tree populations may span decades to centuries. Genetic structure and variability are two factors that may influence the ability of populations to expand.

We studied four disjunct populations of ponderosa pine (*Pinus ponderosa*), in the Bighorn Basin of north-central Wyoming, to examine how genetic structure and variability changed over time. These populations are separated from continuous ponderosa pine forest by distances ranging from 15 to over 100 km. There is strong evidence that the initial colonizers are still present and that there has been no erasure of past trees at the sites, giving us a complete record of population history. All trees in each of the populations were aged using dendrochronological techniques, and needle tissue was collected and used for molecular genetic analysis at nine microsatellite loci. Microsatellite data were paired with tree-age data to carry out population genetic analysis through time. We calculated allele accumulation rates, allelic richness, allele frequencies, and both within and between population differentiation through time. We also carried out parentage analysis, which enabled us to determine the contribution of long-distance dispersal to population growth.

We obtained genotypes and ages for >1100 trees. Results showed that initial colonization occurred between 1530 and 1650 for all populations. During the first century following initial colonization almost every individual (>80%) was the product of long-distance seed dispersal. During this time-interval novel alleles represented a higher proportion of the population, but while long-distance dispersal continued to play a major role in population growth, alleles at most loci became saturated by the late 19th century. Analysis showed that there were high levels of within-population differentiation, but low levels of between population differentiation. While many studies document this finding for conifer species, we show that populations become undifferentiated very early in their development. Furthermore our data show that high levels of immigration may be vital in the early stages of population development if populations are to overcome Allee effects and exceed a threshold size, below which they will not persist. Findings suggest that populations are reliant on long-distance dispersal for the majority of population growth for extended periods following colonization. An understanding of genetic structure and empirical estimates of long-distance dispersal rates and the contribution of immigrants to population growth at early stages of population growth is vital for both conservation and management practices, especially in the context of understanding species capabilities to respond to changing climate.

TACKLING GLOBAL ENVIRONMENTAL CHANGES IN TEMPERATE FOREST ECOSYSTEMS BY USING GENOMIC TOOLS AND RESOURCES: A ROADMAP FOR TRANSATLANTIC COOPERATION

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Nearly 40% of Europe and North America are covered by forests, which provide important ecosystem services, biomass, and wood resources. Their persistence will likely be the acceleration of climate change compared to past natural climatic oscillations, as indicated by more and more evidence.

There is only anecdotal evidence that trees may have inherent capacities to locally adapt and/or migrate to the extent that they may be able to cope with the predicted changes. The extent of the evolutionary change that these long-lived species may be able to express remains an open field of research and requires an interdisciplinary approach combining genetics ecology, genomics, and evolution.

The level of diversity in natural populations is an important source of evolutionary change and has for the time being been very fragmentarily documented. Traits that contribute to adaptation are complex; therefore, whole-genome exploration represents a promising route to locate the relevant gene variation that contributes to adaptation.

The FoResTTraC project, coordinated by Antoine Kremer has brought together research expertise in forest ecosystem genomics from Europe and North America (6 leading European, 3 American, and 2 Canadian partners) to discuss a common research vision and strategy to tackle the most pressing questions posed by global environmental changes on temperate forest ecosystems, using genomic tools and resources. The idea is to create a strategic synergy among research groups and networks on the two sides of the Atlantic, and to try to have the priorities of funding agencies aligned, in order to establish large transatlantic collaborative projects.

After a year and a half of expert consultation (since March 2010), focused on establishing the state-of-the-art with regard to some research themes, on reviewing the existing capacity and infrastructure on forest genomic research in Europe and North America, the FoResTTraC partners have produced a research roadmap that is now open for comments from a wider scientific community, before it is finalized and presented to other stakeholders, including research funding agencies.

POSTER ABSTRACTS

ASSOCIATION MAPPING BETWEEN GROWTH TRAITS AND CANDIDATE GENE VARIANTS IN WHITE SPRUCE CLONES

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Association mapping projects for black (*Picea mariana*) and white spruce (*P. glauca*) were initiated a few years ago with the aim of finding molecular markers linked to growth and wood quality traits. Now that high-throughput genotyping assays have been developed, it is possible to simultaneously assess the genotype of individual trees at hundreds of single nucleotide polymorphisms (SNPs). The large number of sampled genes increases the likelihood of developing diagnostic markers that may explain a significant portion of the phenotypic variation observed in natural and breeding populations. These diagnostic tools could then be integrated into tree breeding and production programs, which traditionally rely only on the phenotypic measurements of quantitative traits such as height, diameter at breast height (DBH), and wood density. They might help shorten the breeding cycle by more efficiently and rapidly selecting trees with desirable attributes, especially for characters at a more mature stage and requiring extensive phenotyping resources.

In this project, we used several SNPs that were previously found to be significantly associated with wood traits in white spruce. These SNPs as well as others present in white spruce genes that were preferentially expressed in secondary xylem were assayed using the SEQUENOM multiplex genotyping platform. A total of 266 high-quality SNPs were used to genotype a population of 325 clones for which nine phenotypic traits were also assessed. These clones were derived from 75 full-sib families produced using a disconnected diallel mating design with 20 parents divided into 4 groups of 5 parents from New Brunswick. Clones were vegetatively propagated using rooted cuttings and planted in tests established at three locations in New Brunswick. Phenotypic data from one test site were analyzed for height, DBH, and wood quality, along with phenotypic means from three test sites for height and volume.

Eight significant associations were found and involved 4 different SNPs with a false discovery rate of 0.2 to account for multiple testing. A significant association explaining 5.6% of the phenotypic variance was detected between DBH measured at 19 years of age and a SNP associated with microfibril angle in a previous association study based on populations from Quebec. With these findings, progress is being made to identify diagnostic DNA markers, and once they are more widely validated, they may be useful selection tools for spruce breeding.

INTERIM MEASURES FOR THE ASSISTED RANGE AND POPULATION EXPANSION OF WESTERN LARCH FOR USE AS A CLIMATE CHANGE ADAPTATION STRATEGY IN BRITISH COLUMBIA: BRINGING SCIENCE INTO THE REALM OF POLICY AND PRACTICE

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Climate change adaptation strategies such as climate-based tree species range and population expansion support British Columbia's Climate Action Secretariat that envisions that "British Columbia is prepared for and resilient to the impacts of climate change." This overarching vision includes the need to "make adaptation a part of the BC Government's business, ensuring that climate change impacts are considered in planning and decision-making across government" and "to assess risks and implement priority adaptation actions in key climate sensitive sectors."

The BC Ministry of Forests, Lands and Natural Resource Operations, Stewardship Division has recently begun to re-focus its policy and practices through a climate change adaptation lens. The Chief Forester's Standards for Seed Use were amended in June 2010 to allow for the limited use of western larch (*Larix occidentalis*) beyond its contemporary range based on recent climate modelling and scientific analysis (Rehfeldt and Jaquish, March 2010). Using bioclimate models, provenance test data, and a computer-simulated decision classification key the presence-absence of tree species can be predicted under future climate scenarios.

The purpose of this particular policy initiative was to develop an interim measure for the assisted range and population expansion of western larch in areas that are projected to be climatically suitable for the year 2030. The intended outcome of this policy initiative is to increase tree species and genetic diversity at the landscape level, maintain or enhance future timber supplies, and reduce tree species vulnerability. Policy changes are considered interim until which time a more comprehensive climate-based seed transfer system can be fully implemented for use across British Columbia.

WATER ACTIVITY: AN INNOVATIVE TOOL FOR LONG-TERM *ex situ* CONSERVATION OF GENETIC DIVERSITY OF ORTHODOX FOREST TREE SEEDS

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Water activity (WA) is a proven measurement for the regulatory control of biological activity of fragile products in the pharmaceutical and food industries. Since the early 2000s, Cemagref, France has adapted this measurement to monitor the quality of conservation of pollen and orthodox forest tree seeds. Scientific collaboration between Cemagref and the Forest Research branch of the Québec Ministry of Natural Resources and Wildlife (Direction de la recherche forestière (DRF) du Ministère des Ressources naturelles et de la Faune du Québec), initiated in 2007, has permitted the operational implementation of this measurement at the Quebec tree seed bank.

WA quantifies the chemical availability of water in compounds, while water content quantifies the total mass of water they contain. Advantages of measuring WA are its ability to predict the biological stability of compounds, its simplicity of use, the speed with which it can be obtained, and its non-destructive character, all of which give WA decisive operational advantages. For 20 years, efficient and economical WA meters have been available, thus facilitating a wider application of WA measurement.

In Quebec, WA measurement is used in day-to-day operations at the Berthier Forest Tree Seed Centre, where it helps to improve management and control of the water status of seedlots while greatly reducing the energy costs associated with seed extraction.

The non-destructive character of WA measurement allows for dynamic monitoring of the water status of seedlots in conservation banks. When a problem is detected, a procedure to adjust the water status can then be applied. We have used this method to assess the quality of seeds stored for approximately 30 years at the DRF. An almost systematic increase in WA, above the recommended limits, was observed, and water content had consequently also increased. For certain lots, notably white spruce (*Picea glauca*) and jack pine (*Pinus banksiana*), this was accompanied by a concomitant reduction in germination. We demonstrated that the increase in WA was due, in particular, to the presence of a high relative humidity in the cold rooms and to the water permeability of the containers used for long term seed storage. A research project is currently underway with Centre de Technologie Minérale et de Plasturgie Inc. to develop a new storage container made of a polymer material that is less permeable to water and fitted with a more efficient cap.

HIGH YIELD SOMATIC VARIETIES: A TOOL FOR INCREASING TIMBER PRODUCTION IN QUEBEC

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Quebec has emphasized the integration of somatic embryogenesis (SE) in the reforestation program to maximize the volume yield of white spruce (*Picea glauca*) plantations compared to naturally regenerated stands, without using genetic transformation.

To achieve this goal, the Forest Research branch of the Québec Ministry of Natural Resources and Wildlife (Direction de la recherche forestière du Ministère des Ressources naturelles et de la Faune (MRNF)) has established different complementary research projects whose objectives are to better characterize white spruce somatic varieties so that they can be efficiently integrated into white spruce breeding and reforestation programs.

The first project, aimed at conducting an early morphophysiological characterization of somatic varieties (SV) of 1- and 2-year-old seedlings in a forest nursery, has enabled us to catalogue more than 800 SV. This will permit a preliminary selection of varieties with a desired set of characteristics (e.g., fewer branches and knots). The amplitude of clonal variation and the degree of genetic control were also evaluated. For example, height presents a high and stable broad sense heritability (H_c^2= 0.60) over two growing seasons under nursery conditions.

The characterization is ongoing as these SV are later being subjected to clonal trials, allowing for genetic correlations to be established between nursery and planting site performance. After four years of growth on two planting sites, SV performance showed a certain stability over the years. The strategy for multiplying the best performing SV for reforestation stock also foresees the use of cuttings as a rapid and economical means of increasing the number of copies. To date, more than 100 SV of white spruce have been successfully integrated into the mass cutting propagation program. Their rooting percentage varies from 71 to 100%.

RADIAL VARIATION OF WOOD TRAITS – PATHWAYS FOR THEIR GENETIC INTERPRETATION

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The description of wood traits in genetic studies is often based on a tree mean value of some kind, typically ignoring the inter-annual variation. Juvenile wood traits in particular are subjected to strong variation from one growth ring to another and they may follow quite diverse patterns from the pith to the bark. We investigated the pith to bark variation of wood traits related to mechanical utilisation of wood. High resolution pith-to-bark profiles for microfibril angle (MFA) and wood stiffness (MOE) were obtained using SilviScan 3 technology on 375 trees from a white spruce (*Picea glauca*) provenance–progeny test in Quebec. Data from the different growth rings from the pith to the bark were fitted with non-linear (exponential, sigmoid) functions that represented the overall trend. Overall, the pith-to-bark trends followed declining (for MFA) or increasing sigmoid (for MOE) patterns that have been reported for spruces and other conifers. In addition, quite extensive variation was observed among the profiles. Evidence was found that progeny may explain part of the variation, but large differences were observed for individual trees. To better understand potential causes and effects that lead to extreme pith to bark profiles, 20 trees showing phenotype patterns with low or high MFA, respectively, were submitted to RNA transcript profiling with a custom microarray comprised of 32 000 oligonucleotides representing more than 25 000 different white spruce genes. This analysis afforded the possibility of identifying transcriptional signatures that help to illuminate the physiological and molecular bases of the phenotypes. The genes that are differentially expressed among the groups of low and high MFA trees may also be used for studying the phenotype-genotype interactions. The results of the study will likely help to understand the mechanisms responsible for wood variation and to improve juvenile wood quality in softwood plantations.

PROPOSED SEED ZONES FOR JACK PINE IN A BRAVE NEW WORLD

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The Minnesota Tree Improvement Cooperative's full-sib second-generation jack pine (*Pinus banksiana*) population was planted onto two sites in 1999. Tree heights were measured after 3, 5, 8, and 10 growing seasons. Tree diameters and stem form were also assessed after 10 years. Cone serotiny (open vs closed), cone stature (straight vs. curved), and gall rust was sub-sampled after 11 growing seasons. A high level of family x site interaction occurred for volume growth. A significant genetic correlation between cone serotiny and volume growth indicated strong ecotypic associations. As a result, future orchards will be produced from two different populations. The top performing families at the Crow Wing County site will be selected for orchards in west-central Minnesota. Top performing families from the St. Louis County site will be selected for deployment in north-east Minnesota. Families selected for fast-growth at each orchard also positively correlate with stem form and low disease incidence. Existing floristic regions of fire-dependent woodland systems are proposed for use as seed zone boundaries.

STUDY ON THE LEVEL OF TRANSCRIPTS OF GENES POTENTIALLY RELATED TO CLIMATE ADAPTATION OF BLACK SPRUCE (*Picea mariana*) IN BUD SET

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Global warming is expected to change the distribution or even threaten the survival of many species including some forests trees. This study examines the genetic basis of adaptation to climate in black spruce (*Picea mariana*). Black spruce is an economically important and widely distributed species in Canadian boreal forests. With the long term aim of potentially assisting the species to survive due to anticipated climate change, we can identify genetic polymorphism playing a role in adaptation.

Using several molecular biology approaches and quantitative trait data such as the date of bud set and height growth, 27 SNPs (Single Nucleotide Polymorphims) were previously identified as potentially related to climate adaptation in black spruce. The SNPs were identified in gene coding regions with similarity to known proteins such as MYBs, APETALA 2, zinc finger, or glycosyl hydrolase. The SNPs may be associated with changes in gene expression levels or in activity of the protein encoded by the gene. The purpose of this study is to quantify transcript levels for the gene containing the 27 SNPs during the period surrounding bud set. The goal is to test whether the expression of these genes influences seasonal cold adaptation.

The accumulation of RNA transcripts may vary between organs, tissues and cells, and also, depending on environmental conditions like photoperiod. Therefore, we aimed to determine where and when the messenger RNAs are expressed for each of the genes. RNA transcript accumulation profiles were obtained in tissues and at different dates during the growth season near bud set. RNA transcript determinations used RT-qPCR (reverse transcription followed by quantitative polymerase chain reaction). None of the 27 transcripts varied significantly with the time of day (8 am vs 3 pm). On the other hand, some of the transcripts varied significantly between the different sampling dates. The most significant and widespread differences were observed between tissue types and organs.

The spatial and temporal correlation that we have observed between RNA transcripts and bud set is of interest because the timing of bud set is directly linked to the synchronisation of dormancy and growth with favourable growth conditions. The terminal bud contains the apical meristem, which is one of the most vulnerable structures to cold. The terminal bud is also responsible for height growth and plays a major role in the coordination of normal tree development. Determining if the expression profiles of genes harboring the SNPs associated with adaptation may be synchronized with bud set could help to identify physiological and molecular mechanisms controlling the trait.

The next step in this project is to study the expression level of different genotypes reported for the 27 different SNPs. For this purpose, a population of 1128 diverse and unrelated trees has been genotyped. A subset of the population will be sampled and tested for differential gene expression. Ultimately, these results may indicate a link between gene diversity and expression with variation in tree phenology and adaptation.

TREE SEED WORKING GROUP

WORKSHOP

TREE IMPROVEMENT AND SEED PRODUCTION BY NB DEPARTMENT OF NATURAL RESOURCES

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The objective of the New Brunswick Tree Improvement Council (NBTIC) is to provide genetically improved seed for reforestation. The provincial nursery currently produces about 25 million seedlings annually. These trees are planted on Crown land by the licensee responsible for the area in question. About 50% of New Brunswick is Crown land which is divided into 10 licenses. The NBTIC program is composed of industry, government, and university members. The Department of Natural Resources (DNR) is responsible for breeding and growing test seedlings. They also manage seed orchards for Crown land reforestation. The DNR provides a chairperson for NBTIC and co-ordinates NBTIC activities. White spruce (*Picea glauca*), black spruce (*P. mariana*), red spruce (*P. rubens*), Norway spruce (*P. abies*), jack pine (*Pinus banksiana*), and white pine (*P. strobus*) are all part of the NBTIC program. To date, 349 field tests have been established on 390 ha. Second-generation selections are completed for black spruce, jack pine, and white spruce. Third-generation selections were started in 2009 for jack pine

Orchard area has been reduced from the first to second generation because management techniques were developed and are more effective. DNR has a total of 20 ha of second-generation clonal orchards (4 species) compared to over 76 ha of first-generation seedling seed orchards of the same species. The average seed yields in DNR orchards are shown in Table 1.

Table 1. Average seed production for first- and second-generation clonal seed orchards

Species	Generation	Seed production/ha	No. trees/ha
White spruce	First	39.3 kg	555
Black spruce	Second	34.0 kg	555
Jack pine	Second	15.0 kg	555

Injections of $GA_{4/7}$ is the principal method used to increase cone production and seed quality is increased through fertilizer application and roguing. Cones are no longer harvested in the seedling seed orchards because there is ample production in the clonal orchards (Table 2).

Table 2. Total seed harvested from clonal seed orchards over various periods of time

Species	Generation	Time period	Weight of seed (kg)
White spruce	First	1989–2009	629
White spruce	Second	2006–2009	22
Black spruce	Second	1994–2009	403
Jack pine	Second	1994–2007	204

Realized gain tests were planted in order to quantify the gains realized from the tree improvement program. At age 15 years, first-generation white spruce had a 25.6% increase in volume/ha over the stand checklots, first-generation jack pine had 19.9% more volume, and second-generation black spruce had 5.7% greater volume than first-generation seedling seed orchard seedlots.

CHALLENGES PROCESSING CONES AND SEED FROM MOUNTAIN PINE BEETLE KILLED STANDS

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The mountain pine beetle (*Dendrotonus ponderosae*) has had a devastating impact on interior forests in British Columbia. By 2010 a cumulative total of 692 million m^3 of timber had been killed, predominantly lodgepole pine (*Pinus contorta*). This created a massive area of literally dead wood that was without a US housing market, was a fire hazard, and would have long-felt implications to the province's future timber supply. Lodgepole pine is a species whose seed orchards are not currently meeting the seed demand and a large effort to collect seed from natural stands was undertaken.

Serotinous lodgepole pine cones provided an opportunity to collect seeds from dead trees. This is a fortunate situation because if we were dealing with the mortality of non-serotinous species we would not have this opportunity. A great deal of evidence in the literature and our own experience indicates that seed remains viable in the cones after tree mortality. We consider the cone an isolated unit separate from the tree and the serotinous cones are simply storage units for the canopy seed bank that has built up over years and even decades. That is the good news! The challenge was that as trees die, foliage is lost and the thermal characteristics of stands change creating enough heat to open these serotinous cones. That is the situation we faced in BC – a race to obtain cones before serotiny was released and seeds disseminated before they could be collected for the required reforestation efforts.

This race resulted in a variety of challenges for cone and seed processing. The first was a massive increase in cone volume for processing which peaked in 2008 with over 15 000 hL of cones being processed that year which was about 2–3X our historic average. Part of our adjustments included a move to Sunday night kilns and weekend shifts in seed processing. It was several years of almost year round processing that resulted in a large amount of wear and tear on employees and equipment. Accompanying this massive effort was an acceptance of older, more weathered cones which were previously not considered worth collecting. In some areas this was a case of necessity, in others a means of maximizing yield. In addition, these collections often contained greater quantities of debris that required additional processing steps.

The loss of serotiny in lodgepole pine cones resulted in changing how lodgepole pine cones were handled. The no-longer serotinous cones required racking in trays to allow air movement and expansion versus the pallet-stacked sacks of serotinous cones generally received from wild stands. Additional risks were the increased probability of fungal contamination (that is generally absent from serotinous cones), heat damage, and the potential for seed deterioration from wetting/drying cycles of the seed depending on how long serotiny had been broken. One of the most persistent trends was the receipt of low moisture content cones that often resulted in much poorer flexing of the cone scales after kilning. This resulted in a larger proportion of seedlots that requiried retreatment to maximize viable seed extraction. These additional kiln treatments complicated scheduling, reduced the number of available kilning opportunities in a processing season, and added additional risk to seed quality. Accompanying this was a large increase in the amount of Quality Assurance efforts directed at assessing cone moisture content and seed extraction efficiency.

In order to maximize potential seedlings, some of the collections were segregated into more than one seedlot of variable quality. This generally was initiated at final cleaning on the gravity table based on an imbibed cutting test assessment. The separation would be made and the potentially viable fractions germination tested to determine whether it was worthwhile keeping the fractions separate or to combine them based on germination, seed volume of the various fractions, and discussions with the client. A realistic example would be the creation of one seedlot at 95% germination and another at 75%.

There were a wide variety of challenges and subsequent lessons from our experiences with seed from dead trees. Traditionally, collections of lodgepole pine from current crops were relatively homogeneous and relatively simple to process. One of our greatest lessons was to invest the time and effort into understanding the collection's unique characteristics in terms of: cone moisture content; status of cone serotiny and degree of flexing; degree of cone weathering; and results of imbibed cutting tests to estimate seed viability and vigour. This information enabled us to better tailor processing to meet each collection's unique processing requirements.

Inter *situ* GENE CONSERVATION

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The goal in forest gene resource conservation is two-fold: the conservation of low frequency alleles and the maintenance of adaptive potential of populations to new environments. The conservation of low frequency alleles is accomplished by *ex situ* and *in situ* conservation, where either seeds are stored in seed banks, or plants grown in clone banks or arboreta (*ex situ*) or tracts of forests are preserved in protected areas, reserves or parks (*in situ*). Both of these types of conservation efforts are more ‘static’ (particularly seed banks) as adaptive variation cannot be maintained in a rapidly changing environment. Protected areas certainly have the ability to respond to natural forces, but the degree to which this happens will be difficult to know or track. A more dynamic approach is the concept of inter *situ* conservation that is comparable to “on farm conservation” in agriculture, where varieties of crops are evaluated across many institutions and farms. In a forestry context, forest plantations with known pedigrees (and therefore known effective population sizes) are replicated over several sites, typically and preferably sampling “new” environments and climates. The dynamic aspect of this concept is the subsequent establishment of new inter *situ* population with open-pollinated seed collected from the previous inter *situ* generation, keeping track, for example, of individual mother trees. Pollen influx from surrounding stands, since it is considered ‘local’, is not a problem as this broadens the genetic base of the inter *situ* population. First generation open or wind pollinated progeny tests are ideal candidates as inter *situ* populations as the pedigree is partially known, the sites are well described and protected, and are representative of larger breeding zones. Challenges in successfully carrying out inter *situ* conservation are the required sizes of the plantations, levels of replication, and when to re-plant inter *situ* stands.

Ex situ CONSERVATION OF ASH

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Emerald ash borer (*Agrilus planipennis*) is an exotic insect that poses an economic and environmental threat to the ash resource in Canada. It was discovered in Windsor in 2002, continues to spread rapidly across southern Ontario, and is now in Sault Ste. Marie, Ottawa, and southeast of Montreal. The five species of ash native to eastern Canada are all susceptible including blue ash (*Fraxinus quadrangulata*) and pumpkin ash (*F. profunda*) that are rare and are only in southern Ontario at the northern fringe of their natural range.

Ash seed are classified as orthodox in their storage behaviour therefore providing the opportunity to store seed. Data from the National Tree Seed Centre for three ash species showed that after 10–27 years in storage seed germination ranged from 66 to 78%. The Seed Centre started collecting ash seed in 2004 in response to the threat posed by emerald ash borer. The objective of these collections is to sample the genetic variation throughout the range of the species growing in Canada. About 5 litres of seed are collected from a minimum of 15 trees spaced at least 50–100 apart in a population. The seed are kept separate by tree. A population can be a single stand, several stands in a defined geographic area or a distribution of trees within a homogeneous environment such as a riparian area along a river or lake.

Over 530 collections of white ash (*F. americana*), black ash (*F. nigra*), and green ash (*F. pennsylvanica*) have been made in the Maritime provinces, and southern Quebec and Ontario. Once seed from white and green ash have dried, the wings are removed by rubbing the samaras in a cloth bag and aspirating the material to remove debris and any light or empty seed. This treatment reduces the volume of seed to store by 60-75% and upgrades the quality of the seedlots. Black ash seed cannot be de-winged. Up until several years ago seed quality was evaluated by conducting viability tests by excising embryos, placing the embryos on moist VersaPak™ in Petawawa Germination Boxes, and incubating at 25°C for 14 days. An embryo was considered to be viable if it remained the same color or showed any sign of growth such as radicle and/or cotyledon development. Seed are now germination tested instead.

Seed are dried to less than 8% moisture content, placed in glass jars, and stored at -20°C. Samples from all collections are stored in the Genetic Conservation collection and any excess seed are stored and made available for research. Samples, for back-up storage, have been sent to the Millennium Seed Bank in Great Britain and the USDA National Centre for Genetic Resources Preservation, Fort Collins, Colorado, USA. Seed collecting must continue in order to obtain samples from throughout the ranges of the species. A conservative estimate of the number of additional seedlots required for each species are: *F. americana* – 230, *F. nigra* – 460, *F. pennsylvanica* – 430, *F. profunda* – 30, and *F. quadrangulata* – 50.

ATTENDEES OF THE
32nd CFGA/ACGF MEETING

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Vancouver, BC
Canada

Paul Charrette
Lakehead University
Thunder Bay, ON
Canada

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Ben Wang
Natural Resources Canada
Chalk River, ON
Canada

Harry Wu
Swedish University of Agricultural Sciences
Umeå
Sweden

PHOTOS

Harry Wu and Gary Hodge remark about the great growth on the red pine compared to radiata pine and loblolly pine.

Carrie Pike and Ashley Thomson awestruck in this black spruce seed orchard.

The guides at Fort William Historic Park explain the history of the site to John Mackay.

Dinner music at Fort William.

Invited speakers from left to right: Gary Hodge, Sally Aitken, Om Rajora, Øystein Johnsen, Bill Parker, John Mackay, Fred Hebard, Harry Wu. Missing, Dan McKenney.

CFGA sponsored students from left to right: Guillaume Tessier (U Laval), Jinhong Kim (UNB), Danielle Bourassa (UNBC), David Roberts (UA), and Blaise Ratcliffe (UBC).

Class of ‘83. The first ‘students’ sponsored to attend a CFGA conference: Michael Stoehr, Sally Aitken, and Om Rajora.

Michael Stoehr, Danielle Bourassa, Ben Wang, Charity Hendry, Sarah Drabble, Michele Fullarton and Dave Kolotelo are amazed as Daren Fellner demonstrates the seed liquid separator at SeedTek.

Darren Derbowka extolling the virtues of hybrid white pine breeding to an interested background audience.

The magnificent Kakabeka Falls.