# PROCEEDINGS OF THE TWENTY-NINTH MEETING OF THE

### CANADIAN TREE IMPROVEMENT ASSOCIATION

PART 1Minutes and Members' ReportsPART 2Symposium

# Climate Change and Forest Genetics



# Changements climatiques et génétiques

# COMPTES RENDUS DU VINGT-NEUFIÈME CONGRÈS DE

# L'ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES

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#### **Conference Logo**

The logo was commissioned by the conference organizing committee on behalf of the Canadian Tree Improvement Association. In commissioning this work of art, the Association recognizes the inter-dependence of natural systems and humanity's shared dependence on natural resources.

Michael Blackstock is a professional writer, visual artist, and forester. He is a Northwest coast native; a member of the Gitxsan house of Geel and carries the name *Ama Goodim Gyet*. Michael was born in 1961 and raised in small communities throughout British Columbia. His mother and father instilled the love of Mother Earth into Michael's upbringing. He now is, first and foremost, a naturalist.

The title of the logo is "Rain Raven." "First Nations believe water was a spirit; it is alive and biotic. Oral history of the Northwest Coast peoples talks about how Raven stole the sun from the Chief-of-the-Skies and brought it to the dark earth, then Raven stole fresh water and brought it to earth. This was a time of great climate change: sun and water were brought to earth. The Rain Raven image is meant to signify this story and respect for water. The Raven beak is in the form of the Gitxsan feast spoon (hoobix) and it holds the water that Raven stole from the Chief-of-the-Skies. The drops that fell from his beak formed rivers and lakes."

### PROCEEDINGS

## OF THE

### TWENTY-NINTH MEETING

### OF THE

# CANADIAN TREE IMPROVEMENT ASSOCIATION

## PART 1

Minutes and members' reports

Kelowna, British Columbia July 26–29, 2004

> Editor J.D. Simpson

Additional copies of this publication are available from:

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### **COMPTES RENDUS**

### DU

# VINGT-NEUFIÈME CONGRÈS

DE

# L'ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES

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

## Procès-verbaux et rapports des membres

Kelowna, Colombie-Britannique 26–29 juillet 2004

> Rédacteur J.D. Simpson

Des copies supplémentaires de cette publication sont desponibles de:

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# BUSINESS MEETING MINUTES

#### CTIA/ACAA 29<sup>th</sup> Business Meeting Minutes

Dave Kolotelo chaired the 29th Business Meeting of the CTIA/ACAA held at The Grand Okanagan, Kelowna, British Columbia on Thursday July 29, 2004. Seventeen members were present.

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Minutes of the 28<sup>th</sup> Meeting (as printed in the proceedings from the 28<sup>th</sup> meeting, Part I)

That the minutes of the 28<sup>th</sup> Business Meeting be approved as published. Motion: Moved by: Kathy Tosh Seconded by: Greg O'Neill Carried.

#### 334 Membership

#### 334.1 Honorary Membership

The names of nominees were presented as follows:

Michael Meagher	Victoria, BC
Joe Webber	Salt Spring Island, BC
Cheng Ying	Victoria, BC

That the nominated Honorary Members be duly elected. Motion: Jack Woods Moved by: Seconded by: Jean Brouard Carried.

#### 334.2 Active Membership

The names of nominees were presented as follows:

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Christine HansonAlberta Sustainable Resources Development Smoky Lake, ABMarek KrasowskiUniversity of New Brunswick Fredericton, NB	Andreas Hamman	University of Alberta Edmonton, AB
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Donna Palama	rek	Alberta Sustainable Resources Development Smoky Lake, AB
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Deogratias Rw	eyongeza	University of Alberta Edmonton, AB
Annette van Ni	ejenhuis	Western Forest Products Ltd. Saanichton, BC
Motion:	That the nominated Active Men	nbers be duly elected.

Motion: That the nominated Active Members be duly elected. Moved by: Bill Parker Seconded by: Greg O'Neill Carried.

#### 334.3 Corresponding Membership

No nominations received.

#### 335 Chair's Report

The 29<sup>th</sup> meeting of the Association was held in Kelowna, BC in conjunction with the following organizations:

Western Forest Genetics Association (WFGA) Northwest Seed Orchard Managers Association (NWSOMA) British Columbia Seed Orchard Association (BCSOA) IUFRO Unit 7.01.04 Resistance Breeding Working Group.

There are large benefits to having joint meetings with other organizations in terms of helping with the meetings organization (not duplicating efforts) and in expanding the base of people interested in attending our meeting. Many organizations can only justify sending individuals to one meeting per year, so it makes educational and fiscal sense to co-ordinate larger joint meetings.

The organizing committee did a wonderful job that spanned 18 months and 15 conference calls. There are also many others involved in putting together this meeting and I have tried to include all the people who put in time for CTIA 2004. Those names underlined also served on the organization committee.

Chair	Dave Kolotelo, Tree Improvement Branch, BCMoF
Vice-chair: Finances, Facilities & Meals	<u>David Reid</u> , Tree Improvement Branch, BCMoF
Welcoming Address	Dale Draper, Tree Improvement Branch, BCMoF
Invited Speakers, Posters & Program	<u>Greg O'Neill</u> , Research Branch, BCMoF
Sponsors	<u>Tim Lee,</u> Vernon Seed Orchard Company <u>George Nicholson</u> , Riverside Forest Products
Sponsored Students and President of WFGA	Michael Otesha Desearch David BOM-F
	Michael Stoenr, Research Branch, BCMOF
Registration & Logistics	<u>Diane Douglas</u> , Tree Improvement Branch, BCMoF

Field Tour and Gifts	<u>Michael Carlson,</u> Research Branch, BCMoF <u>John Murphy</u> , Research Branch, BCMoF <u>Barry Jaquish</u> , Research Branch, BCMoF
Accompanying Persons Program	<u>Vicky Berger</u> , Research Branch, BCMoF <u>Hilary Graham</u> , PRT Group
NWSOMA Meeting & Organizing Committee	<u>Harv Koester</u> , USDI, BLM
Organizing Committee	<u>Randy Johnson</u> , USDA Forest Service <u>Alvin Yanchuk</u> , Research Branch, BCMoF
IUFRO Resistance Breeding	Alvin Yanchuk, Research Branch, BCMoF John King, Research Branch, BCMoF
Tree Seed Working Group	Dave Kolotelo, Tree Improvement Branch, BCMoF
Gene Resources Management session	Don Lester, Genetic Resource Management Jack Woods, Forest Genetics Council of BC
Audio Visual Support and Management	Ron Planden, Tree Improvement Branch, BCMoF Don Summers, DW Summers and Company
Registration Logistics and Design	Cheri Tayler, Tree Improvement Branch, BCMoF Tania Johnson, Tree Improvement Branch, BCMoF
Registration and Logistics at Meeting	Debbie Poldrugovac, Tree Improvement Branch, BCMoF Giselle Phillips, Research Branch, BCMoF
Field Tour Site Hosts	Keith Cox, Skimikin Seed Orchard, BCMoF Tim Lee, Vernon Seed Orchard Company
Field Tour Contribution	Chris Walsh, Tree Improvement Branch, BCMoF
Field Tour Assistance	Dan Gaudet, VSOC John King, Research Branch, BCMoF Rich Hunt, Pacific Forestry Centre, NRC Hadrian Merler, RSI, BCMoF Val Ashley, Research Branch, BCMoF Doug Ashbee, Research Branch, BCMoF
Program assistance	Glenn Howe, OSU – PNWTIRC Marilyn Cherry, OSU - PNWTIRC Sylvia L'Hirondelle, Research Branch, BCMoF Charlie Cartwright, Research Branch, BCMoF
Web Design and Updating	Gary Carleton, Carleton Communications Group Ltd.

Several organizations provided financial support that allowed us to offer the meeting at a first-class resort, invite world-class speakers on the theme of "Climate Change and Forest Genetics", and continue with our important Student Sponsorship program. We also decided to offer students a \$100 discount off the registration fee. The following sponsors provided much appreciated financial support for the meeting:

BC Ministry of Forests, Tree Improvement Branch BC Seed Orchard Association BC Tree Seed Dealers Association Forest Genetic Council of BC Natural Resources Canada Vernon Seed Orchard Company Western Forest Products Riverside Forest Products In closing I'd like to offer some comments concerning CTIA 2004 and some food for thought.

The CTIA executive is primarily concerned with the organization of the subsequent meeting of the organization. This is important and the quality of meetings the CTIA has hosted is an incredible legacy for the organization. Discussion of issues relevant to the organization has been something I have tried to promote between meetings as chair. I think this is important and although time consuming I believe it allows contributions from those not able to attend from across the country. Attendance is often skewed towards the host province. I am hoping that the National survey of Management of Genetic Resources will push for the need for more discussions between meetings.

In my discussions with members there was great support for continuation or even expansion of student sponsorship at CTIA meetings. We even agreed on the addition of two permanent student awards at our business meeting!

The constitution has been discussed for several meetings as needing refinement or upgrading. This is not a pleasant task and a division among CTIA members in discussions of what our objectives are has scared anyone away from taking on this task. In addition to our constitution the functioning of the CTIA is directed by Policies that have been presented at Business Meetings. An example is the Policy of Support for Student attendance at CTIA Meetings that was passed at the business meeting of the 23<sup>rd</sup> CTIA meeting. These policies have not been assembled anywhere as a reference and again this task is not a pleasant one.

The issue of format of proceedings was also a key issue in planning the meeting finances. There was general support to go to electronic proceedings (with some copies for libraries) but some people did want to continue to receive hardcopy proceedings. This is a large cost ( $\approx$  \$8 000) and perhaps there is a better use for this money by the Association.

In terms of venue, as of now we do not have a confirmed location for the next CTIA (2006) meeting, although Quebec City has graciously volunteered to host the 2008 meeting to coincide with their 400<sup>th</sup> Anniversary. Thank you. There are many provinces which have not hosted meetings, but they do not possess the infrastructure (tree improvement program with field trials or geneticists) to think they can host a CTIA meeting. I think there is value in hosting meetings at new venues and possibly expecting that some of these meetings will not profit as opposed to some of the larger meeting venues. I would also hope that members could help other provinces without resources in the organization of future CTIA biennial meetings.

Finally, I think the CTIA has to more closely look at its organization, financial resources, mandate, constitution, and form of communication between meetings. No one member has time for this and I suggest that the CTIA contract some of these duties to a retired CTIA member who is familiar enough with our organization, unbiased, and wishing to put in place the mandate of members at a reasonable cost. Contracts like this have been approved at business meetings in the past, so this is not a precedent setting activity.

The times certainly are changing in terms of communication. We strongly felt having an up-to-date web page greatly helped in promoting the meeting. An 'interesting' side-effect of advertising on the internet was the receipt of several inquiries that appeared similar to the standard "Nigerian gold scam" e-mails that many have probably received over the past two years. Some of the information regarding CTIA 2004 will continue to reside in the following link that I hope you find useful - <u>http://www.for.gov.bc.ca/hti/CTIA/index.htm</u>

Good luck to the next organizing committee and best wishes to all members of the CTIA.

Dave Kolotelo

#### 336 Treasurer's Report

The financial statement for the period of July 2002 to June 2004 was presented by Treasurer Tannis Beardmore and tabled for information and acceptance by the membership (see Attachment #1). The statement shows a balance of \$28,744.58 in the Association's account and GIC's totaling \$8,000.00.

Motion:That the financial statement be accepted as presented.Moved by:Paul CharretteSeconded by:Dave ReidCarried.Carrette

#### 337 Financial Contributions

BC Ministry of Forests British Columbia Seed Orchard Association British Columbia Tree Seed Dealers Association Natural Resources Canada Vernon Seed Orchard Company Western Forest Products Ltd.

Motion:That the CTIA/ACAA executive of the 29th meeting express our sincere appreciation<br/>to these contributors.Moved by:Dave ReidSeconded by:Brian BarberCarried.Carried.

#### 338 Editor's Report

The proceedings were printed and distributed during August 2003 to all active members, honorary members, Canadian universities and libraries, registrants, and financial contributors of the 28<sup>th</sup> meeting. Two hundred and ninety-five proceedings were mailed.

A letter advising of the theme of the 28<sup>th</sup> biennial meeting, context of the proceedings, and a request for a \$20 donation to obtain a copy was sent to all corresponding members, USA addresses, and International addresses. As a result, proceedings were mailed to 8 Canadian, 7 USA and 13 International addresses. Forty-five copies remain on inventory.

The mailing list contains 469 addresses comprised of: 15 Honorary, 78 Active, 110 Corresponding, 59 Canadian libraries, 81 USA, and 126 International.

Dale Simpson

#### 339 Education Committee

As in the past, the organizing committee of the CTIA 2004 meeting decided to keep the tradition going and sponsor students from Canadian Universities with a forestry program. This sponsorship is highly encouraged by CTIA members and financial support (if needed) was offered by the CTIA Treasurer. Eight universities were contacted to nominate a student to sent to the meeting in Kelowna. With the exception of the University of Toronto, all others replied and the following students attended the meeting:

Adriana Almeida-Rodrigues	University of Alberta
Stephanie Beauseigle	Laval University
Jean-Francois Carle	University of Moncton
Jessica Courtier	University of Northern BC
Marianela Ramirez	University of New Brunswick
Claire Riddell	Lakehead University
Nicholas Ukrainets	University of British Columbia

Sponsorship included all costs of attendance, including return flights, accommodation, and meals as well as conference registration.

For the first time this year, CTIA gave out an award for best poster presented by a student. This award went to Cherdsak Liewlaksaneeyanawin from UBC for a poster entitled "Microsatellite analysis of genetic effects of domestication in lodgepole pine" co-authored by Y. El-Kassaby, C. Ritland and K. Ritland.

The prestigious Western Forest Genetics Association Critchfield Award, for best oral presentation by a student was won by Mark Lesser, Lakehead University, a student sponsored by CTIA for the Edmonton meeting in 2002.

Mark Lesser's winning of this award is just one sign that the CTIA sponsorship of students is working to promote student's interest in Tree Improvement and Forest Genetics. Furthermore, in Kelowna, there were at least nine participants attending the CTIA 2004 meeting that were previously sponsored by CTIA as far back as 1983.

Congratulations to all seven sponsored students and the two students winning presentation awards. Thanks to all CTIA members for actively supporting this worthwhile sponsorship program.

Michael Stoehr

#### 340 Working Group Reports

#### 340.1 Tree Seed Working Group

The Tree Seed working group published four editions (# 36 to 39) of the TSWG News Bulletin since our last report. Dale Simpson has done a great job of editing, assembling and distributing the News Bulletin. An electronic format was introduced with edition number 37 allowing us to reach many more people at a fraction of the cost. We are continuing with the idea of having themes for each edition of the News Bulletin. Here are the themes of the past four editions.

- #36 Quality Assurance Monitoring
- #37 Hardwood Seed
- #38 Seed Orchard Practices
- #39 Environmental Impacts on Seed Biology

For our 40th edition (December 2004) the theme is "What are your biggest problems or information needs with respect to tree seed?". We greatly appreciate the contributions that individuals make to our News Bulletin. As an incentive and 'award' we introduced a "News Bulletin Raffle" for everyone (except Dale and myself) who contributed articles to the News Bulletin since the last CTIA meeting. The names (article authors) were placed in a hat and the winner was - Michele Bettez and Fabienne Colas - congratulations. They received one matted and framed rain-raven print. Hopefully this precedent will continue.

The TSW G workshop theme for 2004 was "Quality Assurance in the Seed Handling System" and had the following presentations:

Introduction	Dave Kolotelo
Crop Maintenance and Collection in Seed Orchards	Chris Walsh
Quality Assurance in Cone and Seed Processing	Al Foley
Quality Assurance in Seed Testing	Dale Simpson
Quality Assurance in Seed Storage	Donna Palamarek
Quality Assurance in Seed Preparation	Dave Kolotelo
Quality Assurance in the Nursery	Susan Thorpe Fernando Rey

Volunteer Papers

Thermo-kinetics of water absorption, with special reference to Noble fir seeds

George Edwards

Effect of moisture content during pre-treatment or storage on the germination response of alder, birch, and oak seeds

Conor O'Reilly

I believe the workshop was quite successful and provided a good opportunity to discuss and debate a variety of topics related to tree seed. The only other outstanding issue is our TREESEED Listserver. With my CTIA executive duties virtually completed I will be trying to revise this method of communicating and exchanging information. Thank you to all the contributors and subscribers who have helped make the Tree Seed Working Group a vital and dynamic organization.

A note of sadness for the TSWG was the untimely death of Dr. David Gifford of the University of Alberta on August 15th, 2003. He has passed on, but his research findings and teachings will still be with us. Thank you for your contributions David Gifford.

Dave Kolotelo Chair

#### 340.2 Wood Quality Working Group

Due to Tony Zhang's schedule and Alvin Yanchuk's involvement in organizing the IUFRO Working Group 7.01.04 Resistance Breeding Workshop they were unable to organize a wood quality/technology workshop. An effort will be made to plan a workshop for the next CTIA meeting.

Alvin Yanchuk Tony Zhang Co-chairs

#### 341 Business Arising from Previous Meetings

#### 341.1 Bursary (previous meetings items 317, 305.2, and 328.1)

Michael Stoehr and Bruce Dancik agreed at the last meeting to form a standing committee to propose a terms of reference. Unfortunately, the committee was unable to meet and neither member was able to be present at the business meeting. Alvin Yanchuk passed along a suggestion from Bruce that an award should be named after Carl Heimburger.

Motion: Moved by: Seconded by: Carried.	That the CTIA provide a \$ 500 award to the student who presents the best paper at a CTIA meeting and the award be called the "Carl Heimburger Award". Alvin Yanchuk Greg O'Neill
Motion:	That the CTIA provide a \$ 300 award to the student who presents the best poster at a CTIA meeting and the award be called the "Gene Namkoong Award".
Moved by:	Alvin Yanchuk
Seconded by: Carried.	Al Gordon

#### 341.2 Official Gavel (previous meetings item 318.1 and 328.2)

Dale reported that he had e-mailed Michel Villeneuve, who had agreed at the 2000 meeting to coordinate the crafting of a gavel using wood of improved species from each province. Michel is no longer involved with tree improvement but had passed along the "gavel file" to André Rainville before he left. Dale had e-mailed André prior to the CTIA meeting but had not received a reply. Dale said he would communicate with André to determine the fate of this project.

#### 341.3 Constitution and Bylaws (previous meeting item 329.2)

Jerry Klein was approached to review and update the Constitution and Bylaws but he declined. Nobody at the meeting expressed an interest in conducting this work.

#### 342 New Business

#### 342.1 Survey of Forest Genetic Resources Management in Canada

A workshop was held Monday evening during which the results of the survey were presented. Discussion took place on possible approaches that could be taken to create a representative group to facilitate contact with the federal and provincial governments on policy issues. Jack Woods presented the following pre-amble.

All provinces and territories have an interest in the management of forest gene resources and five provinces (BC, AB, ON, NB, and NS) have forest genetic councils. Contact among provincial and territorial reps and provincial councils is minimal and informal. Formal contact among provincial and territorial reps and councils on policy and organizational matters may be beneficial to all provinces and territories. Federal policy initiatives would benefit from communication with a formal committee representing all provinces and territories.

Motion: That the CTIA endorse a committee of representatives from all provinces and territories, including reps from existing Councils, to be known as the Canadian Forest Genetics Councils Working Group of the CTIA and that this working group advocate and communicate, as needed, on matters related to federal-provincial/territorial policy interactions regarding the management of forest gene resources.

Moved by: Jack Woods Seconded by: Sally John

Discussion followed on how such a group would function. The group could initially meet to discuss membership and identify topic areas to avoid any duplication with other groups. The spirit would be cooperation and information sharing. CTIA is a logical organization to form such a group. There is no national organization that speaks forcefully on tree improvement.

#### Carried

The following was presented to provide further guidance for the working group:

Purposes: 1) to facilitate communication among Forest Genetics Councils in Canada, and 2) to facilitate joint submissions from Forest Genetics Councils to policy makers on matters affecting forest gene management in Canada.

The Canadian Forest Genetics Councils Working Group (CFGCWG) will not take positions independent of the CTIA but will rather serve to facilitate positions to be endorsed by individual councils on a case-by-case basis. CTIA will serve to support the CFGCWG by providing opportunities to meet and other communication tools.

Membership on the CFGCWG shall be selected by: 1) for those provinces/territories with a Council, the Council will select the rep and 2) for those provinces/territories without a Council the active members from that province/territory will select a rep.

 

 Motion:
 That the above guidelines serve as a starting point for the Canadian Forest Genetics Councils Working Group.

 Moved by:
 Kathleen Brosemer

 Seconded by:
 Jack Woods

 Carried
 Carried

 Motion:That Jack Woods and Kathy Tosh approach representatives of provincial Councils<br/>and seek volunteers from other provinces/territories to form the Canadian Forest<br/>Genetics Councils Working Group.Moved by:Brian BarberSeconded by:Paul CharretteCarriedCarried

#### 343 Future Meetings

#### 343.1 Location of 2006 Meeting

Following the 2002 meeting, Mike Butler agreed to host the meeting in Prince Edward Island with assistance from Kathy Tosh and Howard Frame. Due to the current provincial government program review, Mike was unable to confirm the commitment. Kathy Tosh indicated that New Brunswick may be able to host the meeting if PEI is unable to.

#### 343.2 Location of the 2008 Meeting

Jean Beaulieu volunteered to host the meeting in Québec City to coincide with the 400<sup>th</sup> anniversary celebrations of the founding of Québec City.

#### 343.3 Location of the 2010 Meeting

No bid was made at this time.

#### 344 Election of New Executive

The following slate of officers will serve as the executive for the next CTIA/ACAA meeting:

Chairperson:	Kathy Tosh NB Department of Natural Resources
Vice-Chairpersons: Symposium	Judy Loo and Yill Sung Park Canadian Forest Service
Vice-Chairperson: Local arrangements	Mike Butler PEI Department of Environment, Energy and Forestry
Treasurer:	Tannis Beardmore Canadian Forest Service
Editor:	Dale Simpson Canadian Forest Service
Executive Secretary:	Dale Simpson Canadian Forest Service

#### 345 Adjournment

Motion:	That the 29 <sup>th</sup> business meeting of the CTIA/ACAA be adjourned.
Moved by:	Alvin Yanchuk

Attachment # 1

CTIA/ACAA Financil Statement July 2002 – June 2004

#### Balance July 2002

Cash	\$8,864.74
GIC GIC	\$16,000.00 \$8,000.00
Total	\$32,864.74
Credits	
Interest (account) Interest (GICs) Purchase of proceedings GIC matured	\$26.11 \$1,068.48 \$329.54 \$16,000.00
Reimbursement for CTIA 2002 meeting CFS contribution for printing of the proceedings Advance from the BC Ministry of Forests for CTIA 2004 meeting expenses	\$7,000.00 \$2,500.00 \$10,000.00
Total	\$36,924.13
Debits	
Cost of printing the 28 <sup>th</sup> proceedings Mailing envelopes etc. for proceedings Bank charges for the proceedings	\$6,830.80 \$180.49 \$28.00
Expenses for CTIA 2004 meeting: Summerhill Pyramid Winery Grand Okanagan Resort Artwork Printing costs for artwork Dave Reid: for remainer of \$10,000 advance	\$750.00 \$4,005.00 \$400.00 \$1,610.35 \$3,239.65
Total	\$17,044.29
Guaranteed Investment Certificates	
GIC (matures Feb 05)	\$8,000.00
Total	\$8,000.00
Balance June 2004	
Cash GIC	\$28,744.58 \$8,000.00
Total	\$36,744.58

# ACTIVITY REPORTS FROM ACTIVE MEMBERS
# **COOPERATIVE TREE BREEDING IN NOVA SCOTIA**

# Howard Frame and David Steeves

# Department of Natural Resources P.O. Box 68 Truro, NS B2N 6P6

E-mail: hmframe@gov.ns.ca

### Keywords: Picea mariana, P. glauca, P. rubens, P. abies, Pinus strobus, seed orchards, breeding

The Nova Scotia Tree Improvement Working Group (NSTIWG) was established in 1977 and is the coordinating body for tree improvement in Nova Scotia. Active members include the Department of Natural Resources, Bowater Mersey Paper Company Limited, Kimberly-Clark Nova Scotia Inc., StoraEnso, and J.D. Irving, Limited. The Canadian Forest Service has provided support services as well as technical and scientific expertise since the program's inception. Meetings are held in the spring and fall of each year to review progress and plans, and coordination is provided by the Department of Natural Resources. Species of interest include black spruce (*Picea mariana* [Mill.] BSP), white spruce (*P. glauca* [Moench] Voss), red spruce (*P. rubens* Sarg.), Norway spruce (*P. abies* [L.] Karst.), and white pine (*Pinus strobus* L.). There are currently about 21 million trees planted annually in Nova Scotia.

### CHANGES IN WORKING GROUP'S STRUCTURE

Since 2000, the Province of Nova Scotia introduced Forest Sustainability Regulations which restructured the method used to fund private land silviculture. Companies acquiring privately owned wood for processing in Nova Scotia or for export are now obligated to either fund or carry out silviculture on privately owned land. The obligation to implement a silviculture program is based on the amount of wood harvested in any one particular year. With 70% of the land base in Nova Scotia privately owned, the demand for reforestation stock has dramatically increased in response to these regulations.

Accompanying silviculture funding restructuring, came a dramatic reduction in the Department of Natural Resources' tree improvement budget and subsequent reallocation of tree improvement staff. Our industrial cooperative members have had to take the lead role in the day-to-day tree breeding activities formerly carried out by the Department. With first-generation seed orchards able to supply the current seed demand for reforestation, the Department does not plan to move forward with second-generation orchard development. The industrial cooperators, however, are proceeding with second-generation breeding and orchard development. Kimberly Clark has leased the Department's Tree Breeding Centre and is using that facility for orchard propagation as well as housing their woodlands staff.

## SEED ORCHARD PRODUCTION

First-generation white and red spruce clonal orchards have consistently produced excess seed to our needs. Rogued first generation black spruce seedling seed orchards are currently able to produce sufficient seed to meet the Province's reforestation requirements. The second-generation black spruce orchards have not produced any significant amounts of seed. Over the last 10 years, clonal Norway spruce orchards have produced fairly regular crops but trees were injected with GA<sub>4/7</sub> in 2003 to provide some assurance of a cone crop in 2004, which is already evident. All species are projected to have a moderate to heavy cone crop in 2004.

#### PROGRAMS

White Spruce

Breeding values (BV's) for 8-year height, derived from half-sib progeny performance, have been determined for 372 of 459 first-generation white spruce clones tested in 11 field test series established between 1991 and 1999. We anticipate completing measurements in 2006.

These BV's are being used to evaluate 454 random pair mates. As the information becomes available and the full-sib families reach measurement age, second-generation candidates are graded and selected. The goal, which is to select individuals from the 100 best crosses based on mid-parent value, is over 65% completed.

#### Red Spruce

Observation and analysis of 8-year heights in the first of our six, first generation red spruce test series indicated that this may be too early to obtain precise results. This and the temporary lack of an operating plan for this species following restructuring of the NSTIWG, has held up progress in this program.

To date, BV predictions have been made for only 129 of 519 tested clones. The first of the ten-year height measurements will, however, be made fall of 2004 and grading, selection, and propagation work is scheduled to proceed immediately following analysis of these data.

#### **Black Spruce**

Second-generation selections, that were made in several of our first-generation open pollinated family tests, have been and continue to be crossed with a standard 20-tree polymix. To date, two test series comprising 113 half-sib progeny are in the ground. An additional 88 crosses have been completed and are scheduled for planting over the next two years.

#### Norway Spruce

Due to poor seed production among untested Norway spruce clones over the past few years, the openpollinated progeny testing program has ground to a halt. Because of the GA treatments applied in 2003, we hope to be able to establish another test series by 2006.

# NEW BRUNSWICK TREE IMPROVEMENT COUNCIL UPDATE

# Kathy Tosh and Yuhui Weng

# c/o NB Dept. Natural Resources Kingsclear Provincial Forest Nursery 3732 Route 102 Island View, NB E3E 1G3

E-mail: <u>Kathy.Tosh@gnb.ca</u>

**Keywords:** breeding, second generation, genetic gain, seed orchard, black spruce, jack pine, white spruce, red spruce, tamarack

The New Brunswick Tree Improvement Council (NBTIC) completed its 28<sup>th</sup> year of operation in 2004. NBTIC has completed breeding and testing of first-generation white spruce (*Picea glauca* [Moench] Voss) and tamarack (*Larix laricina* [Mill.] Karst.) and has started selection and breeding of second-generation white spruce clones. Testing of second-generation black spruce (*Picea mariana* [Mill.] BSP) and jack pine (*Pinus banksiana* Lamb.) is almost complete with one more series of tests to be planted. First-generation orchards have started to produce substantial quantities of seed and several second-generation crops have been outplanted.

# SEED ORCHARDS AND SEED PRODUCTION

Since 1978, seed orchards have been established by the industrial members of NBTIC who operate reforestation programs on freehold land, as well as by the New Brunswick Department of Natural Resources (DNR) which is responsible for planting programs on Crown land. Over 130 ha of black spruce and jack pine seedling seed orchards were planted over a 10-year period ending in 1987. Clonal seed orchards, primarily of white spruce and tamarack, were also established over this time period, with over 60 ha planted.

Second-generation orchard establishment of black spruce and jack pine began in 1989, with three agencies participating. Orchard establishment is now complete for these species with a total of 34.5 ha. The first planting of second-generation white spruce orchards was started in 2000 and a total of 4.2 ha has been established with four agencies participating.

Seed production in 2002 and 2003 was moderate with a yield of 64 and 60 million seeds, respectively, from NBTIC agency seed orchards. In 2002, half of this seed was collected from first- and second-generation black spruce seed orchards. In 2003, over 39 million seeds were collected in black spruce orchards, of which 34 million seeds were from second-generation orchards. This is the largest collection to date from these second-generation clonal seed orchards. Since 1994, a total of 113 kg of seed have been collected from second-generation clonal seed orchards.

DNR was the only agency that collected in second-generation jack pine orchards in 2002 and 2003, with a yield of 6.6 and 4.8 million seed, respectively. Other NBTIC agencies also had cones in second-generation orchards, but opted not to pick as they had sufficient seed in storage. A good white spruce cone crop was produced in 2002 with almost 12 000 L collected. Seed yield was 19 million in 2002 and 12.5 million in 2003. Many of these first-generation orchards have been rogued 40% or more.

#### BREEDING

The Council completed a complementary polycross breeding program which began in 1987 for white spruce and tamarack. The last series of white spruce tests was planted in 2001 and the last series of tamarack crosses was outplanted in 2002. Polycrossing of second-generation black spruce and jack pine has made tremendous progress with 87 and 90% of the breeding complete, respectively. This is partly due to polycrossing *in situ* on the selections in the family tests as well as the considerable experience that Council members have gained over the 10 years of breeding work. Pair-mating of black spruce and jack pine

commenced in 1994 and 1996, respectively, and will produce material for third-cycle selections. The breeding is 48% complete.

The Council continues to follow the breeding strategy for black spruce that was adopted in 1993. Clones have been uniformly deployed to breeding groups and breeding is conducted in a positive assortative mating design. A total of 15 sub-lines have been established for black spruce and 16 for jack pine.

### SELECTION PROGRESS

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 the results from 10-year progeny test measurements. A total of 175 of 400 trees has been selected to date for the second-generation population. A red spruce (*Picea rubens* Sarg.) tree improvement program was initiated in 2003 and plus tree selection started the winter of 2004.

### TESTING AND DATA ANALYSIS

Testing continues to be an important component of the NBTIC program. Over the past 28 years, 314 tests were planted on over 359 ha. Over the past 14 years, progeny tests have been established to assess the performance of white spruce and tamarack plus trees and second-generation black spruce and jack pine selections. In 2002, three series of progeny tests were planted including the eighth black spruce series, the seventh series of jack pine, and the last series of tamarack progeny tests. In addition, the third series of jack pine full-sib progeny tests was planted. In total, 183 seed lots, planted on 12 sites, was tested on 8.4 ha in 2002. In 2003, the third series of black spruce full-sib progeny tests was established. In the gain test, five seed lots were included: two orchard seed lots, two from natural stands, and one full-sib mix of second-generation material. The tests will be measured at 5, 10, 15, and 20 years, and the results will provide data for genetic gain estimates.

The data analyst position has continued to be funded by the NBTIC. Council members were saddened by the sudden passing of our data analyst, Bryce McInnis, in January of 2003. Bryce had worked for the Council for 6 years and his contribution and dedication to our program has been missed. The position was filled by Mr. Yuhui Weng in August of 2003. A total of ten NBTIC test series were analyzed in 2002 and 2003 which included 8 progeny tests, 1 family test, and 1 gain test.

### PUBLICATIONS AND REFERENCES

- McInnis, B.G.; Tosh, K.J. 2004. Genetic gains from 20 years of cooperative tree improvement in New Brunswick. For. Chron. 80(1):127–133.
- Tosh, K.J. 2003. Nineteenth annual report of the New Brunswick Tree Improvement Council. NB Dept. Natural Resources & Energy, 20 p.

# TREE IMPROVEMENT PROGRESS BY THE NEW BRUNSWICK DEPARTMENT OF NATURAL RESOURCES

# K.J. Tosh and M.S. Fullarton

# NB Department of Natural Resources Kingsclear Provincial Forest Nursery 3732 Route 102 Island View, NB E3E 1G3

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# **Keywords:** cross-pollinations, progeny tests, seed orchards, white spruce, black spruce, jack pine, tamarack, red spruce, balsam fir, Norway spruce

The tree improvement program conductd by the New Brunswick Department of Natural Resources (DNR) is celebrating its 30<sup>th</sup> year of operation. Our efforts continue to focus on the main reforestation species: white spruce (*Picea glauca* [Moench] Voss), black spruce (*Picea mariana* [Mill.] BSP), and jack pine (*Pinus banksiana* Lamb.). Interest in planting red spruce (*Picea rubens* Sarg.) and white pine (*Pinus strobus* L.) has substantially increased over the last couple of years and a tree improvement program for red spruce was initiated in 2004. Secondary species for tree improvement include balsam fir (*Abies balsamea* (L.) Mill.) for Christmas trees, Norway spruce (*Picea abies* (L.) Karst), and tamarack (*Larix laricina* [Mill.] Karst.).

### TREE BREEDING AND TESTING

The DNR is a member of the New Brunswick Tree Improvement Council (NBTIC), a group of cooperators including DNR, the federal government, and six large industrial companies located in NB. All tree improvement work in the province is co-ordinated by the DNR and all co-operators share in the workload.

During the past two years, we have continued to plant black spruce and jack pine progeny tests. One more series of progeny tests should complete second-generation progeny testing in both species. There are now over 735 seed lots outplanted for these two species, meaning we are over 90% complete with progeny testing.

Since 2002, one series of full-sib jack pine tests and 1 series of full-sib black spruce tests were outplanted. These test series came from full-sib crossing done within sub-lines established for each species. These tests will be used for making third-generation selections in 5 to 8 years. A second black spruce gain test was planted in 2003.

# SEED ORCHARDS

All second-generation selections have been identified for black spruce and jack pine and, in 1998, we began selecting second-generation material for white spruce in selection plantations. One hundred and seventy-five trees have been selected since 1998. This will continue until we have made about 420 selections.

In 2003, the first-generation white spruce clonal orchards were rogued again, leaving 50 out of 99 clones in the orchard. A total of 57% of the ramets remain. We have continued to stock existing orchards and establish new ones. The table below summarizes orchard establishment for DNR.

Species	Generation	Туре	Establishment Years	Area (ha)
White spruce	First	Clonal	1985-1987	9.0
	First	Seedling	1978-1982	8.6
	Second	Clonal	2000-2004	5.0
Black spruce	First	Seedling	1980-1987	32.0
	Second	Clonal	1989-1997	11.8
Jack pine	First	Seedling	1979-1986	25.0
	Second	Clonal	1992-1998	7.9
Tamarack	First	Clonal	1984-1986	8.0
Balsam fir	First	Clonal	1990-1992	2.7
Red spruce	One and one-half	Clonal	1999-2001	2.0
Norway spruce	First	Clonal	1999-2001	2.0
		Seed Production Area	1998	2.0

Table 1. Seed orchards established by DNR

# CONE COLLECTION

The second-generation orchards are producing increasing quantities of cones. Table 2 shows cone and seed production from first- and second-generation orchards during the past two years.

Table 2. Cone collection and seed yield from orchards in 2002 and 2003

Species	Orchard	2002		2003	
		Cones (L)	Seeds (Kg)	Cones (L)	Seeds (Kg)
Jack pine	Second-generation clonal	3 022	27.4	2 633	20.1
Black spruce	First-generation seedling	4 592	33.7	-	-
	Second-generation clonal	2 280	6.9	5 012	25.4
Balsam fir	First -generation clonal	2 544	61.9	-	-

# RESEARCH ON GROUND HEMLOCK, REPRODUCTIVE BIOLOGY AND MULTI-VARIETAL FORESTRY STRATEGIES AT CANADIAN FOREST SERVICE – ATLANTIC

# S.I. Cameron, M. Duffy, D. Fleming, J. Letourneau, I. MacEacheron, F. McBain-Hogg, Y.S. Park, J. Phillips, S. Pond, R.F. Smith, P. Stewart-Leblanc, J. Wang and L.D. Yeates

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#### GENETICS OF GROUND HEMLOCK (*Taxus canadensis*) R.F. Smith, S.I. Cameron, J. Letourneau, M. Duffy, D. Fleming, F. McBain-Hogg, J. Phillips, S. Pond P. Stewart-Leblanc, J. Wang and L.D. Yeates

The genetics program for Canada Yew (*Taxus canadensis*) was initiated in 1997. The two main areas of research are: 1) developing ecologically sustainable harvesting protocols in natural stands, and 2) developing a domestication (tree improvement) program to identify and select elite cultivars of the wild species and develop them into a commercially reared crop.

### Sustainable Harvest Research Trials

Commercial harvesting of ground hemlock biomass for taxanes in eastern Canada has increased rapidly during the past few years. In each of 2002 and 2003, approximately one million kg was harvested, whereas in 2004, the figure is close to 4 million kg. Several harvest trials have been established in eastern Canada to quantitatively determine the response of ground hemlock to different harvesting intensities. In 2002, and after consultation with various partners in the Eastern Canadian Ground Hemlock Working Group, the Canadian Forest Service - Atlantic Forestry Centre designed and established a major research study to examine the effects of four harvesting levels on subsequent re-growth of ground hemlock and replicated it on five sites; one in each of the three Maritime Provinces (NB, NS, and PEI) and two in Québec. Although the first full measurement of the trial is not scheduled until the fall of 2005, one site was assessed in the fall of 2004. Two years following pruning treatments, the amount of regrowth on severely pruned plants (7 years of growth removed) was approximately one-tenth of that observed from any of the other three pruning treatments (3 years of growth but following different criteria for selecting the branches to be pruned). These results were consistent regardless of the time of year that the plants were pruned.

#### Tree Improvement Research

*Cultivar Selection* A total of over 4 000 plants, representing over 500 of the 1 300 genotypes in the rangewide provenance trials, were harvested in 2003 and 2004. The first 'final' harvest of four-year-old plants was conducted in the fall of 2004. These plants are now being measured and processed after which taxane levels will be determined. There has been significant variation in both growth and in taxane levels among clones, indicating a significant potential for selecting fast-growing, high taxane producing genotypes.

*Nursery Propagation Using Rooted Cuttings* If a domestication program is to be successful, the time must be minimized between the identification of elite plant materials and when large-scale production is realized. Until such time that tissue culture is sufficiently developed, large-scale rooted cuttings programs will continue to be required. A masters student at the University of New Brunswick, Laurie Webster, started her program which is focused on improving the rooting of recalcitrant genotypes and in reducing the costs of producing rooted cuttings. A technical note (Yeates et al. 2005) on rooting cuttings of ground hemlock was prepared.

*Nursery Culture* One of the objectives of our research is to determine how *T. canadensis* responds to intensive nursery culture. In 2004, four major semi-commercial outplanting trials (10 000 plants at each location) were established in Sault Ste Marie, ON, Gaspé, QC, Grand Lake, NB, and Charlottetown, PEI. An additional cooperative trial using chitosan as both an aid to early establishment and eventually as an elicitor of taxanes, was established with the Institut Agroalimentaire in La Pocatière, Québec.

*Tissue Culture* The most significant advance in the tissue culture research component of the project was the successful production of emblings from bud tissue. The first step in the process to initiate a patent for the SE of Taxus buds, the form for disclosure of IP, was completed.

A graduate from the CESAB (Centre d'excellence en sciences agricoles et biotechnologiques, Grand Falls, NB) biotechnology program, Melissa McGraw, was hired as a co-op student to fulfill the requirements of her course and to begin preliminary work on the bioreactor prototype. She gave her final presentation – a general description of SE in *Taxus* and its application – and graduated in August, 2004.

*Methods to Increase Taxane Yields* For *T. canadensis*, productivity is not limited to assessing the total amount of dry matter weight per unit area per unit time but also to maximize the amounts of taxanes produced. Greenhouse, nursery, and growth chamber studies have been initiated to develop method(s) to increase the taxane content of crop plants (or harvested plant tissues).

#### CONIFER REPRODUCTIVE BIOLOGY R.F. Smith, and J. Letourneau

Research in the conifer reproductive biology project has been winding down. No additional cone induction trials were established. A significant effort was devoted to preparing and publishing a seed orchard management manual (Smith and Adam 2003).

Work on the molecular biology of reproductive development in black spruce was limited to completing sequencing of ESTs and SAGE tags, and preliminary data analyses. Approximately 50 000 and 22 000 SAGE tags from each of female and vegetative (post-dormancy) buds, respectively, were produced. Clones containing a further 30–40 000 SAGE tags for each of these two libraries are currently in frozen storage pending possible renewed funding for sequencing.

Work on conducting full-length sequencing of selected flowering genes is a long-term goal of this project. Efforts to date have been directed at trying to correlate the sequence information generated from ESTs and SAGE tags with similar genes from other species as identified in public databases. Depending on the level or redundancy observed, this may allow us to determine a first estimate of how many genes are being expressed in these tissues and an indication of the differential gene expression between vegetative and reproductive tissues.

### DEVELOPMENT OF MULTI-VARIETAL FORESTRY STRATEGIES USING CONIFER SOMATIC EMBRYOGENESIS Yill-Sung Park and Ian MacEacheron

Multi-varietal forestry (MVF) may be defined as the use of tested varieties in plantation forestry. The implementation of MVF offers many advantages, including: 1) obtaining much larger genetic gain than is possible from conventional seed orchard breeding, 2) flexibility to rapidly deploy suitable varieties with changing breeding goals and environments, and 3) ability to manage genetic gain and diversity in plantation forestry. Despite these advantages, MVF in conifers has rarely been practiced because of the general lack of an efficient vegetative propagation system that can consistently mass produce the same varieties over time. Recently, owing to the refinement in somatic embryogenesis (SE) technology, the implementation MVF with several conifer species has become an alternative to conventional plantation forestry. The implementation of MVF requires three critical phases: 1) development of efficient vegetative propagation and cryogenic storage techniques, 2) development of high-value varieties, and 3) prudent deployment of genetically tested varieties.

Conifer propagation by SE is the key technology for implementing MVF. Currently in eastern Canada, SE technology for spruce species and eastern white pine (*Pinus strobus*) is sufficiently refined for implementation. However, SE for jack pine (*Pinus banksiana*) remained difficult to obtain. During 2002–2003, our work concentrated on improving initiation of SE in jack pine through the manipulation of plant growth regulators and other tissue culture media elements. This work resulted in significant improvement in SE initiation from 0.2

to 3.7%, but it is still very low when compared to an average SE initiation percentage of 55% for eastern white pine. In 2003, we further studied the effects of 2,4-D and CPPU on SE initiation. The results indicated that, in eastern white pine, the 2,4-D containing media produced about 52% initiation but the CPPU containing media produced 36%. However, in jack pine, CPPU had a beneficial effect although it was small. The addition of nickel improved the initiation rate slightly.

Through the SE initiation experiment of summer 2003, with participation of J.D. Irving, Limited and University of Maine, about 2 600 clone lines of eastern white pine were developed. Of these, JD Irving propagated 180 lines for clonal field testing scheduled for summer 2004. Based on this collaboration, the University of Maine now has established a conifer tissue culture laboratory at Island Falls, Maine, USA and is propagating eastern white pine clone lines.

Experiments were also conducted to develop a 'micro-plug' system for the direct *in vitro* germination of somatic embryos and the subsequent mechanized transplanting of micro-plug germinated embyos into greenhouse containers. The micro-plug system consists of a preformed tray of cavities containing 1 cm (diameter) by 2.5 cm (long) plugs filled with rock-wool. The liquid germination media worked well however, we are in process of developing alternative germination media.

The development of high-value varietal lines involves genetic field testing of embryogenic clone lines. To develop an efficient testing strategy, a field test of SE-derived clone lines of eastern white pine was established at the Acadia Research Forest. This test contains 210 clone lines and is planted as two-tree plots with 6 replications. The test was established in 2002 as a mixed planting with tamarack.

Analysis of a clonally replicated test of white spruce was carried out. The preliminary results indicated that MVF with this species would result in much greater genetic gain than from conventional seed orchard breeding. For example, using the best 20 varietal lines (of 370) would result in a 30% increase in 10-year height. This is a huge genetic gain considering that seed orchard seeds would produce 6–7% genetic gain. Figure 1 also shows possible genetic gains through MVF when variable numbers of varietal lines are selected.

These results also illustrate flexibility in possible deployment strategies for MVF. Studies have shown that 10–30 varietal lines are sufficient for obtaining benefits of MVF while providing protection against potential risks due to diseases and insects. However, these results also show that much larger numbers of varietal lines can be included in the varietal mix while achieving a desired level of genetic gain. For example, a breeder may choose a 100-line varietal mix and obtain 15% genetic gain instead of obtaining 34% gain using 10 varietal lines.

We are also working on other MVF deployment strategies to balance genetic gain and diversity, including Mixture of Varieties and Seedlings (MOVAS) and linear deployment of varieties.



Figure 1. Change in genetic gain in relation to the number of varietal lines selected.

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# **GENETIC DIVERSITY RESEARCH AT CANADIAN FOREST SERVICE – ATLANTIC**

# T. Beardmore, B. Daigle, K. Forbes, J. Loo, J. Major, D. McPhee, A. Mosseler, C. Ramirez, M. Ramirez, G. Scheer and D. Simpson

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## POPULATION VIABILITY ASSESSMENTS IN DECLINING TREES AND FOREST TYPES Alex Mosseler and John E. Major

We continued with our efforts to develop and use various biological indicators to measure reproductive success, mating behavior, and genetic diversity to assess the viability of declining tree populations such as white pine (*Pinus strobus*), pitch pine (*Pinus rigida*), red spruce (*Picea rubens*), and Acadian late-successional, old-growth forest types. These studies are aimed at the potentially adverse impacts of certain forest management practices on the viability of populations and forest types at risk. Positive relationships between average stand age and size, and reproductive fitness and genetic diversity in red spruce, indicate that late-successional, old-growth forest types may represent important reservoirs of genetic diversity. Relationships between stand structural features such as stem density of reproductively mature trees show significant, positive relationships with reproductive success and genetic diversity. These findings have important implications for the management of residual populations following harvesting operations in species such as white pine and red spruce, which often occur in small, increasingly isolated, remnant stands. Maintaining a network of reproductively viable and genetically diverse stands may be especially important within an increasingly fragmented landscape where dispersal and gene flow among populations are necessary to minimize the effects of inbreeding and genetic drift and to maintain the reproductive health of natural populations and their ability to function as suitable and adequate seed sources for natural regeneration.

#### INTRODUCTION AND EVALUATION OF PITCH PINE IN ATLANTIC CANADA Alex Mosseler and John E. Major

In 1994, seed was collected from across the Ontario range of pitch pine to assess the genetic and reproductive status of this rare and declining native species. Several genetic tests were established in Nova Scotia and New Brunswick. Based on the excellent growth performance observed in these Maritimes tests, further testing of pitch pine will be expanded to other locations across the Maritimes in order to assess the climatic limitations and general adaptability of pitch pine, and to compare its performance with native seed sources of jack pine (*Pinus banksiana*) and red pine (*Pinus resinosa*) which will be included in these tests. A series of these genetic and species tests was established in 2003 and 2004. The goal of the population genetics study of pitch pine is to conserve the gene pool of a rare and threatened native species, to assess its commercial and ecological potential across eastern Canada, and to understand the ecological and environmental limitations to the northward movement of southern components of Canada's flora as part of the forest sector strategy for adaptation to anticipated climate warming. One of the main impacts of this work will be to assess tree species alternatives for ecological restoration of habitats and difficult-to-manage sites, and as a potential alternative wood supply for forest industries in the event of species losses due to environmental changes or threats.

### ADAPTIVE GENETIC VARIATION, MOLECULAR DIVERSITY AND RESPONSE TO CLIMATE CHANGE John E. Major, Alex Mosseler and Om Rajora

Greenhouse gas levels are expected to double during this century and alter prevailing environmental conditions which alter physiological components of tree productivity. It was hypothesized that there would be significant species x genotype interaction with the projected climate change. A number of studies have been conducted to examine the potential importance of species and genetic x atmospheric  $CO_2$  and genetic x soil

drought interactions in black spruce (*Picea mariana*), red spruce and their hybrids using seed sources from across their sympatric range (Ontario, New Brunswick, and Nova Scotia). Significant results, for instance, show that freezing vulnerability increases under elevated  $CO_2$  conditions and that red spruce seems particularly susceptible to damage. Also, red spruce allocates 40% more biomass to roots than black spruce.

We continue using molecular markers and genomic tools to understand genome organization, develop gene sequence data base and genetic maps, and identify, isolate, and map genes and genetic factors controlling traits related to productivity, adaptation to abiotic stresses, and particularly those relevant to global climate and environment change in red spruce, black spruce, and black x red hybrids. We have produced unique pedigrees of spruce ideal for genome mapping and QTL mapping of morphological and physiological traits related to abiotic stress adaptation. This will provide the foundation for future development of marker-assisted early selection for desirable traits.

### ABOVE- AND BELOW-GROUND BIO-SEQUESTRATION OF A 30+ YEAR-OLD FOREST GENETIC STUDY John E. Major and Kurt Johnsen

Below-ground forestry research is difficult, often neglected and sequestration information is severely lacking. We have been quantifying the above- and below-ground C inventory in a 35-year-old (F1) first generation cross black spruce experiment with distinct differences in above-ground volume growth. Second, in addition to providing a "snapshot" of carbon sequestration of these plots 30 years after planting, these data will be used to parameterize a process model (Johnsen et al. 2001b, Landsberg et al. 2001, Martin et al. 2001) that will permit the examination of black spruce responses beyond the spatial and temporal boundaries of this particular investigation. Along with soil coring, soil and stem respiration, and ground penetrating radar, the data will be used to estimate carbon fixation of a black spruce plantation and how much carbon is added to a system by planting black spruce.

### ACADIAN ALTERNATIVE SILVICULTURE FOREST DIVERSITY PROJECT John E. Major and Alex Mosseler

In 1999, a large 60 ha silvicultural impact test was established at the Acadia Research Forest to demonstrate the impacts of various forest harvesting regimes on biodiversity in general and on the maintenance of longlived, shade-tolerant, late-successional species such as red spruce in particular. We have established a red and black spruce provenance trial under different silvicultural systems. Research on this site involves a multidisciplinary approach where geneticists, entomologists, ecologists, and silviculturists are studying various aspects of harvesting impacts on biodiversity conservation. This larger biodiversity study provides the context for assessing a range-wide genetic sample of red spruce.

### POPULATION GENETICS OF PRODUCTIVITY IN NATIVE WILLOWS Alex Mosseler and John E. Major

In 2004, a project on the productivity of *Salix eriocephala* and *S. discolor* was initiated to assess population genetic variation in native *Salix* species in order to quantify biomass production and related adaptive traits across several sites in central and eastern Canada and to assemble a sample of the native gene pool for conservation/protection of genetic resources and commercial breeding efforts. A short-term (3–5 year) objective will be to select clones for site and habitat restoration (e.g., mines, oil sands, pits and quarries, etc.) and afforestation of difficult-to-manage sites (e.g., riparian zones, abandoned agricultural lands, other poorly drained sites) across Canada. The longer-term (5–10 years) impact of this work is aimed at emerging chemical and energy industries able to use woody biomass feedstocks and the need for dedicated short-rotation plantations to sequester carbon to meet Canada's greenhouse gas emissions reduction commitments under the Kyoto Protocol.

#### GENETIC DIVERSITY AND GENE CONSERVATION Kathleen Forbes, Judy Loo, Donnie McPhee, Carlos Ramirez, Marianela Ramirez and Dale Simpson

Research on genetic diversity of natural populations of forest trees continued to focus on developing conservation strategies for native tree species. The Canadian Forest Service (CFS) genetic resources group maintains and facilitates the informal New Brunswick Gene Conservation Working Group and has

developed gene conservation strategies for four New Brunswick tree species (Loo et al. 2003). The CFS group is also working to raise awareness of the need for conservation of forest genetic resources and to develop a gene conservation working group at the national level. A meeting was hosted by CFS at Montebello, Québec, in February 2004 to explore the need and potential for such a working group (Beardmore et al. 2004). A survey was conducted in 2003 to identify the tree and shrub species requiring conservation attention across the country and 72 tree species (and six varieties) and 142 shrub species (and eight varieties) were indicated to be either in need of conservation measures or more information before designation could be made, in at least one jurisdiction within the species range (Simpson et al. 2004).

Isozyme analyses were completed for a genetic diversity study of eastern hemlock (*Tsuga canadensis*), using foliage samples, to determine whether genetic diversity measures differ between mature and regenerating trees in 16 populations throughout the Maritime provinces. None of the enzyme loci, having clear results, exhibited any genetic diversity, either in mature trees or seedlings. It was difficult to achieve clear results, so seed was collected for further analysis using isozymes, as well as for conducting germination trials, and seed storage studies. Seed from 15 trees from each of five populations were analysed and results indicated very low genetic diversity, with only one locus showing some variability. Seed will be collected in Québec to determine whether the phenomenon of extremely low genetic diversity is limited to the Maritime provinces or is more widespread.

Research continued on American beech (*Fagus grandifolia*) resistance to beech bark disease and on developing vegetative propagation techniques for production of resistant genotypes, in collaboration with Marek Krasowski at the University of New Brunswick. The disease is caused by a combination of two causal agents: the felted beech scale (*Cryptococcus fagisuga*) and a fungus (*Nectria coccinea var. faginata*). Alone, neither the insect nor the fungus results in serious damage, but together they are devastating. Trees exhibiting resistance are actually resistant to the scale insect, rather than the fungus, but in the absence of the insect infestation, the fungus does not attack the trees. Putatively resistant trees are documented on an ongoing basis.

Graduate student, Marianela Ramirez studied the feasibility of producing beech plantlets using tissue culture protocols, following up on work initiated by a previous student. Each of the vegetative propagation methods attempted to date yielded only partial success (J. Simpson, Masters thesis; K. Ellis, unpublished report). Buds were collected from diseased and putatively resistant trees in February 2003 and 2004 for continuation of micropropagation studies. None of the trials were successful in producing surviving plantlets, although there was a fair degree of success with production of roots on plantlets.

In summer 2002, more than 1 000 seedlings were grown from disease-free trees to provide root-stock for a grafting experiment. Scions were collected and grafted in late February and early March 2003 from 25 disease-free and 5 diseased beech trees. Beech scale eggs were collected in July for inoculation of the grafts in late summer to test for genetic resistance to the scale. Grafts were examined in July, 2004 and all but two of the putatively resistant genotypes showed very low or no scale survival. The scale insect became established with high survival on all of the grafts from susceptible trees as well as grafts from two of the putatively resistant ones. Where scale eggs were placed on or below the graft union, as well as above it, the scale became established on and below the graft union, even on resistant trees where the scale did not survive above the union, indicating the presence of a component in the bark of the resistant trees that inhibits scale development. The experiment was repeated in 2004.

In spring, 2004, four putatively resistant and one susceptible, roadside beech trees at each of two locations, were control-pollinated with pollen collected from putatively resistant and susceptible trees. If successful, the crosses will provide material to screen for resistance to determine the inheritance of the trait.

Restoration using genetically diverse, local seed sources complements *in situ* and *ex situ* gene conservation efforts for species such as bur oak (*Quercus macrocarpa*), that have declined significantly in a portion of the natural species' range. Bur oak restoration trials, established in 1998 and 1999, to determine the feasibility of this approach for gene conservation, were evaluated in early summer 2003. Seven trials were established in 1998 on a variety of sites including a coal mine spoil, where 72.5 % of the trees were surviving in 2003, but growth is slow; two islands in the Saint John River where annual flooding occurs and cattle are pastured in the summer. In spite of efforts to restrict movement of the cattle, they caused heavy mortality and just 20% of the trees are still surviving. Survival and growth was best, at 98% survival and height ranging from 35 to 220 cm, at Cherry Hill, a planting carried out in collaboration with Greg Adams at J.D. Irving, Limited. The site is maintained as a conservation and seed production area with near ideal conditions. Four additional trials, established in 1999, were also assessed in 2003. Survival

ranged from 65% on a wet black spruce cutover site, where deer and rodents had browsed about 50% of surviving trees, to 90% in an old field with heavy grass competition. A site with partial shade, where competition from grass, raspberry, goldenrod and alder is severe, exhibited 86% survival with 66% of the seedlings exceeding 50 cm in height.

Graduate student, Carlos Ramirez, continues genetic diversity and quantitative variation studies on weeping pinyon (*Pinus pinceana*), conducting isozyme analyses, germination trials, and evaluation of needle wax quantity, growth parameters, and drought tolerance with the purpose of developing a conservation strategy for the species (endemic to Mexico and endangered). This PhD study is one piece of a larger collaborative research project supported by the government of Mexico and overseen by a subgroup of the North American Forest Genetic Resources Working Group (FAO's North American Forestry Commission). The objective is to understand ecology, management, and genetic issues and to develop a conservation plan for the species, which has value to local economies because of the high nutritional quality of the seed (edible pine nuts). The species is considered to be a keystone species because it is often the only tree species occurring in semi-arid ecosystems, where associated species are spiny shrubs and cacti. The germination component of the study has been completed and results from a series of germination trials indicated that the seed coat forms a physical barrier that severely inhibits germination if the seed coat is not manually cracked. Initial results of genetic diversity studies indicate highly significant differences among the three regions in Mexico where weeping pinyon occurs.

A gene conservation project for white elm (*Ulmus americana*) was initiated in 2001. The elm population was devastated throughout the 1960s, 1970s, and 1980s by Dutch Elm Disease. Trees are still succumbing to the disease when they are attacked by elm bark beetles which carry the disease however, large living trees can be still found scattered around the countryside. Are these trees escapes from the disease or are they resistant to the disease? Over several years 25 trees were located in New Brunswick. Cuttings were collected and grafted with a grafting success rate of at least 80%. Open-pollinated seed was also collected from many of these trees. The grafts were established in a clone bank where they will be challenged with the fungus that causes Dutch Elm Disease when they have reached an appropriate size.

#### SEED RESEARCH

#### Tannis Beardmore, Kathleen Forbes, Garry Scheer and Dale Simpson

Research is ongoing in evaluating the use of cryopreservation (storage at -196°C using liquid nitrogen) for the long-term storage of germplasm (e.g., seed, embryonic axes, buds, and pollen). Cryopreservation is an option for the storage of seeds or seed parts and embryonic axes) which are intolerant of long-term storage using conventional means (4°C and -20°C). In particular, efforts are focusing on working with Canadian tree species of concern. For example, butternut (Juglans cinerea) is being killed throughout North America by butternut canker caused by the fungus Sirococcus clavigignenti-juglandacearum. To date, there is no control for this fungal disease and no natural resistance has been documented Research is focusing on identifying methods for preserving the existing genetic variation of butternut, specifically cryopreserving buds. Cells within the meristem will survive cryopreservation and callus growth can be initiated from these cells. However, limited success has been met with producing viable plantelets following cryopreservation. Methods have been developed for the cryopreservation of bur oak (Quercus macrocarpa) embryonic axes. This method involves gradually lowering the temperature the embryonic axes are exposed to (from  $4^{\circ}C$  to  $-20^{\circ}C$ ). Attempts have been made to cryopreserve Garry oak (Q. garryana) embryonic axes, however, these axes are intolerant of cryopreservation and none of the tissue was found to be viable following cryopreservation. Garry oak acorns most likely represent the most extreme recalcitrant-like seed phenotype that we have in trees native to Canada.

Research was also completed on a project examining the ability to induce recalcitrant silver maple (*Acer saccharinum*) seed to become orthodox. Mature silver maple embryonic axes were treated with abscisic acid (ABA) and/or tetcyclacis (TC) to examine the effect of these treatments on the axes' tolerance to desiccation. Axes were placed on nutrient media containing 0, 20, or 60 µM ABA and/or 10-6 M TC for 2 weeks, after which axes were removed from the media and desiccated to approximately 5% water content. After desiccation all ABA- and/or TC-treated axes exhibited root growth ranging between 12 and 97% when placed on growth media. Only 97, 63, and 22% of the desiccated 20 and 60 µM ABA/10-6 M TC-and 10-6 M TC-treated axes exhibited shoot growth, respectively. In addition, 55 and 12% of the desiccated 20 and 60 µM ABA/10-6 TC-treated axes, respectively, germinated after cryopreservation. The control axes (desiccated immediately after collection from the tree) did not tolerate desiccation or cryopreservation. ABA content was examined in the embryonic axes and it was found that only the 20 and 60 µM ABA/10-6 M TC-treated axes maintained a high ABA content (57 and 62 pmole ABA/embryo, respectively). The ABA content of all other treated axes declined to 31 pmole/embryo and lower. These

results suggest that inhibiting ABA degradation may have a role in developing tolerance to desiccation in silver maple axes and that ABA/TC treatment of axes may be useful for the storage of axes. While this work provides information on the nature of recalcitrance it is too time consuming to apply as a treatment for inducing desiccation tolerance and subsequent long-term storage of seed.

A project was recently initiated examining the variation in seed dormancy and the orthodox/recalcitrant-like nature of red maple (*A. rubrum*) across its range in North America. In the more southernly regions of its range red maple exhibits a recalcitrant-like seed behavior, while in the more northern regions mature seed is orthodox and may be dormant.

### NATIONAL FOREST GENETIC RESOURCES CENTRE Dale Simpson and Bernard Daigle

The National Tree Seed Centre (NTSC) is a key activity under the National Forest Genetic Resources Centre. The NTSC's mandate is to provide seed of known origin and quality for research and to store germplasm for *ex situ* conservation. Staff have been busy over the past two years collecting and processing seed, conducting germination tests, setting up and evaluating research trials, and supervising students conducting research for their theses.

Seed collection continues to be a major activity in order to expand the Centre's inventory. Collecting focuses on natural populations of native tree and shrub species with emphasis on making single-tree collections. During 2002 and 2003 about 435 seedlots were collected and stored from 40 species as well as 120 seedlots from four species were donated from several agencies. Germination tests were conducted on over 1 700 seed lots and 157 requests for seed for research were processed to provide 1 900 seed lots. The web site, <a href="https://www.atl.cfs.nrcan.gc.ca/seedcentre/seed-center-e.htm">www.atl.cfs.nrcan.gc.ca/seedcentre/seed-center-e.htm</a>, continued to receive a large number of visits and the number of seed requests continued to increase as a result.

Developmental research is conducted in order to determine appropriate pre-treatment and/or germination conditions for species for which there is little known or to optimize current protocols. International Seed Testing Association (ISTA) rules for sugar maple (Acer saccharum) seed prescribe 8 weeks of chilling prior to germination at 20°C. Testing trials at the Seed Centre using local seed indicated 12-15 weeks of chilling was required but less time was needed for an Ontario seed source. A trial was established using seed obtained from New Brunswick (NB), Québec (QC), and Ontario (ON) provenances in which 2 constant germination temperatures (15°C and 20°C), 3 seed soaking times (0, 72 hrs, and 14 days), and 3 moist chilling durations (8, 12, and 16 weeks) were evaluated. Seed from NB imbibed about 10% more water than seed from QC and ON. Eight weeks of moist chilling were adequate for the QC and ON seed lots but 12 weeks were required for the NB seed. Soaking seed for 72 hours was beneficial for the NB seed but soaking did not improve germination of QC and ON seed. Soaking seed for 14 days tended to cause increased mortality. Germination was slightly higher at 15°C than at 20°C. Sufficient seed was available to allow the remainder to be stored for one year at -20°C and repeat the experiment to evaluate any impact storage may have on germination for seed treated in the same manner as above. ISTA rules for testing eastern hemlock (Tsuga canadensis) seed prescribe 28 days of moist chilling followed by 28 days in a germinator at 15°C. This length of chilling time is inadequate to alleviate dormancy of NB seedlots therefore the procedure adopted was to moist chill seed for 24 weeks and germinate at diurnal temperatures of 20/30°C which provided satisfactory results. A trial, using local seed, was established where seed that had been moist chilled for 4 week increments was germinated at either 10/15°C, 15°C, or 15/25°C. Just over 10% of the seed chilled for 4 weeks germinated at 15°C where as 65% germinated at 15/25°C. After 16 weeks of chilling, germination at these two temperature regimes was about the same (90%). Therefore, the evidence indicates that 4 weeks (28 days) of moist chilling is inadequate to alleviate dormancy in local eastern hemlock seed but germination at 15°C is sufficient. Further testing should be conducted to determine if there is geographic variation in chilling requirements. Being able to store seed for many decades is important when conducting ex situ gene conservation activities. The Seed Centre has been germination testing and storing seed for over 25 years and has accumulated a sizeable database for many species with seed collected from many geographic locations and different years. Germination data were evaluated for five hardwood and ten softwood species. Generally, after 25 years of storage germination exceeded 60% for all species and for some was higher than 90% (Simpson et al. 2004).

The "OECD Scheme for the Control of Forest Reproductive Material Moving in International Trade" is applied to certify the origin of seed which is destined for the international market. The Scheme is a means to promote and facilitate international trade and movement of forest tree seed, plants, and vegetative propagules. Participation in the Scheme is voluntary. Certified seed carries with it a Certificate guaranteeing the provenance and implicit that all rules and regulations were followed in the collection and

processing of the seed. Certification does not cover seed quality, i.e., germination. The Scheme, which was adopted in 1974, consists of four categories of reproductive material: Source Identified, Selected, Untested Seed Orchards, and Tested. Certification work has been conducted almost exclusively in British Columbia because of the demand for seed from tree species located there. Most all OECD certified seed shipped from Canada has been in the Source Identified category with small quantities in the Untested Seed Orchards category. Over the past two years, 500 kg of certified seed were exported.

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# TREE IMPROVEMENT PROGRESS BY THE DIRECTION DE LA RECHERCHE FORESTIÈRE

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#### WHITE SPRUCE TREE IMPROVEMENT André Rainville

In the past two years, four first-generation white spruce (*Picea glauca* [Moench] Voss) seed orchards (out of 18 established) were evaluated in order to maximize genetic gain by collecting cones only from the best clones. By collecting cones from the best ten clones, additional gains are expected to be 12% over the gain from a general collection from all of the orchard clones.

Measurements of two full-sib progeny tests at 8 years were used to recommand the best crosses to be performed in the future, thus fulfilling needs of reforestation by cuttings. Gains of 11–15% over the cross means can be achieved by reproducing the 10 best crosses and propagating them by cuttings. Control crosses to evaluate GCA of the remaining 140 trees of the base population were completed and seeds will be sown in the spring of 2004. A part of the crosses needed to evaluate SCA has been done and will also be completed in 2004.

On a more operational level, analysis performed on ecological data was used to refine the seed transfer model (Li et al. 1996) and redefine the utilization territory of all the first-generation white spruce seed orchards. On a request from the forest industry, improved material, identified from tests located in the southern part of Québec, has been established in 5 tests in the north, in the black spruce-sphagnum moss cover type. While waiting for the first results, 167 trees were also selected in natural stands in the same region to create a new seed source.

### NORWAY SPRUCE TREE IMPROVEMENT Marie-Josée Mottet

An exceptional flowering year in 2003 allowed us to almost complete our Norway spruce (*Picea abies* [L.] Karst.) polycross program, as well as to move forward with the specific crosses program, done with 19 genotypes that demonstrate increased resistance to the white pine weevil.

The best families and provenances were identified for the St. Lawrence River improvement zone (sugar maple-basswood domain) from mean heights at five years in three open-pollinated progeny tests. For 10% of the best families, genetic gain varied from 8 to 17%. For the Laurentian improvement zone (sugar maple-yellow birch domain), ten-year results will be available in 2004. All of the new data will permit us to validate the proposed improvement zones. Tree selections are planned in these tests in 2004 and 2005, mainly for Polish progeny recently introduced into Québec that show superior growth gains as well as increased tolerance to the white pine weevil.

In collaboration with the Canadian Forest Service (CFS), a study of the effect of the white pine weevil on the wood quality of Norway spruce was completed using data from two other plantations at Duchesnay, in addition to the one at Valcartier. The analysis of mechanical and wood density resistance criteria led to

concluding that damage by the weevil, in general, has little effect on these variables and that Norway spruce possesses superior qualities to planted white spruce.

### JACK PINE AND SCOTS PINE TREE IMPROVEMENT Roger Beaudouin

In 2002, roguing of the twelfth and last first-generation jack pine (*Pinus banksiana* Lamb.) seed orchard was carried out. For the four orchards and the test located in improvement zone "A", the genetic gain was calculated using dominant trees and by taking into account site index quality of the related family test. The gain in merchantable volume varies from 2.6 to 8.8 m<sup>3</sup>/ha at 40 years, when seeds are harvested from the retained families.

In 2002–2003, selection of 300 jack pine elite trees in zone "A" for the second-generation was completed. Total height of the best families makes up the principal selection criterion. Seventy-two clones form the elite population, in which biparental controlled crosses will be done. These clones are part of a 109 clone seed orchard established to produce improved seed for reforestation in zone "A". The 228 other clones make up the support population, in which polycross controlled crosses will be carried out.

A total of 1 603 grafts were done in March 2003 at the Duchesnay grafting centre near Québec, in order to establish half of the zone "A" support population. With all varibles blended, the rate of grafting success after one year of growth was 92%. Forty-five percent of the grafts were top grafted and 55% were side veneer grafts. The survival rate of the top grafts was equal to that of the side veneer grafts. On two-year-old root stock with a mean height of 35–40 cm, the growth in 2003 of top grafts is 33% better (13.3 cm) than the growth of side veneer grafts (10.0 cm).

A detailed description of eleven visual symptoms observed on the terminal shoot, the buds, and the needles of Scots pine (*Pinus sylvestris* L.), as well as their frequency and the period they appeared indicates that the main cause of the loss of apical dominance, the formation of multiple leaders, forks and large branches, and the slowing of height growth is attributable to a soil deficiency of boron. Foliar analyses showed that the mean concentration of boron varied from one site to another, from 7.1 to 15.2 ppm. Soil analyses indicated that the boron concentration of each sample was very low, varying from less than 0.1 to 0.3 ppm. These values are inferior to those recommended for optimal growth. Microscopic examination of needles also showed that important structural changes are produced in needles following boron deficiency. A forest research bulletin on this subject was published in 2004.

In 2003, three Scots pine seed sources that were genetically improved for reforestation were tested (rogued provenance tests, the Cleveland Township clonal orchard, and the Groenendaal clonal orchard in Belgium). On the other hand, two comparative plantations, including control plots and fertilized plots were established in order to identify boron dosages on different sites to supply optimal growth conditions.

#### BLACK SPRUCE TREE IMPROVEMENT Mireille Desponts

In 2003, the first phase of the black spruce (*Picea mariana* [Mill.] BSP) program was completed. The last prescription for roguing a seed orchard was prepared and selection of elite trees in first-generation progeny tests was completed in all of the tree improvement zones. Setting up new clonal seed orchards and establishing improved populations for the next generation will be completed in 2005. Four clonal tests, composed of cuttings, were established in 2003.

Analyses of progeny test data, paired with data from two juvenile nursery tests, will continue in 2004, when nine progeny tests, paired to three juvenile tests, will be measured. The juvenile nursery tests, maintained and systematically thinned, will serve several purposes especially for reproduction studies and carrying out  $F_2$  controlled crosses or backcrossing for which the pedigree will be known for three generations.

#### LARCH TREE IMPROVEMENT Martin Perron

During the past two years we have continued implementation of the improvement strategy for secondgeneration European larch (*Larix decidua* Mill.), Japanese larch (*L. kaempferi* [Lamb.] Carr.), and their hybrid (*L. x marschlinsii* Coaz). The reason for using the latter species is to increase wood quality and volume of hybrid larch. To that end, parents of European and Japanese larch were selected in 2003 and 2004, especially with respect to growth and indirect characteristics that influence wood quality. To accomplish this, data from 39 experiments were re-analysed, but tree selection was carried out in only 13 of them. Moreover, the hybrid individuals were removed using molecular markers specific to the species. Selection was done especially with respect to height growth, then mass selection was done using trunk straightness, crown quality (branch size, angle, and number), and the absence of damage (diseases). Selected trees also had to have a height/diameter ratio of less than one.

The 80 Japanese larch that were selected to make up the breeding population for the second-generation represent 0.34% of candidate trees. They come from the 28 best sources (provenances and progeny) among the 137 evaluated (20.4%). The 80 European larch that were accepted represent 0.39% of the total and they originate from 45 sources out of 280, or 16%.

Furthermore, the genetic improvement strategy for exotic larches was amended and improved following reception of comments from geneticists who are members of the CAGAF (Comité d'amélioration des arbres forestiers [Québec]; Forest Tree Improvement Committee).

### HYBRID POPLAR IMPROVEMENT PROGRAM Pierre Périnet and Marie-Josée Mottet

The hybrid poplar improvement program, started in 1969 by Gilles Vallée, is still very active testing the populations obtained since the beginning of the project, producing new hybrid material, and maintaining collections of poplar species (*Populus maximowiczii*, *P. trichocarpa*, and *P. nigra*). In addition to selection of Septoria-resistant clones for the St. Lawrence River valley area in southern Québec, the program is mainly oriented towards the production of *P. maximowiczii* hybrids well-adapted to forest sites located in bioclimatic domains 3, 4, and 5 (sugar maple-yellow birch, balsam fir-yellow birch, and balsam fir-paper birch domains).

From 1999 to 2004, 19 clonal tests were established mainly in southern Québec (domains 1, 2, 3, and 4) from Témiscamingue to Témiscouata for clonal evaluation of a hybrid population obtained in 1993. In northern Québec, 8 clonal tests were established from 2000 to 2003 with *P. maximowiczii* hybrids, particularly with *P. X rollandii X P. maximowiczii* (DNxB)XM), from the 1985 to 1992 breeding programs.

In 2005, the first two clonal tests from the 1996 breeding program will be established. More than 800 clones were selected from three 5-year-old progeny tests planted in 1997 in the Saguenay-Lac-Saint-Jean area. More selection is planned in three other progeny tests planted in 2000 in the Bas-Saint-Laurent area with the hybrid population from the 1998 breeding program.

A new population was bred in 2004 for the Abitibi-Témiscamingue area, using parents from local and Alberta balsam poplar provenances, selected hybrid poplar clones, *P. maximowiczii*, and progenies of *P. deltoides* or *P. X canadensis* from northern locations. Up until now, successful results have been obtained from most of the crosses.

In 2001, Septoria cankers were found for the first time since 1970 on hybrid poplars in the Témiscamingue area. Infected trees, representing different hybrids of *P. maximowiczii*, *P. balsamifera*, and *P. trichocarpa*, and *P. X jackii* in tests planted from1985 to 1993, are located in Notre-Dame-du-Nord and Angliers. Adjacent hybrid poplars (*P. X jackii*) planted in 1970 in Notre-Dame-du-Nord (1 km apart), are still Septoria-free. We expect a spread of the disease, at least in parts of bioclimatic domains 3 and 4. We don't know to what extent these recent infections might be related to climate change.

Technology transfer is routinely achieved with forest industry partners for operational planting of hybrid poplar, with provincial nurseries for steckling production (1-0 rooted cuttings), and with scientists from the Réseau Ligniculture Québec. The provincial list of selected clones for planting is revised regularly, based on the results of clonal tests. The poplar research team contributes to different projects on Septoria canker, wood quality, and molecular genetics studies with scientists from Université Laval, University of Alberta, Forintek, and the CFS.

### HARDWOOD TREE IMPROVEMENT André Rainville

Over the last two years, most of the effort in hardwood tree improvement have been to establish white ash (*Fraxinus americana* L.) provenance-progeny tests. Fifty-nine progenies from 14 provenances were

represented in ten tests and will be used to evaluate genetic variation in quality and growth characteristics of white ash across its distribution in Québec and to identify improved material for reforestation.

Five year results of inspections performed in butternut (*Juglans cinerea* L.) conservation plantations revealed no statistical difference among provenances and progenies for susceptibility to butternut canker but interesting observations were noted about the epidemiology of the disease. Eradication of infected trees or diseased parts of trees within the plantations has been a very effective control method.

#### REALIZED GENETIC GAIN TESTS André Rainville, Mireille Desponts, Roger Beaudoin and Guy Prégent

This new project was initiated in 2002 with the objective of evaluating long-term gains in productivity (volume) and quality associated with planting improved seedlings from first-generation seed orchards. Results will be used to validate estimations of gains from progeny tests and for use in growth models being used to calculate annual allowable cut. In the first two years, seed from five black spruce seed orchards was sown and sites were prepared for the first plantings in 2005. In the spring of 2004, seed was sown from six black spruce and four jack pine seed orchards in seed zone "A" and tests will be established in 2006.

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# **RECENT RESULTS OF TREE REPRODUCTION IN QUÉBEC**

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Keywords: seed orchard management, pollen, pollen conservation, mass pollination, seeds, germination, stratification, *Picea glauca*, *Picea mariana*, *Larix decidua*, *Larix kaempferi*, *Larix marschlinsii* 

This report summarises the research carried out and the results obtained during the past two years on sexual reproduction of trees by the Direction de la recherche forestière.

### SEED ORCHARD MANAGEMENT

Larches (Larix decidua Mill., Larix kaempferi [Lamb.] Carrière)

Research on larch seed orchard management is being carried out on grafts grown in 100 L pots in shelters. A bagging method was developed to harvest pollen from the entire tree, which facilitates the harvest of a large volume of optimal-quality pollen. The pollen can either be used directly to produce hybrid seeds or be cold-stored in a pollen bank for later use. Our results show that stored pollen can maintain its initial viability for at least four years.

We currently use an electrostatic pistol for mass pollination, which allows us to rapidly carry out a very large number of crosses with a small crew. We are making headway with a method to extract seeds from European larch (*Larix decidua*), which is normally difficult.

Black Spruce (*Picea mariana* [Mill.] BSP)

We have developed a pollination trailer that has platforms at different heights, to make it easier to pollinate trees up to 6 m in height. Using a series of windbreak screens on the sides of the trailer and directly around the tree crown, we are able to significantly improve mass pollination efficiency using the electrostatic pistol, demonstrated by the increased number of filled seeds per cone. A second experiment on black spruce will confirm the result; the same protocol will be repeated on white spruce (*Picea glauca* [Moench] Voss).

### GERMINATION OF CONIFER SEEDS AND SEEDLING PRODUCTION

White spruce seeds are delivered, to the nursery, stratified with their moisture content reduced to 15%. We have demonstrated that during the germination process, stratified seeds have a significant advantage over untreated seeds or seeds that are simply primed. This advantage is even more obvious in extreme temperature conditions (10° and 30°C). Moreover, in case of frost, stratified seeds have better resistance than do untreated seeds. These two results clearly illustrate the advantage that nursery managers have in using stratified white spruce seeds for seedling production.

Next we will be working on methods to modify the seeding factor for nursery produced white spruce, black spruce, and jack pine (*Pinus banksiana* Lamb.) seedlings. We want to determine a seeding factor as a function of the percent germination for the seed lot. An initial trial was undertaken in a greenhouse. A second trial was started in 2004 at four provincial nurseries.

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# **RECENT ADVANCES IN TREE AND FOREST GENOMICS**

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Keywords: comparative and functional genomics, ectomycorrhiza, genome mapping, marker-aided selection, mitochondrial DNA, molecular ecology, molecular population genetics, phylogenetics, phylogeography, poplars, spruces, tree improvement, wood formation, xylem.

### INTRODUCTION

The last two years have seen many new developments. D. Khasa and J. Mackay have established their research groups at Laval University. J. Cooke, who was formerly at the University of Florida in Gainesville, has joined us. Collaborations have intensified with colleagues from the Canadian Forest Service (CFS), J. Beaulieu, N. Isabel, and A. Séguin, who have the rank of adjunct professors in our group. The synergetic effects have been tremendous. One good example is the *Arborea* project, which was launched in 2002 with the aim to better understand the functional genomics of wood formation and defence mechanisms in white spruce (*Picea glauca*) and poplars (*Populus* spp.). This initiative is a major undertaking which regroups all of us and is lead by J. Mackay. It is funded by Genome Québec and Genome Canada, and it also involves S. Reagan from Carleton University and E. Retzel from University of Minnesota.

During the last two years, the group has welcomed several postdoctoral researchers, including C. Bomal, A. Guérin, J.-L. Jany, N. Pavy, C. Guillet-Claude, F. Lafarguette, T. Laurentiu, and Q. Yu. Many graduate students were involved in various research projects, including at the MSc level S. Beauseigle, H. Bérubé, F. Boileau, E. Campagnac, C. Côté, A. Gagné, J. Godbout, M.C. Gros-Louis, A. Noël, and P. Pollefeys and at the PhD level, F. Bedon, G. Bois, M. Bouillé, I. Gamache, J.P. Jaramillo-Correa, S. Legay, B. Pelgas, F. Pitre, S. Plante, and R. Sibout. Many of these students were co-supervised by adjunct professors J. Beaulieu, N. Isabel, and A. Séguin.

#### PHYLOGEOGRAPHY J. Bousquet

Over the past two years, we have pursued the study of the phylogeography of Canadian conifers. In collaboration with scientists from the CFS and Forêt Québec (J. Beaulieu, N. Isabel, and M. Perron) and funding from Natural Sciences and Engineering Research Council (NSERC) and CFS, PhD student J.P. Jaramillo-Correa (supervised by J. Bousquet and J. Beaulieu) developed and characterized a number of sequence-based mtDNA markers in conifers (Jaramillo-Correa et al. 2003). Because they are maternallyinherited, these markers are less affected by gene flow and their geographical patterns of variation are more likely to reflect history. PhD student I. Gamache (supervised by ecologist S. Payette and J. Bousquet) tested this hypothesis by investigating the population structure of Picea mariana at the tree limit in northern Québec. While there was essentially no difference in gene diversity between subarctic and boreal populations, there were large differences in mtDNA gene diversity among boreal populations and between boreal and subarctic populations implying limited gene flow in mtDNA (Nm<1 as opposed to Nm>15 for nuclear genes). Populations from the subarctic were monomorphic in their mtDNA suggesting a founder event during the colonization of the Holocene (Gamache et al. 2003). Another study lead by Jaramillo-Correa showed that Picea rubens was essentially monomorphic for mtDNA but for a different mitotype than subarctic populations of P. mariana. Overall, P. mariana was more diverse and contained the variation observed in P. rubens (Jaramillo-Correa and Bousquet 2003) which confirmed a similar trend of a progenitor-derivative species relationship at nuclear gene loci (Perron et al. 2000).

More recently, range-wide genetic variation of *P. mariana* was studied using these mtDNA markers (Jaramillo-Correa with J. Bousquet and J. Beaulieu). This study was funded by NSERC and CFS and it

was made possible thanks to the collaboration of colleagues of the Canadian Tree Improvement Association who kindly participated in the sampling effort. Low levels of mtDNA diversity were detected within the 32 populations surveyed but 10 mitotypes were found across Canada. A highly significant subdivision of population genetic diversity was detected, which was associated with geography ( $G_{s_T} =$ 0.671,  $N_{s_T} = 0.726$ ). The distribution of mitotypes revealed four partially overlapping areas presumably colonized from three southern and one northeastern glacial refugia (Jaramillo-Correa et al. 2004). A similar range-wide study was also initiated for *Pinus banksiana*. It is being carried out by MSc student J. Godbout (supervised by J. Bousquet and J. Beaulieu). A highly variable minisatellite locus was discovered in the mtDNA of this species and the pattern of intra- and interpopulation variation is under investigation. Again, a highly significant subdivision of population genetic diversity has been detected. Similar studies have also been initiated for *P. glauca* and other major transcontinental conifers for which informative mtDNA variation has been detected.

#### MOLECULAR ECOLOGY AND POPULATION GENETICS D. Khasa

With funding from NSERC and Ligniculture Québec and collaboration with P. Périnet from Forêt Québec and Alpac Forest Industries in Alberta (B. Thomas), several poplar SSR markers have been optimized and used for parentage analysis of poplar clones (Khasa et al. 2004). Some of these microsatellite markers and other gene markers including SNPs (in collaboration with J. Bousquet and N. Isabel) have been shown to be species-specific to identify five commercially important poplars and their interspecific hybrids in North America (Khasa et al. 2004, in prep.). These diagnostic markers will be used to assess potential gene flow from plantations of exotic poplar species into coexisting stands of native species and between native species potentially hybridizing naturally (*Populus deltoides* and *P. balsamifera*). On another front and in collaboration with CFS, MSc student M.-C. Gros-Louis, supervised by J. Bousquet and N. Isabel, has been developing nuclear and organellar diagnostic markers for the recognition of larch species and their hybrids. These markers are being used to certify hybrid progenies (M. Perron et al., Forêt Québec) and to estimate the extent of gene flow between exotic plantations and natural populations of indigenous species. This work has been supported by the Canadian Biotechnology Strategy (CBS), Forêt Québec, and Ligniculture Québec. The comparative study of population genetic diversity and breeding systems in western and subalpine larches is also nearly completed (Nadeem et al. 2003, Khasa et al. 2004, in prep.).

The molecular ecology of mycorrhizal fungi is also an important aspect of our research program. Using both an enriched genomic library for single- or low-copy sequences and data mining strategies, we have been successful in developing single-locus codominant microsatellite markers for several ectomycorrhizal fungi including *Laccaria bicolor* (Jany et al. 2004) and *Hebeloma* species (Jany et al. 2003). These sets of hypervariable markers will be valuable in environmental genomics studies such as monitoring the persistence of introduced mycorrhizal strains into the environment and the potential introgression of alien genes into the gene pool of native ectomycorrhizal species, or the assessment of their population genetic structure and mating system. Other strategies relying on the sequence analysis of ITS-rDNA have also been used to study mycorrhizal and root endophytic fungi of containerized *P. glauca* seedlings in commercial forest nurseries in northern Alberta (Kernaghan et al. 2003). In collaboration with Syncrude Canada and NSERC, a two step approach aimed at selecting both plant and mycorrhizal genotypes has helped us determine potential mycorrhizal plant systems for use in saline-alkaline habitats (Kerngahan et al. 2002).

#### FUNCTIONAL GENOMICS J. Mackay

J. Mackay has established a functional genomics research program that will focus primarily on issues related to wood formation and properties. A large-scale initiative lead by J. Mackay, project *Arborea* (www.arborea.ca) funded by Genome Canada, is now underway with a major focus on putative regulatory genes of secondary xylem (wood) formation in both poplar and spruce and defence response in poplar (Mackay et al. 2003). Several genes have been isolated that are similar to sequences described in model angiosperm plants and have been implicated in the regulation of primary and secondary vascular development. Gain-of-function experiments were initiated for several of these genes through over-expression studies in spruce or poplar in collaboration with A. Séguin from CFS. Preliminary results from these studies provide early indications that several of these genes are involved in regulating plant growth and development and affect vascular development either directly or indirectly. An integrated RNA analysis laboratory has been established by J. Mackay and J. Cooke to support diverse studies of gene expression from high-resolution studies of individual genes to large-scale analyses of thousands of genes using macroarray and microarray technologies. They are now beginning to use RNA profiling methods to assess

the molecular function of targeted regulatory genes and conduct a variety of gene expression studies related to wood formation in spruce and poplar.

Within Arborea, a large-scale EST sequencing effort has been completed in *P. glauca*. High quality sequences have been obtained for approximately 35 000 ESTs from 17 cDNA libraries created from diverse organs and tissues. Part of the new sequence information is now publicly available at <a href="http://web.ahc.umn.edu/biodata/spruce">http://web.ahc.umn.edu/biodata/spruce</a> and the remaining sequences will be released in coming months. An analytical pipeline has been established by N. Pavy and collaborators for the comparative analysis of ESTs from large databases (Pavy et al. 2004). Using conifer *knox-1* genes, an analytical pipeline has also been established for studying the functional diversity of transcription factors. This study is lead by C. Guillet-Claude in collaboration with J. Bousquet and N. Isabel. The phylogeny of plant *knox-1* genes has been estimated and indicates that conifer *knox-1* genes have evolved independently from angiosperms. They could further be divided into four classes. Signs of functional evolution were numerous, with accelerated rates after duplications and significant rate shifts at many amino acid positions. The same sites and additional sites in introns and promoter regions are monitored at the intraspecific level from up to 120 haplotype sequences for each gene. Conifer *knox-1* genes were also mapped and accelerated molecular evolution correlated with major translocations (Guillet-Claude et al. 2004).

#### STRUCTURAL AND EVOLUNTIOARY GENOMICS J. Bousquet

In collaboration with N. Isabel, linkage maps of *P. glauca* were published (Gosselin et al. 2002). Over the past two years, PhD student B. Pelgas and MSc student S. Beauseigle (supervised by J. Bousquet and N. Isabel) have focused their efforts to further map the genome of *P. glauca*, establish base maps for *P. mariana*, and develop a set of anchor markers for spruce and conifer mapping. These will be useful to construct composite maps from various pedigrees within species and estimate map synteny between divergent spruce species including *P. mariana*, *P. glauca*, and *P. abies* in collaboration with European partners from INRA. Two types of anchor markers have been developed with variable success: microsatellites and expressed sequence tag polymorphisms (ESTPs). To accelerate the developement of ESTPs, we relied on DNA pool sequencing (DPS) to sample polymorphism. We showed that DPS is a highly reliable strategy to increase marker recovery, whether from the detection of indels or SNPs (Pelgas et al. 2004). More than 100 codominant markers are now available for establishing consensus maps in *P. glauca* and *P. mariana* with amenable genotyping strategies such as AGE, DGGE, or CAPS (Pelgas et al. 2004). Composite maps are near completion in *P. mariana* and *P. glauca* and synteny is currently being estimated among major spruce species. This joint project with N. Isabel funded by a NSERC-genomic grant and the CBS.

Studies have also been pursued to characterize the patterns of DNA variation at the intraspecific and interspecific levels in the genus *Picea*. PhD student M. Bouillé (supervised by J. Bousquet) showed that transpecies shared polymorphisms at the DNA level are numerous among distantly related spruce species, indicating long allele coalescence time and large historical population sizes (Bouillé and Bousquet 2004). Thus, these studies suggest a unifying mechanism for the maintenance of the high levels of neutral genetic diversity observed in conifers and in trees characterized by outcrossing mating system and high gene flow. We are also in the process of completing the estimation of the phylogeny of the complete genus *Picea* using a half-dozen genes from the nuclear, chloroplast, and mitochondrial genomes. The results obtained indicate that none of the traditional morphological classifications is entirely right. At the same time, the comparison between mtDNA and cpDNA phylogenies suggests cases of ancient lateral gene transfer.

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# ADVANCES IN FOREST GENETICS, GENOMICS AND BIODIVERSITY AT THE CANADIAN FOREST SERVICE – LAURENTIAN FORESTRY CENTRE

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This report summarizes the progress of the Canadian Forest Service - Laurentian Forestry Centre's research in forest genetics, genomics, molecular ecology and biodiversity for the period 2002–2004. Our activities have mainly focused on breeding work in three spruces, eastern white pine (*Pinus strobus*) and Canada yew (*Taxus canadensis*). We have also continued to spend time on population genetics studies of forest tree species and in structural genomics in collaboration with J. Bousquet from Laval University (LU) and as part of our participation in the activities of the Canada Research Chair in Forest and Environmental Genomics (Tier I). Research was also done in collaboration with our colleagues from Forintek Canada Corporation and Laval University on genetic variation and control of wood characteristics. Some of us have also joined the functional genomics research program Arborea (Mackay et al.), a major initiative, funded by Genome Canada for studying functional diversity of transcription factors. On another front, in collaboration with J. Bousquet and M. Perron et al. (Forêt Québec), nuclear and organellar diagnostic markers for the recognition of larch species and their hybrids has been developed.

Research accomplishments were made possible with the generous contributions of dedicated collaborators and staff who deserve our thanks. They are: René Pâquet, Daniel Plourde, Jean-Paul Bilodeau, Yves Dubuc, Manuel Lamothe, France Gagnon, Marie-Claude Gros-Louis, Esther Pouliot, Sylvain Boisclair, Michèle Bernier-Cardou, Pamela Cheers, and many summer students.

### GENETICS OF INDIGENOUS SPRUCES Jean Beaulieu

White Spruce

Over the last two years, we completed most of the study initiated in collaboration with Tony Zhang (Forintek Canada Corp.) and André Rainville (MRNFP) to estimate genetic parameters of white spruce (*Picea glauca*) wood traits in order to develop a breeding program for growth performance and quality endproducts. A genecological test, including 40 open-pollinated families from the Great Lakes-St. Lawrence region and replicated on two sites, was thinned in 2001. Two 210 cm logs were taken from each of the 320 36-year-old trees sampled and transported to the Forintek Canada Corp. facilities in Sainte-Foy, Québec. Logs were then either processed into 2 x 4 studs or cut in sections for analysis of veneer properties and decay resistance. A first paper, (Yu et al. 2003), investigated genetic variation in decay resistance and its correlation to wood density and growth. We found that decay resistance was inversely related to growth rate of the fungi on heartwood blocks. Narrow-sense heritabilities to decay resistance to brown rot and white rot were 0.21 and 0.27, respectively. Phenotypic and genetic correlations between the growth rate of brown rot and wood density were positive, whereas it was negative but not significant for white rot. A second paper, (Zhang et al., in press), investigated genetic variation in veneer quality and its correlation to growth. The narrow-sense heritability for veneer density, veneer modulus of elasticity, and veneer roughness was 0.62, 0.13, and 0.14, respectively. The phenotypic and genetic correlations between ring width and veneer density or veneer modulus of elasticity were negative. This means that selection for tree volume growth would lead to a decrease in wood density and veneer stiffness. Study of the genetic variation of solid wood mechanical properties is presently under way. The white spruce reforestation program is one of the most important in Québec with close to 30 million seedlings planted annually. This indigenous species is valued for its high yield (over 420 m<sup>3</sup>/ha were obtained at 38 years with a fast growing progeny test established at Valcartier, for instance) and its general tolerance to insects and disease.

A study to determine the extent of the differences in tracheid length among 30-year-old white spruce openpollinated families was carried out. Narrow-sense heritability was estimated as well as its genetic correlation with growth traits and wood density. Heritabilities at both the individual and family levels were low. Tracheid length was negatively correlated to growth traits but appeared to be independent of wood density. Vegetative propagation of the trees with the longest tracheids would be the most promising avenue to increase the average tracheid length in white spruce.

The development of pedigree populations was continued. Single-pair matings were carried out in order to have access to a large number of unrelated families for the genomics studies. White spruce individuals belonging to  $F_1$  families were bulked up using rooted cuttings. A partial diallel cross using trees from families with high, medium, and low breeding values for height growth was completed. Seeds were sown in 2004 and trees will be propagated in the future using cuttings and replicated tests will be set up for genomics studies.

#### Black Spruce

The study on patterns of adaptive genetic variation and seed source transfer in black spruce (*Picea mariana*), initiated during the previous period, was completed and results were published (Beaulieu et al. 2004).

The development of pedigree populations was continued. Backcross,  $F_2$  (self), and  $F_2$  (intercross) families were obtained using controlled crosses. Vegetative propagation of these families (between 600 and 1 200 individuals per family) began in 2001, thanks to the collaboration of Michel Rioux from the Saint-Modeste cutting centre. Six tests were established using backcross vegetative propagules. Other pedigree families were also propagated using cuttings and are presently grown in a nursery at the Valcartier Research Forest. The controlled crosses for the development of third-generation black spruce pedigree populations have also been completed during the period under review.

#### POPULATION GENETICS AND PHYLOGENETICS OF FOREST TREE SPECIES Jean Beaulieu, Marie Deslauriers and Nathalie Isabel

After developing and publishing mitochondrial DNA (mtDNA) PCR-RFLP markers for spruces (Jaramillo-Correa et al. 2003), range-wide genetic variation of black spruce was studied using mtDNA PCR-RFLP markers in a study led by Juan Pablo Jaramillo-Correa, a PhD student supervised by Jean Bousquet and Jean Beaulieu (Jaramillo-Correa et al. 2004). This study was funded by grants from Natural Sciences and Engineering Research Council (NSERC) and Canadian Forest Service (CFS), and it was made possible thanks to the collaboration of colleagues of the Canadian Tree Improvement Association who kindly participated in the sampling effort. Thirty-two populations, each represented by 15 to 30 individuals, were analyzed. Low levels of mitochondrial diversity were detected within populations. A total of ten mitotypes was found across Canada. A highly significant subdivision of population genetic diversity was detected, which was associated with geography ( $G_{sT} = 0.671$ ,  $N_{sT} = 0.726$ ). The distribution of mitotypes revealed four partially overlapping areas presumably colonized from different glacial refugia. We inferred at least three southern and one northern glacial refugia for black spruce during the last glaciation.

A similar study was also initiated for jack pine (*Pinus banksiana*). It is being carried out by Julie Godbout, a M.Sc. student supervised by Jean Bousquet and Jean Beaulieu. Juan Pablo Jarmillo-Correa is also participating in the study. A highly variable minisatellite locus was discovered in the mitochondrial DNA of this species and the pattern of intra- and inter-population variation is presently under investigation. Again, a highly significant subdivision of population genetic diversity has been detected.

The level of genetic diversity of 16 white spruce populations, including six populations from old-growth forest and 10 populations from second-growth forest, was estimated using allelic variants at eight ESTP loci (Beaulieu et al. 2003). A total of 23 alleles was detected in the 16 populations. All alleles present in the second-growth forest populations were also present in the old-growth forest populations and one variant

was present in the old-growth forests only. The proportion of rare alleles in the old-growth forest populations was more than twice that in second-growth populations. Moreover, the population structure of old-growth was remarkably different from that of the second-growth forest, which was confirmed by both F statistics and cluster analysis.

With funding from Canadian Regulatory System for Biotechnology, NSERC, Ligniculture Québec, and the collaboration of P. Périnet from Forêt Québec several gene markers (N. Isabel and J. Bousquet) and SSR markers (D. Khasa and J. Bousquet) have been shown to be species-specific to identify five commercially important *Populus* spp. and their interspecific hybrids in North America. These diagnostic markers will be used to assess potential gene flow from plantations of exotic poplar species into co-existing stands of native species and between two native species potentially hybridizing naturally, *P. deltoides* and *P. balsamifera*. In parallel, with collaboration from LU, MSc student M.-C. Gros-Louis supervised by J. Bousquet and N. Isabel has been developing nuclear and organellar diagnostic markers for the recognition of larch species and their hybrids (Gros-Louis et al., submitted). These markers are being used to certify hybrid progenies (M. Perron et al.) and to estimate the extent of gene flow between exotic plantations and natural populations of indigenous species. This work has been supported by the Canadian Biotechnology Strategy, Forêt Québec and Ligniculture Québec.

An international symposium on silviculture and the conservation of genetic resources was organized as a side event of the XII<sup>th</sup> World Forestry Congress on September 21, 2003 in Québec City. Eleven papers were given at that symposium and the proceedings were published (Beaulieu, editor 2004).

### GÉNÉTIQUE DE L'ÉPINETTE DE NORVÈGE, DU PIN BLANC ET DE L'IF DU CANADA Gaëtan Daoust

### Épinette de Norvège

Pour l'épinette de Norvège, les efforts concertés du Service canadien des forêts (SCF) et du Ministère des ressources naturelles, de la faune et des parcs du Québec (MRNFPQ) ont permis de poursuivre l'avancement du programme conjoint d'amélioration génétique. En 2002 et 2003, 200 croisements polycross de première génération ont été réalisés dans le parc d'hybridation de l'Arboretum Serge-Légaré à Valcartier. Ces lots de semences nous permettront d'initier en 2005 la deuxième série de tests polycross (E411-II). La première série (E411-I), comprenant 90 lots sera établie au printemps 2004 dans 5 régions écologiques différentes.

Au cours de la même période, plusieurs croisements bi-parentaux d'épinette de Norvège ont été réalisés et seront ensemencés au printemps 2004 à la pépinière de Berthierville (E408-II). Ce test complètera la première série établie en 2000 et comprenant près de 200 croisements bi-parentaux. Des croisements bi-parentaux ont également été réalisés par la Direction de la production des semences et des plants (DPSP) du MRNFPQ dans le parc d'hybridation afin d'alimenter en semences de grande qualité génétique le programme de bouturage. Pour ces croisements, nous avons recommandé des génotypes sélectionnés pour leur résistance au charançon du pin blanc.

Les résultats des tests de descendances de la Série E390 ont permis de formuler des recommandations précises à la DPSP pour la récolte de sources performantes provenant de plantations commerciales, de tests de provenances et du parc d'hybridation. En se basant sur nos tests de descendances provenant de pollinisation libre, les clones du parc d'hybridation ont pu être classés en 3 classes selon leur potentiel de gain de croissance. Ces semences seront utilisées dans le programme de reboisement du MRNFPQ. Plusieurs collaborations ont été entreprises au cours des dernières années avec des compagnies forestières ou des groupes de recherche tel que l'Université du Québec en Abitibi-Témiscamingue afin d'acquérir de l'information sur la croissance et la rusticité de l'épinette de Norvège dans des régions plus nordiques.

Une étude sur la variabilité génétique de l'épinette de Norvège en test clonal a été réalisée et les résultats ont permis la réalisation d'un mémoire de fin d'études (Page 2003). L'étude entreprise afin d'étudier l'impact du charançon sur la productivité et la qualité du bois a été complété. L'étude a permis de faire ressortir le potentiel de cette espèce lorsqu'on la compare à l'épinette blanche tant pour la productivité que pour les caractéristiques du bois. Le faible défilement des tiges d'épinette de Norvège représente un atout majeur pour cette espèce.

#### Pin Blanc et Pins Hybrides

Au cours de la dernière période, les divers tests décrits dans le compte-rendu précédent ont été établis. Plus précisément, 3 tests comprenant 130 descendances uni-parentales (E601-II); 3 tests comprenant 90 descendances bi-parentales (E602) et 3 tests comprenant 36 descendances bi-parentales (E603, dialèlle 6 x 6) ont été établis dans différentes régions écologiques. Les données prélevées durant leur développement en pépinière (3 ans) nous permettront de faire une première classification du potentiel des génotypes sélectionnés produisant des semences dans notre parc d'hybridation de Cap-Tourmente et dans certains vergers à graines du MRNFPQ. Deux autres séries de tests comprenant des hybrides produits dans nos collections (E605) ou des pins exotiques haploxylons (E604) ont également été établis.

Les 5 clones hybrides *griffithii* x *strobus* obtenus du programme ontarien dans les années 80 ont été affectés très sévèrement par les rigueurs climatiques survenues durant l'hiver 2002-2003 à la Station forestière de Valcartier. La majorité des plants, qui atteignaient plus de 4 m de hauteur en moyenne, n'ont pas survécu à cet hiver rigoureux. Toutefois les mêmes clones hybrides établis à Cap-Tourmente, en bordure du fleuve Saint-Laurent et protégés des vents dominants n'ont pas été affectés par le froid.

Un bilan des travaux de génétique et d'amélioration du pin blanc au Québec a été présenté lors du White Pine Blister Rust Workshop tenu en mars 2003 à Ottawa (Daoust et Beaulieu 2003). Dans le cadre d'un projet d'études en statistique forestière à l'Université Laval, les données d'une série de tests de descendances, douze ans après plantation, ont été analysés. Les résultats obtenus démontrent une forte corrélation entre les hauteurs à 6 ans et à 16 ans après plantation, des héritabilités familiales relativement élevées pour la hauteur et le volume et des différences, pour le site le plus nordique des trois testés, quant au choix des provenances et des descendances recommandées (Gagnon et al. 2004). Une étude sur la stabilité somatique au niveau de loci de microsatellite nucléaire et chloroplastiques a été réalisé chez le pin blanc. Aucune variation à l'intérieur des ramets de 12 clones n'a été détecté chez les 12 loci étudiés (Cloutier et al. 2003).

#### If du Canada

L'étude des 3 populations de Charlevoix s'est poursuivi par une deuxième échantillonnage en 2002 sur environ 30% des individus étudiées en 2001. Pour le contenu en taxanes, aucune différence entre les populations n'a été observé encore une fois. Les variations entre les individus demeurent importantes et les corrélations phénotypiques entre 2001 et 2002 pour le contenu en taxanes sont significatives. Ces résultats confirment le statut des individus sélectionnés en 2001 pour leur forte concentration de taxanes. Des tests clonaux comprenant 50 individus pour chacune des 3 populations ont été mis en place dans Charlevoix par le projet sous la responsabilité de la Société d'aide au développement de la collectivité de Charlevoix.

#### STRUCTURAL GENOMICS AND PHYLOGENOMICS Nathalie Isabel

In collaboration with J. Bousquet, linkage maps of white spruce have been published (Gosselin et al. 2002). Over the past two years, PhD student B. Pelgas and MSc student S. Beauseigle, supervised by J. Bousquet and N. Isabel, have focused their efforts to further map the white spruce genome, establish base maps for black spruce, and develop a set of anchor markers for spruce and conifer mapping. These will be useful to construct composite maps from various within-species pedigrees and to estimate map synteny between divergent spruce species including black, white, and Norway spruce. Two types of anchor markers have been developed with variable success: microsatellites and expressed sequence tag polymorphisms (ESTPs). To accelerate the development of ESTPs, we relied on DNA pool sequencing to sample polymorphism. We showed that DPS is a reliable strategy to increase marker recovery, whether for the detection of indels or SNPs (Pelgas et al. 2004). More than 100 codominant markers are now available for establishing consensus maps in white and black spruce with amenable genotyping strategies such as AGE, DGGE or CAPS (Pelgas et al. 2004). Composite maps are near completion for black and white spruce and synteny is currently being estimated among major spruce species (Pelgas et al., in progress). This joint project with J. Bousquet was funded by a NSERC-genomic grant and the Canadian Biotechnology Strategy.

Within the context of the Arborea project, an analytical pipeline for studying the functional diversity of transcription factors was developed using conifer *knox-1* genes (candidate gene for somatic embryogenesis). This study is lead by C. Guillet-Claude in collaboration with J. Bousquet and N. Isabel.

The phylogeny of plant *knox-1* genes has been estimated and indicates that conifer *knox-1* genes have evolved independently from angiosperms. They could further be divided into four classes. Signs of functional evolution were numerous, with accelerated rates after duplications and significant rate shifts at many amino acid positions. The same sites and additional sites in introns and promoter regions are monitored at the intraspecific level from large sampling (up to 120 haplotype sequences for each gene). Conifer *knox-1* genes were also mapped and accelerated molecular evolution correlated with major translocations (Guillet-Claude et al., submitted).

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# MODELLING AIRBORNE POLLEN DISPERSAL TO AID REDUCTION IN POLLEN CONTAMINATION

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**Keywords:** pollen dispersal, pollen contamination, gene flow, seed production, genetically engineered plants, modelling

Pollen contamination can significantly reduce the genetic quality of seed used for reforestation and, ultimately, reduce wood yield per unit land area. Significant resources have been directed to Ontario's tree breeding program in order to produce seed of high genetic quality in seed orchards. This report describes the development of a computer model of pollen contamination that could be used to manage pollen contamination in seed orchards.

## BACKGROUND

This work originated in the early 1990's with a series of field studies and developmental computer modelling efforts. In 2000, work was re-initiated with funding from Forest Genetics Ontario and was aimed at model development and application for a particular seed orchard. Extensive further work was done on both the model and the processing of input data for the model as well as an assessment of the field measured values. Assumptions made during model development in the early 1990's were tested and required modifications. Re-development with more rigorous assumptions indicated that the model generally over-estimated measured values. Although conservative results are acceptable when attempting to model environmental phenomena, especially in the context of anti-contamination methods development, at that time we believed that results would be more acceptable to the tree seed community if the degree of model conservatism was minimized. An evaluation of all aspects of the work indicated that some further work was necessary in refining the model, and possibly comparing model predictions against a second independent data set of dispersal data and dispersal model.

## RATIONALE FOR LATEST DEVELOPMENTS

Funding for this further work was obtained from the Canadian Food Inspection Agency (CFIA). CFIA regulates the introduction of plants with novel traits into Canada including trees. This also includes the introduction of genetically modified plants. The key element in the use of modelling is the ability to replicate multiple years of gene-flow data at one-site or at many sites, thus allowing the variability in outcrossing (OC) to be quantified. A quantification of variability in OC allows confidence intervals or containment success probabilities to be assigned to, for example, isolation zone widths or flowering desynchronization methods. Such a probabilistic or "risk-assessment" type of approach to OC is more realistic than guidance based on limited site- and time-specific experimental data given the inherent variability in OC. This is an approach recently developed and utilizes the power of dispersal modelling more fully. Such an approach has been recognized by CFIA as adding additional useful information to their regulatory risk assessment process.

# MODEL COMPLETION AND TESTING

The dispersal code was updated with contemporary stochastic Lagrangian algorithms. The resultant Langevin equation model (LEM; codified as POLDISP v.2.0) has been proven to emulate scalar dispersal within vegetative canopies more accurately than other commonly used dispersal models. POLDISP

compared favourably against an independent tracer data set and against an "off-the-shelf" air pollution dispersal model (US EPA's ISCST3 regulatory air pollution model, Fig. 1).

Run 5 Red

Figure 1. Observed (Aylor and Ferrandino 1989, Bound.-Layer Meteorol. 46: 251-) and modelled results of concentration profiles of marked Lycopodium spores at 2 m downwind from a release point at 0.4 m height under conditions u\* = 0.38 m s<sup>-1</sup> and L = -10,000 m. Results for the POLDISP model (=LEM) are shown where the deposition factor is fully applied (LEM – dep). Results are also shown for the ISCST3 model. The dotted, horizontal line indicates the approximate height of the canopy. Arrow on left-hand axis indicates the release height.

Concentration (spores m)

Presently available datasets for pollen dispersal and OC are lacking some of the information required to suitably test the model but those used thus far suggest the model is behaving well.

CFIA requested that this model be applied to wheat. There are many varieties of wheat, each with their own OC characteristics; i.e., pollen production/anther, anther extrusion, pollen viability, and settling velocity, etc. For generalized regulatory purposes a "worst-case" OC scenario was developed and OC at various distances from a source into a receptor field was simulated for nine locations across the major wheat growing regions of Canada and for 30 years at each location. At each location, OC at each distance downwind was combined for all 30 years to provide a mean and 95% CI. This was also repeated for all locations combined providing a "cross-Canada" result. OC data were then further processed to determine the probability of success of isolation by distance (isolation zones) for each of four threshold OC levels (0.1, 1.0, 5.0, and 10.0%) which, at this point, were chosen arbitrarily and for demonstration purposes only until CFIA decides on a "regulatory" threshold level, if one single level can be chosen.

The model is now ready to be used to optimize anti-contamination methods at orchards across Ontario and, if desired, further afield. Although further refinements are possible, we envisage a process of continual refinement through its practical application.

# NORTHEAST SEED MANAGEMENT ASSOCIATION (NESMA)

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The past two years have been challenging and rewarding. Although the second-generation activities have demanded most of our attention, we have also made significant accomplishments in our first-generation programs. We continue to make progress toward accomplishing our goal to satisfy our partner's seed demand with improved seed from our first-generation orchards. NESMA has, to date, supplied over 130 million improved seed to its partners. An exhaustive second-generation tree breeding program, fueled by the Living Legacy Trust, has progressed considerably over the last two years. Successful breeding work has concluded in most of our breeding programs. A significant amount of effort and personnel were required to carry out the breeding work efficiently and in a cost effective manner. Improvements were made to overcome technical challenges, worker safety, and lower costs. Education and awareness of forestry, tree improvement, and our company continue to play a significant role.

## FIRST GENERATION PROGRAMS

NESMA currently manages eight black spruce (*Picea mariana* [Mill.] BSP) and five jack pine (*Pinus banksiana* Lamb.) orchards located throughout northeastern Ontario.

Vegetation management, geared to competition reduction, is practiced at the orchards as required. Annual orchard fertilizing regimes are established based on the analysis of annual soil and foliage samples which are collected at all of the orchards in the fall of each year.

A combined Cone Crop Monitoring Study (CCMS) and a flower induction study were initiated at the Aidie Creek Jack Pine Seed Orchard in 2002. The study will provide flower counts on hormone treated trees in a managed jack pine orchard. It will also be used as a cone crop size predictive tool based on the monitoring of female flowers through their developmental stages and will provide insight as to the nature and impact of cone and seed predators.

To compare cone collection costs between orchard trees with and without crown management, a Crown Management Decision Support System (CMDSS) was developed and implemented at the Ramore Jack Pine Seed Orchard. Since 1994, crown management has afforded cone collection cost savings of 23%. Operational crown management, in conjunction with cone collection, is carried out annually at most of the orchards. In 2002, a total of 56.85 hL of cones were collected. A bumper cone crop in 2003 produced a total of 197.17 hL of cones from the orchards.

Roguing of the black spruce and jack pine orchards to approximately 20% of the original 400 families has been completed in all but two orchards (Lastheels and Curtis Black Spruce Seed Orchards).

Field colour-coding stratification of the final orchard population, according to family breeding values at the Island Lake Breeding Zone 1 and 4 Black Spruce Orchards, was completed in 2003. This allows for distinguishing between four classes of cones based on their "genetic worth".

At the Edward Bonner Orchard and at two genetic tests, two formulations of NEEM botanical insecticide were applied for the control of white pine weevil. This testing is being carried out to develop a reliable control method for the weevil and other insects which damage the leader of test trees.

An operational flower induction trial was also carried out at the Edward Bonner Black Spruce Seed Orchard. Three treatments were applied: 1)  $GA_{4/7}$  hormone injections, 2) high-rate ammonium nitrate fertilizer, and 3) a combination of  $GA_{4/7}$  and ammonium nitrate.

NESMA has developed a strategy to move the white pine (*Pinus strobus* L.) tree improvement program forward by developing three genetic tests, one of which may be converted to a seed orchard after testing

has been completed. This strategy will capitalize on seed collection efforts undertaken by the Ministry of Natural Resources, Nipissing Forest Resource Management Inc., Ottawa Valley Forests, and Westwind Stewardship Inc. In 2002, three first-generation white pine genetic tests were established in Evanturel, Gratton, and Gurd Townships. Competing vegetation management, re-tagging, and re-planting of frost heaved seedlings were the focus during the 2003 season. Plus-tree cone collections for an additional three tests were made in 2003. Seeding will take place in early 2004.

# SECOND GENERATION PROGRAMS

Tree breeding work was carried out in ten black spruce and jack pine programs involving 24 test sites distributed across northeastern Ontario. Rigorous analysis of the first-generation selections was made in order to strengthen the genetic gains and minimize possible inbreeding of the second-generation selections.

Access was created to and around all of the trees selected for breeding in order that the tree tops could be safely accessed with orchard ladders. An innovative tree bending technique was utilized on trees that were too tall for the modified 20 foot orchard ladders. The weather, staff procurement, training, and limited time proved to be challenges. In 2002 alone, over 100 people were recruited and trained in the art of tree breeding by experienced NESMA staff. An amalgamation of two jack pine programs (Durban Pj 25 and Aidie Creek Pj 4) to form the Durbie Creek Pj 4 program led to slightly reduced personnel requirements in 2003.

To date, all of the programs, with the exception of the Island Lake Pj 3, have produced sufficient seed to establish second-generation genetic tests. In addition to the regular breeding programs, special crosses with trees in the Edward Bonner black spruce program are expected to yield 25 - 30% gains. A vegetative propagation program is being developed.

Three Elite second-generation tests for the Island Lake, Breeding Zone 4 and the Edward Bonner, Breeding Zone 2 programs were established in the fall of 2003 with seeds collected from the elite controlled crosses made in 2001 and 2002. Seeds collected from open-pollinated trees in the infusion population were used to establish two infusion tests in each of the programs. Table 1 summarizes breeding activities conducted in 2002 and 2003.

	Breeding		Ye	ear
Program	Zone	Species	2002	2003
Island Lake	1	Black spruce	32	39
E. Bonner	2	Black spruce	60	6
Ramore	3	Black spruce	41	43
Aidie Creek	5	Black spruce	47	40
Ramore	2	Jack pine	80	53
Island Lake	3	Jack pine	31	55
Aidie Creek	4	Jack pine	66	
Durbie Creek	4/25	Jack pine		78
Durban	25	Jack pine	72	
Hambleton	21	Jack pine	46	59
Total crosses			475	373

#### Table 1. A summary of controlled crosses by program

Pollen collected from across the northeast was sent to a processing facility near Englehart where they were dried, processed, and returned to the field in a timely fashion. In 2002, 686 pollen collections were handled while in 2003 the lab processed 454 collections. Some of the pollen was sent directly back to the field for breeding but much of it was vacuum freeze-dried and placed in storage.

Flower induction, to promote female flowering, was carried out at all of the test sites in the spring after rapid shoot elongation of black spruce and in mid-July during bud differentiation in jack pine.

Cones were collected from the Elite controlled crosses and from the infusion open-pollinated crosses in September of 2002 and 2003. The cones were sent to the Ontario Tree Seed Plant for processing. Further x-ray testing and "float" testing was done by NESMA to validate the viability of the seed prior to seeding. To date, over 4 million seeds have been collected from the tree breeding program.

Site acquisitions and field work (root-raking, brush pile burning, and cultivating) were made at five locations in preparation for future test sites.

Additional plus-tree selections, to fill in the "geographic holes" left by the first-generation selections, were made in the Island Lake Sb 1, Ramore Sb 3, Island Lake Sb 4, Ramore Pj 2 and Aidie Creek Sb 5 Breeding Zones.

NESMA will continue its efforts to establish second-generation genetic tests and manage first-generation orchards that will provide our partners with enhanced wood yields by using genetically improved seed. NESMA's future plans include breeding at the second-generation jack pine genetic test located near Chapleau, Ontario (established in 1987) and the development of a white spruce tree improvement program.

# FOREST GENETICS RESEARCH AT THE ONTARIO FOREST RESEARCH INSTITUTE

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## WHITE PINE GENECOLOGY STUDY Pengxin Lu, Dennis Joyce and Bob Sinclair

Results from a short-term genecology study of eastern white pine (*Pinus strobus* L.) have been summarized. Seed movement guidelines were proposed based on revealed trends among provenances in growth potential, cold hardiness, and growth rhythm. A validation trial using 42 more evenly distributed provenances from eastern Ontario was established near Timmins in 2002. This trial will further test the adaptation and growth of southern provenances in a more northerly environment.

## SCREENING EASTERN WHITE PINE FOR BLISTER RUST RESISTANCE Pengxin Lu, Bob Sinclair and Tim Boult

In the past two years, a major effort was made to screen eastern white pine seedlings for blister rust resistance. Seeds were collected from putatively resistant trees of eastern white and interspecific hybrids from Ontario's white pine clone banks and field progeny trials. An artificial blister rust inoculation procedure was developed which, in conjunction with the modern growth facilities of Ontario Forest Research Institute (OFRI), enables blister rust to be inoculated on Ribes and white pine seedlings at about 100% infection rate and without the limitation of growing season. Four inoculation experiments have been carried out since, involving more than 12 000 6- to 18-month-old seedlings from > 250 open-pollinated families. Data collection was completed for the first 3 inoculations. The inherited disease resistance was evaluated based on seedling mortality. Preliminary results indicated that pure eastern white pine seedlings generally lacked genetic resistance to blister rust and suffered high mortality (> 97%) when heavily infected. Although the selected eastern white pine parent trees had been exposed to blister rust in previous tests, they did not transmit strong genetic resistance to their offspring. In contrast, some open-pollinated families of  $F_2$  and  $F_3$ eastern white pine backcross hybrids, especially those between eastern white pine and Himalaya blue pine (Pinus wallichiana A.B. Jacks.), have demonstrated stable inherited blister rust resistance, as shown by a significant improvement in seedling survival rates (> 50%) compared with those of pure Pinus strobus (< 3%) at the end the experiments. For some hybrid families, only about a 50% survival rate was expected because of the heteroallelic stages of hybrid female patents and little blister rust resistance expected from eastern white pine trees.

To retain more desirable characteristics of *Pinus strobus* in the hybrids and to improve their growth and adaptation in more northerly environments, an attempt was made to increase the genomic composition of *Pinus strobus* in the hybrids through back-crossing. Controlled-pollinations were conducted in 2003 and resulted in about 85 backcrosses. Seedlings from a few  $F_3$  backcrosses were obtained, which have shown promise in blister rust resistance and adaptation.

In the near future, more eastern white pine and its interspecific hybrids are to be screened for blister rust resistance through artificial seedling inoculation. Segregation data on seedling mortality from open- and control-pollinated hybrid families will be used to infer gene-reaction modes. Vegetative propagation of selected seedlings is being conducted through rooted-cuttings for clonal testing both in blister rust resistance and field growth performance.

#### ROOTED-CUTTINGS OF JACK PINE Pengxin Lu and Wayne Bell

In 2003, jack pine (*P. banksiana* Lamb.) was vegetatively propagated through rooted-cuttings at a relatively large scale. The method of Browne et al. (1997) was modified by eliminating the plant hormone treatment applied to cuttings and enhancing rooting environment control (i.e., relative humidity and temperature). A rooting rate of 70–80% was achieved using proliferated dwarf shoots taken from 2-year-old seedlings. The simplified rooting procedure markedly reduced operating time in cutting handling and, therefore, labor cost.

Using 2-year-old seedlings from 25 open-pollinated families as cutting donors, more than 7 500 rooted cuttings were produced with 4–5 ramets per seedling. Field trials are to be established in 2004 to compare growth performance between jack pine rooted-cuttings and seedlings.

#### WOOD DENSITY STUDY IN JACK PINE AND BLACK SPRUCE Paul Charrette, Tony Zhang and Pengxin Lu

Collaboration among the Superior Woods Tree Improvement Association (SWTIA), Forintek Canada Corporation, and OFRI investigated genetic parameters between wood density and growth traits within breeding populations of jack pine and black spruce (*Picea mariana* [Mill.] BSP) in northwest Ontario. Increment cores were collected by SWTIA from two field progeny trials for each species and wood quality attributes including wood density were measured in Forintek's laboratory using an x-ray densitometer. Data analyses and interpretation were conducted at OFRI. Preliminary results indicated that wood density is under moderately strong genetic control, which is weakly and negatively correlated with growth traits, such as height, DBH, and volume in black spruce, but strongly negatively correlated with growth traits in jack pine. Implications of wood density to selections in tree improvement depend on the expected end products. If the breeding objective is to increase fibre production for pulp, the decrease in wood density due to selection for faster growth may well be compensated for by increased volume. Whereas, if the expected final product is solid lumber, wood density needs to be taken into consideration because selection criteria based on growth traits only (height and DBH) may result in economic loss due to deteriorating wood quality, especially in jack pine. Correlation breakers existed for both species, some of which are desirable candidates for increasing both growth and wood density.

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# TREE SEED AND GENETICS RELATED ACTIVITIES AT THE PETAWAWA RESEARCH FOREST 2002–2004

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Keywords: seed dispersion, species introduction, moist chilling, repair mechanism, peak pollen release

Tree seed and genetics related activities have continued at the Petawawa Research Forest during the last two years. Cooperation with the Liaoning Provincial Academy of Forestry Science in the introduction of Canadian tree species to Northeast China resulted in a large-scale planting trial of jack pine and Petawawa helped with the procurement of 50 kg of seeds from Ontario latitude 46° N and vicinity for nursery sowing in 2003. The cooperation work is continuing and expanding to source trials of other tree species. On May 28, 2004, a delegation from the Liaoning Provincial Government headed by Mr. Xu Ming, Deputy Minister of Science and Technology, visited the Petawawa Research Forest and "An Offer of Cooperation Agreement between the Research Forest and the Liaoning Provincial Academy of Forestry Sciences" was signed. Results from more than 20 years of seed source trials of Canadian eastern white pine (*Pinus strobus*) provided sufficient confidence for the provincial forestry authority to initiate a large-scale operational planting trial. Petawawa was asked to procure a large quantity of eastern white pine seeds from latitude 46° N or proximity in Ontario if there are good seed crops in 2004. Details of the tree seed and genetics related activities are described below.

The collection of baseline data on natural seed dispersion in eastern white pine and red pine stands has yielded additional information. For assurance of genetic and physiological quality of seeds of local major tree species for research and operational purposes, seed collections have been initiated in good seed crop years with a view to establishing an in-house seed bank.

# RESEARCH AND DEVELOPMENT

**Repair Mechanism** 

It was found during an artificial aging study that moist chilling can induce natural repairing mechanisms in white spruce (*Picea glauca*) seeds as it was reported in black spruce (*Picea mariana*) seeds (Wang and Berjak 2000). This hypothesis was further proved by evidence from other germination data (Fig. 1 and Table 1).

Figure 1 demonstrates the natural repair mechanism induced by moist chilling in three white spruce seed lots from Nova Scotia. The cones were collected by one agency and each of the three cone collections was divided in half and processed by two different seed processing facilities. The germination tests of all seed lots were conducted at the National Tree Seed Centre located at the former Petawawa National Forestry Institute, Chalk River, Ontario. Seed dormancy was defined as the difference in germination percentage of non-chilled and 21-day moist chilled seeds. The degree of dormancy of seed processed at facility A varied from 13% in seed lot A, 15% in seed lot B to 31% in seed lot C. Germination test results of the non-chilled seeds processed by the two seed processing facilities (A and B) varied with 6% in lot A, 16% in lot B and 13% in lot C. This difference in germination is most likely due to differences in handling and processing procedures used at the two facilities. However, when moist chilled, these differences in germination between the two facilities were essentially eliminated in lots A and B and reduced to 6% in lot C. Furthermore, the effect of moist chilling on seed germination consistently showed a 6–15% increase for the three seed lots that were processed by facility A than by B. If dormancy in seeds processed by facility A is considered true, then the 6-15% improvement in germination in the three lots can be attributed to the natural repair mechanism induced by moist chilling.



Figure 1. Effect of moist chilling on germination of three white spruce seed lots extracted from cones by two different processing facilities (A and B).

Table 1 shows that moist chilling induced natural repair mechanisms in white spruce seeds through comparison of seed germination results from cones picked in Alberta by hand and by a mechanized cone thrasher designed for lodgepole pine cone collection.

	% germination after 21 days at 20/30°C with 8 h light				
Seed lot	Collection method	21 day chill	No chill	Gain from chilling	
А	Hand picked	90	73	17	
	Mechanized	76	45	31	
	Loss from cone damage	14	28	14	
В	Hand picked	90	75	15	
	Mechanized	75	51	24	
	Loss from cone damage	15	24	9	

Table 1.	Effect of moist chilling on germination of Alberta white spruce seeds extracted from cones
	collected by hand and by a mechanized cone thrasher

It can be seen from Table 1 that germination increased 14% for lot A and 9% for lot B as a result of chilling seed collected using a mechanized cone thresher vs. hand collection. Damaged seeds repaired themselves during the 21 day moist chilling. Apparently, seeds were not injured severely enough to prevent them from repairing themselves. This natural repair mechanism, induced by moist chilling, has been previously demonstrated in black spruce (*Picea mariana*) by Wang and Berjak (2000). This is another important reason for nursery operators to use moist chilling as a pre-sowing treatment.

## Peak Pollen Release

The Petawawa Research Forest has sporadically maintained a record, since 1950, of the date of pollen shedding for some of the major native tree species. Local origin trees are tracked for their pollen release in the spring and the 'peak' day recorded. Figure 2 shows the pattern of the peak pollen release of black spruce, white spruce, jack pine (*Pinus banksiana*), and red pine (*P. resinosa*). Other species monitored include tamarack (*Larix laricina*) and white pine along with some exotics.

The year to year variation for these species is as much as 31 days with an example of an early year being 1998 and a late year being 1997.



Figure 2. Peak pollen release at Petawawa by Julian day (Sw = white spruce, Sb = black spruce, Pj = jack pine, Pr = red pine).

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# MANITOBA'S TREE IMPROVEMENT PROGRAM

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Keywords: jack pine, black spruce, white spruce, AAC determinations, GIS

Manitoba Conservation – Forestry has established tree improvement programs for the three main coniferous reforestation species in Manitoba: jack pine (*Pinus banksianna* Lamb.), white spruce (*Picea glauca* [Moench] Voss) and black spruce (*Picea mariana* [Mill.] BSP). Breeding zones for these three species are shown in Figs. 1 and 2. The three forest companies holding Forest Management Licences (FML's), Tolko Manitoba, Louisiana-Pacific Canada Ltd. (LP), and Tembec Forest Resources, cooperate in some of these programs within their FML's. A Tree Improvement Trust Account has been set up to allow cost sharing for work completed in these co-operative breeding zones. This report will highlight the major activities completed in the past 2 years.



Figure 1. Location of jack pine and white spruce breeding zones within Manitoba.

## TOLKO MANITOBA – MANITOBA CONSERVATION CO-OPERATIVE PROGRAMS

The first roguing of the Prospector black spruce seedling orchard in the Saskatchewan River Breeding Zone was completed in 2003. In 2003, 7.8 hL of cones were collected at the Prospector orchard in conjunction with crown management. The 10-year height measurements of the three black spruce family tests in the Nelson River Breeding Zone were completed in fall 2002 and spring 2003. Analysis of the height data has been completed and roguing of the Axis Lake seedling seed orchard will be completed in 2004. Maintenance continued on the three family tests and one seedling seed orchard established in the Highrock Breeding Zone in 1997/98.

There was a good white spruce cone crop in 2003 in the Saskatchewan River Breeding Zone with 12.1 hL of cones collected from the Reader Lake clonal orchard.

#### TEMBEC FOREST RESOURCES – MANITOBA CONSERVATION CO-OPERATIVE PROGRAM

The 15-year height, diameter, and form measurements were completed in 2003 on the three black spruce family tests in the Lake Winnipeg East Breeding Zone. The measurement data will be contracted for analysis in the fall of 2004 with the final roguing of the Brightstone seedling orchard to follow. In 2003, 7.0 hL of cones were collected in conjunction with crown management.

#### LP CANADA LTD. – MANITOBA CONSERVATION CO-OPERATIVE PROGRAMS

Maintenance continued on the black spruce family tests and seedling seed orchard that were established in the Mountain Breeding Zone in 2001.

Cone induction treatments using gibberellin injections were completed in the spring of 2002 and 2003 at the Birds Hill white spruce clonal orchard. Cones were collected from the orchard in the fall of 2002 (12.75 hL) and 2003 (8.0 hL). Two family tests were sprayed with Methoxychlor and/or pruned for weevil control in 2002 and 2003.

#### MANITOBA CONSERVATION PROGRAMS

The two black spruce mass selection seed orchards in the Interlake Breeding Zone were rogued for the second time based on data analysis of family performance as well as neighbourhood comparison, spacing, condition, and form.

Maintenance continued at the Northern and Interlake mass selection jack pine orchards as well as the jack pine clonal orchard in the Mountain Breeding Zone. The Southeast Breeding Zone pedigree jack pine orchard at Hillside suffered some minor damage due to a late spring snowfall in 2004 causing the breaking of crowns. Damage was especially evident in older, crown managed trees. A contractor collected cones in the Interlake (9.2 hL) and Hillside (4.7 hL) orchards in 2002.

A white spruce seed production area was established for the Southeast Breeding Zone in 2001–2002.

Over the past several years all tree improvement data have been supplemented with the inclusion of spatial information. All data for the provincial and industry co-operative programs have been entered using the ESRI Arc View Geographic Information System (GIS) program. This allows for such things as the mapping of plus tree locations for either top or bottom ranked families in a breeding zone, mapping and analysis of mortality or disease incidence in tests or orchards, and production of maps for the roguing of seed orchards.

In conjunction with the Manitoba Conservation Forest Inventory and Resource Analysis Section, trials are underway to determine size and frequency of plots to be established in plantations originating from improved seed to determine genetic gain. Plots will be established and measured over a number of years to determine an accurate genetic gain to be applied to wood supply analysis.



Figure 2. Location of Black Spruce Breeding Zones within Manitoba.



Figure 3. A segment of roguing replication data from Prospector Seed Orchard in Breeding Zone 11.5.

# WEYERHAEUSER – TREE IMPROVEMENT IN SASKATCHEWAN

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**Keywords:** seed orchard, progeny tests, cross-pollinating, top grafting

Conifer tree improvement programs in Saskatchewan are managed and funded totally by Weyerhaeuser. The jack pine (*Pinus banksiana*) program was initiated in 1978 and was followed by white spruce (*Picea glauca*) in 1982.

# JACK PINE PROGRAM

A grafted first generation orchard is exceeding all operational seed requirements. A small local market for improved jack pine seed has emerged over the last three years. Seed sales along with reduced cone collections have enabled us to improve management of our seed inventories.

Cross-pollination in ten of twelve breeding sub-lines is near completion. A protocol for "top-grafting" onto the crown of established orchard and breeding bank trees has been successfully developed and has allowed us to relocate a significant portion of our breeding work to the orchard property. Since 2001, a series of full-sib progeny tests has been established annually as seed becomes available from the sub-lines.

The final series of cross-pollinated progeny tests, sub-lines eleven and twelve from orchard trees, has been measured and the data were analyzed by Dr. Dudley Huber. Selections will be made in 2005 for inclusion into a second-generation seed orchard.

# WHITE SPRUCE PROGRAM

A grafted first-generation orchard is now meeting all operational seed requirements with a generous surplus of seed which is available for sale. Crown management was undertaken by removing an additional whorl from the tops of the taller trees. The last top pruning was undertaken in 2001. At that time, the top two to three whorls were removed to control the height, leaving tree height at a mean of four m.

Data are currently being collected from open-pollinated progeny tests. Selections will be grafted for a second-generation seed orchard to be established starting in 2006.

The conservation of genetic diversity in public owned forests is a concern commonly addressed. Dr. Sally John compiled a report outlining a strategy for ensuring maintenance of adequate levels of genetic diversity in forest trees on Saskatchewan's Prince Albert Forest Management Lease to include diversity maintenance in regenerated stands, seed orchard populations, breeding populations, and a plan for long-term conservation.

Weyerhaeuser Saskatchewan will ensure that diversity is maintained by: 1) setting standards for minimum diversity levels in wild seed collections and orchard seed lots, 2) instituting a monitoring program to quantify diversity levels of all future seed orchard crops, 3) increasing the size of the white spruce seed orchard population, and 4) working with the Saskatchewan government to develop an ecologically sound system of *in situ* conservation areas of special interest.

# BLACK SPRUCE PROGRAM

There are no immediate plans for a black spruce tree improvement program. In the meantime, we will continue collecting black spruce seed from wild stands.

# PUBLICATIONS AND REFERENCES

Corriveau, L.S. 2003. Annual tree improvement report. 8 p.

John, S.E.T. 2004. Maintaining genetic diversity on Weyerhaeuser's Prince Albert FMA. 2 p.

# AAFC-PFRA SHELTERBELT CENTRE - TREE IMPROVEMENT SUMMARY

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

The PFRA Shelterbelt Centre has, over the past 80 years, had an active program dedicated to improving tree species for the Canadian prairies. The objective of the program is to develop genetically diverse trees and shrubs that are functional agroforestry plantings. In addition, considerable effort is focussed on improved, clonally propagated Populus hybrids. The Centre also maintains germplasm collections of prairie woody plant genera. Improved trees and shrubs developed in the program are used for farm, field, and roadside shelterbelts, wildlife habitat planting, riparian buffers, phyto-remediation, and afforestation.

## LARIX IMPROVEMENT

Seed representing four species Larix sukaczewii Dyl, Larix sibirica Ledeb., Larix cajanderi Mayr., and Larix gmelinii Rupr. was obtained from the Swedish University of Agriculture Sciences. This seed originated from collections of 1 005 individual Larix trees distributed over 16 regions and 45 stands in Russia. The seeds have been distributed to researchers in Russia, Scandinavia, Japan, and Canada. This collection represents a very wide genetic and geographic distribution of Larix. The Shelterbelt Centre was selected as the Canadian participant in the project. Seed from this project has been propagated and is planted in a block planting and riparian buffer strip. Seedlings have also been made available to the University of Saskatchewan for planting in an afforestation project. The objectives of this project are to evaluate the adaptability of exotic Larix populations to prairie growing conditions, to study genotype-environment interactions of the Larix species, and to provide genetic material for future selection and breeding.

# **POPULUS IMPROVEMENT**

Populus improvement is a key activity at the Shelterbelt Centre. The program focuses on trait improvement related to biomass accumulation, cold hardiness, drought resistance, nutrient uptake, pest resistance, and wood quality. The Centre maintains an extensive clonal library, a breeding arboretum, and 40 clone tests situated across the prairie provinces. In 2003, a new clone, Katepwa, was released for agroforestry applications in the Canadian prairies.

## Breeding Program

The current breeding program was initiated in 2001. The objectives are to develop hardy, fast growing, drought, and pest resistant hybrid poplar suitable for afforestation and agroforestry activities in the prairie provinces. The breeding project is conducted in partnership with Alberta Pacific Forest Industries. Controlled crosses were done to produce inter-specific hybrids of selected Populus deltoides Marsh. var occidentalis Rydb., Populus balsamifera L., Populus maximowiczii A. Henry, Populus nigra L., Populus laurifolia Ledeb., Populus tristis Fisch., and P. x canadensis Moench. Nursery evaluations and field testing will be conducted on control-pollinated (CP) seed lots to identify superior Populus genotypes. Since 2002, a total of 129 CP families have been produced in the program. The seedlings have been established in nursery trials at the Shelterbelt Centre. These plantings total over 15 000 individual genotypes. The plants (ortets) in the nursery trial will be used as a source of clonal copies (ramets) for field trials.

## **Clone Tests**

One of the major areas of research being undertaken at the centre is testing of hybrid *Populus* clones. At this time, over 300 clones are being tested at 40 locations across the prairie provinces. All clone trials are planted in replicated trials. The objective of these tests is to evaluate performance and adaptation of *Populus* clones in afforestation and agroforestry applications. These tests have illustrated the variability in performance of clones and the importance of science based evaluation prior to large scale use of any particular clone.

#### Characterization of *Populus* Cold Hardiness

The ability of *Populus* to withstand winter damage depends mainly on how quickly they become cold hardy in the fall and how resistant they are to losing their hardiness in spring, rather than the ultimate level of cold hardiness attained in winter. We have examined patterns of low temperature acclimation in the fall and late winter or early spring de-acclimation in 21 clones of hybrid Populus commonly used or newly introduced to the prairies, using our newly established cold hardiness testing facility. All clones developed an extremely high degree of low temperature tolerance, but the dates of initiation, rates of development, and dates and patterns of de-acclimation differed strongly among the clones. Three general patterns of acclimation were observed in the fall: a very early initiation (without much exposure to low temperatures) accompanied by an extremely fast rate of acclimation, a later initiation (after exposure to low temperatures) but accompanied by a fast rate of acclimation, and a late initiation (after exposure to low temperatures) but with a slow rate of acclimation. De-acclimation patterns in spring could also be grouped into three general patterns: a very early group which were unable to re-acclimate after exposure to low temperatures, a very late group which de-acclimated only when risks of low temperatures were minimal, and an intermediate group which were able to re-acclimate quickly whenever low temperatures occurred. The patterns of acclimation in fall did not necessarily correspond to the patterns of de-acclimation in spring, e.g., hybrids that acclimated early in fall did not necessarily de-acclimate late in spring. These results indicate that both fall and spring hardiness patterns are important for successful planting of hybrid Populus clones.

## ADAPTATION OF AGROFORESTRY SPECIES TO CLIMATE CHANGE

There is little information on the impacts of climate change on fragmented natural or created ecosystems, such as shelterbelts or other tree-based buffers that are integral components of the Canadian agroecosystem. Tree or shrub species and genotypes that currently exist in these environments will be impacted by climate change, yet we do not know how they will respond or whether there is sufficient genetic diversity within the populations to be able to adapt to the predicted changes. A number of studies are being conducted to address issues of climate change impact on agroforestry species across Canada. Techniques and technologies are being developed to help to rapidly screen populations or genotypes for adaptability to the different aspects of climate change. A detailed examination of species populations in the most vulnerable Canadian agro-ecosystem, the prairies, for their adaptability is focused on *Populus deltoides* Marsh. var occidentalis Rydb., Fraxinus pennsylvanica Marsh. var subintegerrima (Vahl.) Fern., *Quercus macrocarpa* Michx., and *Prunus virginiana* [A. Nels.] Sarg. The four year project addresses issues related to: vulnerability to winter damage, vulnerability to drought, and impacts on nutrition.

# EX SITU CONSERVATION OF WOODY PLANTS

The Centre is involved in evaluation and conservation of woody genetic resources used in agroforestry. This includes sampling native woody plant populations and establishment of *ex situ* conservation nurseries. Collections have been completed for *P. virginiana*, *Cretaegus rotundifolia* Moench, *Shepherdia argentea* Nutt., *Symphoricarpos occidentalis* Hook., *Rosa woodsii* Lindl., *Q. macrocarpa*, *F. pennsylvanica*, *P. deltoides* var. *occidentalis*, *Prunus nigra* Ait., and *Prunus americana* Marsh. A new initiative to expand the collections outside the prairie region is being developed.

# WESTERN BOREAL ASPEN CORPORATION

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Western Boreal Aspen Corporation (WBAC) began as an aspen tree improvement cooperative in 1992. Since then, the program has grown to include poplars and research and development of silviculture techniques for plantation establishment. The focus of our program is to ensure that future hardwood fibre needs can be met. Current members are: Ainsworth Lumber, Daishowa-Marubeni International, Footner Forest Products, and Weyerhaeuser Company. WBAC is a member of the Minnesota Aspen and Larch Genetics Co-operative and also works cooperatively with the Canadian Forest Service and former members. WBAC's mission statement is: to develop genetically improved aspen and to support research towards meeting companies' fibre needs. As a secondary focus, the corporation also supports poplar tree improvement.

WBAC has initiated a comprehensive genetics program for pure native aspen, including provenance trials, a grafted breeding orchard, clonal testing, and associated clonal archives. The group is also conducting a hybrid breeding program involving *Populus tremula* from Europe and *P. davidiana* from eastern Asia. WBAC members have begun testing hybrid poplars developed elsewhere and will initiate a balsam poplar testing and selection program. Tree improvement efforts focus on the testing and selection of superior clones for operational deployment. Initial candidate materials are collected from wild populations, but as progress is made with controlled breeding, candidates for clonal testing will be selected from progeny tests.

The group has initiated basic silviculture research involving density trials, site preparation, and planting stock production using seed, root suckers, greenwood cuttings, and tissue culture, as well as applied research associated with breeding (pollen handling, flowering induction, controlled crossing, seed processing, and seedling production). Establishment of aspen field trials began in 1995. Hardwood trial establishment and ongoing trial monitoring and maintenance are now a routine part of cooperators' in-kind contributions.

Notable achievements in the recent past: 1) development of an effective and economical method of mass vegetative propagation for operational deployment, 2) refinement of flower induction treatments in the potted orchard, 3) an early analysis of a 2001 clonal aspen trial, 4) a presentation titled "Aspen, adaptation, and climate change. Is Alberta aspen adapted to a fossil climate?" at the CIF/SAF October 2004 meeting, and 5) the hosting of a successful tree improvement industry meeting to discuss public education and awareness strategies about the industry in 2003.

Breeding and crossing efforts in 2001 and 2002 were very successful, following several years of difficulty. In 2003, WBAC successfully completed about half of its first series of controlled crosses in the native aspen breeding program. After completion of all the factorial crosses in 2004, seed was sown for progeny trials. One trial will be planted in 2005 and the remaining stock will be used for vegetative propagation to install several clonal-based progeny trials in 2006. Table 1 summarizes the various trials that have been established.

Туре	Number of trials	Number of Treatments	Type of treatments
Clonal tests	17	337	Clones
Exotic and hybrid aspens	25	165	Families
Provenance trial	5	43	Seed lots
Silviculture trials	8	Various	Browse, density, and fertilizer

Table 1. Summary of various trials established by WBAC

# **GENETICS AND TREE IMPROVEMENT PROGRAM, 2002–2004** ALBERTA SUSTAINABLE RESOURCE DEVELOPMENT

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**Keywords:** tree breeding, provenance studies, species testing, seed orchards, clone banks, progeny testing, selection, climate change

This report summarizes the progress of the Public Lands and Forests Division (PLFD) genetics and tree improvement program for the period 2002-2004. A large part of the work was done in collaboration with forest industry, Alberta Agriculture and Rural Development, University of Alberta, North Peace Applied Research Association, Canadian Forest Service, U.S. Forest Service, and British Columbia Ministry of Forests.

#### PROGRAM DEVELOPMENT

The Standards for Tree Improvement in Alberta (STIA) were released in manual form for implementation in 2003. The manual provides a regulatory framework and standards which guide traditional reforestation activities, enables industry to plant genetically improved stock on Crown Land, and directs conservation efforts necessary to preserve the genetic diversity of Alberta's Crown forests. The Forest Genetics Alberta Association (FGAA) was formed with Alberta Sustainable Resource Development as one of the founding members. The objectives of the FGAA include cooperative project development and management for genetics and tree improvement work in Alberta involving the Alberta Tree Improvement and Seed Centre (ATISC) and industry. The Alberta Forest Genetic Resources Council (AFGRC) continued its work. Major initiatives of the council during the report period included review and endorsement of STIA, updating of the policy position on GMOs, technical review of reforestation with non-native species, and sponsorship of a Canada-wide survey of forest genetic resources management practices and policy in cooperation with Ontario and British Columbia.

## GENETIC IMPROVEMENT

## Assembly of Breeding Stock

Geographic and superior parent tree selections, made as part of Sustainable Resource Development (SRD)/Industry cooperative breeding projects, included 23 lodgepole pine (Pinus contorta var.latifolia) and 16 jack pine (Pinus banksiana). Companies participating in these projects include Manning Diversified Forest Products Ltd., Northland Forest Products Ltd., and Tolko Industries Ltd. (High Level Lumber Division).

#### Genetic Testing

Tests of white spruce (*Picea glauca*) and lodgepole pine open-pollinated families from Cypress Hills Provincial Park were established in 2003. The higher elevation portions of the park were untouched during the last glacial advance and it is presumed unique 'outlier' island populations of white spruce and lodgepole pine may have persisted. These populations show promise for selection and breeding for climate change adaptation in northern Alberta. Sixty-four white spruce and 20 lodgepole pine families were established in separate tests on four sites using an alpha design.

Eleven-year assessments of white spruce half-sib open-pollinated (OP) progeny trials for Breeding Region E (northeast boreal lowland) and Breeding Region H (northwest boreal lowland) were completed in 2002. Survival, tree condition, height, leader growth, and white pine weevil incidence were measured or assessed. The test results will form the basis for the first genetic roguings of the seed orchards associated with these breeding regions to be completed in the fall of 2004.

White spruce half-sib progeny trials for Breeding Region D1 (Slave Lake area boreal) were measured in 2003 at 21 years. Survival, tree condition, height, dbh, and incidence of white pine weevil were measured or assessed. A third genetic roguing will be undertaken in the Region D1 seed orchard based on the results.

## Seed Orchards and Clone Banks

Seed Orchards In 2003, the most abundant cone crops to date were produced in the white spruce seed orchards located at the Alberta Tree Improvement and Seed Centre (ATISC). The Breeding Region D1 seedling orchard, comprised of 989 trees representing 89 families and established in 1982/83, produced 250 hl of cones yielding 315 kg of seed. The average number of cones per tree was about 4 600. The trees in the orchard averaged about 7 m in height and all were topped by approximately 2 m to facilitate cone collection. Until 2003, the 1999 crop was the largest. In that year, 142 hl of cones were collected yielding 213 kg of seed. The Breeding Region E white spruce clonal seed orchard, established in 1989, produced 78 hl of cones with a yield of 111 kg of seed in 2003. The orchard contains 1 161 trees representing 94 clones and, on average, each tree produced about 1 300 cones. Trees greater than 3 m in height (approximately 40% of orchard trees) were topped to assist with cone collection and to promote growth of a bushier crown. The most abundant crop in previous years was produced in 1999 when 14 hl of cones were collected yielding 16 kg of seed.

In 2003, the Breeding Region E1 (northeast boreal lowland) white spruce clonal orchard was expanded to accommodate the needs of the regional oil sands industry. The project is currently a cooperative venture between Northland Forest Products Ltd. and SRD. The size of the orchard was increased from 510 to 870 positions. To date, 487 ramets representing 59 parents are established in the orchard. Another 20 parent tree selections from the northern part of the breeding region are planned over the next five years to provide a better geographic balance to the breeding and orchard populations and to produce enough seed to meet oil sands industry requirements.

Forty percent of the trees in the Breeding Region H white spruce clonal orchard, established in 1989 at the Edwand site 15 km north of ATISC, were killed by wildfire in 2002. Complete loss of the orchard was avoided due to regular mowing of the site and pruning of lower branches on the grafts. This maintenance prevented the fire from spreading to the tree crowns. Re-grafting from the clone bank is underway and the orchard will be redesigned and replanted. A more substantial fireguard will be cleared and maintained around the site, located in an over-mature jack pine stand.

The interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) clonal orchard for Breeding Region F1 (southern montane), located at the Crop Diversification Centre at Brooks, was re-designed in 2003 to balance the number of ramets per clone. Eighteen grafts representing 11 clones were removed from the orchard and either discarded or re-established in the clone bank, also located at Brooks. Ten grafts representing 7 clones were removed from the clone bank and re-established in the orchard. Currently, the orchard is comprised of 138 ramets representing 45 clones.

*Clone Banks* Over the report period, 110 new clones were established in ATISC clone banks. The bulk of these represent white spruce and lodgepole pine but other species include black spruce (*Picea mariana*), jack pine, red pine (*Pinus resinosa*), limber pine (*Pinus flexilis*), Scots pine (*Pinus sylvestris*), and tamarack (*Larix laricina*). Two Douglas-fir clones and one western larch (*Larix occidentalis*) clone were established in the clone banks for these species located at the Crop Diversification Centre at Brooks.

# GENETICS AND TREE IMPROVEMENT RESEARCH

#### **Provenance Studies**

Twenty-four- and 27-year assessments were completed on five and two trials, respectively, in the Albertawide white spruce provenance series. This series includes 13 trials established throughout Alberta. Twenty-seven seed sources were evaluated for survival, total height, and dbh.

Statistical and genetic analyses were carried out on 24-year measurements of white spruce provenance trials available from eight test sites. Large differences were found among provenances and test sites for growth traits. On an individual site basis, variation among provenances accounted for 6.3–9.45% of the total variance for height and 4.3–14.0% of the total variance for dbh. Variation among provenances across sites was moderate for height and low for dbh with both traits accounting for 5.5 and 3.5% of the total variance respectively. The provenance X site variance component was significant for both traits and amounted to 50.7 and 85.6% of the provenance variance for height and dbh respectively indicating the importance of the genotype X environment interaction. Height and dbh growth were related to latitude and elevation of seed origin. However, the strength of these relationships differed among test sites. No specific geographic pattern could be established for provenance survival.

Statistical and genetic analyses were also carried out on 15-year measurements of black spruce provenance trials established at seven test sites. For growth traits, differences among provenances were low and for test sites substantially large in comparison to white spruce. On an individual site basis, variation among provenances accounted for 0.9–7.2% of the total variance for height and 1.7–8.5% of the total variance for dbh. Variation among provenances across sites was also low and accounted for 0.9% of the total variance for both height and dbh, with 65.2 and 68.8% of the total variance for height and dbh respectively, being due to variation among test sites. The site X provenance variance component was 59.5 and 96.0% of the provenance variance component indicating the importance of the genotype X environment interaction. Geographic patterns for height were generally weaker than those of white spruce. No geographic patterns were observed for survival.

In 2002, three white spruce 'northern areas' provenance-progeny trials were assessed at 18 years from seed. The 125 families in the test series represent 23 provenances ranging in longitude from  $111.28^{\circ}$  (Fort McMurray) to  $119.78^{\circ}$  (Clear Prairie) and in latitude from  $55.62^{\circ}$  (Saddle Hills) to  $58.62^{\circ}$  (Assumption). Survival, height, dbh, and white pine weevil attack incidence were assessed. Overall survival for sites ranged from 79-89%. For the combined sites ANOVA, variation in height was significant for sites, provenances, and families but site by provenance and site by family interactions were not significant. Individual and family heritabilities were 0.15 and 0.47, respectively. Differences in survival were significant for sites only. White pine weevil incidence across sites ranged from 4-28%. For the site with the highest weevil incidence, individual and family heritabilities were 0.17 and 0.43, respectively. The quadratic regression of weevil incidence on longitude of seed source was significant ( $r^2=0.45$ ) with incidence of attack increasing with longitude.

Climate Change and Genetic Variation of Forest Tree Species Populations

Based on 1961-1990 climate normals, a climate model for Alberta has been developed using the method of thin-plate splining. By this method, the data from fixed-point climate stations are transformed into continuous surfaces allowing the estimation of climatic variables at any point in Alberta. As a result, questions relating to climate-tree relationships at experimental sites and sources at considerable distances from climatic stations can be addressed.

An archive containing monthly mean daily temperature, monthly mean daily maximum temperature, monthly mean daily minimum temperature, and monthly mean precipitation data has been produced. Depending upon the variable, between 1 260 to 1 433 climatic stations are represented. The splining technique was applied to these primary variables. In addition, 13 variables considered important in the distribution and response of trees to climate were derived from the primary four. The derived variables include mean temperature of the coldest month, degree-days above 5°C, and annual moisture index.

Using the model to estimate the climate at provenance origins and at experimental sites, response and climate transfer functions are being developed with provenance trial data to evaluate growth and survival responses of species and seed sources to climate and climate transfer.

With the climate model as a base, future climate was estimated from global climate models (GCM). Estimates for changes in the four primary variables were obtained from the Canadian Climate Centre

Model 2 and the Hadley Centre Model 3. The changes estimated by these GCMs were added to the current values for Alberta to produce estimated values for the decades starting in 2030, 2060 and 2090. The thin-plate splining technique was applied to these data. Five derived variables were also calculated including: mean annual temperature, mean annual precipitation, mean temperature of the coldest month, number of degree-days > 5°C and annual moisture index. Provenance trial data is being used to explore the implications of predicted climate change on conifer species and populations.

# TREE GENE RESOURCES CONSERVATION

Work continued on developing a forest tree gene conservation plan for Alberta in cooperation with Alberta Parks and Protected Areas. A list of tree species native to Alberta was finalised. It recognises 29 native tree species of which five species (western red cedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), western larch (*Larix occidentalis*), western white pine (*Pinus monticlola*), and Pacific yew (*Taxus brevifolia*) are designated to be rare species. Two species, limber pine (*Pinus flexilis*) and white bark pine (*Pinus albicaulis*), have been recognised as "*watch list*" species because of special concern regarding declining populations due to an increasing threat from white pine blister rust. The plan would address conservation of representative wild populations of all native tree species in natural subregions and seed zones based on gap analysis and risk assessment. With the implementation of STIA, gene conservation has become an integral part of forest management planning. A procedural framework for implementing these in a forest management plan for southern Alberta was developed and is to be implemented as a pilot study.

Conservation activities consisted of special seed collections of limber pine from seven populations in cooperation with the Canadian Forest Service. Several ecologically significant populations of jack-lodgepole hybrid pine were collected in cooperation with Alberta Parks and Protected Areas.

## PLANT PROPAGATION, WOOD, SEED AND POLLEN TECHNOLOGY

## Plant Propagation

In 2003 and 2004, stock production at ATISC included approximately 89 700 seedlings. This includes 30 900 for establishment of genetic trials, the major project being the rearing of 28 000 Siberian stone pine (*Pinus sibirica*) seedlings for a research study with the BC Ministry of Forests (BCMoF) to test for species introduction, adaptability, and resistance to white pine blister rust (*Cronartium ribicola*) in Alberta and BC Under the conservation program, 51 800 seedlings and small potted stock were reared for projects including promotional trees for National Forestry Week, Arbour Day, and Junior Forest Wardens, white spruce and lodgepole pine reforestation and reclamation plantings in Cypress Hills Provincial Park, and establishment of wild fire resistant plantings of paper birch (*Betula papyrifera*) and tamarack for the community of Fort McMurray. Rootstock production for the grafting program was approximately 7 000 seedlings.

Graft production consisted of 2 673 grafts for establishment in seed orchards and clone banks. This included 1 290 white spruce, 932 lodgepole pine, 102 black spruce, 254 jack pine, 78 interior Douglas-fir, 2 western larch, and 15 red pine.

## Wood Technology

Samples from a total of 115 parent trees were received for fibre length and wood density determination including: 30 jack pine, 83 lodgepole pine and 2 white spruce.

#### Seed Technology

Over the report period, 665 seed lots were added to the genetics seed bank. The bulk of these (316) were the Siberian stone pine seed lots provided by BCMoF. A total of 92 seed lots were gene conservation collections of limber pine (41), alpine larch (*Larix Iyallii*) (25), Rocky Mountain alpine fir (*Abies bifolia*) (21), and white bark pine (5). The majority of the remaining seed lots were single-tree collections from selected parent trees included in the various SRD/industry tree improvement programs. The major species were lodgepole pine (92), jack pine (33), and white spruce (89). Other species seed lots added to the seed bank included Ponderosa pine (*Pinus ponderosa*) (18), Scots pine (11), black spruce (4), western larch

(4), Siberian larch *(Larix sibirica)* (2), tamarack (2), Colorado blue spruce *(Picea pungens)* (1), and Douglas-fir (1). To date, the seed bank contains 5 481 seed lots.

Reference seed lots, representing 2% of the seed bank entries, have been monitored for 23 years. Average germination of lodgepole pine seed lots has remained stable. Initial average germination for the species in 1981 was 88% and in 2003, germination was 87%. Germination of white spruce seed lots has declined slightly from an initial mean germination of 92% in 1981 to 86% in 2004. Mean germination of aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), and plains cottonwood (*Populus deltoides*) has declined from 34–50% after 12 years of testing.

#### Pollen Technology

Five clonal white spruce pollen collections were completed in the Breeding Region E orchard and viability tests (electrical conductivity and germination) were completed. These collections represent the top five families for 11-year height as assessed in 2002 in the half-sib OP progeny trials.

#### REFORESTATION SEED PROGRAM

The Reforestation Seed Program is responsible for the registration, storage, distribution, and tracking of tree seed used for public land reforestation. At the end of the reporting period, there were 18 tree species represented in seed storage by 1 690 individual lots for a total of 41 900 kilograms of seed. During the reporting period, 2 009 kg of seed were withdrawn for seedling production, direct seeding, research projects, and quality testing and 216 new seed lots were collected and registered for public land use. An emergency generator was added to the seed storage facility to further safeguard against temperature fluctuations due to power failure.

The Standards for Tree Improvement in Alberta, implemented on May 1, 2003, cover regulations that deal with the collection, processing, testing, registration, storage, withdrawal, transportation, and deployment of all tree seed for public land use. The seed movement guideline requiring that wild seed be deployed within 80 km and ±150 m of its origin has been replaced with a seed zone system. There are presently 84 seed zones based on geographic subdivisions of the Natural Regions and subregions of Alberta.

# ISABELLA POINT FORESTRY LTD.: ACTIVITIES 2002–2004

# S.E.T. John and J.S. Brouard

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**Keywords:** breeding, seed orchard, progeny, provenance, experimental design

Isabella Point Forestry Ltd. is a small consulting firm providing technical expertise in forest genetics. During the period 2002–2004 we focused on a range of activities in Alberta, British Columbia (BC) and Saskatchewan. Activities included program planning, trial design and data analysis, and seed orchard research for tree breeding programs in Alberta and Saskatchewan, and investigation of the interaction of silviculture and disease with genetics in north-central BC.

## ALBERTA AND SASKATCHEWAN

#### Program Planning and Implementation

Working with individual companies and industrial cooperatives, we provide technical direction for tree breeding programs in lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.), jack pine (*P. banksiansa* Lamb.), white spruce (*Picea glauca* [Moench] Voss), black spruce (*P. mariana* [Mill.] BSP), aspen (*Populus tremuloides* Michx.), and poplar (*Populus* spp.) hybrids. Our role includes preparation and updating of detailed breeding and work plans; parent selection; orchard design; test design, layout, and data analysis; and seed orchard operational research on topics such as flower stimulation, phenology, selfing, and crown management. In hardwoods, our work also focuses on reproductive biology, vegetative propagation, and silviculture. We focus particularly on sound and flexible strategy development, ensuring a broad and appropriate program genetic base, and error control through appropriate trial design and layout.

Sally John has represented a group of forest companies as councillor on the Alberta Forest Genetic Resources Council since its inception in April 2000.

#### BRITISH COLUMBIA

## Cross-disciplinary Research

A small project, initiated by industry through the Babine Enhanced Forest Management Pilot Project (EFMPP), and continued with funding provided by the Morice and Lakes Innovative Forest Practices Agreement (IFPA), the Forest Investment Account (FIA), and Forestry Innovation Investment Ltd. (FII), considers some possible interactions of genetics with silviculture and disease.

*Does Genotypic Rank Depend on Inter-tree Spacing?* This component investigates the interaction between tree espacement and genotype as it affects growth performance. If ranking of genotypes in genetics trials was found to depend on inter-tree spacing, this could influence optimum trial design. A trial involving six families of contrasting crown architecture was established in 2001 near Burns Lake, BC, using a Marynen (or plaid) factorial design, with inter-tree spacing ranging from 0.6 to 2.4 m. Preliminary results are expected by 2006.

Does Resistance to Comandra Rust Have a Genetic Basis? Through earlier assessments of a 14–15-yearold lodgepole pine (*P. contorta* Dougl.) Ministry of Forests progeny test site for comandra rust (*Cronartium comandrae* Peck.) infection, we found that variation in susceptibility to this rust has a highly significant genetic basis, and that infection rates by family ranged from 0 to 69% in the study population. Latitude, longitude, and elevation did not explain variation in resistance. However, cluster analysis succeeded in partitioning the study population into nine geographical groups in such a way that 32% of the variation in infection is attributable to the grouping. Data and results from this study have been used by BC Ministry of Forests genetics staff to identify several provenances as resistant to comandra rust.

A series of trials established on five sites in the Bulkley Valley in spring 2004 is expected to provide precise estimates of rust resistance of families produced in the Bulkley Valley seed orchard. This is expected to facilitate targeted deployment of rust-resistant stock on high-risk sites.

# FOREST GENETICS AT THE FACULTY OF FORESTRY, THE UNIVERSITY OF BRITISH COLUMBIA, 2002–2004

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# GENE CONSERVATION, ECOLOGICAL GENETICS AND CLIMATE CHANGE S.N. Aitken

In the Centre for Forest Gene Conservation, we are developing in situ and ex situ gene conservation strategies, evaluating the degree of current protection, and characterizing the genetic structure and evolutionary ecology of forest trees. Funding for most of this research comes from the Forestry Investment Account of BC through the Forest Genetics Council. Research Associate Andreas Hamann has been analyzing the extent to which B.C.'s current protected areas sustain adequate populations to maintain natural genetic variation using Geographic Information Systems and spatial analysis. Washington Gapare completed his PhD research in 2003 characterizing micro- and macro-spatial patterns of variation from core and peripheral populations of Sitka spruce (Picea sitchensis). He genotyped large numbers of individuals from eight populations for eight expressed sequence tagged polymorphisms to determine optimum strategies for ex situ sampling. PhD student Andy Bower is investigating adaptive trait variation for the threatened species whitebark pine (*Pinus albicaulis*) in a range-wide common garden experiment. He is also assessing the relationship between inbreeding and infection by the introduced fungus causing white pine blister rust. The proposed theoretical tradeoff between gene flow and adaptation in peripheral populations is being explored empirically by PhD student Makiko Mimura, using Sitka spruce as a model species. She has conducted field and growth chamber common garden experiments to quantify adaptation to local climate and is estimating gene flow using microsatellite markers and two-generation analysis. Not all of the focus is on conifers. PhD student Mohammed Iddrisu (co-supervised by K. Ritland) is investigating the mating system and effect of habitat fragmentation on bigleaf maple (Acer macrophyllum). Summer student Karolyn Keir has been successfully adapting microsatellite primers developed for Cornus florida for use with Pacific dogwood (C. nuttallii). We plan on using these markers for a project on mating system and genetic structure.

A strategic grant was obtained (by S.N. Aitken and A.D. Yanchuk, BC Ministry of Forests [MoF]) in 2003, funded jointly by Natural Sciences and Engineering Research Council and the BIOCAP Canada Foundation, entitled "Adapting forest gene resource management to climate change". Research Associate Tongli Wang has been predicting future productivity of lodgepole pine (*Pinus contorta*) populations under global warming scenarios and predicting the optimal redeployment of orchard seed under these scenarios using data from lodgepole pine provenance trials established by Keith Illingworth. Postdoctoral Fellow Pia Smets is characterizing the norms of reaction of seedlings from a number of these provenances under different temperature and carbon dioxide regimes. In a related project, Andreas Hamann is predicting the future distribution of current "climatic envelopes" for tree species and ecological zones under different climate change scenarios.

A new area of research initiated in 2004 is a project on the ecological genomics of cold adaptation. This association study, to be undertaken partially by MSc student Jason Holliday, will investigate nucleotide sequence variation and expression in candidate genes for cold hardiness-related traits in populations of Sitka spruce.

## APPLIED FOREST GENETICS AND BIOTECHNOLOGY Y.A. EI-Kassaby

During the past two years I have been involved in a wide array of research projects nationally and internationally. Nationally, the research topics focused on molecular analyses and modeling of various components of the forest tree domestication process, while others have highlighted forest gene resources management and conservation applications for commercially and ecologically important species, including the potential impacts of climate change. This work was conducted in collaboration with scientists from the

BC MoF and Canadian Forest Service. Two graduate students completed their M.Sc. thesis projects: "Genetic diversity and population structure of the potential biocontrol agent, *Valdensinia heterodoxa*, and its host *Gaultheria shallon* (salal)" (Jennifer Wilkin) and "Genetic structure and mating system in arbutus (*Arbutus menziesii* Pursh)" (Jaclyn Beland). As a part of the forest genetics team in the Faculty of Forestry, I participated in developing the "Forest Genetics" chapter to be published in the revised version of the Forestry Handbook, a widely-distributed resource published by the UBC Forestry Undergraduate Society. Internationally, as a member of the Panel of Experts on Forest Gene Resources of the Food and Agriculture Organization of the United Nation, I drafted a proposal regarding a global survey of the status of forest biotechnology and along with a group of international forest geneticists we completed a study on the "Criteria and indicators for sustainable forest management: assessment and monitoring of genetic variation". Additionally, issues related to seed orchard genetics are being investigated with the Tree Breeding Division, Forest Genetic Resources Department, Korea Forest Research Institute.

#### POPULATION GENETICS AND GENOMICS OF BC CONIFERS K. Ritland

My major activity has been a co-principle investigator (with three others at UBC) on the Genome British Columbia Forestry project, which spans four years (April 2001–March 2005). We are developing genomics tools (ESTs, microarrays) for spruce and poplar and applying these tools to understand the genomic basis of traits relating to forest health and wood quality. Several of my students have been involved in other forestry related projects, including Lisa O'Connell (PhD 2003, "Evolution of inbreeding in western redcedar"), Yanik Burube (MSc 2003, "Microsatellite genetic structure of yellow cedar"), Hugh Wellman (MSc 2004, "Molecular breeding in western hemlock"), Mohammad Iddrisu (PhD ongoing, "Mating system and genetic structure of bigleaf maple"), Cherdsiak Liewlaksaneeyanawin (PhD ongoing, "Evolutionary genomics of lodgepole pine"), and Dilara Ally (Ph.D. ongoing, "Evolution of clonality in aspen"). I also participated in developing the "Forest Genetics" chapter of the revised Forestry Handbook (see El-Kassaby's section).

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# TREE SEED ACTIVITIES AT THE BCMOF TREE SEED CENTRE

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**Keywords:** cone and seed processing, stratification, gene conservation, seed testing, extension

A primary focus of my work over the past two years has been in the publication entitled "Seed Handling Guidebook" which emphasizes the Seed Handling System as a chain of events (custody) from cone collection to the sowing of seed in the nursery. The guidebook is included under references and copies are available free of charge from myself. I served as co-chair of the Canadian Tree Improvement Association (CTIA) meeting in Kelowna and have served on a variety of advisory committees. Extension efforts have focused on strengthening the Tree Seed Working Group News Bulletin and performing extension to various groups within the seed handling system. Research has focused primarily on improving the quality of our products through operational Quality Assurance (QA) monitoring.

The advancement of the concept of the Seed Handling System has been my primary focus for the past two years. This has been done by extending the concept at a variety of meetings and continuing to quantify our operational experiences with British Columbia (BC) species. Seed has been the focus, but understanding the interactions with insects and fungi are very important to increasing overall seed efficiency (from production to sowing). We continue to perform fungal assays on high priority species and continue to co-operate with the Pest Management group within Tree Improvement Branch.

# SEED PRODUCTION

Priorities for operational improvement have focused on western white pine (*Pinus monticola*) due to significant differences between germination test results on lab samples and operational quantities of seed. Results are quite promising and result from the following changes: extending cold stratification to 17 weeks, targeting moisture content to 37% (34 to 40% acceptable operational range), reducing maximum stratification unit to 750 g (vs. 3 000 g for other species), using larger bags to improve aeration, and monitoring and mixing seed within a bag on Monday, Wednesday, and Friday. The target moisture content concept (non-destructive) has been extended to our QA monitoring of all species at time of shipping and to the dryback procedure in use for Amabilis fir (*Abies amabilis*), subalpine fir (*A. lasiocarpa*), and Noble fir (*A. procera*) seed lots.

Our QA program continues to perform germination tests at time of shipping (approximately 200 requests). Requests are made to nurseries for germination and comparisons made between germination at the nursery, operational seed preparation at shipping, and laboratory testing. This program helps to identify priority areas in need of further investigation. An interesting observation was a comparison in stratification moisture content between wild and seed orchard produced crops, specifically in spruce. For both Sitka (*Picea sitchensis*) and interior spruce (*P. engelmannii x glauca*), a lower stratification moisture content of approximately 5% was noted for seed orchard produced crops. Fortunately no decrease in germination was noted, but I think it is important to track the differences we see in seeds obtained from these two sources. Monitoring will continue.

# TESTING

During this time period a new Retest schedule was introduced. This was based on an analysis of species deterioration rates (obtained by averaging individual seed lot deterioration rates [(Current Germination Capacity – Initial Germination Capacity) / time interval]. Other factors including sample size, degree of change in estimate since 1997, number of seed lots per species, and age of seed lots also played a role in the retest frequency entered into our local database program (CONSEP). The intent is to update these

estimates at 5-year intervals and future emphasis will be moving from the species level to identification of seed lots showing greater than average deterioration.

The Tree Seed Centre has been very active during this period with large improvements to the process control of many testing functions. I take very little credit for this, but it is noteworthy.

## GENERAL

I have been involved in a review of QA practices at the Tree Seed Centre. It is intended to review current practices, identify excesses and gaps in our efforts, and try to fill in gaps in our understanding of conifer tree seed as part of our stewardship mandate. Priority areas for Cone and Seed Processing (CSP) include improving the standardization of cone and seed evaluation methods (i.e., cutting tests) and quantifying variability in extraction efficiency by species. Inventory Management (IM) priorities are aimed at a continuation of existing sowing request QA activities including germination and pellet content assessment of western red cedar. An additional priority is the handling, testing, and re-distribution of returned seed not sown at nurseries. Emphasis for this program is on seed orchard seed lots and especially where deficits exist (i.e., lodgepole pine (*P. contorta*)). Testing (TST) priorities include the introduction of seed weight tolerances into our information system and no longer allowing germination test results to be based on three replicates (formerly accepted by the Association of Official Seed Analysts).

My commitment to various committees and organizations has been large during the past two years: 1) Co-Chair CTIA 2004 Meeting in Kelowna (and dealing with all CTIA specific issues), 2) Chair of CTIA Tree Seed Working Group involved co-ordinating of a workshop at 2004 CTIA and spearheading the News Bulletin with Dale Simpson, 3) various Forest Genetic Council Technical Advisory Committees: Coastal, Interior, Gene Conservation, Pest Management, proposal review committees, and various species committees, 4) technical review committee for new Chief Forester's Standards for Seed Use, and 5) member of International Seed Testing Association's Forest Tree Seed and Shrub Committee.

It has been an eventful and exciting two year period. I'm hoping I have more of a research component to report for the next proceedings.

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# BRITISH COLUMBIA MINISTRY OF FORESTS FOREST GENETICS RESEARCH AND TREE BREEDING PROGRAM

# Mike Carlson, Charlie Cartwright, Barry Jaquish, John King, Sylvia L'Hirondelle, Greg O'Neill, John Russell, Michael Stoehr, Chang-Yi Xie, Alvin Yanchuk

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# **Keywords:** tree breeding, insect resistance, disease resistance, adaptation, climate change, wood quality, genecology, somatic embryogenesis

The British Columbia Ministry of Forests' (MoF) forest genetics research program still maintains a strong focus on forest genetic resource management research, policy, and tree breeding. The significant downsizing in the MoF appears to be over and we have been able to maintain a relatively strong program comprised of 32 personnel (research scientists, technicians, station staff, and administrators) at headquarters in Victoria and at our two research stations, one coastal and the other in the interior. The breeding and research program is now largely funded through 'external' funds (i.e., non-MoF vote) and planned with the assistance of the Forest Genetics Council (FGC) through the FGC business planning process. Plans are now developed annually by the tree breeders in conjunction with industry clients and focus on 10 commercial species in British Columbia (BC) in over 40 separate research and development programs. Some of these programs now also include work on hardwoods and true fir species. Research is still being carried out in the areas of insect, disease, and deer resistance, climate change, and wood quality. Work on gene conservation is largely being undertaken with an FGC sub-program on gene conservation with the Centre for Forest Gene Conservation at The University of British Columbia.

Dr. Cheng C. Ying, our well-known provenance forester and senior scientist at Research Branch, retired in 2003. For more than 20 years Cheng oversaw the enormous provenance program. Over the last few years, he initiated the genecology study of hardwoods in coastal BC and created the basis for the genetic improvement of these species. Cheng still maintains activity in the Research Branch, under the Emeritus Scientist program.

Dr. Joe Webber, our flowering physiologist at the Glyn Road Research Laboratory, Research Branch, also retired after 30 years. Joe, as many of you know, worked on many aspects of seed orchard biology, flowering enhancement, and pollen and seed orchard management. Joe is now active as a consultant and living on Saltspring Island.

# TREE BREEDING

#### Coastal Douglas-fir (Pseudotsuga menziesii [Mirb] Franco var. menziesii)

*Low-elevation Coastal Douglas-fir* Controlled pollinations for advanced-generation breeding for general combining ability (GCA) and full-sib testing progressed well, with over 200 crosses completed in 2004. Series 1 subline tests, planted in 1999 for third-generation seed orchard selections, were maintained and measured (height at 5 years). Series 2 subline tests (planted in 2003), also containing eight sublines, were weeded and fertilized. Realized gain trials, planted in 1996, were brushed.

*High-elevation Douglas-fir* The high-elevation Canfor Douglas-fir seed orchard (> 700 m), at Sechelt, is being progeny tested at two test sites on Northern Vancouver Island (Mt. Cain and Sutton Creek) and age 5 heights were used to rogue from below (20%). Breeding values, calculated based on year 7 heights measured in 2004, will also be used for additional roguing, with final selections to be made based on age 12 measurements. Breeding values, adjusted for selection at age 7 and forecast to rotation age 60 for the

top 30 clones, range from 2 to 10, based on best linear projection (BLP) calculations. As the age-age correlation increases, these values will increase correspondingly.

*Sub-Maritime (Transition Zone) Douglas-fir* The newly established clonal seed orchard 181 at Puckle Road (Saanich) will be progeny tested using a polycross (GCA) test. For this purpose, all clones in the orchard were control-pollinated in the spring of 2003 using a polymix made of submaritime pollen (and some high-elevation coastal pollen). Of 94 parent trees in the orchard, seed was obtained from 91 and sowed for the production of test stock at Cowichan Lake Research Station. Progeny tests will be established in the spring of 2005 at three sites in the transition zone at elevations above 800 m.

#### Interior Douglas-fir (*Pseudotsuga menziesii* var. glauca [Beissn.] Franco)

Controlled crossing in the Interior Douglas-fir program is focusing on the low-elevation Nelson seedplanning unit (SPU). This new zone is largely the result of merging the old Shuswap-Adams, Mica, and West Kootenay low-elevation zones. In 2003, a total of 171 crosses were completed in the Nelson zone and 188 were completed in lower priority zones. Approximately 200 pollen lots were collected, processed, and stored for future breeding.

Five, 15-year-old progeny test sites and two 20-year-old sites in the old Mica and Cariboo Transition zones, respectively, were maintained and measured. Data analyses for these sites are on-going. Determination of wood relative density was completed for the Mica Revelstoke Dam. Three, 10-year-old research sites from the Douglas-fir elevation transect study were maintained and measured.

The pilot *Armillaria* root disease resistance-screening project is now three years old and is near completion. This joint Canadian Forest Service (CFS)/MoF project uses artificial inoculation to screen 88 open-pollinated families of Interior Douglas-fir. To date, family mortality attributed to *Armillaria* ranges from 0 to 82%, so the results are extremely encouraging.

#### Interior Spruce (*Picea glauca x engelmannii*)

Interior spruce tree improvement began in the mid-1960s and has progressed to the point where much of the current spruce planting stock (over 75 million seedlings per year) originates from rogued first-generation seed orchards. Second-generation full-sib progeny tests are also in place for three seed planning units (SPUs). Over the years, refinement of interior spruce SPUs continued as relevant new information became available from genetic tests. Today, six large SPUs are in place, and selection, breeding, and testing activities are on-going in each unit.

Thirty-three (30 Thompson Okanagan and three Prince George) pollen lots were collected, processed, and stored for future crossing. Thirty-five crosses were completed in the new expanded Prince George (PG) zone. Controlled crossing for the PG zone is now 98% complete and we look to establish new second-generation progeny tests in 2006. Six, 20-year-old progeny test sites in the old Quesnel Lake, Finlay, and Bulkley Valley (BV) seed zones were maintained and measured, and four, five-year-old sites in the Fort Nelson zone were maintained. Eight, second-generation sites in the PG, BV, and East Kootenay SPUs were maintained.

The Shuswap Adams polycross test at the Skimikin Seed Orchard, which was augmented with cagereared leader weevils in fall 2002, was evaluated for leader damage. When evaluation was completed, the site was clear-felled and wood disks were collected from each family to determine the relative density of the wood. In June 2003, spruce leaders containing weevil eggs were collected near Vernon and more than 10 000 weevils were reared in cages at CFS's Pacific Forestry Centre, Victoria. In early fall, adult weevils were released in the Thompson Okanagan Series I polycross test at Skimikin. Plantation damage assessment will commence in summer 2004.

Three spruce provenance tests (Quick, Verdun, and Bowes Ck.), that were established in the mid-1960s, were maintained, pruned, and relabelled. These tests represent the oldest interior spruce plantings with genetic structure in BC. Two, 10-year-old realized gain tests, in the old Hudson Hope zone, were maintained and measured. In these tests, the superior seed lots were 17% taller and had 15% greater survival than the composite wild stand seed lot. Six field plantings of somatic emblings were maintained and measured.

A new genecology study was established in the new PG SPU to: 1) further refine seed zone boundaries and seed transfer rules, 2) characterize growth and physiological differences between PG class A and wild stand seed lots, and 3) confirm weighting coefficients used for estimating breeding value. One raised bed site was established for intensive study at the Kalamalka Forestry Centre and three wild sites were established in the Prince George area.

#### Western Larch (Larix occidentalis Nutt)

This program was initiated in 1987 and has advanced very rapidly. To date, 609 parent trees have been selected and 607 wind-pollinated families have been established in progeny tests across 14 sites. The oldest of these progeny tests is 13 years old. Two grafted seed orchards were established in 1990 and are now in full production. Presently, over 70% of the 6.5 million western larch seedlings planted annually in BC originate from seed orchards. Second-generation controlled crossing is over 40% complete and three realized gain field tests will be planted in spring 2004. In 2003, 82 controlled crosses were completed and about 160 pollen lots were collected, processed, and stored for future crossing. One progeny test site (EK Semlin Ck.) was thinned and wood disks were collected for relative density determination.

## Western Redcedar (Thuja plicata Donn ex. D. Don)

Breeding for first-generation polycross testing, involving just under 1 000 parents for the Maritime Low SPU, is complete. To date, six annual series of tests have been established totaling 39 sites. The last series will be established in spring 2005. Breeding values for volume at rotation are currently available for series 1 based on seven-year heights (150 parents), and series 2 and 3 based on five-year heights (248 parents). Approximately 50 parents from the first three series have been selected for advanced generation and established into breeding orchards. Information on the durability of the parents in the first three series is currently being developed. Sixty within-family selections for high needle monoterpene concentration/deer resistance were made at the Holt Creek (southern Vancouver Island) family/population study. These selections will be bred using a combination of selfing and outcrossing, and resultant progeny will be screened for monoterpene concentration and deer resistance. Both backward and forward selections can then be incorporated into a deer-resistant production population.

# Yellow-cedar (*Chamaecyparis nootkatensis* [D. Don] Spach)

First-generation cloned progeny tests have been established and the focus is currently on maintenance and measurements. The first two series of trials were measured at five years in the field and clonal values from 3 000 clones were released. The third series will be measured in 2005. Advanced generation selections from the Western Forest Products clonal program and from MoF genetic trials have been established in three breeding orchards, including one high-elevation site. Maintenance continued in 2004 in these orchards, as well as monitoring of the performance and production of pollen for collection for advanced generation breeding.

Various technical support projects were maintained and measured. These include clonal maturation, clonal competition, pollen viability, and deer resistance studies. Provenance trials were also maintained. Analyses of 10-year data from interior population test sites revealed responses similar to those on coastal sites; significant population variation for growth with no discernible adaptive responses. Survival was high, with little evidence of cold-related damage for all populations except northern California. Breeding continued for a deer resistance heritability study.

#### Western Hemlock (*Tsuga heterophylla* [Raf.] Sarg.)

Low-Elevation Maritime The primary focus of the program at this point is advanced generation breeding. Data for height at age five have been collected from 11 trials and preliminary analysis has been performed. A full-scale formal report is awaiting further results, that are due in fall 2004, from nine trials established by American co-operators. Over 100 forward selections have been made and propagated but are being used for studies of methodologies for breeding juvenile material while confirmation from the later round of testing is awaited. Early results suggest that, to a large degree, our whole co-operative can be treated as one breeding zone with some Oregon parents performing well even on harsher test sites.

A secondary aspect of the hemlock forest genetics program involves provenance studies. Although tested parents from the breeding program are generally deployed in areas where they have been tested, smaller

trials with a wider representation of plants from across the species' range are valuable for establishing the limits of safe transfer. As well, areas not warranting investment in a breeding program still need rules for transferring wild stand collected seed. Height at age five data were collected from three sites in the autumn of 2003 and a further five sites will be measured in 2004. As well, age ten results from eight tests were reported at a Western Forest Genetics Association meeting in Whistler, BC in July 2003.

At the same meeting, research was reported supporting hemlock's resistance to dwarf mistletoe. Trees from ten provenances were infected with the parasitic plant. No significant variation by provenance or family was noted, although one provenance clearly had half the rate of successful attack of the others.

Similarly, there was a wide range of response by family, but analysis suggests that these results might not be repeatable, probably because too few replications were used to detect the effect and so it could not be confirmed. The inoculations and scoring of the progress of infection was in the hands of forest health specialist Dr. Simon Shamoun of the CFS and two of his graduate students, Sue Askew and Lea Reitman.

*High-Elevation Maritime Hemlock* The high-elevation breeding program covers maritime hemlock regeneration from 600 m elevation to the transition to mountain hemlock. A last round of first-generation testing has just been sown which will bring the number of high-elevation selections tested to about 325. Ten-year-old data for height and diameters are expected for 91 parents with revised breeding values for these to follow by April 2004. Projected volume gains of selected available seed lots should rise from around 6% currently to over 10% with these results.

Second-Generation Family Testing of Lodgepole Pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.)

The intercrossing of tested/selected first-generation lodgepole pine parent trees for second-generation family testing is complete for all five SPUs (low-elevations PG, BV, TO, NE, and CP). Factorial crossing sets comprise subpopulations bred for stem volume growth rate and wood relative density for each SPU (130 full-sib families per SPU). In 2002, the PG test series was planted. In 2004, the BV series will be planted, and the TO and NE series will be sown/grown for planting in 2005. The last test series, the CP, will be planted in 2006.

In addition to the BV SPU test series grown, lifted, and packaged in 2003, research trial planting stock for three trials was grown, lifted, and packaged for planting in 2004. These included the Comandra Rust Screening Trial for the Vernon Seed Orchard Company and two provenance screening trials, one each by Weyerhauser Co. and West Fraser Ltd. The fall 2003 lift and tagging for these four trials set a record for the pine team in that 83 000 test seedlings were lifted, of which 63 000 were individually tagged. A job well done by Future Forest Services Ltd. of Vernon.

Lodgepole Pine "Forward " Selections for Expanding Seed Orchard Production

The planting of lodgepole pine throughout the BC interior has greatly increased beginning in the early 1990s. Today, 90 million seedlings are planted annually. Our first-generation tested seed orchards were designed to meet earlier seedling needs. Regrafting to expand orchard seed production capacity began in 1996. Since then, more than 60 000 grafts have been made and several new orchards have been planted to meet approximately two-thirds of the total seed needs. The majority of parents grafted in this expansion effort have come from first-generation progeny test family arrays aged 15-18 years from seed, so called "forward " selections. These superior trees were identified using a two-step process. First, Dr. Chang-Yi Xie from the MoF Research Branch in Victoria took the ten-year measurement data sets and developed a selection index score for every tree in each particular test series (30 000 trees per test series). Each tree's final score or BV depends on the relative superiority of its family compared to other test families and its own superiority within the family (compared to its sibs). Site means, block means, and plot means are also estimated and are used to adjust individual tree scores (BVs). The analysis product is a listing of the top 500 BV-ranked trees across the sites in a particular test series. The second step was to visit one or more sites with the ranking list and examine trees in descending rank order. Tree height at age ten years is only one criterion used for tree selection. Height and diameter growth rates sometimes change with competitive interactions after age ten. Also, stem form and branching habits have significant impacts on log/wood values and can be assessed at ages 15-18 years. So, in addition to current tree height, stem diameter and form, branching characteristics including branch numbers, diameters, lengths, and stem growth cyclicity (monocyclic vs. polycyclic internodes) are all considered in selecting seed orchard parent trees. With this, the final year of forward selection, over 400 progeny test trees will have been selected and

grafted (60–80 ramets each). Overall organization and direction of seed orchard expansion projects has been the responsibility of Select Seed Company (a corporation owned by the BC Forest Genetics Society, and reporting to the Forest Genetics Council).

## Sitka Spruce (*Picea sitchensis* [Bong.] Carriere)

Activities in 2004 included establishment of the first phase of the F1 breeding program. Parents were designated by their resistance to the spruce weevil (*Pissodes strobi*) and their source (mainly Haney or Big Qualicum). Some susceptible parents were also used for inheritance studies. The trials have been established in the Adam's River drainage, North Vancouver Island and at Jordan River, South Vancouver Island. We have another site that we hope to establish on the Queen Charlotte Islands in spring 2005.

Breeding to establish future series is continuing. The F1s will replace the clones and families in our research efforts to understand the mechanisms behind resistance.

Parent trees have been screened for a more detailed investigation of resistance mechanisms. All of these have been field assessed for resistance to weevil attack after weevil augmentation, but a more detailed microscopic evaluation is being attempted in order to classify according to putative mechanism. This is particularly valuable for "constitutive" type of mechanisms such as sclereid cells or constitutive resin cells and we are working to extend these techniques to look at some of the "inducible" mechanisms, particularly traumatic resin cell production.

On-going trial assessments for weevil attack have continued. These have included some of the hazard assessment trials. The hazard assessments were a series of trials established to test resistance over a variety of ecological and potential weevil-hazard areas. The main objective is to aid in determining deployment guidelines. Some of these guidelines were assessed in previous years to investigate elevational and latitudinal transfer of weevil-resistant sources. To further this objective, we have sowed a small series of high-elevation selections in 2004, along with some resistant sib lots to be established in 2005.

Good progress has been made on the first draft of a report outlining the weevil resistance program. This report will present many of the details of the program, an outline of our scoring system for resistance, and a ranking of all our parents to date. The report will also present deployment guidelines for resistant material. In addition to last year's book chapters, we have published an article in the Journal of Forestry entitled: "Genetic resistance of Sitka spruce (*Picea sitchensis*) populations to the white pine weevil (*Pissodes strobi*): distribution of resistance."

#### Coastal White Pine (*Pinus monticola* Dougl. ex D.Don)

In 2004, we sowed and grew the first phase of the F1 breeding program. Parents for this effort came from the CFS screening program and were designated as either slow canker growth (SCG) or difficult to infect (DI). SCG or "slow-rusting" trees occur more frequently than DI. Also included as parents were some of the best of the Texada trees and some Idaho, Interior, and Dorena trees. We are identifying several sites to establish these trials and are looking at sites on Texada Island and near Sechelt.

We collected several more scions from the CFS screening list — two from Texada Island and a few from Vancouver Island, and several of the major gene resistance (MGR) trees. Much of this was to make up for the grafts made the previous year that did not survive. Grafting this year was done for us at Kalamalka Forestry Centre and grafts will be established in the North Arm clonal archive, Cowichan Lake seed orchards, and a newly established breeding orchard at Puckle Road (Saanich).

In September 2003, we toured the Interior provenance trials established by Rich Hunt (Root Rot Series) and Mike Meagher (Provenance Series) in the 1980's. We looked particularly at the Dorena MGR seed lots to see how well they held up in the Interior and, overall, they did not appear too bad. We are now in the process of measuring all these trials. These measurements will help us to evaluate the resistance of these sources and assess their transferability across the province. We are also currently doing a first assessment of the full-sib block trial initiated by Patti Brown, of Canadian Forest Products Ltd. This trial allows the comparison, in field conditions, of various CFS selections such as "low spotters", and "slow rusting" against Dorena and wild unselected lots. Although the data are still being gathered, some preliminary results are very encouraging. Some of the wild unselected blocks are 90% infected, whereas some of the Dorena x Dorena and Dorena X SCG are only 5% infected.

Our paper entitled "The five needle pines in British Columbia, Canada: Past, present and future " by J. King and R. Hunt was published as a special USDA Forest Service Research Report, and a chapter for a special edition of the Journal of Forest Ecology and Management entitled "Genetic approaches to the management of blister rust in white pines" is well underway.

#### Queen Charlotte and Coast/Interior Transition Spruce

The Queen Charlotte program allows us to assess Sitka spruce growth free from weevil attack and supports the seed orchard program (SO 142 and its replacement). Our major difficulty has been in getting "free-to-grow" conditions, in this case not free from weevil or brush but from deer browse, and a good deal of our maintenance effort is on browse protection. In 2004, we spent a good deal of time cleaning up, tagging, and removing browse protection from the trials we established there. During fall of 2004, we hope to do a major measurement of our Queen Charlotte Islands sites. The transition program is designed mainly to test seed transferability in a very environmentally heterogeneous area with pressures from both frost and weevil. In the past year most of the activities for this program have concentrated on routine site maintenance, although a small weevil-resistance plot was established in the Bish River area near Kitimat. A series of measurements and assessments are planned for this summer.

## FOREST GENETICS AND SEED TRANSFER RESEARCH

The Role of Site, Genotype and Seed Transfer on Branching and Wood Quality in Lodgepole Pine

Progeny test data from nine populations were used to examine the relationships of site climate, seed source climate, and transfer distance climate with branching and wood quality traits. A manuscript is in preparation.

#### Potential Genetic Gain Using Somatic Embryogenesis of Interior Spruce

Age 7 height of seedlings and 313 embling lines from 11 families was compared. Genetic gain was estimated for mass clonal selection and clone within-family selection, and compared with gain expected from seed orchards. A manuscript has been submitted for review.

#### Climate-Based Site-Specific Seed Transfer to Maximize Productivity

Age 20 lodgepole pine tree volume and survival data from 140 populations tested at 60 test sites were used to estimate the climate of the optimum and acceptable ranges of seed sources for regeneration. Presentations were made to compliance and enforcement officers in the MoF, for consideration of penalties to excessive seed transfer in BC. These data and supporting documents will be used to support the new Chief Forester Standards on Seed Use in BC.

#### Potential New Distributions of Lodgepole Pine Populations in BC

Ranges of climate variables of lodgepole pine populations were determined and mapped spatially. Layers of each variable were overlaid on each other to identify and map the location of climates of distinct individual populations ('climatypes'). Data from global circulation models were used to map the shift in the location of those climates in BC in 2025, 2055, and 2085 to illustrate where climates of current lodgepole pine populations will be found in the future. At this point, these simulations are being used for internal discussion on what the next steps may be for seed transfer under various climate change scenarios.

#### Genecology of Interior Spruce

Interior spruce seedlings from 141 wild stands and 9 seed orchards were planted in spring 2003 at three field sites in central BC and one nursery bed in Vernon, BC, to examine the species' geographic distribution of genetic variation. Results will be used to refine wild stand and seed orchard seed transfer for current and future climates and to help standardize breeding values of elite genotypes from disparate breeding programs.

#### Field Test Designs

As follow-up work on the use of incomplete block designs for genetic testing for parental selection (e.g., Fu, Y.B. et al. 2000. For. Gen. 7:287–293), we have also initiated a study to examine the most efficient field designs for forward selections. Complementary designs were not investigated as we are no longer considering them in most of our programs.

#### Population Structure With Negative Genetic Correlations

Dr. Leopoldo Sanchez, from INRA, Orleans, France spent two months with us in 2003 to further develop our breeding strategies in relation to population size and structure when there may be a negative genetic correlation. Collaborative work with Dr. Sanchez is continuing and we hope to further refine our breeding approaches with the use of his model and address some important questions with respect to optimum selection strategies currently being considered in animal breeding.

#### Forest Genetics and Biotechnology

We continue to be actively involved in various stakeholder group meetings, as well as in collaborative policy development programs related to the use of various biotechnologies in forestry. Alvin Yanchuk participated in the CFS workshop on Environmental Safety in Montebello, Québec, and spent six weeks at FAO, Rome to assist with various projects related to the status of biotechnology in forestry and additional writing projects on gene conservation.

#### Genetic Improvement of Hardwood Species in Coastal British Columbia

Utilization and management of hardwood resources in the coastal region of BC received considerable attention in the 1990s. This attention focussed on the management of three hardwood species with commercial potential, namely red alder (*Alnus rubra* Bong.), bigleaf maple (*Acer macrophyllum* Pursh), and black cottonwood (*Populus trichocarpa* Torr. & Gray). One consensus derived from problem analyses then was the major knowledge gap on the lack of genetic information on variation of adaptive and commercially important traits that would hinder the effective management of these species. Against this backdrop, genetic research was initiated in the mid-1990s.

Red alder and bigleaf maple, because of their high wood quality, have potential for value-added products such as furniture manufacture. One problem with these species is the difficulty in finding quality logs (large diameter with good stem form) in natural stands. Long-term provenance-progeny testing was initiated in 1995 for red alder and in 1997 for bigleaf maple with the objectives: 1) establishing seed transfer guidelines for selection of productive seed sources for planting and 2) evaluating the potential to grow better quality logs in plantation settings. A breeding strategy will be developed for red alder based on 10-year test results that will be assessed the fall of 2004 and selection will start spring 2005.

Black cottonwood is the only native poplar species with the potential of short-rotation (less than 10 years) fibre production through clonal forestry. The outstanding yield (mean annual increment >  $30 \text{ m}^3/\text{ha/yr}$ ) of the selected black x eastern cottonwood (*P. deltoides*) hybrid clones in Oregon and Washington demonstrated this potential. However, most of these hybrid clones developed in the United States were not hardy when planted at sites on BC's mid- and north-coast. A short-term common garden study of black cottonwood with about 800 clones from 170 provenances has been completed. Three long-term provenance-clonal tests located in three geographic regions, namely south-coast, north-coast, and interior, will be established spring 2005.

#### MOLECULAR GENETICS PROJECTS

Determination of Selfing Rate in a Top-Pruned Interior Spruce Seed Orchard

In the past, seed orchards have routinely been top-pruned to facilitate easier and safer orchard management. This practice, however, brings male and female cones closer together, potentially increasing the chance of selfing, which in turn may reduce seed set and reduce progeny vigour. DNA markers are available to test for increased selfing in these top-pruned orchard ramets.

Before candidate clones are identified for study, orchard clones must be genotyped. For this purpose, total DNA was extracted from vegetative buds of each producing clone (78 clones in total) in interior spruce orchard 305. Several chloroplast DNA (cpDNA) markers were applied using the PCR technique to screen for unique clones, ones that are unambiguously identifiable among all orchard clones. These easy to score clones have been chosen for further study in 2004.

The results of the genotyping revealed that, with two primer pairs only, 12 out of the 78 trees in the orchard can be unambiguously identified (Table 1). Marker S2spF/R is, especially, extremely variable, yielding 14 alleles (different bands) alone. However, as some of the clones in orchard 305 are too small to conduct pollinations on or have been subsequently culled, genotyping was intensified using all 5 available polymorphic markers. The separation power increased so that 18 clones can be identified using 5 primer pairs (Table 2). This array of available clones will be used to make final selections for further study based on the expected cone crop of these clones in year 2004.

Primer S2spF/R	Pairs 89F/62R	No. Clones	Clone ID
А	b	1	575
В	b	1	3079
С	С	1	3180
Е	d	1	1289
F	b	1	692
G	d	1	3243
Н	d	1	3146
J	d	1	1299
К	а	1	3004
L	С	1	661
Ν	d	1	3254
0	d	1	2005

 Table 1. Unique genotypes of interior spruce clones growing in Kalamalka Orchard # 305 identifiable using 2 genetic markers.

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S2spF/R	89F/62R	84.1F/R	8F/8R3	7RR/7F3	No. Clones	Clone ID
а	b	d	b	b	1	575
b	b	d	d		1	3079
С	С	d	b		1	3180
d	С	d	bc	b	1	3173
е	С	d	b	а	1	3263
е	d	d	b	b	1	1289
f	b	d	b	b	1	692
g	d	d	b	b	1	3243
h	b	d	С	b	1	1364
h	b		b	b	1	2008
h	d		b		1	3146
j	С	d			1	2702
j	С	е	b	а	1	3324
j	d	d	b	С	1	1299
k	а	d	С		1	3004
I	С	С	С	а	1	661
n	d	d	b		1	3254
0	d		b		1	2005

 Table 2.
 Unique genotypes of interior spruce clones growing in Kalamalka Orchard # 305 identifiable using 5 genetic markers.

# Determination of Selfing in a Clonal-Row Spruce Orchard

In the past, seed orchards needed to be licensed to ensure that orchard management plans and orchard composition was up to standard. A seed orchard license was a pre-requisite for seed lot registration. This licensing requirement no longer exists and more emphasis is placed on the seed lot rating protocol. However, in an orchard with a new and innovative design, mating dynamics should be checked to ensure they conform to expectations. One type of orchard with a new and innovative design is a clonal-row orchard, where many ramets of the same clone are planted in a row at relatively close spacing. This type of orchard will make orchard management more efficient, as all ramets of a single clone are in close proximity for surveys, pollinations, cone harvesting, and other orchard management activities. However, this arrangement may also increase selfing levels as many "closest-neighbour" trees are genetically identical and will have very similar flowering phenologies, (receptivity and pollen shed). Clearly, before seed lots from this type of orchard are registered, it is prudent to evaluate selfing as increased levels of selfing in a seed lot large enough to raise 2.2 million seedlings per year may have serious implications. Not only will seed set be lowered, but also the vigour of the resulting seedlings is compromised.

Paternal analyses of seed from ten easily identifiable clones was conducted. For this purpose, all 30 clones of orchard 620 were genotyped using polymorphic chloroplast DNA (cpDNA) markers. Briefly, for genotyping, total DNA was extracted from all clones using vegetative buds. Potentially polymorphic sections of cpDNA were amplified using PCR and compared among the 30 clones. Ten clones with a unique multi-locus genotype pattern were chosen for further analysis. From the ten selected clones, a clonal bulk sample of seeds was collected and their embryos were analyzed individually to see if they have the same cpDNA pattern as their maternal parent (seed trees). The identical pattern in the embryo

indicates a selfed seed, as cpDNA is carried by the male gamete only. A total of 50 seeds per clone were assayed for a total of 500 seeds.

Based on the 10 sample clones, there was no evidence that there is an overall increased level of selfing in this particular clonal-row orchard. Individual selfing on a clonal basis ranged from 0 to 14% with an average of 4.4%. Results for the sample clones are listed in Table 3. It is important to realize that these selfing levels are unambiguous as only unique orchard clones were selected for this study.

Clone	Selfed seed	Total seed	Percent selfed seed
311	5	50	10
381	4	50	8
339	0	50	0
386	7	50	14
408	3	50	6
447	0	50	0
422	0	50	0
377	0	50	0
410	0	50	0
419	3	50	6
Average			4.4

Table 3. Levels of natural selfing in 10 clones of a clonal-row spruce orchard # 620 at Kalamalka