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

PART 1	Minutes and Members' Reports
PART 2	Symposium

Adaptation, Breeding and Conservation in the Era of Forest Tree Genomics and Environmental Change

QUÉBEC 2008

L'adaptation, l'amélioration et la conservation à l'ére de la génomique forestière et des changements

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

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

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PROCEEDINGS

OF THE

THIRTY-FIRST MEETING

OF THE

CANADIAN FOREST GENETICS ASSOCIATION

PART 1

Minutes and members' reports

Quebec City, Quebec
August 25–28, 2008

Editor
J.D. Simpson

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COMPTES RENDUS

DU

TRENTE-ET-UNIÈME CONGRÈS

DE

L' ASSOCIATION CANADIENNE GÉNÉTIQUE FORESTIÈRE

1^re PARTIE

Procès-verbaux et rapports des membres

Québec, Québec
25–28 août 2008

Rédacteur
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pour
l'Association canadienne génétique forestière
2009

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BUSINESS MEETING MINUTES

CFGA/ACGF 31st Business Meeting Minutes

Jean Beaulieu chaired the 31st Business Meeting of the CFGA/ACGF held at the Loews Le Concorde, Quebec City, Quebec on Monday August 25, 2008. Twenty-four Active Members and 1 Honorary Member were present.

358 Minutes of the 30th Meeting

(as printed in the proceedings from the 30th meeting, Part I)

Motion: That the minutes of the 30th Business Meeting be approved as published.
Moved by: Dave Kolotelo
Seconded by: Yill Sung Park
Carried.

359 Membership

359.1 Honorary Membership

The name of one nominee was presented as follows:

Roger Beaudoin

Motion: That the nominated Honorary Member be duly elected.
Moved by: André Rainville
Seconded by: Gaëtan Daoust
Carried

359.2 Active Membership

The names of thirteen nominees were presented as follows:

Sylvie Carles	Université Laval Quebec, QC
Lee Charleson	Ministry of Forests and Range Victoria, BC
Jeannette Coote	Mountain Forest Section Renewal Company Swan River, MB
André Deshaies	Ministère des Ressources naturelles et de la Faune Quebec, QC
Mireille Desponts	Ministère des Ressources naturelles et de la Faune Quebec, QC
Claude Gagné	Ministère des Ressources naturelles et de la Faune Quebec, QC
Krystyna Klimaszewska	Canadian Forest Service Quebec, QC
Mohammed Lamhamedi	Ministère des Ressources naturelles et de la Faune Quebec, QC
Michée Lemieux	NS Department of Natural Resources Strathlorne, NS

Matthew LeRoy	Ministry of Forests and Range Victoria, BC
Donatien Lévesque	Ministère des Ressources naturelles et de la Faune Quebec, QC
Martin Perron	Ministère des Ressources naturelles et de la Faune Quebec, QC
Anne Savary	Ministère des Ressources naturelles et de la Faune Quebec, QC
Armand Séguin	Canadian Forest Service Quebec, QC

Motion: That the nominated Active Members be duly elected.
Moved by: Greg O’Neill
Seconded by: Gaëtan Daoust
Carried.

359.3 Corresponding Membership

No nominations submitted.

360 Chair’s Report

The 31st meeting of the association was held for the third time in its history in Quebec City. It was hosted by Université Laval and co-organized by Université Laval, Natural Resources Canada and the Ministère des Ressources naturelles et de la Faune du Québec. The organization of the meeting was truly a “joint” effort from all the committee members. This was a joint meeting with the International Union of Forest Research Organizations (IUFRO) Working Groups 2.04.01 (Population, Ecological and Conservation Genetics) and 2.04.10 (Genomics). Moreover, it was held in conjunction with the 2nd Forum on the Conservation of Forest Genetic Resources.

On behalf of the 2008 **Executive Committee (Jean Bousquet, André Rainville, John MacKay, and Jean Beaulieu)**, I would like to thank the many individuals who contributed to the success of this 31st CTIA meeting. The various organizing committees did a great job in putting the conference together over the last two years. In particular I would like to thank the following people and all individuals that are too numerous to list here and their respective employers who generously supported the conference as well as the field trips.

International Scientific Advisory Committee for the Main Conference
Sally N. Aitken (Canada)
Santiago c. González-Martínez (Spain)
Pär Ingvarsson (Sweden)
Nathalie Isabel (Canada)
Mathias Kirst (USA)
Antoine Kremer (France)
Andrew Lowe (Australia)
Tim Mullin (New Zealand)
David B. Neale (USA)
Christophe Plomion (France)
Kathy Tosh (Canada)
Giovanni G. Vendramin (Italy)
Alvin Yanchuk (Canada)

Local Organizing Committee
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Sauphie Senneville (Université Laval)
Monick Debroux (Azymuths)

CTIA Tree Seed Working Group Committee
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Laurence Tremblay (Ministère des Ressources naturelles et de la Faune du Québec)
André Rainville (Ministère des Ressources naturelles et de la Faune du Québec)

CTIA Wood Quality Working Group Committee
S.Y. (Tony) Zhang (FPInnovations – Forintek)
Alvin Yanchuk (British Columbia Ministry of Forests and Range)
Jean Beaulieu (Natural Resources Canada)

CTIA Sponsored Students' Committee
Gaëtan Daoust (Natural Resources Canada)
and Kathy Tosh, Denise Tousignant, Leonard Barnhardt, John Russell, and Jack Wood for their kind help in selecting the best oral and poster presentation by a student.

Arborea-Treenomix Joint Workshop Committee
John MacKay (Université Laval)
Jörg Bohlmann (University of British Columbia)
Kermit Ritland (University of British Columbia)
Jean Bousquet (Université Laval)

CON**FOR**GEN
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David Reid (British Columbia Ministry of Forests and Range)
Dale Simpson (Natural Resources Canada)
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The support of 14 sponsors allowed us to invite internationally renowned speakers and to continue our student sponsorship program. The following sponsors provided much-appreciated financial support for the 2008 meeting:

Natural Resources Canada (Canadian Forest Service and Canadian Wood Fibre Centre)
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I am very grateful to these sponsors for their generous contributions. It would not have been possible to organize such an international event without their support.

Lastly, I would like to thank all the participants. There were about 250, including the keynote and invited speakers as well as the voluntary speakers and the students who made this 2008 conference a success. It is their participation and contributions to the meeting that made it enjoyable and successful. Good luck to the next CTIA organizing committee and I hope to see you in Thunder Bay in 2010.

Jean Beaulieu
Chair

361 Treasurer’s Report

The financial statement for the period of June 2006 to August 2008 was presented by Dale Simpson on behalf of Treasurer Tannis Beardmore and tabled for information and acceptance by the membership (see Attachment #1). The statement shows a balance of $14,448.00 in the Association’s account plus GIC’s totaling $16,000.00. Dale reported that Tannis wished to resign and hoped that somebody would be willing to take on this position.

Motion:	That the financial statement be accepted as presented.
Moved by:	Sally Aitken
Seconded by:	Greg O’Neill

Carried.

362 Financial Contributions

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Centre for Forest Research
The Prostate Center at Vancouver General Hospital
FPInnovations
Poplar Council of Canada 2007
Larix 2007
Les Industries Harnois Inc.
Solplast Inc.

Motion:	That the CFGA/ACGF executive of the 31st meeting express our sincere appreciation to these contributors.
Moved by:	Jack Woods
Seconded by:	Dave Kolotelo

Carried.

363 Editor’s Report

The proceedings were printed and distributed during September 2007 to all Active members, Honorary members, Canadian universities and libraries, registrants, and financial contributors of the 30th meeting. Two hundred and eleven proceedings were mailed. Eighteen copies on CD-ROM were sent to Active members who requested this alternative medium.

A letter advising of the theme of the 30th biennial meeting, context of the proceedings, and a request for a twenty dollar donation to obtain a copy was sent to all Corresponding members, USA addresses, and International addresses. As a result, proceedings were mailed to 4 Canadian, 5 USA, and 5 International addresses. Twenty-five copies remain on inventory.

The mailing list contains 417 addresses comprised of: 17 Honorary, 78 Active, 84 Corresponding, 61 Canadian libraries, 66 United States, and 111 International.

Dale Simpson

364 Education Committee

The Organizing Committee of the CTIA 2008 meeting sponsored students from seven Canadian universities with an accredited undergraduate forestry program. This sponsorship is encouraged by the CTIA members and financially supported by conference registration fees and contributions provided by sponsors. Sponsorship covered costs of attendance, including transportation, accommodation, and conference registration.

The seven universities were asked to nominate a student to attend the meeting in Quebec City. They nominated the following students:

Fougère Augustin	Université de Moncton
Stanislav Bashalkhanov	University of New Brunswick
Jordan Bemmels	University of British Columbia
Leonardo Galindo	University of Alberta
Julie Godbout	Université Laval
Phillippe Stankowski	Lakehead University
Ryan Thorgeirson	University of Northern British Columbia

The Gene Namkoong Award is presented to a student for the best poster. The winner was Julie Gravel-Grenier from Université Laval. The title of her poster was “Utilization of family genetic variability of white spruce (*Picea glauca*) to improve rooting of plant cutting root systems”, and was co-authored with Mohammed S. Lamhamedi, Jean Beaulieu, Sylvie Carles, Hank A. Margolis, Michel Rioux, Debra Christiansen, and Line Lapointe.

The Carl Heimburger Award is presented to a student for the best oral presentation. Karolyn R. Keir from the University of British Columbia received the award for a paper entitled “Beautiful, charismatic but lacking diversity: The story of Pacific Dogwood (*Cornus nuttallii*) as told through microsatellite and chloroplast sequence data”, co-authored with Sally Aitken.

Gaëtan Daoust

365 Working Group Reports

365.1 Tree Seed Working Group

The primary focus of the Tree Seed Working Group (TSWG) has been in exchanging information through our biannual Newsbulletin and through the biennial Tree Seed Workshop. Since our last report we have published four editions of the News Bulletin (#44–47) on the following topic areas.

#44	Seed Dormancy
#45	Seed Storage
#46	Seed Transfer
#47	Seed Use Efficiency

The News Bulletin has been well received and now reaches 260 people worldwide electronically. Appreciation is extended to all who contribute to the News Bulletin. Anyone who wishes to be on the distribution list can contact me (Dave.Kolotelo@gov.bc.ca) or Dale Simpson (dsimpson@nrcan.gc.ca). Past electronic versions of the News Bulletin can also be downloaded in pdf format from the following link: http://www.for.gov.bc.ca/hti/treeseedcentre/tsc/tswg.htm

The 2008 TSWG conference activities included a pre-conference field trip to the Berthier Forest Nursery, our dedicated workshop, and several contributions to the poster session that were presented to the group. The TSWG organizing committee consisted of Fabienne Colas, Michèle Bettez, Laurence Tremblay, and André Rainville. The pre-conference field trip consisted of tours of the nursery, indoor larch orchards, and the Tree Seed Centre. The workshop theme was “Preserve and Multiply Forest Genetic Resources” and included eight oral presentations and five posters as presented below.

1. Meeting provincial seed production objectives in British Columbia: Systems for ranking priorities and determining orchard size	Jack Woods
2. Zoom in on seed production in Québec	Anne Savary
3. French forestry pattern: From seed to plant	Nicolas Vionnet
4. The *ex-situ* genetic conservation plan for British Columbia tree species	Dave Kolotelo
5. Conservation of genetic diversity by tree seed centres	Dale Simpson
6. Water activity: A new tool for moisture management of seedlots in tree seed centres	Fabienne Colas
7. An integrated approach to preserve and multiply improved material of white spruce by somatic embryogenesis	Laurence Tremblay
8. Seed size and family effects on vegetative propagation rates of stockplants for the mass cutting propagation of white spruce in Québec	Denise Tousignant
1. Genetic parameters of seedling juvenile characters of 75 open-pollinated white spruce (*Picea glauca* (Moench) Voss) families	Sylvie Carles
2. *Fraxinus* germplasm collections in the United States	Robert P. Karrfalt
3. Clonal variation in *Picea abies* stockplants produced by somatic embryogenesis and their use in cutting propagation programs	Denise Tousignant
4. Utilisation of family genetic variability of white spruce (*Picea glauca*) to improve rooting of plant cutting root systems	Julie Gravel Grenier
5. Effects of cone number and thinning on nutrient content in needles of Korean pine	Tae Hoon Kim

The workshop was well attended and provided a forum for some interesting discussion on the preservation and multiplication of our genetic resources. Look forward to seeing you at our next workshop in Thunder Bay in 2010.

Dave Kolotelo
Chair

365.2 Wood Quality Working Group

The workshop, organized by Tony Zhang, Alvin Yanchuk and, Jean Beaulieu, was entitled "Managing for wood quality attributes and economic value" with Dr. Harry Wu as a keynote speaker, and the following volunteer speakers presented a wealth of information on the topic.

Overcoming adverse genetic correlation between wood quality and quantity traits through development of breeding objectives in advanced breeding generation of radiate pine	Harry X. Wu
Development of an economic weight-based selection strategy for maximum economic value	Jean Beaulieu
Effect of cyclophysis on intra-ring components of growth and wood density of jack pine	Young-In (David) Park
Genetic control of wood properties of white spruce: Trends with cambial age	Patrick Lenz
Genetic variation in physical and chemical wood quality traits in *Pinus taeda*	Fikret Isik

In situ wood quality assessment in Douglas-fir	Yousry A. El-Kassaby
Genetic parameters for tropolones associated with *Thuja plicata* heartwood durability	John Russell
Trees: Structural marvels but very imperfect wood factories	Rowland D. Burdon
Evaluation of the resistograph for wood density estimate and the use of multi-trait selection index for genetic selection in jack pine	Paul Charrette

The workshop was well attended and provided good opportunities for discussions. We look forward to our next workshop in Thunder Bay in 2010.

Tony Zhang
Alvin Yanchuk
Co-chairs

365.3 Canadian Forest Genetics Councils Working Group

The Working Group was created at the CTIA meeting held in Kelowna in 2004 to form a Council composed of representatives from provinces and territories which could respond to issues having a national context. Since no issues have arisen Jack Woods recommended that the Working Group be eliminated.

Motion: That the Canadian Forest Genetics Councils Working Group be discontinued.
Moved by: Yousry El-Kassaby
Seconded by: Jack Woods
Carried.

366 Old Business

366.1 Canadian Institute for Science and Technical Information (former business meeting item 354.1)

At the last Business Meeting Bruce Dancik suggested that this organization may be able to store and archive historical documents and proceedings of the Association. Shortly after the meeting Bruce received a reply from the Institute stating that CISTI doesn't collect "forestry" material because Natural Resources Canada collects such information and has jurisdiction in this area. Dale Simpson confirmed that he has all the documents and proceedings stored in a vault and will continue to store the material.

366.2 Change name of Canadian Tree Improvement Association (former business meeting item 354.2)

Following the last meeting two documents were prepared; one supporting changing the name and the other supporting retaining the name. These documents were electronically circulated to Active Members for their consideration and vote. A total of 48 out of 86 Active Members voted (56%). The result was 22 voted to retain the name Canadian Tree Improvement Association and 26 voted to change the name to Canadian Forest Genetics Association.

Motion: That the results of the September 2006 poll be accepted to rename the Canadian Tree Improvement Association/Association Canadienne pour l'amélioration des arbres to the Canadian Forest Genetics Association/l' Association canadienne de génétique forestière.
Moved by: Sally Aitken
Seconded by: Jack Woods
Carried.

367 New Business

367.1 Printing of Proceedings

It cost about $ 8,000 to print and distribute the Proceedings of the last meeting. Proceedings of most conference are now available electronically. Publishing the Proceedings electronically would save the Association a significant amount of resources.

Motion:	That the Proceedings of CFGA/ACGF be provided by electronic means.
Moved by:	Brian Barber
Seconded by:	Kathy Tosh
Carried.	

367.2 New Logo

Jean Bousquet suggested that a new logo be designed that would incorporate the Association's new name. Kathie Brosemer agreed that the organizing committee for the next meeting would look after this.

367.3 CFGA/ACGF Web Site

Discussion ensued on the need for the Association to have a permanent web site. The site could contain a complete set of the Proceedings as well as other documents. Announcements of future meeting would also be posted there. The question arose as to who would be able to host such a site. Brian Barber said the the BC Ministry of Forests and Range could do this.

Motion:	That the BC Ministry of Forests and Range set up and maintain a web site for the CFGA/ACGF with funds provided by CFGA/ACGF.
Moved by:	Brian Barber
Seconded by:	Dave Kolotelo
Carried.	

Dale Simpson will explore ways and means of having all Proceedings scanned.

368 Future Meetings

368.1 Location of the 2010 Meeting

Kathie Brosemer confirmed to host the meeting in Thunder Bay.

368.2 Location of the 2012 Meeting

Vancouver.

368.3 Location of the 2014 Meeting

There was no volunteer.

369 Election of New Executive

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

Chairperson:	Kathie Brosemer Forest Genetics Ontario
Vice-Chairperson: Symposium	Pengxin Lu Ontario Ministry of Natural Resources
Vice-Chairperson: Local arrangements	Paul Charrette Lakehead University
Treasurer:	Michele Fullarton NB Department of Natural Resources
Editor:	Dale Simpson Canadian Forest Service
Executive Secretary:	Dale Simpson Canadian Forest Service

370 Adjournment

Motion: That the 31st business meeting of the CFGA/ACGF be adjourned.
Moved by: Sally Aitken

Attachment # 1

CTIA/ACAA
Financial Statement
June 2006 – August 2008

Holdings – June 27, 2006

Cash balance	$30,143.89
Total GICs	$23,000.00
Total Holdings	**$53,143.89**

Credits

Interest (checking account)	$12.73
Interest (GICs)	$1,743.29
Purchase of proceedings	$301.48
GIC (matured June 27/08)	$7,000.00
Conference credits[1] (registrations, donations, etc.)	$23,199.18
Total credits	**$32,256.68**

Debits

Printing proceedings (including mailing supplies)	$8,030.71
Conference debits[1] (speakers travel expenses, Delta room charges, etc.)	$39,921.86
Total debits	**$47,952.57**

Guaranteed Investment Certificates

GIC (matures February 27, 2009)	$8,000.00
GIC (matures June 29, 2009)	$8,000.00
Total GICs	**$16,000.00**

Holdings – August 18, 2008

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

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[1] Note, 2006 CTIA conference debits and credits span 2 financial statements (2004-06 and 2006-08).

Summary of 2006 CTIA conference:

2004-06:
Debits: $2,902.71
Credits: $16,087.59
= total credit of $13,184.88

2006-08
Debits: $39,921.86
Credits: $23,199.18
= total debit of $16,722.68 **Overall conference debit: $3537.80**

ACTIVITY REPORTS FROM ACTIVE MEMBERS

COOPERATIVE TREE BREEDING IN NOVA SCOTIA

Howard Frame and David Steeves

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Keywords: *Picea mariana*, *Picea glauca*, *Picea rubens*, 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 (NSDNR), Abitibi-Bowater (formerly Bowater Mersey Paper Company Limited), Northern Pulp (formerly Neenah Paper), New Page Corp (formerly StoraEnso), and J.D. Irving Limited. The Canadian Forest Service has provided support services as well as technical and scientific expertise from the program's inception. Coordination is provided by NSDNR. Meetings are held in the spring and fall of each year to review activities and plans. 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.)

With first-generation seed orchards able to supply the current seed requirements for reforestation within the province, NSDNR stepped back from second-generation orchard development and breeding activities around 2000. Industrial cooperative members assumed the lead role in the day-to-day tree breeding activities formerly carried out by NSDNR. However, the tree improvement data analyst was seconded to perform a provincial wood supply simulation. With a data analysis function at the center of any tree improvement program, forward momentum was restricted. The industrial cooperators proceeded with second-generation white spruce selection, breeding, and orchard development as information was provided by the analyst. Black spruce breeding and orchard establishment was also carried out by industry with NSDNR providing data analysis and breeding value calculations, as time permitted.

In 2008, with the department's wood supply analysis completed, tree improvement analyses have been brought back into the forefront with emphasis on red spruce. The push for this year will be to get the second-generation program off the ground with selection and grafting beginning in the fall of 2008.

The NSTIWG continues to benefit from strong commitment from all members, even during challenging economic times in the forestry sector.

BREEDING AND SELECTION

Since 2006, second-generation selection for white spruce has been completed. Based on parental breeding values (mid-parent values), derived from half-sib progeny tests, 103 of 459 white spruce specific crosses from a random pair-mate design were selected from which 410 trees (approximately four trees / cross), growing in selection plantations, were chosen on the basis of size and form characters to constitute the second-generation breeding population. These selections have been segregated into 20 breeding groups to contain relatedness in anticipation of commencement of progeny testing. Of these selections, 60 unrelated have been chosen for deployment into second-generation clonal seed orchards.

With breeding values now predicted for over 95% of our first-generation red spruce clones, ranking of specific crosses and selection of individual trees in the selection plantations for the second-generation breeding population will commence in the fall of 2008. The objective is to make approximately 500 selections from between 165 to 170 of the 250 specific crosses derived from our first-generation breeding effort (random pair-mate design). Current economic challenges are forcing the NSTIWG cooperators to reconsider how best to move forward with breeding work. Since 2006, only limited breeding activity has taken place.

TESTING

GCA testing for juvenile height of the 462 clones of the first-generation white spruce population is now complete. This component of the program consisted of 42 field tests planted between 1991 and 1999. The last of these was measured in the fall of 2006.

All but one test series of red spruce half-sib progeny remains to be measured. When complete, 29 tests established between 1991 and 1999 will have provided breeding value estimates for 494 first-generation red spruce clones.

Because of the slowdown in breeding activity, there have been no additional second-generation black spruce and white spruce progeny tests established since 2006. To date, half-sib progeny of 227 of 363 black spruce clones are in 20 tests planted between 2000 and 2006 and 33 of the 382 second-generation white spruce clones are being tested in four tests planted in 2006.

SEED ORCHARDS

Orchard expansion into the second-generation continues with Northern Pulp establishing both a black spruce (complete) and a white spruce orchard (90% complete) at their East Mines facility. Abitibi-Bowater has proceeded to establish a white spruce orchard (50% complete) at their Melvern Square property and have prepared a site for a red spruce orchard. J. D. Irving have shown interest in establishing a second-generation red spruce orchard as material becomes available. The NSDNR has established a second-generation black spruce orchard at their Strathlorne Forest Nursery property (75% complete). With the discovery of significant archeological artifacts on the NSDNR tree breeding and orchard property (200 ha), it has become increasingly important to develop a strategy to migrate our working group’s plant material and maintain an uninterrupted seed supply from cooperative orchards to offset the loss of this property to substantial archeological interests.

TREE IMPROVEMENT PROGRESS BY THE NEW BRUNSWICK DEPARTMENT OF NATURAL RESOURCES

K.J. Tosh and M.S. Fullarton

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Keywords: cross-pollinations, progeny tests, seed orchards

The tree improvement program in NB continues to focus on the main reforestation species, white spruce (*Picea glauca* [Moench] Voss), black spruce (*Picea mariana* [Mill.] 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 New Brunswick Department of Natural Resources (DNR) is a member of the New Brunswick Tree Improvement Council (NBTIC), a group of cooperators including the federal government and four 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.

In the past two years, we have continued to plant black spruce and jack pine polycross tests. The last series of polycross tests for jack pine will be planted in 2008. The last black spruce series was completed in 2006.

Since 2006, one series of full-sib black spruce and one series of black spruce polycross tests were planted. The full-sib tests came from crossing done within sub-lines established using information from polycross progeny tests. Older tests will be used for making third-generation selections within the next two years.

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. Selections are being made in selection plantations. Three hundred and sixty-seven trees have been selected since 1998. This will continue until we have made 400–500 selections.

We have continued to stock existing orchards and establish new ones. The table below summarizes orchard establishment for DNR.

Table 1. Seed orchards established by DNR.

Species	Generation	Type	Establishment Year	Area (ha)
White spruce	1st Gen.	CSO[1]	1985–1987	9.0
	1st Gen.	SSO	1978–1982	8.6
	2nd Gen.	CSO	2000–2007	4.1
Black spruce	1st Gen.	SSO	1980–1987	38.0
	2nd Gen.	CSO	1989–1997	11.8
Jack pine	1st Gen.	SSO	1979–1986	27.0
	2nd Gen.	CSO	1992–1998	7.9
Tamarack	1st Gen.	CSO	1984–1986	8.0
Balsam fir	1st Gen.	CSO	1990–1992	2.7
Red spruce	1st Gen.	CSO	2007–2008	1.6
	1.5 Gen.	CSO	1999–2001	2.0
Norway spruce	-	SPA	1998	2.0

[1] CSO = clonal seed orchard, SSO = seedling seed orchard, SPA = seed production area

CONE COLLECTION IN ORCHARDS

The second-generation orchards are producing a substantial amount of cones providing ample seed for our production nursery operation. Table 2 shows production from first- and second-generation orchards.

Table 2. Cone collection and seed yield from orchards in 2006 and 2007.

Species	Orchard	2006		2007	
		Cones (l)	Seeds (kg)	Cones (l)	Seeds (kg)
White spruce	1st Gen. CSO	10,112	141.10	-	-
White spruce	2nd Gen. CSO	142	1.97	-	-
Black spruce	1st Gen. SSO	2,392	22.44	-	-
Black spruce	2nd Gen. CSO	10,236	43.60	9,448	55.27
Balsam fir	1st Gen. CSO	3,168	109.98	-	-
Jack pine	2nd Gen. CSO	-	-	1,644	14.83

NEW BRUNSWICK TREE IMPROVEMENT COUNCIL UPDATE

Kathy Tosh, Michele Fullarton and Yuhui Weng

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

The New Brunswick Tree Improvement Council (NBTIC) was formed in 1976. It is comprised of industrial, scientific and government agencies. NBTIC has completed breeding and testing of first-generation white spruce (*Picea glauca* [Moench] Voss) and tamarack (*Larix laricina* [Mill.] Karst.) and is well into selection and breeding of second-generation white spruce clones. Polycross testing of second-generation black spruce (*Picea mariana* [Mill.] BSP) and jack pine (*Pinus banksiana* Lamb.) selections is now complete. Second- generation black spruce and jack pine orchards are producing substantial quantities of seed and are meeting the reforestation demand for Crown land.

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 Natural Resources 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 planted. The first planting of second-generation white spruce orchard started in 2000 and a total of 11.6 ha has been established. Three agencies are participating in the establishment of second-generation white spruce orchards. NBTIC members have also established 9 ha of red spruce and 10.5 ha of white pine clonal seed orchards.

Seed production in 2006 was substantial, with a yield of 172 million seeds, one of the larger cone harvests in NBTIC history. Most of the seed was collected in clonal black spruce and white spruce orchards (~85%). As expected, the seed yield for 2007 was a modest 54 million seed from NBTIC agency seed orchards with the majority of the seed collected was from black spruce second-generation orchards (~93%).

BREEDING

The Council completed a complementary polycross breeding program which began in 1987 for first-generation white spruce and tamarack. The last series for each species was planted in 2001 and 2002, respectively. Polycrossing of second-generation black spruce and jack pine is now complete and the last jack pine series will be planted in 2008. Third-generation jack pine selections are on hold due to a reduction in demand for this species in the reforestation program. Black spruce third-generation selections will begin in 2010, based on 10 year full-sib test results. The full-sib testing is 73% complete for black spruce and 62% complete for jack pine.

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 23 sub-lines has been established for black spruce and 21 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 results from 5-,10-, and 15-year polycross progeny test measurements. A total of 367 of 400–500 trees have been selected to date. A red spruce tree improvement program was initiated in 2003 and 100 plus trees have been selected to date.

TESTING AND DATA ANALYSIS

Testing continues to be an important component of the NBTIC program. Since 1976, 334 tests have been planted on over 376 ha. Since 1989, 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 2006, the 6th series of black spruce full-sib progeny tests was planted. Another black spruce full-sib series (7th) and a jack pine full-sib series (4th) was planted in 2007.

The data analyst position has continued to be funded by the NBTIC. A total of 10 NBTIC test series were analyzed in 2006 and 2007 which included 5 progeny tests, 3 full-sib progeny tests and 2 gain tests.

OTHER

The realized genetic gain trials of the first-generation jack pine, black spruce, and white spruce programs are over 15 years old. The realized gains for the improved seedlots were reasonably high and generally comparable to the theoretical gains predicted from genetic tests. Comparisons in growth functions (i.e., height-DBH relationship, tree size distribution) between the improved and unimproved seedlots suggest the importance of incorporating tree improvement into growth and yield models.

PUBLICATIONS

Shao, S.L.; Jin, Z.F.; Weng, Y.H. 2008. Lignin characteristics of *Abies beshanzuensis*, a critically endangered tree species. J. Wood Sci. 54:81–86.

Weng, Y.H.; Tosh, K.J.; Park, Y.S.; Fullarton, M.S. 2007. Age-related trends in genetic parameters for jack pine and their implications for early selection. Silvae Genet. 56(5):242–252.

Weng, Y.H.; Tosh, K.J.; Park, Y.S.; Fullarton, M.S. 2007. Application of nursery testing in long-term white spruce improvement programs. North. J. Appl. For. 24(4):296–300.

Weng, Y.H.; Parker, W.H. 2008. Adaptive variation in fall cold hardiness of aspen from Northwestern Ontario. Tree Physiol. 28(1):143–450.

GENETIC DIVERSITY AND GENE CONSERVATION RESEARCH AT THE ATLANTIC FORESTRY CENTRE, 2006–2008

J. Loo, B. Daigle, K. Forbes, D. McPhee, S. Pond and D. Simpson

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GENETIC DIVERSITY AND GENE CONSERVATION

J. Loo, K. Forbes, D. McPhee and C. Ramirez

The research focus is in three areas: genetic resistance of American beech (*Fagus grandifolia*) to the beech scale (*Cryptococcus fagisuga*); understanding and conserving genetic resources of eastern Canadian tree species of concern: hemlock (*Tsuga canadensis*), bur oak (*Quercus macrocarpa*), ash (*Fraxinus* spp.), butternut (*Juglans cinerea*); and genetic variability of an endangered Mexican pine species (*Pinus pinceana*).
Protein analysis of the inner bark of beech trees susceptible and putatively resistant to the beech scale was carried out in collaboration with Dr. J. Koch at the US Forest Service lab at Delaware, Ohio. Twenty protein spots that differed between the two groups were chosen for sequencing. It is evident that differences exist in proteins of susceptible and resistant trees, but the biological significance of the differences remains to be determined. A new grafting technique using heating cables at the graft union, known as the hot callus method, has increased grafting success to more than 50%, representing a substantial improvement over previous survival rates. Putatively resistant beech trees are grafted each year, to obtain a sufficient number of ramets for resistance screening. Progeny of controlled crosses among putatively resistant trees, susceptible trees, and between resistant and susceptible trees have been screened one year in the greenhouse for resistance to beech scale and a second year of results will be collected. In collaboration with Dr. M. Krasowski at University of New Brunswick the progeny test was outplanted at two locations in summer 2008 and additional resistance screening will be conducted over the next two years.

A hemlock nursery test with families and provenances from throughout the Maritime provinces is yielding information about genetic variation in phenology and growth traits. This will complement allozyme results as well as results of studies in Quebec, where diversity of microchondrial and chloroplast DNA markers is being examined, to complete a comprehensive assessment of genetic variability of the species, to inform a genetic conservation guideline. Bur oak restoration trials will be assessed for survival at 10 years, over the next two years. Plots have been established and are monitored to assess progress of butternut canker in New Brunswick (NB) and presence/absence of emerald ash borer. A survey was carried out to assess the health of butternut populations throughout the species range in NB and results indicated that the canker has spread through much of the NB range. Assistance was provided for a range-wide microsatellite diversity study initiated by Dr. J. Romero-Severson at Notre Dame University in Indiana.

C. Ramirez completed his PhD studies on *Pinus pinceana* genetics and returned to Mexico. He found that the genetic variance was greatest among regions for most traits and allozyme diversity was also highest among regions, with less but significant variability at the population and family levels. Recommendations were made for genetic conservation. The larger collaborative project continues, with the objective of developing and ultimately implementing a conservation strategy for the species. The project is a task of the Forest Genetic Resources Working Group of the FAO North American Forest Commission. J. Loo also taught short courses in conservation genetics at two Mexican universities fulfilling obligations of the Working Group.

Work has continued to develop and strengthen CON**FOR**GEN (Canadian Programme for Conservation of Forest Genetic Resources) by establishing a steering committee with representation from all provincial and one territorial jurisdictions, as well as a standing technical committee and several subcommittees. CON**FOR**GEN members contributed to populating CAFGRIS (Canadian Forest Genetic Resources Information System), work has been initiated to write a series of conservation guidelines for tree species of concern, and plans are underway to extend the genetic resources gap analysis across Canada. The second forum was organized and held in conjunction with the 2008 CTIA meeting, with the theme "Applied Forest Genetic Conservation".

CONSERVATION AND RECOVERY OF ENDANGERED HARDWOOD SPECIES THROUGH SOMATIC EMBRYOGENESIS

S.E. Pond and S. Tucker

Conservation and recovery of endangered species is important to ensure forest sustainability. Tree species can be endangered by natural disturbances (pests such as insects and diseases and climate change) or man made disturbances. Somatic embryogenesis (SE) is an integral part of conserving genetic resources that are present in the forest now and an important tool for reintroducing the species in the future. We are developing SE protocols for American beech and butternut.

American Beech

Beech bark disease (an exotic insect-disease complex of the woolly beech scale, (*Cryptococcus fagisuga* Lind.) and an ascomycete, (*Neonectria faginata* (Lohman et al.) Castl.) infects American beech in the Maritime provinces and much of the north-eastern United States, and is still spreading. There is no practical chemical control for the beech scale and natural predators are not effective in stopping its spread. Some trees show resistance to beech scale attack. Vegetative propagation of these resistant trees using SE will help conserve the gene pool and restore disease-free beech to the forest. Embryogenic tissue (ET) was induced from zygotic embryos and young expanding leaves of mature trees (Pond 2008). We are currently refining protocols for induction and germination.

Butternut

Butternut is native to eastern North American forests from eastern Ontario, New Brunswick, the New England States (except northern Maine), south to Georgia and west to Missouri. However, Butternut canker (an exotic fungal disease caused by *Sirococcus clavigignenti-juglandacearum* V.M.G. Nair, Kostichka and Kuntz) threatens the species. A single canker can kill small trees while coalescing multiple cankers girdle larger trees. Some trees in the natural population are reported to have a resistance to this fungus. Vegetative propagation of these putatively resistant trees will help conserve the gene pool and restore disease-free butternut to the forest. We were able to initiate ET from non-zygotic tissue which was easily cryopreserved, thawed, and regrown. The ET produced somatic embryos. A few embryos germinated normally and were planted in the greenhouse. Work is ongoing to improve SE induction rates and proliferation and germination of the somatic embryos.

NATIONAL FOREST GENETIC RESOURCES CENTRE

J.D. Simpson and B.I. Daigle

The National Tree Seed Centre is the key activity of the National Forest Genetic Resources Centre. The Seed Centre collects, stores, and provides seed of know origin and quality for research. Another role is long-term storage of seed for *ex situ* genetic conservation. The Centre aims to store seed from throughout the ranges of tree and shrub species in Canada. Seed from most species is stored at -20°C. On-going activities include collecting and processing seed; testing such as germination, moisture content, and 1000-seed weight; setting up and evaluating research trials; and supervising students conducting research projects for theses.

The Seed Centre celebrated its 40th anniversary in 2007. To mark the occasion several activities and events were undertaken to promote the Seed Centre: new seed display panels were developed and produced that included seed encased in epoxy resin, a silhouette of the tree, and a color picture of a reproductive structure for each species; a poster highlighting the three main activities of the Seed Centre (Collection, Collaboration, and Conservation); a revised Impact Note used to promote the Seed Centre; a mail out to clients who received seed over the last 10 years; an article titled "The National Tree Seed Centre Celebrates 40 Years" in the September/October issue of The Forestry Chronicle; and participation in several shows and fairs.

Seed collection is an important activity in order to expand the geographic sampling for species and to add new species to the inventory. Seed is primarily collected from individual trees because many scientists prefer these types of collections, it is a desirable means to sample genetic variation, and it allows for evaluation of variation in storage ability among genotypes. A minimum of 15 trees are sampled in a population. During 2006 and 2007, 520 seedlots from 28 species were collected and stored. An additional 250 seedlots from 9 species were either donated or purchased. Over 6300 seedlots are available for research. The Seed Centre's genetic conservation collections are stored long-term and material will only be made available for unique research or for restoration. Samples are placed in this collection from species or populations that are unique or threatened as well as from other collections to preserve genetic variation from throughout the range of a species. Sample sizes range from 1000 to 10 000 seed depending on species and seed size. Some species in storage are: white spruce (*Picea glauca*), eastern hemlock, tamarack (*Larix laricina*), and limber pine (*Pinus flexilus*) - threatened by white pine blister rust. Just over 3900 seedlots from 30 species are in storage at -20°C.

The Seed Centre is leading a project on collecting and storing ash seed. Ash trees are being attacked and killed in Ontario by an introduced insect, the emerald ash borer, and it is spreading. It seems that all ash species are susceptible to this insect and the existence of the ash resource is threatened. There is an opportunity to collect seed and store it to preserve the genes of ash species so that this material can be used to develop trees resistant to the insect and/or for small restoration plantings after the insect infestation has passed through an area. The material can also be evaluated to determine the pattern of genetic variation within the species and may help identify unique areas for additional sampling. Seed from throughout the range of six species in Canada is being collected from a minimum of 15 individual trees in a population and stored at -20°C. Collecting will continue over a period of years when there are good seed crops. Seed from about 400 collections is in storage.

Short-term operational research projects are conducted on a regular basis to improve seed processing techniques and germination testing prescriptions. Black ash (*Fraxinus nigra*) seed is difficult to germinate and the treatments used to alleviate dormancy require many months. Seed are shed in the autumn and consist of an immature embryo. The seed must be first exposed to conditions that allow he embryo to mature then treated to alleviate dormancy in the embryo. Germination prescriptions vary but all use a combination of warm and cold treatments. When treatments prescribed in the literature and by seed testing rules were applied to seed collected in New Brunswick, germination was low with many viable seed failing to germinate. This prompted setting up a trial evaluating various durations and combinations of warm and cold treatments. Six single-tree collections were used. Three combinations of warm and cold treatments were used: initial cold pre-treatment (0, 60, 90, and 120 days), followed by a warm treatment (90 and 120 days), followed by moist chilling (150, 180, and 210 days). One of the seedlots exhibited shallow dormancy and responded to all treatments. Exposing seed to 60 days of cold pre-treatment, 120 days of warm, and 180 days of moist chilling resulted in 89% of the filled seed germinating.

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GENETIC DIVERSITY: IMPACTS AND ADAPTATION

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POPULATION VIABILITY ASSESSMENTS IN DECLINING TREES AND FOREST TYPES

Alex Mosseler and John 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 of 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 a significant, positive relationship 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 is necessary to minimize the effects of inbreeding and genetic drift and 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 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 tests, further testing of pitch pine was expanded to 10 other locations across the Maritimes in 2003 and 2004 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 were included in these tests. The goal of our 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. Assisted migration of southern components of our flora may become an important forest management option where rapid climate change scenarios may overcome a species’ ability via genetic adaptation or tracking environmental changes through appropriate rates of dispersal and re-colonization.

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 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 and adaptation to abiotic stresses, 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 carbon inventory in a 35-year-old (F1) first-generation cross black spruce experiment with distinct differences in above-ground volume growth. 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 long-lived, shade-tolerant, late-successional species such as red spruce in particular. We established a red and black spruce provenance trial under different silvicultural systems. Research on this site involves a multi-disciplinary 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 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 for commercial breeding efforts. In 2008 and 2009, 30 superior clonal selections were distributed to various agencies based on preliminary biomass harvest results. Our comparative species tests have since been expanded to include seven native species including *S. amygdaloides*, *S. bebbiana*, *S. discolor*, *S. eriocephala*, *S. humilis*, *S. interior*, and *S. nigra* collected from natural populations across eastern and central Canada. A short-term (3 – 5 years) 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) and other poorly drained sites across Canada. The longer-term (5 – 10 years) impact of this work is aimed at the ability of emerging chemical and energy industries to use woody biomass feedstocks. Our current focus is the identification of clones with good rooting ability in *S. bebbiana* and *S. humilis* which often show poor rooting ability. These species are of special interest because they are better adapted to drier sites and may allow us to expand willow culture to drier sites.

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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, measurements were made in five white spruce (*Picea glauca*) half-sib progeny tests and six full-sib progeny tests at 8–10 years. Results were used to select 158 parents for the next generation which were reproduced by grafting. These measurements also served to identify the best crosses to be performed in the future, thus fulfilling reforestation needs by cuttings. Height gains of 22–28% over unimproved sources can be achieved by reproducing the 10 best crosses and propagating them by cuttings. In an effort to capitalize on clonal variation, 184 clones derived from somatic embryogenesis were planted in two tests in 2007 and 2008, and 200 clones are in production for the next 3 years. Five half-sib progeny tests and four full-sib progeny tests were also planted to evaluate the remaining 140 trees of the first-generation base population.

Clones of 14 first-generation seed orchards (out of 18 established) have been evaluated in progeny tests. Cones are now collected from only the best clones for seedling production, which gives an additional height gain of 10% over general collections in seed orchards. As these best clones have never been introduced into the breeding program, 71 of them (representing 3 seed orchards) were grafted in 2008 and will be used later in control crosses.

LARCH TREE IMPROVEMENT

Martin Perron

In spring 2007 and 2008, our intra and interspecific 10 x 10 factorial design with five European larch (EL, *L. decidua* Mill.) and five Japanese larch (JL, *L. kaempferi* [Lamb.] Carr.) was pursued. The scientific goal of this mating design (Experiment 1) and our strategy for the second breeding cycle of *Larix* x *marschlinsii* were published recently (Perron 2008). Since spring 2007, 60% of this mating design has been completed (Perron 2008) but spring 2008 was a bad flowering year. Therefore, that spring, we only collected pollen for future breeding (main second breeding cycle of *Larix* x *marschlinsii*).

In 2007, we hosted the LARIX 2007: 6th International Symposium of the IUFRO Working Group S2.02.07 (http://www.mrnf.gouv.qc.ca/carrefour/english/larix.asp). The Proceedings and Field Trip Guide are available on the Web pages.

JACK PINE TREE IMPROVEMENT

Mireille Desponts

In 2008, the establishment of new jack pine (*Pinus banksiana*) clonal seed orchards and clone banks for breeding for the next generation was completed and controlled crosses for the southern zone of Quebec began. For each breeding zone, polycross and controlled crosses will be carried out using about 300 individuals trees from elite as well as base populations.

BLACK SPRUCE TREE IMPROVEMENT
Mireille Desponts

In spring 2007 and 2008 the mating design for the second-generation of improved black spruce (*Picea mariana*) for Eastern Quebec (Gaspésie and Bas-Saint-Laurent) was completed. Establishment of clonal seed orchards and clone banks for breeding has been completed also for all breeding zones. A new research program on genetics of wood quality has been developed and will begin fall 2008. We plan to integrate wood quality traits in the future selection criteria for black spruce.

NORWAY SPRUCE TREE IMPROVEMENT
Marie-Josée Mottet

Four half-sib Norway spruce (*Picea abies*) progeny tests were established in 2007. The first-generation testing will be completed in 2009 by the establishment of four full-sib progeny tests. To introduce new material and take advantage of weevil resistance information, selections have been made in two 14-year-old open-pollinated progeny tests in the regions of Bas Saint-Laurent and Gaspésie. The 164 clones that will constitute the first part of the next generation population, were planted in the spring of 2008 at the Duchesnay Experimental Station. A new seed orchard will be established in the next several years with the best 50 clones.

Preliminary 10-year results from three provenance tests in the western boreal forest indicate that Norway spruce and white spruce don't perform as well as black spruce in this region. Exceptionally favourable sites should be found in order to obtain successful plantations.

HYBRID POPLAR IMPROVEMENT PROGRAM
Pierre Périnet and Marie-Josée Mottet

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

New clones were selected in 2007 from hybrid populations bred between 1992 and 1995. In spring 2008, 27 clones were introduced to provincial nurseries for propagation. This new plant material, including some Septoria-resistant clones, should be available for planting in 2011. Selection of superior trees in progeny tests from the Bas-Saint-Laurent region (1998 breeding) was also completed in 2008. Once propagated in stoolbeds, clones will be evaluated in farm field tests for early selection.

In the global context of climate change, *Septoria musiva* monitoring, through leaf spot and canker identification in plantations, is maintained in areas traditionally free of the canker (regions 3, 4, and 5) but where some leaf spots or cankers have been unfortunately found since 2001. Resistant clones are already available for Southern Quebec and new ones showing good resistance to the canker will soon be released for planting in Northern areas. Steady collaboration between the pathologist and breeder allows us to carry out studies for resistance to Septoria canker and for monitoring the spread of the disease.

Several projects are taking place within the Réseau Ligniculture Québec network in collaboration with scientists, forest industry partners, and provincial nurseries for steckling production (1-0 rooted cuttings). The poplar research team contributes to different projects on Septoria canker, wood processing and utilization, and molecular genetics studies with scientists from Université Laval, Concordia University, University of Alberta, Forintek, and Canadian Forest Service.

In 2007, we hosted the CPC/PCC 2007, Poplar Culture: a Collaborative Effort from Clone to Mill, the Annual Meeting of the Poplar Council of Canada in the Témiscouata region and Quebec City. The Conference Handbook and the Field Trip Guide are available on the Carrefour de la Recherche forestière Web pages.

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

This project, initiated in 2002, aims at evaluating long-term gains of productivity (volume) and quality associated with first-generation seed orchards. In 2006, three black spruce tests and three jack pine tests were established in the central improvement zone. In 2007, two more black spruce tests were planted in the Gaspésie region, while efforts were turned to establishing four jack pine tests in the black spruce region.

On the other hand, we also measured four 10-year-old black spruce tests from the northern zone in 2005. These tests included seedlings from three unrogued local seed orchards. Gains of 5–10 % in height were obtained when seed orchard material was compared to natural sources.

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

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In Quebec, the integration between different steps in the reforestation process, from seeds to outplanting, is unparalleled. The Direction de la recherche forestière (DRF – the Research Directorate) of the Ministère des Ressources naturelles et de la Faune (MRNF – Ministry of Natural Resources and Wildlife) has a multidisciplinary research group which includes expertise in forest genetics, seed orchard management, seed technology, mass cutting propagation, somatic embryogenesis, seedling production, environmental monitoring, planting techniques, and forest plantation silviculture. In close collaboration with other research agencies (universities, research centres), this group is responsible for acquiring, integrating, and transferring research results related to seed and plant production to the 24 Quebec forest nurseries (6 government and 18 private), the provincial seed centre, and the cutting propagation and somatic embryogenesis centres. DRF projects must answer research and development needs and are subject to rigorous evaluations of both pertinence and scientific value by independent committees. The evaluation of these projects, as well as those submitted by other research institutions, is administered by an independent agency (Fonds québécois de la recherche sur la nature et les technologies). The present report outlines the salient facts and results of the principal studies related to seed and plant production: seed germination and sowing factors, assessment of water activity, production of hybrid larch seed, genetics of seedling production, characterization and early selection of hybrid poplar clones, vegetative propagation by rooted stem cuttings and somatic embryogenesis (SE), as well as controlled crosses of plants produced by SE.

SEED AND POLLEN

Seed Germination and Sowing Factors

In Quebec in 2007, 80% of the conifer seeds sown were from genetically improved sources. This sizable proportion is a result of the significant investment that the MRNF has made in the areas of genetic improvement and seed orchard establishment. For the principal commercial species, white spuce (*Picea glauca* [Moench] Voss), black spruce (*Picea mariana* [Mill.] BSP), and jack pine (*Pinus banksiana* Lamb) we investigated the possibility of reducing the number of seeds allocated to seedling production. This study has led to a 20% reduction in the number of seeds required to produce white spruce and jack pine, without adversely affecting the percentage of occupied container cavities (Colas et al. 2007a, 2007b).

Production of Hybrid Larch Seed

As a result of technology developed by the DRF research group, hybrid larch seeds (HL, *Larix* x *marschlinsii* Coaz) have been successfully produced in covered seed orchards at the Berthier forest nursery since 2006 (Colas et al. 2007a, 2008). Thanks to the operational production of new grafts, seed produced from this seed orchard is from the best genetic selections available in Quebec. The seeds are subsequently multiplied by cutting propagation to get the maximum benefit from the superior quality seed.

Water Activity

In 2006, the DRF, in collaboration with the Institut de recherche pour l'ingénierie de l'agriculture et de l'environnement (Cemagref, Nogent sur Vernisson, France), initiated a project financed within the framework of the 61^{e} Commission permanente de la coopération franco-québécoise. The objective of the project is to implement water activity assessment as a means of managing the moisture status of seeds and pollen. This simple and non-destructive technique, which is currently in use at two French forest seed centres, will permit quick, reliable, and economical control of the quality of pollen and seed used in Quebec reforestation programs. In addition, it will limit the systematic recourse to standard germination testing conducted at the Centre de semences forestières de Berthier (Forest Seed Centre, Berthier). Preliminary results involving the application of water activity measurements have permitted characterisation of the moisture status of black spruce and jack pine seeds in an effort to improve their long-term conservation in seed banks (Baldet et al. 2007a, 2007b; Colas et al. 2007a). The application of this technique to other species is presently being studied.

SEEDLING PRODUCTION

Genetics and Seedling Production

Since May 2004, researchers from different research centres (Université Laval: Dr. Hank Margolis and Canadian Forest Service: Dr. Jean Beaulieu) have been collaborating with us on a project to determine whether the root mass of large dimension white spruce seedlings can be genetically improved. The results of this study, comparing the growth of 1+0 and 2+0 seedlings from 75 half-sib families under nursery conditions, showed significant family variability for several morphological (height, root collar diameter, root length, surface area and dry mass, needle length, and surface area) and physiological (N, P, and K content) variables evaluated at the end of the growing season (Carles et al. 2006, 2007, 2008). With the exception of seedling height, the percentage of the variation explained by family variability decreased between the first and second growing seasons as shown by: 1) a reduction in the maternal effect associated with seed size, 2) a reduction in diameter growth due to lateral competition between adjacent seedlings in the containers, and 3) a reduction in root growth due to the restrained cavity volume (320 cm^3). The percentage of variation in seedling height explained by family variability remained constant (28%) at the end of both growing seasons. This implies that, regardless of the effort that is made to homogenize cultural practices, approximately ¼ of the variability will remain as a result of genetics. In addition, genetic correlations between the above- and below-ground characteristics of the seedlings indicate that selection for the best performing families on the basis of root collar diameter is also, indirectly, a selection for families with the highest root dry mass. The efficiency of this indirect selection was 70.5% and 59.3% at the end of the first and second growing seasons, respectively. By contrast, the efficiency of indirect selection for families on the basis of height is strongly negative (-61.5%) in terms of root dry mass at the end of the second growing season.

Growth of cuttings, taken from 3+0 seedlings representing the same 75 half-sib families, is presently being followed in a nursery study. The family variability of the rooting ability of the cuttings as well as the morphological and physiological characteristics of the B+1 and B+2 plants are being evaluated.

Another aspect of this study will permit comparison of 1+0 and 2+0 seedlings from the 10 most currently used seed orchards in Québec. This portion of the study will clarify whether certain lots of white spruce seed exhibit different growth characteristics under nursery conditions.

Early Characterisation and Selection of Hybrid Poplar Clones

Innovative studies, involving cultural techniques and the early characterisation and selection of hybrid poplar clones have been applied under forest nursery conditions. The criteria used for the early selection of clones include growth, carbon sequestration, frost sensitivity, drought resistance, and stem form (Lamhamedi et al. 2007). Combined with the optimisation of certain nursery cultural techniques, this characterisation may contribute to the establishment of certification criteria for hybrid poplar clones. The goal of the research is to favour rapid establishment and superior juvenile growth of poplar plantations.

MASS CUTTING PROPAGATION

In Quebec, cutting propagation is used for large scale multiplication of the best controlled crosses. In 2007, objectives for the production of conifer seedlings via cutting propagation reached a peak of 5.15 million (Tousignant et al. 2007a). Cutting propagation in outdoor double walled enclosures has benefited from many improvements, notably the management of environmental conditions (temperature, light, and vapour pressure deficit) (Tousignant et al. 2007a, Rioux et al. in press). In 2006, the MRNF installed new propagation facilities at the Berthier and Grandes Piles forest nurseries, in addition to those at the Cutting Propagation Centre at the Saint Modeste nursery. We are actively involved in the ongoing transfer of knowledge and expertise with these new centres.

Characterisation of Controlled White Spruce Crosses

In the case of white spruce, the use of seeds of different sizes and from different controlled crosses leads to a certain heterogeneity in the growth of stock plants used for cutting propagation. A study of the sources of this heterogeneity confirmed that both seed size and controlled cross had significant effects on stock plant growth (Lamhamedi et al. 2006a) and cutting yield. For example, the average yield from stock plants originating from the smallest seeds was only 57% of that from stock plants grown from the largest seeds. However, the rooting ability of the cuttings was not significantly affected by these factors. The segregated culture of stock plants originating from smaller seeds enables the nurseryman to adapt the growing regime to meet the cultural objectives.

SOMATIC EMBRYOGENESIS

As a result of advancements in genetic improvement programs, the MRNF has access to very high quality genetic material. One of the uses for this material is the controlled crossing of elite individuals for the purpose of producing clones by SE. The MRNF is progressively integrating SE techniques into breeding programs. As a result, SE, which is used for the principal commercial forest species, will permit the large scale multiplication and accelerated use of the best performing clones. Due to the advancement of its genetic improvement program, the first species being targeted is white spruce. The aim of our research is to improve SE techniques, establish clonal tests, produce stock plants for cutting propagation, and study clonal variation. The approach used by the MRNF is distinguished by its thorough examination of the possibilities offered by this technology in the scheme of forest seedling production (Lamhamedi et al. 2006b, Tremblay et al. 2006).

The implementation of this technology at an operational scale is already in progress, thanks to close collaboration between the Saint Modeste forest nursery (DGPSP – Direction génerale des pépinières et des stations piscicoles) and the DRF. This collaboration permits the optimization of SE techniques under both laboratory and forest nursery conditions (Tremblay et al. 2007a, b, c), with very positive results. In just two years, more than 38 000 plants, representing 515 white spruce clones, have been produced at Saint-Modeste, and are intended for clonal tests. A total of 1,119 clones, from 41 recommended controlled white spruce crosses, were placed in cryopreservation. These very encouraging results were obtained using a standard *in vitro* protocol, optimized and specific to white spruce, regardless of the crosses used. As a result of continuous refinement of the different steps in the process, SE techniques are progressively being scaled up to an operational level, notably by experiments leading to the development of a bioreactor as well as large scale germination (large containers in a closed circuit with continuous monitoring of environmental variables) and direct seeding (sowing) of mature somatic embryos.

To complement the above studies, we developed a method of individually characterizing the morphophysiology of white spruce clones during the different stages of the SE process and under nursery conditions[1]. The method is applicable to clones being used for clonal tests of growth variables, nutritional status, and shoot and needle architecture. These variables will later be correlated with the observed performance after outplanting so that the desired clones can be selected as early as possible. The suitability of these clones for use in the mass cutting propagation program is also evaluated. In effect, the MRNF is banking on two complementary vegetative propagation techniques, cutting propagation and somatic embryogenesis, for selecting and multiplying the best performing genetic material. A preliminary study on the use of somatic Norway spruce (*Picea abies* [L.] Karst.) stock plants showed the importance of clonal effects on stock plant cutting yield, shoot architecture, and rooting ability of the cuttings. Studies presently in progress also show that white spruce somatic stock plants from 76 clones being used for clonal tests had rooting percentages of between 71% and 100%.

Production of Seeds from Somatic Embryogenesis Clones

Quebec has some of the oldest clonal tests planted in Canada. We have been able to show precocious male and female flowering of somatic white spruce and black spruce clones. Studies of flower development, pollen quality and seed production by somatic clones show that these clones may be used as seed trees (Colas and Lamhamedi 2006), or as parent trees in controlled crossing for the production of elite seed. To our knowledge, these results are unique in the field of SE of coniferous species.

CONCLUSION

The production of high quality seedlings is the result of the acquisition and development of new knowledge and its continuous transfer and implementation at an operational scale (Tousignant et al. 2007b). In close collaboration with researchers and graduate students from different research centres, the seed and plant production research group has emphasized the transfer of expertise and knowledge and the integration of research results in Quebec forest and horticultural nurseries. This transfer has been realized through scientific and technical publications, visits and conferences, as well as workshops and training sessions on particular themes. During these events (Collective, 2007) researchers, technicians, nurserymen, and graduate students (from different research centres in Quebec or elsewhere) are invited to present their research results. The content of these conferences is principally directed towards forest nurserymen in Quebec, as well as managers who work in the field of artificial regeneration.

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FUNCTIONAL AND DIVERSITY GENOMICS AT UNIVERSITÉ LAVAL

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INTRODUCTION

Over the last two years, a number of major projects have been initiated and pursued at the national and international levels. Owing to satisfactory progress, the Canada Research Chair in Forest and Environmental Genomics (Tier 1, lead by J. Bousquet) has been renewed for a second period of seven years (2008 to 2015). The deployment of the second phase of our major spruce genomics program *Arborea* (lead by J. MacKay and J. Bousquet) has proceeded smoothly. The emphasis is on expanding spruce genomic resources and developing molecular breeding tools to improve growth and wood characters of white spruce (*Picea glauca*) and black spruce (*P. mariana*). It is supported by Genome Canada and Genome Québec for a period of four years. It is also supported by the Canadian Biotechnology Strategy (CBS), the Canadian Forest Service (CFS) and the Fonds québécois de la recherche en nature et technologie (FQRNT). It involves research scientists from Université Laval (N. Gélinas, P. Rigault), the University of Alberta (J. Cooke), the CFS (N. Isabel, A. Séguin), the Canadian Wood Fibre Centre (J. Beaulieu), the Quebec Ministry of Natural Resources (MRNFQ) (M. Perron, A. Rainville, M. Desponts), Agriculture and Agri-Food Canada (A. Bertrand), and Paprican (L. Wallbäcks).

Work related to the Phylogeographical Atlas of Canadian Conifers (lead by J. Bousquet) has also levelled off with the support of the Natural Science and Engineering Research Council of Canada (NSERC). Collaborators in this project are from CFS-Québec (J. Beaulieu), the BC Ministry of Forests and Range (B. Jaquish), University of Alberta (F.C. Yeh), University of Guelph (A. Fazekas), CFS-Fredericton (J. Loo), and the Nova Scotia Department of Natural Resources (M. Lemieux). An international program on the phylogeography and conservation genetics of endemic conifers of Mexico (lead by D. Khasa and J. Bousquet) has also been pursued in collaboration with J. Beaulieu (CFS), L. Eguiarte (University of Mexico and F.T. Ledig (University of California at Davis) with financial support from the Quebec and Mexican governments.

During the last two years, the group has hosted B. Boyle as senior research associate as well as several postdoctoral researchers, including C. Bomal, J.P. Jaramillo-Correa (now at CIFOR – INIA, Spain), M.-C. Namroud, and N. Pavy. Many graduate students were involved in the various research projects, including at the MSc level: H. Bandali, V. Chiflot, S. Girardi, G. Guigou Cairus, S. Omondi, P. Talbot, H. Yin, and at the PhD level: R. Adeleke, A.R. Atangana, F. Bedon, M. Bouillé, J. Godbout, D. Hinsinger, S. Legay, P. Lenz, C. Ouinsavi, B. Pelgas, F. Pitre, J. Prunier, and C. Rochon. Many of these students were co-supervised by adjunct professors J. Beaulieu, N. Isabel, and A. Séguin (CFS).

PHYLOGEOGRAPHY

J. Bousquet

We pursued the development of mtDNA markers and have succeeded in resolving the large-scale geographical structure of broadly distributed or endemic conifers in various parts of the world. Because they are maternally inherited, mtDNA markers are less dispersed than cpDNA or nuclear markers and their geographical patterns of genetic variation are more likely to reflect history. New contributions include the work on western larches (Khasa et al. 2006) in collaboration with B. Jaquish (BC Ministry of Forests and Range), and that on lodgepole pine (*Pinus contorta*) (Godbout et al. 2008) in collaboration with A. Fazekas (University of Guelph), F.C. Yeh (University of Alberta), and C. Newton (Vizon Scitech). Although based on nuclear microsatellites, the larch study permitted the identification of at least two distinct lineages for each of western larch (*Larix occidentalis*) and alpine larch (*L. lyallii*), presumably arising from refugia located east and west of the Rocky Mountains. The signature of recent bottlenecks was evident in subalpine larch (Khasa et al. 2006). The range-wide lodgepole pine mtDNA study revealed a highly structured genogeography: at least four genetically distinct and geographically circumscribed maternal lineages could be recognized with various zones of contact between them. The differentiation among lineages coincided with the various mountain ranges in the Pacific Northwest. A lineage also coincided with the possible existence of a glacial refugium on the coastal islands of British Columbia (Godbout et al. 2008).

For eastern and central Canada and the northeastern United States, range-wide phylogeographical studies are progressing on a number of fronts for *Abies balsamea*, *Larix laricina*, *Pinus strobus*, *Taxus canadensis*, and *Tsuga canadensis*, as part of the Phylogeographical Atlas of Canadian Conifers. A study involving cpDNA and mtDNA markers has been initiated regarding the hybrid zone in Alberta between *Pinus contorta* and *P. banksiana*, in collaboration with F.C. Yeh.

Another major study was completed in Scots pine (*P. sylvestris*) with sampling covering most of the natural distribution of the species from Western Europe to Asia, in collaboration with J. Beaulieu (CFS), K. Naydenov, and F. Tremblay (Université du Québec, Abitibi-Témiscamingue). Genetically distinct maternal lineages (mtDNA) were identified in Asia Minor and most surprisingly, in northern central Europe. These results suggest the revisiting of scenarios of post-glacial recolonization and gene conservation in the region (Naydenov et al. 2008).

Two phylogeographic studies of Mexican endemic spruce and fir taxa have been completed (Jaramillo-Correa et al. 2006, 2008). Most investigated species harbored genetic signatures typical of relictual populations that were once much larger. The genogeographic structures were often complex with lack of congruence between maternal and paternal lineages. The overall genetic picture is one of severely restricted taxa suffering from Holocene collapse, drift and loss of genetic variation. With respect to the accumulating evidence for further climate warming in the region, the outlook for the future appears grim unless drastic conservation measures are taken. Some of these studies were commissioned by the Forest Genetics Resources Working Group of the North American Forest Commission of the FAO, and they involved collaborations with J. Beaulieu (CFS), L. Eguiarte (University of Mexico), and F.T. Ledig (University of California at Davis).

MOLECULAR ECOLOGY AND ENVIRONMENTAL GENETICS

D. Khasa

Work on spontaneous introgression from exotic poplars into native congeners in eastern Canada has made significant progress, in collaboration with N. Isabel and co-workers (CFS), P. Périnet (MRNFQ), and with the support of the Canadian Biotechnology Strategy, Ligniculture Québec, and NSERC. Using species-specific SSR markers, previously defined for five important poplars and their interspecific hybrids (Khasa et al. 2005), MSc student G. Guigou Cairus assessed the rate of gene flow from hybrid poplar plantations containing exotic components (i.e., *Populus maximowiczii*, *P. nigra,* and *P. trichocarpa*) into natural populations in Quebec (i.e., *P. balsamifera* and *P. deltoides*). Fortuitous hybrids between exotic and native species were detected, as well as between the native species *P. balsamifera* and *P. deltoides*. Site-to-site variation as well as year-to-year variation were noted, and introgression was more severe in peripheral populations (Guigou Cairus 2008). Under the leadership of N. Isabel (CFS), these studies have been expanded to other regions of Canada. At the international level and with funding from AUF and NSERC, the group has contributed to the development of novel microsatellite DNA markers for threatened African endemic tree species, including *Milicia excelsa* (Moraceae) and its closely related allie, *Milicia regia* (Ouinsavi et al. 2006). When used in a population survey, these markers revealed regional heterogeneity in the population genetic structure of *M. excelsa* (Ouinsavi et al. 2008).

The molecular ecology and ecophysiology of mycorrhizal fungi remains an important aspect of our research program. Using single-locus codominant microsatellite markers developed for *Laccaria bicolor* (Jany et al. 2006), MSc student A. Gagné monitored the persistence of an introduced mycorrhizal strain into the environment (Gagné et al. 2006). Other strategies relying on PCR-RFLP and DNA sequence analyses (e.g., of SSU rRNA genes) have been used to study the effects of soybean intercropping on the arbuscular mycorrhizal diversity of poplar (Chifflot et al. 2008).

Based on *in vitro* and *in vivo* studies, three promising ECMF species (i.e., *Hebeloma crustiniliforme*, *Suillus tomentosus,* and *L. bicolor*) were selected for use in the reclamation of saline-alkaline habitats and more specifically, oil sand tailings (Bois et al. 2006a,b). Field trials have been established on Syncrude Canada sites (Fort McMurray, Alberta) using greenhouse grown conifer seedlings (*Picea glauca* and *Pinus banksiana*) with the three above-mentioned ECMF. One of the main conclusions of the study was that the inoculation of conifer seedlings with an ECMF consortium comprising *H. crustiniliforme*, *S. tomentosus,* and *L. bicolor* resulted in the best survival and growth (Bois et al. 2006b). Inoculation of alders (*Alnus crispa*) with a mixed *Frankia*-ectomycorrhizal fungal inoculant for use in land reclamation has been shown to be promising under commercial nursery production conditions (Quoreshi et al. 2007a). A review of actino-mycorrhizal alder technology was completed (Roy et al. 2007). A study on the effectiveness of mycorrhizal inoculation in the nursery on root colonization, growth, and nutrient uptake of aspen and balsam poplars was completed (Quoreshi and Khasa 2008). We also completed an evaluation of the field performance of conifer and hardwood species five years after nursery inoculation in the Prairie Provinces (Quoreshi et al. 2007b). With the financial support of Syncrude Canada, the CONRAD consortium, and NSERC, a research project involving the isolation, identification, and selection of arbuscular and ericoid mycorrhizal fungi for reclamation of oil sand tailings is now underway.

FUNCTIONAL GENOMICS

J. MacKay

Our functional genomics research program focuses primarily on wood formation and the genetic basis of wood properties. A major aim is to identify gene sequences to be incorporated into association studies related to wood properties, which in turn should lead to practical tools and methods to enable the selection and breeding of trees based on DNA markers. This research is part of the *Arborea* genomics initiative (*Arborea* II) funded by Genome Canada, Genome Québec, and several other partners from the public sector. A particular emphasis has been placed on putative regulatory genes of secondary xylem (wood) formation in both poplars and conifers (MacKay et al. 2004, Bedon et al. 2007, Legay et al. 2007). This work involved the collaboration of A. Séguin (CFS) and J. Grima-Pettenati (CNRS, France). This program is also complemented by a number of investigations on the macro-phenotypic variation in wood characters of hybrid poplars, in collaboration with T. Zhang (FP Innovations) and P. Périnet (MRNFQ), and the support of Ligniculture Québec (Pliura et al. 2006, 2007; Yu et al. 2008).

Random cDNA sequencing efforts have also been pursued in white spruce and augmented with new higher throughput sequencing technologies (454 sequencing platform). In total, high quality sequences have been obtained for over 200 000 ESTs from 30 different cDNA libraries created from diverse organs and tissues. Sequence information from partial analyses is publicly available in the database ForestTreeDB (Pavy et al. 2007) which can be easily queried via www.arborea.ulaval.ca . More extensive data analyses and databases are being developed in collaboration with P. Rigault (Centre de recherche du Centre hospitalier, Université Laval (CRCHUL)).

This gene discovery effort has enabled the development of gene microarrays to support several ongoing gene expression studies. These investigations included secondary xylem transcript profiling experiments conducted in a 30-year-old white spruce progeny trial, aiming to characterize genetic variation in terms of gene expression and to identify genes whose expression correlates with phenotypic variation in such traits as cellulose microfibril angle (MFA), cell wall thickness, and wood stiffness (MOE). These wood property traits have been determined by Silviscan analysis in collaboration with J. Beaulieu (CFS) and P. Watson and L. Wallbacks (FP Innovations). In collaboration with J. Cooke (Univ. of Alberta), our program is also investigating physiological aspects of development that may impact upon wood properties and end uses, such as nitrogen availability in poplars (Pitre et al. 2007a,b) (NSERC).

STRUCTURAL AND POPULATION GENOMICS WITH IMPLICATIONS FOR MOLECULAR BREEDING

J. Bousquet

In collaboration with N. Isabel (CFS) and with funding from the CBS and NSERC, composite genetic maps of black spruce, white spruce, and Norway spruce (*Picea abies*) (also involving V. Acheré and S. Jeandroz from University of Nancy, France) were assembled with nearly 1,000 markers each, including markers of anonymous position (AFLPs), SSRs, and markers of expressed genes (ESTP). Dozens of anchor markers were positioned, which allowed to compare the genome macro-structure between these species and with Douglas-fir (*Pseudotsuga menziesii*) and loblolly pine (*Pinus taeda*) (Pelgas et al. 2006, Bousquet et al. 2007). Chromosome fission, segmental duplications, and translocations were identified. As part of *Arborea* and in collaboration with C. Paule (Univ. of Minnesota), large-scale searches of SNPs in gene assemblies derived from ESTs have also been conducted in white spruce, with over 12 000 SNPs identified (Pavy et al. 2006). The conditions to minimize the rate of false positives were determined and the categorization of SNPs in various classes was achieved. A registry of SNPs (Pavy et al. 2006) has been created as part of ForestTreeDB (accessible via www.arborea.ulaval.ca). These SNPs are most useful for gene mapping as well as in association and population studies aimed at finding gene SNPs of adaptive significance.

As part of the *Arborea* collaborative project we have completed the testing of a high-throughput SNP genotyping approach where thousands of SNPs identified from resequencing expressed genes or from *in silico* identification in EST gene assemblies were genotyped for hundreds of individuals. The genotyping strategy relies on the Illumina bead-array technology and the highly multiplexed GoldenGate allele-specific extension assay, where up to 1,500 SNPs can be genotyped at a time (Pavy et al. 2008). A success rate in excess of 70% of the SNPs with more than 98% of trees genotyped for each successful SNP was obtained for both white spruce and black spruce, which compares well with success rates reported in the the literature for humans and mice.

In collaboration with N. Isabel and J. Beaulieu (CFS), current *Arborea* studies with SNP arrays involve the mapping of thousands of expressed genes on the genome of white spruce and black spruce, co-localization studies with QTLs, association studies, and identification of outlier SNPs in natural populations. Collaboration is pursued with K. Ritland (University of British Columbia) and J. Cottrell (UK Forestry Commission) to test these SNPs in other spruce species. The mapping of several hundred genes revealed that the genomes of white spruce and black spruce are highly conserved, in terms of gene synteny and macro-colinearity (Pavy et al. 2008), which may ease the transfer of molecular breeding applications between these species. Genetic differentiation among white spruce natural populations was also monitored for more than 500 gene SNPs. These populations were also grown in common garden studies (CFS) where they were characterized for a number of quantitative characters. A few dozen outlier SNPs could be identified as candidates for local adaptation. Post-hoc relationships were found between SNP differentiation, gene functions, population phenotypic attributes, and bioclimatic parameters of population sources (Namroud et al. 2008). These gene SNPs are currently being monitored at the range-wide level and will be tested in association studies.

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ADVANCES IN FOREST GENETICS, GENOMICS AND BIODIVERSITY AT THE CANADIAN FOREST SERVICE, QUEBEC REGION

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This report summarizes the progress of the Canadian Wood Fibre Centre and Laurentian Forestry Centre (LFC), both of the Canadian Forest Service (CFS, SCF), for research in forest genetics, genomics, molecular ecology, and biodiversity for the period 2006–2008. Our activities have mainly focussed on research on spruce genomics and environmental genomics of poplars. This work was made possible through grants obtained from the Canadian Biotechnology Strategy – Genomics Funds and through our participation in the *ARBOREA* project (Mackay, Bousquet and others, Université Laval), a major initiative, funded by Genome Canada and Genome Quebec, to develop molecular breeding in softwood trees. We have also continued to invest considerable time and effort in population genetics studies of forest tree species as part of our participation in the activities of the Canada Research Chair in Forest and Environmental Genomics (Tier I), via a Natural Sciences and Engineering Research Council strategic grant for the development of a Phylogeographic Atlas of Canadian conifer tree species (J. Bousquet and J. Beaulieu), as well as a grant from the Ministère développement économique, innovation et exportation du Québec to collaborate with Mexico (D. Khasa, J. Bousquet, and J. Beaulieu). On another front, studies of gene-flow among poplar and larch species were made possible by grants from the Canadian Regulatory System for Biotechnology (N. Isabel). Parts of this project were conducted in collaboration with Université Laval (J. Bousquet, D. Khasa), Forêt Québec (P. Périnet and others), Agri-Food Canada, Saskatchewan (B. Schroeder), and other members at LFC (A. Séguin). Other research activities on wood characteristics of a tree species growing in the Amazon region were also made in collaboration with Roger Hernandez of Université Laval. A project was carried out on the genetics of white spruce root systems thanks to funding from the Fonds québécois de recherche sur la nature et les technologies (H. Margolis, M. Lamhamedi, and J. Beaulieu).

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TREE GENOMICS

During the last two years, grants were obtained from the Canadian Biotechnology Strategy to initiate one project on QTL mapping and an association study of growth-related traits and another one on association mapping of wood traits, both in white spruce (*Picea glauca*). These grants were also used as co-funding for two of the research activities of the *Arborea* Project, a major initiative, funded by Genome Canada and

Genome Quebec for developing molecular breeding in softwood trees and co-led by J. Mackay and J. Bousquet (Université Laval). For wood traits, a discovery population of 495 trees was assembled as well as a validation population of 200 trees. Increment cores were collected from these trees and phenotypic values for a variety of wood traits and properties were assessed at FPInnovations – Paprican's facilities in Vancouver. Hence radial profiles of over 25 wood physical properties and about 10 wood chemical traits were obtained for each of the 695 trees. An Oligo Pool All array including 1,536 single nucleotide polymorphisms (SNPs) harboured by 625 candidate genes for wood formation was built and the discovery population was genotyped using the Illumina GoldenGate Genotyping assay. Candidate genes were either selected based on expression profiling studies and on literature reports or were detected *in silico* from the gene sequences. Association studies are underway and the first gene variants associated with wood traits in white spruce were identified. Preliminary results show that identification of candidate gene variants that can explain a significant part of variation in some wood traits is possible. Models to predict wood chemical properties using NIR and Raman spectroscopic scans were also developed. A publication is in preparation with *ARBOREA* and FPInnovations colleagues.

Over 500 SNPs representing 345 genes including many regulatory genes were used to identify genes targeted by selection in six natural white spruce populations. Eight of these genes were identified as targeted by selection by comparing the differentiation in allele frequencies among populations (F_{ST}) using both infinite alleles and Bayesian. Moreover, 47 other genes showed a trend of local adaptation to environmental conditions, most of them being regulatory genes. These results will help manage white spruce genetic resources. This is the first time that such a study was carried out for a tree species in Canada. Results were published (Namroud et al. 2008).

Quantitative Genetics of Wood Properties

A model of stand diameter distribution was developed for unthinned white spruce plantations using data collected in a network of commercial plantations established in Quebec. Modelling stand diameter distributions is essential for assessing the structural heterogeneity of a stand and calculating some useful stand yields such as stand volume and diameter-specific recoverable products and values. This was achieved through collaboration with S.Y. Zhang of FPInnovations – Forintek and G. Prégent of Ministère des Ressources naturelles et de la Faune (MRNF). Results will be presented in a scientific paper.

Breeding objectives to achieve further value must be defined on the basis of economic weights of selection traits. To estimate these economic weights, a vertically integrated enterprise simulation model was developed in which pulp and structural lumber product flows and cost/income flows are followed from plantation establishment to production of lumber and pulp. Several biological and production parameters such as wood chemistry and physical properties, labour costs and productivity, tree spacing, and many other parameters can influence production costs and wood/products flow. In the model, white spruce plantation production scenarios, which are chosen using yield tables, are automatically optimized using net present value (NPV). Intensive scenarios of thinning operations are simulated in light of the province of Quebec's legislation and are applied on precise stem diameters, depending on the types of thinning chosen. Stem diameter distributions are generated combining a Weibull's distribution percentile recovery method and volume production tables. Harvesting costs and productivity are calculated using customizable models. Recovery models are used to determine for each stem the proportion of lumber, pulp, and sawdust produced. The complete enterprise model was developed using a Microsoft EXCEL spreadsheet. Economic weight-based breeding objectives will in the near future be determined using Monte Carlo sensitivity using the @RISK for EXCEL software.

Traditional wet chemistry methods to measure wood characteristics are lengthy, tedious, and costly. Often, these time and cost constraints limit the acquisition of chemical wood properties (lignin, carbohydrates, and extractives) that may be valuable. Therefore, rapid, cost-effective methods are necessary for measuring the large sample sizes that are essential for data acquisition that assists with effective decision-making. Empirical models were built to predict chemical composition (lignin, carbohydrates, and extractives) in white spruce using near infrared (NIR) and Raman spectroscopy. This work was done through our participation in the *ARBOREA* project. Results will be presented in a scientific paper.

The first efforts to develop molecular breeding for wood traits in black spruce (*Picea mariana*) were also deployed. A discovery population of 400 samples was assembled by collecting wood increment cores in a progeny test in New Brunswick, thanks to the collaboration established with the New Brunswick Tree Improvement Council (Kathy Tosh). Wood increment cores were also collected on 100 more trees in a range-wide provenance trial in Quebec (Mireille Desponts), Ontario (Paul Charrette), and Alberta (Leonard Barnhardt) to build a validation population to get access to essential pedigree material. Foliage was also collected on the 500 trees to extract DNA in order to genotype them. Radial profiles of wood density, stiffness, and microfiblil angles were obtained through a contract with FPInnovations – Paprican in Vancouver. The search for SNPs in gene sequences amplified using primer pairs developed for white spruce was achieved for over 1,500 contigs, using white spruce cDNA sequences available through our partnership with *ARBOREA*. The development of an Oligo Pool All (OPA) array to genotype the discovery population was initiated and will be completed in the next period as well as the association studies needed to identify the gene variants that have the potential to be used as selection tools.

QTL Mapping and Association Study of Growth Related Traits

In conjunction with the *ARBOREA* project two complementary approaches, QTL mapping and association study, are being used for large-scale mapping of candidate genes related to growth and particularly those regulating bud set. For both approaches, macro and molecular phenotyping are currently in progress. The Discovery QTL population composed of 500 siblings was clonally replicated. A mixed strategy was employed, in which all clones remained under natural outdoor conditions at the Valcartier Experimental Station of the CFS during the bud flush, growth, fall acclimation, and overwintering periods of the 2006–2007 and 2007–2008 seasons. During the bud set period, however, one half of the replicates were moved to Soils and Crops Research and Development Centre (SCRDC) of Agriculture and Agri-food Canada (AAFC, Quebec) under controlled conditions of constant temperature and declining photoperiod. Macro-phenotyping data for bud flush, growth, and bud set that were collected at both experimental sites during springs and summers of 2006, 2007, and 2008 are currently being used to perform QTL analyses. A manuscript reporting the results of the first two years is in preparation (Pelgas et al.).

Additional clonal replicates from the discovery population were used to assess the biochemical changes occurring during bud set and growth cessation in response to declining photoperiod. This experiment was performed under controlled environmental conditions at the SCRDC of AAFC, Quebec during the summer of 2006. Spruce trees were sampled 8 times from mid-July to the end of September, i.e., from a photoperiod of 15.7 hours to 12.2 hours daylight. Soluble sugars, soluble amino acids, and starch were quantified in apical bud, current year needles, current year stem, 2-year old stem, 3-year old stem, and roots. Soluble proteins will also be quantified. A manuscript reporting these results is in preparation (Dhont et al.).

The association populations (Discovery and Validation) were assembled from 1,000 unrelated families. Seedlings were clonally replicated by rooted cuttings during the summer of 2006. Cuttings were submitted to a series of growth and rest periods under controlled conditions (alternation between cool chamber and greenhouse) at the LFC during the winter of 2006. Seedlings were transferred to natural outdoor conditions during the summer of 2007 and repotted. Collection of phenotypic data for bud flush started in spring 2008 and will be continued for bud set during the summer of 2008. Phenotypic data will be collected once again in 2009.

Additional seedlings from the association population were used to assess the biochemical changes that occur at the onset of bud set, in response to an abrupt switch from long (16 hours) to short (8 hours) photoperiod. This experiment was performed under controlled environmental conditions at the University of Alberta (Dr. Janice Cooke). Trees were sampled 7 times: on "day 0" (abrupt switch to short photoperiod) and then 1, 3, 7, 14, 28, and 70 days after the switch to short day conditions. Soluble sugars, soluble amino acids, and soluble proteins will be quantified in apical bud, current year needles, current year stem, 2-years old stem, and roots at the SCRDC of AAFC, Quebec in 2008–2009.

Other Quantitative Genetics Related Studies

During the last review period, a GIS application was developed to estimate and map relative risks of maladaptation when black spruce and jack pine (*Pinus banksiana*) seed sources are transferred for reforestation in Quebec. This application was upgraded this year to include the impact of climate change on estimates of relative risks. The application was transferred to the MRNF division responsible for seed and seedling production.

Biophysical site index models were developed for seven conifer species used for reforestation in Quebec. These models make it possible to predict their site index (average height of dominant trees at 25 years) for any location based on climatic and soil data. A new GIS application was developed to estimate and map plantation yield of these seven species. This application uses biophysical site index models in combination with existing growth and yield models. Plantation yield can be estimated for any age and spacing combination and is mapped for each land class using the Quebec land region classification system.

A series of five scientific papers on the genetics of wood properties of *Calycophyllum spruceanum* was published during the review period. The papers report results of studies carried out by a PhD student who graduated in 2007, using provenance trials established in the Peruvian Amazon and who was supervised by R. Hernandez (Université Laval) and co-supervised by one of us. Traits analyzed were wood density, shrinkage, mechanical properties, and colour. It was shown that most of the traits studied were under moderate to strong genetic control and genetic correlations were also estimated to see the impact of the selection for one trait on the others. It appears that good progress could be made by selection and breeding.

Phylogeography

A novel polymorphic region of the Scots pine (*Pinus sylvestris*) mitochondrial genome was found, i.e., the intron 1 of *nad7*, with three variants caused by insertions-deletions. Primers were designed to amplify this region. From 986 trees distributed among 54 populations, four distinct multi-locus mitochondrial haplotypes (mitotypes) were detected based on the three *nad7* intron 1 haplotypes and two previously reported size variants for *nad1* intron B/C. Population differentiation was high (G_{ST} = 0.657) and the distribution of the mitotypes was geographically highly structured, suggesting at least four genetically distinct ancestral lineages. A cosmopolitan lineage was widely distributed in much of Europe throughout eastern Asia. A previously reported lineage limited to the Iberian Peninsula was confirmed. A new geographically restricted lineage was found confined to Asia Minor. A new lineage was restricted to more northern latitudes in northeastern Europe and the Baltic region. The study was carried out in collaboration with Francine Tremblay (Université Québec à Temiscaming) and Jean Bousquet (Université Laval) teams and published in BMC Evolutionary Biology.

Range-wide variation in Chihuahua spruce (*Picea chihuahuana*) was investigated using maternally inherited mitochondrial (mtDNA) and paternally inherited chloroplast (cpDNA) DNA markers. Among the 16 mtDNA regions analyzed, only two mitotypes were detected, while the study of six cpDNA microsatellite markers revealed eight different chlorotypes. The average cpDNA diversity (H = 0.415) was low but much higher than that for mtDNA (H = 0). The distribution of mitotypes revealed two clear non-overlapping areas ($G_{ST} = N_{ST} = 1$), one including northern populations and the second one including the southern and central stands, suggesting that these two regions may represent different ancestral populations. The cpDNA markers showed lower population differentiation (G_{ST} = 0.362; R_{ST} = 0.230), implying that the two ancestral populations continued to exchange pollen after their initial geographic separation. Results indicate that most of the modern *P. chihuahuana* populations are now effectively isolated with their genetic diversity essentially modeled by genetic drift. These gene diversity trends are consistent with the hypothesis of a population collapse during the Holocene warming. We recommended that conservation efforts for this species should take into account the distribution of both mitotypes and chlorotypes. They should focus on the most southern populations and the northern and central stands exhibiting high levels of genetic diversity. Results were published in Molecular Ecology in collaboration with J. Bousquet's group (Université Laval).
Other phylogeography studies on a variety of species such as balsam fir (*Abies balsamea*), black spruce and jack pine are also underway and involve graduate students.

ENVIRONMENTAL GENOMICS

The genomic variation of trees in natural environments may have important consequences for forest health and sustainability. During this reporting period, a new grant was obtained from the Canadian Regulatory System of Biotechnology (2006–2009) to continue our previous work on genomic exchange between exotic and native tree species. Part I of the project (2004–2006) focused exclusively on the first step of the introgression process, namely the rate of hybrid formation between exotic trees in plantations and adjacent natural populations of native species. The current project, Part II, has a much wider view and will address both the first stage and the more advanced stages of the introgression process. It comprises three main activities: 1) determining the frequency of hybrid formation between exotics and native species in different ecological contexts, 2) detailing the establishment of advanced-generation hybrids in nature, and 3) estimating the extent of long-distance gene flow and local adaptation for populations of native trees. The results of this project will be included into a model to simulate the possibility for introgressed genes to spread throughout the range of native trees.

Conclusion of Part I (2006)

One article describing twelve species-specific genotyping markers for poplar was published (Meirmans et al. 2007). Two manuscripts reporting the frequency of formation of first-generation hybrids between exotic and native species are presently in peer review (poplar, Meirmans et al., submitted) or in preparation (larch, Meirmans et al., in preparation). Genotypes for over 6,200 offspring for poplar and over 6,000 offspring and juveniles for larch were collected for these studies. In larch, the rate of hybridization between exotics and natives was generally very low, with approximately two percent of seeds having hybrid origin. The frequency of hybrids was approximately the same for naturally established recruits (2%), suggesting the absence of strong selection against hybrid trees in the natural environment. In poplar the rate of hybridization varied widely between species and populations, ranging from 3% to over 60%.

In addition to these experimentally obtained data, computer simulations were performed to model gene flow from exotic trees into natural populations of wild relatives. This population genomic model reinforced the idea that the extent of introgression of a given genotype will be affected by the degree of natural selection acting on the phenotype. Additionally, when natural populations are connected by a high rate of migration, the resulting frequency of an introgressed gene was found to be much higher throughout the range of the native species. A manuscript on the simulation model and main results has been submitted to Evolutionary Applications and is currently under review.

Part II (2006–2009)

The objective of Activity I is to determine the potential gene flow between cultivated poplars and their natural congener, *Populus balsamifera*, on the fragmented landscape of the Canadian prairies. We first determined the parentage of 30 common shelterbelt hybrids as many were derived through open-pollination. DNA sequencing of eight unlinked nuclear gene regions and a chloroplast intron confirmed the presence of exotic alleles from *P. laurifolia* and *P. nigra* with variable prevalence, in addition to *P. deltoides* alleles. Second, we sampled four different sites where mature natural populations of *P. balsamifera* grew adjacent to mature poplar shelterbelts. Seeds are being genotyped for 42 species-specific markers. Preliminary analysis of the foliage revealed the presence of exotic alleles in natural populations of *P. balsamifera* suggesting that gene flow between exotic and native poplars may have already occurred. This will be confirmed through genotyping 40 SNPs specific for one of the four species and will be completed during summer 2008.

The risk of introgression does not only depend on the rate of hybridization between plantations and natural populations, but also on the ability of hybrids to establish themselves in the natural populations. In Activity II we broadly surveyed patterns of introgression across a sympatric zone of two native poplars (*P. balsamifera* and *P. deltoides*) within which the exotic *P. nigra* has been extensively planted for centuries. Thirty eight SNPs that appeared fixed within each species were used to genotype 636 trees of different age classes, sampled from 15 sites that varied in the extent of anthropogenic disturbance. The extent of admixture within sampled trees was then assessed through Bayesian clustering of these genotypes. Hybrids were present in eight of the populations, with 2.99% of all sampled trees showing some degree of admixture (hybridity).

After introgression occurs between exotic and native tree species, would these novel genes remain local, or would they spread throughout the range of the native species? Activity III is addressing this issue by studying the structure of natural populations and the evidence for local adaptation. We will therefore analyze the amount of population structure and determine whether candidate genes for commercially important traits are experiencing diversifying selection. *P. balsamifera* population samples from throughout Canada will be genotyped for over 100 of both SNPs of presumably neutral genes and SNPs of genes underlying traits that are likely to be used for GMTs (Genetically Modified Trees), such as tolerance to biotic and abiotic stress. All samples were collected during the summer 2007, the DNA was extracted, and the genotyping process completed.

All of the activities within Part II necessitated the development of new genotyping markers so we produced more SNPs (intraspecific or interspecific) for several poplar species (*Populus* spp.). This has been done in the first half of the project. We designed specific DNA primer pairs for about 100 new selected genes and specific primer pairs were used to sequence several individuals in which variations were detected. We used Perl scripting in conjunction with the SNPdetector to detect highly-reliable SNPs directly from DNA sequence files. To date more than 3,500 individuals were sequenced on both sides in order to identify the SNPs that are used in the different activities. In all activities, genotyping of the SNPs are done with the Sequenom iPLEX Gold technology. This valuable resource is reported in a paper that will be submitted shortly.

ÉPINETTE DE NORVÈGE

Au cours de cette période nos efforts ont consisté principalement à maintenir les divers dispositifs expérimentaux et collections en place, à poursuivre la collecte de données et à s'assurer de rendre le matériel et les données disponibles à Marie-Josée Mottet de la Direction de la recherche forestière du Ministère des ressources naturelles et de la faune du Québec (DRF-MRNFQ) pour l'avancement du programme conjoint d'amélioration génétique.

L'étude entreprise conjointement par la DRF-MRNFQ, Forintek et le SCF afin d'étudier l'impact du charançon sur la productivité et la qualité du bois a été complétée (Daoust et Mottet 2006) ainsi que celle touchant aux caractéristiques du bois des sciages (Mottet, Daoust et Zhang 2006). Les travaux de cette étude ont été présentés au Forum sur la répression des ravageurs forestiers en décembre 2007 (Daoust, Mottet et Zhang, 2007).

PIN BLANC ET PINS HYBRIDES

Le programme d'amélioration génétique du pin blanc au Québec est toujours sous la responsabilité du SCF et l'intérêt de la part du MRNFQ pour cette espèce est de plus en plus grandissant. En effet, la demande de plants pour le reboisement triplera au cours des prochaines années afin de suppléer le manque de régénération naturelle obtenu suite aux traitements sylvicoles utilisés au cours des dernières décennies.

Au cours de la dernière période des efforts constants et soutenus ont été nécessaires afin de protéger les dispositifs expérimentaux contre les attaques du charançon du pin blanc et la rouille vésiculeuse afin de nous permettre, dans un premier temps, une évaluation sans biais des caractéristiques de croissance juvénile. Ces dispositifs expérimentaux représentent des tests d'aptitudes générale et spécifique issus de la première génération d'arbres-plus sélectionnés dans les peuplements naturels du Québec. Les résultats de ces tests nous permettront de fournir des recommandations au MRNFQ afin qu'il poursuive les éclaircies de ses vergers à graines ou qu'il effectue des récoltes dirigés de semences.

Les résultats de nos premiers tests génécologiques, établis au début des années quatre-vingt, ont permis l'identification des meilleures familles et de sélectionner les arbres-élites pour la préparation par le MRNFQ d'un premier verger de deuxième génération. Le greffage a été réalisé à Saint-Modeste en 2008 par une équipe du MRNFQ. Par rapport aux 250 familles testées, les familles sélectionnées présentent une croissance moyenne supérieure de 14% à 15 ans.

Depuis la fin des années 90, nous avons débuté un programme de production d'hybrides basés sur 5 clones de *Pinus wallichiana* x *strobus* qui avaient été développés en Ontario et qui nous avaient transféré dans les années 80 par Louis Zsuffa. Ce programme de rétrocroisement avec le pin blanc vise essentiellement à retenir la résistance de ces clones à la rouille vésiculeuse tout en assurant une bonne proportion des caractères adaptatifs du pin blanc pour répondre à nos conditions climatiques. À ce jour, plusieurs familles d'hybrides ont été produites et testées à différents endroits au Québec. En 2007, nous avons commencé à la Station de recherche de Valcartier des essais d'inoculation artificielle sur ces hybrides. Les semis seront suivis au cours des prochaines années. Les résultats récents obtenus par Pengxin Lu, Ontario Forest Research Institute, pour ces mêmes genres d'hybrides sont très encourageants (P. Lu et al. For. Ecol. and Mgt. 214 (2005) 344-357).

Toujours au niveau des pins hybrides issus de notre programme, de très bons résultats ont été obtenus par Rich Hunt, un de nos collaborateurs au centre du Pacifique du SCF avec nos hybrides *P. strobus* x (*P. wallachiana* x *strobus*). En effet, après deux essais d'inoculation artificielle (2003 et 2006), les 3 familles testées ont montré un taux de résistance moyen de 32%. Cette résistance est exprimée par la proportion des semis sans chancre. De plus, environ 20% des autres semis testés présentaient des chancres à croissance lente. Pour fin de comparaison, les hybrides de *P. strobus* x *P. peuce* testés ne présentaient, à toutes fins pratiques aucune résistance significative.

En collaboration avec Krystyna Klimaszewska du SCF, plus de 300 lignées d'hybrides de *P. strobus* x (*P. wallachiana* x *strobus*) ont été produits par embryogénèse somatique. Plusieurs de ces lignées sont en production et certaines ont été inoculées artificiellement pour tester leur résistance à la rouille vésiculeuse. Ce matériel nous permettra d'effectuer des tests clonaux et de sélectionner avec une grande certitude les lignées résistantes qui pourront être propagées éventuellement à grande échelle.

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NORTHEAST SEED MANAGEMENT ASSOCIATION (NeSMA): SUMMARY OF 2006 AND 2007 FIELD OPERATIONS

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NeSMA is a partnership of nine forest companies and the Ontario Ministry of Natural Resources operating in north-eastern Ontario. The company has tree improvement programs for black spruce (*Picea mariana* [Mill.] BSP), jack pine (*Pinus banksiana* Lamb.), and white pine (*Pinus strobus* L.) This report summarizes the main activities of the company during the 2006 and 2007 field seasons. The majority of the work was in the 13 first- generation seed orchards. The second-generation work concentrated on maintaining existing sites and establishing a black spruce clonal archive.

FIRST-GENERATION PROGRAMS

A cone crop management cycle is employed for each orchard to maintain a predictable and consistent supply of seed for the forest company partners. An assessment protocol has been developed to estimate the volume of cones available for collection each fall. In most orchards the trees are stratified by their Breeding Value and the cones are collected separately according to three categories.

The volume of cones collected in 2006 and 2007 was 331 hL which yielded over 81 million viable seeds. Crown management is done in conjunction with cone collection to facilitate collection and to manage the height of the trees. A total of 18 936 trees received crown management treatment.

Flower induction and fertilization are annual treatments in almost all seed orchards; nearly 23 000 trees received flower induction treatment during the two operating seasons.

The conversion of the Aidie Creek Black Spruce Farm Field Test to a seed orchard was completed in 2007 with the fifth thinning.

In 2006, a second series of 3 genetic tests was established for the white pine program. Erosion and vegetation control measures were undertaken with fertilization at one test in 2007. In addition, height and condition data were collected from the first series of tests and vegetation control maintenance completed.

A drought in 2005 resulted in the death of many trees at the black spruce orchards at the Island Lake Tree Improvement Area. The 2006 assessment showed that the Breeding Zone 1 orchard had lost 61% of the trees, 24% were healthy and 15% were damaged but likely to recover. The Breeding Zone 4 orchard figures were 48.5%, 27%, and 24.5%, respectively.

The Crown Management Decision Support System study concluded in 2006. Information was recorded since 1994 to determine if the cost of crown management begun early in jack pine orchards could be justified by the lower cost of cone collection. The calculations showed the annual accumulated cone harvesting savings ranged from a low of -$1,350.00 in 1994 to $99,496.66 in 2006.

SECOND-GENERATION PROGRAMS

The Jack Pine Pilot Program genetic tests, established in 1998, were thinned to avoid loss of live crown and to facilitate making final selections for orchard production and advanced generation breeding.

In 2006, the cones resulting from controlled crosses made in the Hambleton jack pine seed orchard were collected and processed. Sufficient seed is now available for the establishment of elite and infusion tests.

The black spruce elite selections grafted in 2004 were maintained at a holding area at the Aidie Creek Seed Orchard. A total of 2707 grafts representing 4 programs were planted in the Eby Township archive in the spring of 2007.

Three elite tests and two infusion tests received chemical and mechanical treatments in successive years to manage competing vegetation. In addition, 5 elite tests were assessed for height, condition, and survival.

TECHNOLOGY TRANSFER

The company continues to make people aware of forest genetics in general, and its programs in particular, through its website media data base, field tours, guest lectures at high schools and colleges in the area, and supporting events such as science fairs and forestry education in local schools.

FOREST GENETICS AT FACULTY OF FORESTRY AND THE FOREST ENVIRONMENT, LAKEHEAD UNIVERSITY, 2006–2008

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SPECIALISTS OR GENERALISTS

Since 2001, many of the range-wide provenance trials established by Canadian Forestry Service and cooperators for white spruce (*Picea glauca* [Moench] Voss) (410 Series), black spruce (*Picea mariana* [Mill.] BSP) (353 Series), and jack pine (*Pinus banksiana* Lamb.) (255 Series) have been remeasured for height, dbh, and survival by our group. Table 1 presents numbers of seed sources for each of the three species and percents of variance expressed among provenances for each variable at each test. While the tests have been generally neglected and many show little differentiation among sources, at least one test for each of the three species had 60%, or close to 60% of the height variance distributed among sources. These numbers approach the values reported for Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) which is widely accepted as a specialist species. Although some of these data have been published previously, this three-species comparison is useful due to the similarities in layouts and growing conditions across the three series of trials. The results demonstrate that the three species seem to have very similar adaptive strategies. Dale Simpson, Canadian Forestry Service, and many others have provided the guidance to make this work possible.

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Table 1. Intraclass Correlation Coefficients of Jack Pine, Black Spruce and White Spruce from Canadian Forestry Service Range Wide Provenance Tests

Jack Pine (41 yrs.)

Location	No. Provs.	Height (%)	Diameter (%)	Survival (%)
Caramat, ON	49	18.3	19.0	62.4
Espanola, ON	49	28.7	23.2	37.4
Fraserdale, ON	81	33.5	33.0	38.0
Kakabeka, ON	72	9.7	25.9	20.9
Petawawa, ON	98	16.8	20.9	30.5
Red Lake, ON	56	9.2	10.3	50.7
Swastika, ON	81	32.3	21.4	42.9
Baskatong Lake, QC	64	31.7	38.2	21.3
Clova, QC	64	50.4	38.6	51.8
Blackville, NB	72	16.8	20.9	30.5
Bald Mountain, NB	72	0.0	0.0	12.4
Blackberry, MN	89	30.5	40.2	49.5
Cloquet, MN	91	48.1	59.5	47.3
Allegan, MI	94	57.9	61.0	39.9
Dunbar, MI	67	31.9	40.3	9.7
Kellogg, MI	94	0.0	23.9	19.7

	No. Provs.	Height (%)	Diameter (%)	Survival (%)
Mean	74.6	26.0	29.8	35.3
Std. Dev.	16.0	17.2	16.2	15.4
Median	72	30	25	38
Max	98	58	61	62
Min	49	0	0	10

Black Spruce (36 yrs.)

Location	No. Provs.	Height (%)	Diameter (%)	Survival(%)
Reno, AB	23	43.5	30.2	1.0
Snowden, SK	44	44.1	24.7	1.4
Mafeking, MB	48	16.3	0.0	12.6
Blackberry, MN	99	30.5	26.0	13.1
Rosemont, MN	100	15.3	8.1	8.6
Dryden, ON	63	42.2	19.6	19.3
Raith, ON	56	13.5	30.0	0.7
Longlac, ON	56	7.2	0.0	0.0
Chapleau, ON	64	43.2	35.9	36.6
Petawawa, ON	56	35.2	11.4	20.5
Mount-Laurier, QC	86	54.2	40.5	12.5
Chibougamau, QC	88	14.5	0.7	23.5
Valcartier, QC	94	15.0	7.2	0.0
Lac-St.-Ignace, NB	85	10.5	2.2	5.6
Black Brook, NB	74	36.0	27.0	5.2
Fredericton, NB	94	59.6	52.9	9.2
Dromore, PE	75	27.0	33.7	3.1
East Dalhousie, NS	76	28.7	25.3	4.3
Pleasant Valley, NS	74	50.2	50.6	6.6
Roddickton, NF	64	23.4	40.9	12.5

	No. Provs.	Height (%)	Diameter (%)	Survival (%)
Mean	71.0	30.5	23.3	9.8
Std. Dev.	20.3	15.8	16.7	9.4
Median	74	30	26	8
Max	100	60	53	37
Min	23	7	0	0

White Spruce (24 yrs.)

Location (ON)	No. Provs.	Height (%)	Diameter (%)	Survival (%)
Dryden	79	4.5	7.5	30.5
Fort Frances	65	17.3	21.0	17.0
Kenora	51	21.3	21.1	7.8
Red Lake	79	35.9	32.8	3.9
Nipigon	80	20.6	37.7	14.3
Thunder Bay	78	15.2	9.2	0
Manitouwadge	83	1.9	8.4	0.1
Sudbury	88	49.5	38.1	18.1
Chapleau	81	7.1	8.5	0
Minden	80	28.5	29.8	1.8
Hearst	89	60.4	56.0	16.7
Kirkland Lake	81	1.1	0	0
Owen Sound	77	30.0	25.4	22.0
Cornwall	67	35.4	41.4	0.8
Chalk River	76	30.1	33.4	0
Chalk River	74	35.3	37.1	5.6

	No. Provs.	Height (%)	Diameter (%)	Survival (%)
Mean	76.8	24.6	25.5	8.7
Std. Dev.	9.3	16.9	15.5	9.7
Median	79	25	28	5
Max	89	60	56	31
Min	51	1	0	0

MANITOBA'S TREE IMPROVEMENT PROGRAM

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

Manitoba Conservation – Forestry has established tree improvement programs in breeding zones across the province 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). Tree improvement work in many of these zones is accomplished through cost-sharing agreements with the three forest companies holding Forest Management Licences: Tolko Manitoba, Louisiana-Pacific Canada Ltd. (LP)/Mountain Forest Section Renewal Company (MFSRC), and Tembec Forest Resources. This report highlights the major activities completed from the summer of 2006 to the spring of 2008.

TOLKO MANITOBA – MANITOBA CONSERVATION CO-OPERATIVE PROGRAMS

Rsults from the analysis of 16-year measurements of the four black spruce family tests in the Saskatchewan River Breeding Zone were received in June 2006. All trees from the bottom 50% ranked families were rogued from Prospector Seed Orchard in the fall of 2006 (bottom 25% families and other trees had previously been removed based on 10-year measurements). As well, there were 4 families with high family by environment interaction that were removed. There was also some selective roguing done to improve spacing within the orchard. The number of families represented in the orchard was reduced from 288 to 190. Of the 6,839 trees remaining in the orchard, 61% are from top quartile families and 39% are from second quartile families. The cone induction treatments that were done in half the orchard in the spring of 2005 resulted in a noticeable difference in the volume of cones in the fall of 2006. A total of 25.75 hL of cones was collected from the orchard in conjunction with orchard roguing and crown management. It is interesting to note that 6 of the 10 families from Breezing Zone 11.2 that were included in the Zone 11.5 family tests ranked in the top 25% in the 15-year analysis. At its nearest point, Zone 11.2 is about 140 km south-east of Zone 11.5.

In the Nelson River Breeding Zone, an infestation of white pine weevil was found at the Axis Lake black spruce seedling seed orchard in July 2006. The infected tree tops were cut and removed from the orchard to reduce future infestations. This control proved to be effective as very little weevil damage was found in 2007.

The 10-year family test measurements in the black spruce Highrock Breeding Zone were completed in the fall of 2006 (see Figure 1). One-third of the replications in the seedling seed orchard were also measured based on a recommendation from the forest geneticist. The analysis results were obtained in March 2007. Extensive needle loss and browning in the Simonhouse seed orchard was noted in the spring of 2007. The provincial forest biologist could not determine the cause of the extensive damage. The orchard was fertilized in August 2007 to improve the health of the trees going into the winter. The orchard was inspected in May 2008 and appears to have come through the winter with no further damage.

A cone collection (12.9 hL) was completed at the Reader Lake white spruce clonal seed orchard in the Saskatchewan River Breeding Zone in the fall of 2006.

Due to the economic conditions in the forest industry, only the highest priority work to maintain the programs for the Tolko Tree Improvement Co-operative was completed in 2007. The 15-year test measurements for this zone that were scheduled for 2007 have been postponed until 2009.

The GIS database was used to assist Tolko in selecting areas for general cone collections in two black spruce breeding zones until the orchards in those zones produce enough seed to meet forest renewal requirements. Family ranking data from the 10-year family test measurements was associated with the plus trees and mapped to identify potentially good collection areas based on congregations of top-ranked plus trees. There were also areas that were recommended to be avoided due to a high density of bottom-ranked plus trees.

TEMBEC FOREST RESOURCES – MANITOBA CONSERVATION CO-OPERATIVE PROGRAM

In the Lake Winnipeg East black spruce breeding zone, selective roguing of 390 trees was completed in Brightstone orchard in the summer of 2006. Cone induction treatments using gibberellin injections were conducted in half of the orchard in the spring of 2007. A large cone crop was collected in the other half of the orchard in conjunction with crown management in the fall of 2007. This built up the seed supply in storage allowing further roguing to be done in the orchard to increase genetic gain. In the spring of 2008, all trees from the bottom 40% families were removed from the orchard, based on the 15-year analysis. A total of 568 trees were removed, leaving 3,766 trees with 53% from first quartile families, 37% from second quartile families, and 10% from the top end of the 3rd quartile of families.

LP CANADA LTD./MFSRC – MANITOBA CONSERVATION CO-OPERATIVE PROGRAMS

The Tree Improvement Co-operative for black and white spruce in the Mountain Breeding Zone was transferred from LP Canada to the Mountain Forest Section Renewal Company (MFSRC) in the summer of 2007. The Memorandum of Understanding signed by MFSRC also included the jack pine program for this zone.

Maintenance (mowing) continued on the black spruce family tests and seedling seed orchard that were established in the Mountain Breeding Zone in 2001. The 10-year measurements are scheduled for 2010.

In the Mountain White Spruce Breeding Zone, 8.2 hL of cones were collected from the Birds Hill clonal seed orchard in the fall of 2006. Cones collected from top 25% families were kept separate forming an elite seedlot. Due to the hot dry summer, cones opened early reducing the collectable volume. A large cone crop was collected in the fall of 2007 which will provide enough seed to meet renewal requirements for about 8 years. Estimated genetic gains, based on family breeding values and volume of cones per family, were calculated. The elite seedlot (top 25% families) is estimated to have a gain close to 3 times that of the other seedlot; this seed will be used in higher value, more productive planting areas. The orchard suffered increased mortality starting in 2006 (41 dead) and continuing into 2007 (181 dead). Forest health staff could find no apparent cause for the mortality. A survival survey in the spring of 2008 indicated that these losses have subsided. In the spring of 2008 the orchard was further rogued removing all trees in the bottom 50% families based on the 15-year analysis.

MANITOBA CONSERVATION PROGRAMS

The Lake St. George black spruce mass selection seed orchard (MSSO) in the Interlake Breeding Zone was rogued in the fall of 2007 in conjunction with a small cone collection. This roguing removed 815 trees leaving 21% of the original number of trees in the orchard. To increase cone production, trees in one-half of the orchard were injected with gibberellin in the spring of 2008.

Jack pine cone collections were conducted at the MSSO in the Interlake Breeding Zone, the Cranberry Portage MSSO in the Northern Breeding Zone, and the Hillside Pedigreed seed orchard in the Eastern Breeding Zone. The Cranberry Portage MSSO was brushed in the spring of 2008.

The province cooperated with the Canadian Forest Service in the establishment and maintenance of Forest 20/20 hybrid poplar trials and bioenergy plantations.

The province is also cooperating with CON**FOR**GEN (Canadian program for the conservation of forest genetic resources) and CAFGRIS (Canadian Forest Genetic Resources Information System).

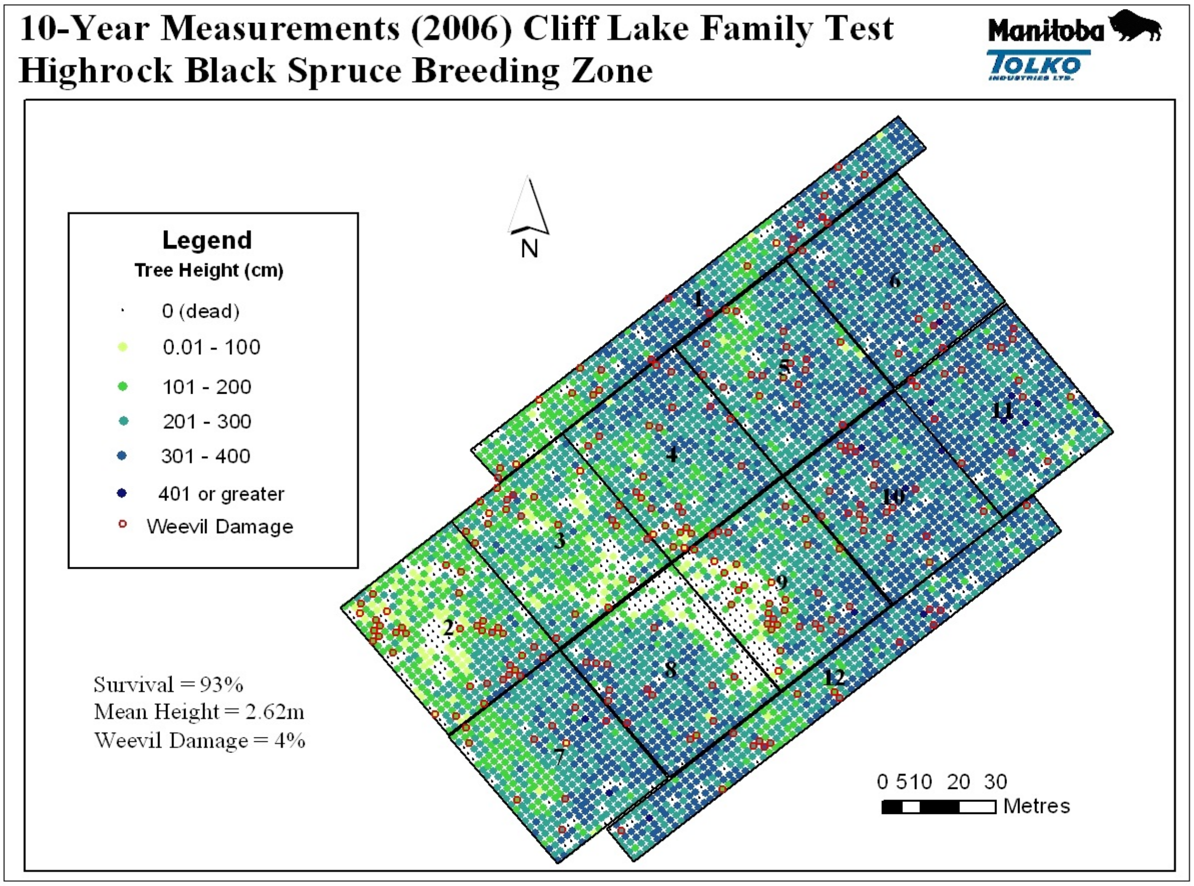

Figure 1. Variation of tree height at Cliff Lake family test

AAFC-PFRA SHELTERBELT CENTRE – TREE IMPROVEMENT SUMMARY

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

Since 1947, the AAFC-PFRA Agroforestry Division (PFRA Shelterbelt Centre) has had an active program dedicated to improving tree species for the Canadian prairies. In 2007 the Centre celebrated 60 years of tree improvement. The objective of the program is to develop genetically diverse trees and shrubs that are functional agroforestry plantings. These projects increase our knowledge on the adaptation of tree and shrub populations to prairie growing conditions and provide genetic material for selection and breeding. Genetically improved trees and shrubs enhance the value and function of agroforestry systems by improving adaptation to climate variation, disease and insect resistance, and increasing biomass accumulation of woody species. Highlights for the 2006–2008 period include the initiation of the *Salix* improvement program, establishment of the AgCanBap *Populus balsamifera* L. collection and examination of genetic variation, development of a poplar clone mix, and release of *Populus* x 'Okanese'. The Division also continued to be active in development of hybrid poplar and maintaining germplasm collections of prairie woody plant genera. Improved trees and shrubs developed in the program are used for shelterbelts, wildlife habitat planting, riparian buffers, phyto-remediation, and afforestation. From 2006 to 2008, the tree improvement program focused on genera of *Larix*, *Quercus*, *Populus*, and *Salix* as well as evaluation of long term genetic trials.

LARIX IMPROVEMENT

The objectives of the *Larix* improvement program are to evaluate the adaptability of exotic *Larix* seed populations to prairie growing conditions, study genotype x environment interactions, and develop improved larch seed strains for prairie agroforestry. Since 1985, the *Larix* improvement program has introduced over 350 new populations, evaluated the populations in seed source trials at 15 locations in Manitoba, Saskatchewan and Alberta, and developed an improved seed strain, 'Lindquist', for agroforestry planting in the Canadian Prairies. Both clonal and seedling seed orchards have been established. *Larix* species of interest include *L. sukaczewii* Dyl., *L. sibirica* Ledeb., *L. gmelinii* Rupr., and *L. decidua* Mill. Since 2006, 15 year growth data of half-sib progeny from selected trees in the Altai region of southern Siberia were collected and 20 year growth data collected from a Russian origin seed source study.

POPULUS IMPROVEMENT AND CONSERVATION

Populus Breeding

Since 1947, the poplar improvement program has been active testing populations from breeding programs, selecting and producing new hybrids, and maintaining collections of poplar species and hybrids. In 2007 a new poplar hybrid 'Okanese' was released. This hybrid originated from a cross between *P.* x 'Walker' and *P.* x *petrowskyana*. Okanese was selected from a seedling row in 1986 and has been tested since that time as 'WP-69V-86'. 'Okanese' exhibits excellent hardiness, above average growth, and resistance to common insects and diseases.

The poplar breeding program has involved controlled-pollinations using *P. deltoides* Marsh. var *occidentalis* Rydb., *P. balsamifera*, *P. maximowiczii* A. Henry, *P. nigra* L., *P. laurifolia* Ledeb., *P.* x *petrowskyana* Schneid, and *P. tristis* Fisch. Seedlings from the controlled crosses have been planted in clonal trials throughout the prairies. The individual families and genotypes are being screened using several criteria. Regardless of end use we are primarily concerned with biomass gain. *Populus* biomass, is a function of many internal plant processes and their interaction with the environment. However, we are most interested in traits with high heritability and strong correlation with biomass accumulation that can be easily and efficiently scored early on during growth. Tree stem height and basal diameter have been strongly correlated with biomass as has leaf area. In the prairie region, water use efficiency (WUE) is linked to tree growth. We indirectly estimate WUE through carbon isotope discrimination. Therefore, initial screening of trees is based on tree height, basal diameter, leaf area, and carbon isotope discrimination.

Ex situ Conservation of *P. balsamifera*

The distribution of *P. balsamifera* throughout its Canadian range has been determined as accurately as possible and populations that are physically isolated from the contiguous range have been identified. Our primary purpose was to obtain a genetically representative sample of balsam poplar germplasm over a wide geographic region for climate change research and *ex situ* conservation. The collection, named AgCanBap, includes 39 populations and 585 genotypes from five north-south transects in the native range of *P. balsamifera* in Canada and Alaska. We have chosen to preserve the assembled genotypes in common gardens. In 2006 and 2007 common gardens were established at the University of British Columbia, Vancouver and AAFC-PFRA, Indian Head, Saskatchewan. In 2008 a third common garden was established at Prince Albert, Saskatchewan.

Examining Genetic Variation in Populations of *P. balsamifera* Using Ecotilling.

This project, in collaboration with the University of British Columbia, aims to elucidate the extent of genetic variation within and among *P. balsamifera* populations in resource-use traits using single nucleotide polymorphism (SNP). The ability of individuals within a species to adapt to different environments resides in their genetic diversity. This diversity, most commonly manifested as simple nucleotide polymorphisms, can provide clues to the adaptive strategies and population histories that have played roles in species' evolution. In addition, natural DNA polymorphisms form the basis for the intraspecific variation that is of great relevance to breeders. The study uses trees from 45 populations of the species range AgCanBaP collection. The populations exhibit great phenotypic variation both in common garden and greenhouse conditions. The candidate genes of most interest in the study are those involved in carbon gain, water-use, and nitrogen uptake and use. Variation is being analyzed both within a single tree (heterozygosity) as well as between individual trees in reference to *Populus trichocarpa* (Nisqually 1), whose genome has recently been sequenced. The availability of a sequenced genome made it possible to direct our attention to candidate genes of interest. This study will greatly assist in the understanding of physiological, ecological, and evolutionary genetics of *P. balsamifera*.

Clone Trials

One of the major areas of research being undertaken at the Division is testing of hybrid *Populus* clones. At this time, over 300 clones are being tested at 40 trial locations across the prairie provinces. 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. Data from these clone trials are used to determine the makeup of poplar clonal mixes used in agroforestry plantings.

SALIX IMPROVEMENT

Salix is an important genera for agroforestry systems particularly in riparian zones. Very little is known about the genetic variation of productivity-related traits of Canada's native *Salix* populations across their geographic range. In collaboration with the Canadian Forest Service, Fredericton, NB and Institut de recherche en biologie in Montreal a *Salix* improvement project for biomass production was initiated in 2005. Since 2006, collections of *Salix eriocephala* Michx. and *Salix discolor* Muhl., from natural habitats in eastern Canada and Saskatchewan, have been propagated and common gardens established at the AAFC-PFRA Shelterbelt Centre, Indian Head and University of Saskatchewan, Saskatoon. The collection includes 90 clones representing 24 populations of *S. discolor* and 127 clones representing 23 populations of *S. eriocephala*. In 2008, a Salix breeding program using *S. discolor*, *S. eriocephala*, and *S. interior* was initiated. Inter- and intra-specific crosses have been completed (Table 1).

Table 1. Objectives of AAFC-PFRA *Salix* breeding program

Growth and Yield	Increased biomass accumulation
Architecture	Tall clones with either few, thick or many, thin shoots per stool
Adaptation	Improved adaptation to low temperature
	Improved water use efficiency
	Improved nutrient uptake
Pest	Resistance to common willow diseases and insects

GENETIC RESOURCE COLLECTIONS

The Agroforestry Division is involved in conservation of woody genetic resources used in agroforestry. This includes sampling native populations and establishment of common gardens. These collections facilitate access to genetic resources for breeding and the study of genetic diversity. The collections provide for *ex situ* conservation of native woody plant populations which supports Agriculture and Agri-Food Canada's actions in biodiversity. The gene pool of collections is conserved in common gardens or gene banks established within the native range of the species. The purposes of the gardens are to conserve the collection, provide data on adaptation and performance of the collection, and provide genetic material for genomic studies. The vegetative collections include 756 populations and 24 500 individual genotypes of the following species: *Prunus virginiana* L. var. *melanocarpa* (A. Nels.) Sarg., *Cretaegus rotundifolia* Moench, *Shepherdia argentea* Nutt., *Symphoricarpos occidentalis* Hook., *Rosa woodsii* Lindl., *Quercus macrocarpa* Michx., *Fraxinus pennsylvanica* Marsh. var. *subintegerrima*, *P. deltoides* var. *occidentalis*, *P. balsamifera, Prunus nigra* Ait., and *Prunus americana* Marsh.

EVALUATION OF LONG TERM GENETIC TESTS

The AAFC-PFRA Agroforestry Division has established over 200 genetic trials in the prairie region. Genera included in the tests are *Pinus*, *Larix*, *Quercus*, *Populus*, *Salix*, *Prunus*, *Hippophae*, *Celtis*, *Symphoricarpus*, *Rosa*, *Shepherdia*, and *Fraxinus*. These tests are evaluated every five years for survival, growth, adaptability and pest resistance. From 2006–2008, 19 trials were evaluated. Information from the tests describe genetic variability within genera, are used to recommend new species, and/or to provide data to support release of new clones or seed strains.

GENETICS AND TREE IMPROVEMENT PROGRAM, 2006–2008 ALBERTA SUSTAINABLE RESOURCE DEVELOPMENT

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This report summarizes the progress of the Forestry Division’s genetics and tree improvement program for the period 2006–2008. A large part of the work was done in collaboration with forest industry, Alberta Agriculture and Food, Alberta Tourism, Recreation and Parks, University of Alberta, Forest Genetics Alberta Association, North Peace Applied Research Association, Manning Forestry Research Fund, Canadian Forest Service (CFS), and B.C. Ministry of Forests and Range (BCMoFR).

PROGRAM DEVELOPMENT

Mountain pine beetle (MPB) (*Dendroctonus ponderosae* Hopkins) infestation on lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) in Alberta spread rapidly in 2006 posing a serious threat to natural and managed pine forests. A plan was developed for the protection of genetics field trials, seed orchards, and conservation areas and for special seed collections for genetic conservation and reforestation purposes. This work is being carried out with special funding from the MPB initiative which also provides additional resources for expansion of lodgepole pine seed orchards and breeding for insect and disease resistance.

Cooperative tree improvement agreements were developed between the Alberta Tree Improvement and Seed Centre (ATISC) and industry participating in the Forest Genetics Alberta Association (FGAA). Agreements for northwestern Alberta projects were finalised and those for northeastern Alberta are under review to finalise.

A scientific review of genetic gain from tree improvement programs and its integration into growth and yield models was carried out at the request of the Alberta Forest Genetic Resources Council. A consultant’s report on the subject was completed and is being reviewed for science and policy issues.

The Alberta Forest Genetic Resources Council continued its work and the following significant accomplishments were made over the report period: the provincial gene conservation plan was reviewed and endorsed, the CON**FOR**GEN initiative was reviewed and endorsed, a forest genetics research gap analysis was completed and research needs were prioritized, and the Council’s communications initiative was expanded.

GENETIC IMPROVEMENT

Breeding Plans

The Alberta tree improvement program consists of 20 approved tree breeding projects involving white spruce (*Picea glauca* [Moench] Voss), lodgepole pine, black spruce (*Picea mariana* [Mill.] BSP), Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), and western larch (*Larix occidentalis* Nutt.). A standard format for describing and documenting breeding plans and related activities was developed for ATISC projects and work is in progress to complete pending breeding plans which are referred to as Controlled Parentage Program Plans. Breeding region delineation and digitization work was completed for all projects and all available documentation is being consolidated to complete the reports which will be periodically updated.

Assembly of Breeding Stock

Forty lodgepole pine parent trees were selected to augment the lodgepole pine breeding program as part of the Alberta Sustainable Resource Development (ASRD) Mountain Pine Beetle strategy. The selections sampled a geographic area extending from southeastern Alberta (Crownest's Pass) to northeastern Alberta (Clear Hills).

Seventy-five forward selections from the Breeding Region K1 (lower foothills) lodgepole pine progeny trials were made based on 11-year height measurements. Selections were made in anticipation of continued high mortality at the test sites due to infection by armillaria root rot (*Armillaria mellea*).

A small controlled crossing program is being initiated for white spruce Breeding Region G2 (northwestern outlier foothills). In collaboration with the Canadian Wood Fibre Centre (CWFC) in Fredericton, NB about 200 white spruce varietal lines will be developed from 20 full-sib crosses using somatic embryogenesis (SE). Trees will be produced for field testing while the corresponding embryogenic tissue will be cryopreserved at CWFC. ATISC is also cooperating with CWFC on SE for lodgepole pine.

Companies participating in these projects are Sundre Forest Products Ltd., Manning Diversified Forest Products Ltd., and Tolko Industries Ltd. (High Level Lumber Division).

The elevation distributions of parent trees in the base populations of all breeding regions are being analyzed. Work was completed on two projects: white spruce breeding region G2 and lodgepole pine breeding region J, both in the northwestern outlier foothills. ArcView was used as a GIS tool to create and summarize elevation data and a 'shapefile', containing approved breeding region line work, was stratified and combined with a 100 x 100 m digital elevation model to create a single file. The file for each project was summarized based on a 50 m elevation range.

Results showed that Breeding Region G2 covers approximately 3 072 904 ha with a mean elevation of 710 m and an elevation range of 415–930 m. The 400–450 m class is the least represented, comprising 0.0008% of the total area; the 651–700 m class is the most represented, comprising 22% of the total area. A total of 104 white spruce parent trees were selected for the G2 base population and the analysis indicates that these selections are modestly biased toward the higher elevation classes. Thirty percent of the selections are in the 751–800 m class, representing about 13% of the total area, while the 651–700 m class is represented by only 8% of the selections. The results are being used to make adjustments to the base population so that it better represents the breeding region elevation landscape.

For Breeding Region J, results showed the area covered to be approximately 2 651 050 ha with a mean elevation of 780 m and an elevation range of 390–1,030 m. The 1,001–1,050 m elevation class is the least represented, comprising 0.002% of the total area while the 701–750 m elevation class is the most represented, comprising 21% of the total area. A total of 80 lodgepole pine parent trees, including seven parent trees from British Columbia (BC) ranging in elevation from 1,050–1,250 m, were selected for the J base population. The summary showed that, as in the G2 project, the parent tree selections are modestly biased towards higher elevation classes. Changes are being made to adjust the base population to better conform to the breeding region's climatic and elevation profile.

Genetic Testing

Three series of white spruce half-sib open-pollinated (op) progeny trials were assessed in 2006: Breeding Region E (northeast boreal lowland) and Breeding Region H (northwest boreal lowland) trials were assessed at 15 years from seed and Breeding Region D1 (Slave Lake boreal) trials were assessed at 24 years from seed. Survival, tree condition, height, and white pine weevil incidence were measured or assessed in all trials and dbh was also measured in the Region D1 trials.

Also in 2006, Breeding Region K1 (southern upper foothills) lodgepole pine op progeny trials were measured at 11 years from seed. Individual and family mean heritabilities for 11-year height were 0.36 and 0.67, respectively. The two sites in this series are both heavily infested with armillaria root rot. Analysis of variance indicated no significant family differences in susceptibility to this disease but incidence was positively correlated with elevation of family origin (r=0.30, p=0.004).

In 2007, Breeding Region G2 (northwestern outlier foothills) half-sib op progeny trials were measured at 7 years from seed. Survival, growth, vigour, climatic and pest damage (specifically white pine weevil damage) were measured or assessed.

The Cypress Hills lodgepole pine op family trials were also assessed in 2007 at 6 years from seed. These trials are not associated with a specific breeding program. The families in the trial represent unique 'outlier' island populations of lodgepole pine that were untouched by the last glacial advance and that show promise for selection and breeding for climate change adaptation in northern Alberta.

The first in a series of three jack pine (*Pinus banksiana* Lamb.) half-sib op progeny trials for Breeding Region P1 (northeastern lowlands boreal) was established in 2007 with 87 families represented in the alpha design trial.

Seed Orchards

Seed production over the report period from the five white spruce orchards located at ATISC was approximately 300 kg; essentially all of the seed production was from 2007. The last substantial crop was in 2003 when a total of 447 kg of seed was produced.

The Breeding Region D1 (Slave Lake area boreal) white spruce seedling orchard at ATISC was rogued for the fifth time in the spring of 2008, primarily to reduce seed tree numbers. Established in 1982–83, the orchard was originally comprised of 3,438 trees representing 150 families. Subsequent to the fifth roguing, the orchard was reduced to 524 trees and 82 families. Based on 21-year progeny trial results, genetic gain in 21-year height is predicted to be approximately 10% given the current orchard composition. If pollen contamination is taken into account, gain is reduced to about 8%.

Chloroplast DNA markers developed in BC are being used to estimate pollen contamination in two white spruce clonal seed orchards: Breeding Region E (northeast boreal lowland) and Breeding Region G2 (northwestern outlier foothills). Using cpDNA extracted from dormant bud tissue, all parental clones in both orchards were haplotyped using a set of five markers (loci) specific to spruce. Forty-four haplotypes were detected in the 92 clone Region E orchard; frequencies ranged from unique (0.01) to 0.18. In the 100 clone Region G2 orchard, 55 haplotypes were detected with frequencies ranging from unique (0.01) to 0.1 Background stands or plantations that are likely sources of contaminant pollen are also being sampled to estimate the frequency of haplotypes common to both the background stands and the orchard. Seeds from a given orchard seedlot will then be haplotyped using cpDNA isolated from seed embryos. Seed embryos can be defined as either detectable contamination (i.e., genotypes not present in the orchard) or as within-orchard pollination events adjusted for the presence of orchard-sized genotypes in the background pollen.

PROVENANCE RESEARCH

White Spruce

Analysis of 24-year height, dbh, and survival data from the Alberta white spruce provenance trials was completed. The analysis had several objectives: to examine the amount and pattern of genetic variation in relation to climate, to determine the response of provenances to climate change, and to assess the potential implications of this response for future forest productivity in Alberta. Detailed results are available in Rweyongeza et al. (2007a). This study showed that white spruce in Alberta occurs in three distinct regions: northern (> 57°N), central (54–57°N) and southwestern (< 54°N). Within regions, variation in growth potential is clinal in relation to winter temperatures and continentality. Models are being developed to guide seed transfer within these regions.

As part of climate change adaptation research, a pilot study of provenance variation in water use efficiency was initiated. Wood core samples of selected provenances from low and high moisture index areas were collected on two of the eight sites in the Alberta range-wide provenance trials to analyze carbon (^{13}C vs. ^{12}C) assimilation as a measure of drought tolerance. Results will be compared with provenance growth potential and provenance climate to allow identification of superior drought hardy provenances for future climates.

The spruce genecology and climate change field trials, established at two sites in Alberta, were assessed in 2006 at 3 years from seed. The trials contain white spruce, Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and hybrid provenances from BC, Alberta (AB), Northwest Territories (NT), Yukon (YT), and the western United States (US). Analysis of the Hay River site (59°08'N) showed that climatic-related dieback over the first growing season was the lowest (<10%) among provenances from northern BC, YT, NT and AB (outside the Rocky Mountain region). Provenances from AB and BC from within the Rocky Mountain region had an average of 10.1% and 33.0% dieback incidence, respectively. Among the other provenances, dieback incidence was the highest (average 19.8–83.1%) for provenances from warmer regions in BC and the US. The correlation between provenance mean dieback incidence and provenance degree days below 0°C (NDD) was 0.72 ($P < 0.0001$) indicating that provenances from warmer climates were more susceptible to winter-related dieback than those from cooler climates. Concomitantly, dieback incidence and continentality (January and July mean temperature differential) were negatively correlated ($r= -0.58$, $P < 0.0001$) indicating that provenances from regions with extreme seasonal temperatures were less susceptible to winter damage than those from less extreme seasonal climates. The correlation between mean chlorosis incidence (seasonal yellowing) and latitude of seed origin was -0.57 ($P < 0.0001$) as exemplified by the common occurrence of chlorosis among US provenances.

Analysis was undertaken on a Canada range-wide white spruce provenance trial located in central Alberta. The trial contains 43 provenances from across Canada ranging from 44°26' to 66°02' N latitude, 57°37' to 139°00' W longitude, and 30 to 1,280 m above sea level. At 24 years from seed, the average height of five local Alberta provenances at the test site was 5.97 ± 0.28 m. In order of performance, the five provenances exceeding this local population height by more than two standard errors were Gold Creek (Manitoba), Angle Inlet (Manitoba), Burt Twp (Ontario), Daserat (Quebec), Candle Lake (Saskatchewan), and Kapuskasing (Ontario). The mean height of these provenances ranged from 6.55 ± 0.33m (Kapuskasing) to 7.06 ± 0.32m (Gold Creek). A second degree polynomial regression indicated that 24-year height (H24) was significantly ($P < 0.0001$) related to latitude ($r^2= 0.45$) and longitude ($r^2 = 0.76$) of seed origin. Generally, the best provenances for this central Alberta site were from southeasten Manitoba, near the Ontario border; the Ottawa Valley; and southwestern Quebec. Provenances with the lowest H24 were from YT, NT, BC, Newfoundland and Nova Scotia; growth potential of these provenances was similar to that of high elevation provenances from the Rocky Mountains in Alberta.

Lodgepole and Jack Pine

Alberta provenances of lodgepole pine, jack pine and their putative natural hybrids were compared using height growth potential and the response of 15-year height to a multivariate climate transfer index. Results were published Rweyongeza et al. (2007b). The study showed that population differentiation for lodgepole pine is determined by climate, which varies along an elevation gradient. Consequently, elevation seed transfer models are being developed to guide seed transfer for lodgepole pine reforestation in Alberta.

Tamarack

Provenance x site (PS) interaction in tamarack (*Larix laricina* [Du Roi] K. Koch) was analyzed for total height at 8, 15 and 20 years from seed. Using the additive main effect and multiplicative interaction (AMMI) model and multivariate climate indices, it was found that provenances were closely associated with test sites of similar climate. Thus, the PS interaction was mainly caused by climate differences between provenances and test sites. Provenances and test sites located in climatically marginal areas at high latitudes in northern Alberta and at high elevations in the Rocky Mountains contributed the most to the PS interaction. The correlation between the first AMMI axis and the first climatic principal component axis ranged from 0.5 to 0.7.

Ponderosa Pine and Douglas-fir

Ponderosa pine (*Pinus ponderosa* P. Laws. ex C. Laws.) is not native to Alberta and a previous provenance trial established at ATISC in 1980 failed due to winter damage. However, predictions show that in the future, Alberta's climate will be warmer and drier making it possible for the species to flourish, especially at high elevations in the southern part of the province. Thus, the Rocky Mountain ponderosa pine (*Pinus ponderosa* var. *scopulorum*) has been identified as a potential species for natural range expansion and for controlled introductions as part of a strategy to manage the effects of climate change on Alberta's forests. A provenance testing program, to commence in 2009, will include 18 US provenances, 6 BC provenances, and one local Alberta landrace. Test sites will be established in each of the following ecological zones: Upper Montane, Lower Montane, Lower Foothills, and Dry Mixedwood-Central Parkland transition. Seven provenances of interior Douglas-fir will also be included in the trials. This species is also considered to have potential for range expansion into west central Alberta.

TREE GENE RESOURCES CONSERVATION

The “Gene Conservation Plan for Native Trees of Alberta” was completed in cooperation with Alberta Parks Division and was endorsed by the Alberta Forest Genetic Resources Council. The plan is under final review for publication and describes a technical framework including concepts for tree gene conservation, gap analysis, and implementing, coordinating and maintaining a provincial network of *in situ* reserves for 28 native tree species in Alberta. The gap analysis work is currently underway in cooperation with Dr. Andreas Hamann, University of Alberta. The conservation plan is expected to be implemented over a seven-year period. A formal protocol will be used to establish priorities by species. The initial emphasis is on white spruce and lodgepole pine, for which large tree improvement programs are in place, and on limber pine (*Pinus flexilis* James) and whitebark pine (*P. albicaulis* Engelm.), species that are on the provincial tracking list because of the increasing threat from white pine blister rust (*Cronartium ribicola*). A working group has been formed and is undertaking initial inventory, status reporting, and conservation and restoration work for limber and whitebark pine which are known to have suffered serious mortality and population decline in southwestern Alberta.

In co-operation with Alberta Parks Division, seed was collected from one population of whitebark pine in Willmore Wilderness Park west of Grande Cache and material for clone banking was collected from 10 balsam poplar (*Populus balsamifera* L.) in Miquelon Lake Provincial Park southeast of Edmonton and 10 river valley cottonwoods from the balsam poplar and western plains cottonwood (*Populus deltoides* ssp. *monilifera* (Aiton) Eckenwalder) contact zone in Dry Island Buffalo Jump Provincial Park north of Drumheller. ATISC also conducted an additional seed collection for whitebark pine from Table Mountain west of Pincher Creek, a Rocky Mountain juniper (*Juniperus scopulorum* Sargent) collection from the Crowsnest Pass, and seed collections from 11 lodgepole pine populations in areas of high risk to mountain pine beetle in western Alberta.

A somatic embryogenesis project for propagation and conservation of whitebark pine was initiated with Drs. Yill-Sung Park and Krystyna Klimaszewska of the CFS. The objective is to determine the feasibility of using SE techniques as an additional *ex situ* conservation strategy for white bark pine. The work involved collections of immature cones from single trees at three locations and three collection times. The results were promising and showed about 14% initiation rate in the best collection. The work will be continued in 2008 with additional collections of whitebark pine being made and limber pine collections initiated.

Over the report period, 11 new white spruce clones were established in the clone bank at ATISC and 20 Engelmann spruce selections from the subalpine region in southwestern Alberta were grafted for *ex situ* conservation purposes.

PLANT PROPAGATION, WOOD AND SEED TECHNOLOGY

Plant Propagation

In 2007 and 2008, ATISC produced 87 000 seedlings for the conservation program; 76 000 seedlings and small potted stock were reared for National Forestry week, Arbor Day, and Junior Forest Wardens; 6,000 seedlings were grown for rootstock including 3,500 lodgepole pine for the Mountain Pine Beetle conservation effort; 2,000 white spruce and lodgepole pine seedlings were grown for reforestation and reclamation planting in Cypress Hills Interprovincial Park; and 1,300 jack pine seedlings were grown for a series of progeny trials for Breeding Region P1 (northeastern lowlands boreal). Stock production pilot studies were carried out with ponderosa pine and western larch seedlings to determine optimum rearing regimes for these species in preparation for stock production for future progeny trials and climate change studies.

Graft production consisted of 1,800 grafts for establishment in seed orchards and clone banks including 800 grafts made as part of the lodgepole pine conservation program funded by the MPB initiative. Species grafted included white spruce, lodgepole pine, black spruce, jack pine, interior Douglas-fir, western larch, tamarack, Scots pine (*Pinus sylvestris* L.), and red pine (*Pinus resinosa* Ait.).

Wood Technology

Fibre length and wood density were determined on 50 trees over the report period: 10 balsam poplar and, as part of the ASRD mountain pine beetle strategy, 40 lodgepole pine.

Seed Technology

Over the report period, 308 seedlots were added to the genetics seed bank. To date, the seed bank contains 5,982 seedlots.

Collections from research tree improvement programs included the following species: white spruce (58), western larch (35), lodgepole pine (14), jack pine (7), Douglas-fir (3), black spruce (2), tamarack (2), and Scots pine (1).

As part of the ASRD mountain pine beetle strategy, 40 single-tree lodgepole pine collections and 11 bulk lodgepole pine collections were completed. White bark pine was identified as a 'species-at-risk' and 95 single tree collections and 1 bulk collection were completed. Nineteen single-tree collections and 1 bulk collection of Rocky Mountain juniper were completed.

Reference seedlots representing 2% of the seed bank entries have been monitored for 27 years. Initial average germination for lodgepole pine in 1981 was 88.3% and in 2007 average germination was 89.1%. Initial average germination for white spruce in 1981 was 91.7% and in 2006 average germination was 86.1%. After 15 years of testing, mean germination of aspen (*Populus tremuloides* Michx.) has declined 77%, balsam poplar has declined 55%, and plains cottonwood has declined 61%.

REFORESTATION SEED PROGRAM

The Reforestation Seed Program is responsible for the registration, storage, distribution, and tracking of tree seed used for public land reforestation. During the reporting period, 1,870 kg of seed were withdrawn for seedling production, direct seeding, research, and quality testing and 289 new seedlots and 12 new vegetative lots were collected and registered for public land use. At the end of the reporting period, there were 19 tree and shrub species represented in seed storage by 2,086 individual lots for a total of 45 210 kg of seed.

All seedlots are germination tested approximately every 5 years and moisture content is determined on a representative sample. Over the report period, germination testing was completed on 314 seedlots and moisture content was determined for 24 seedlots.

ASRD's Reforestation Seed Program was reviewed as part of a provincial reforestation audit carried out by the Auditor General of Alberta. Based on the recommendations, the following areas of the ATISC seed program are being strengthened: the tracking mechanism and monitoring of seed from storage to nursery to planting site, development and implementation of a spatial assessment system for the provincial seed inventory to meet reforestation needs, and development of a contingency plan for continuation of reforestation with minimum disruption in case of significant loss of the provincial seed inventory at ATISC due to a possible catastrophic event. The review and recommendations provided an opportunity for a thorough review of reforestation seed management and work has started on developing a Provincial Seed Use Plan.

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WESTERN FOREST PRODUCTS – COASTAL SEED DELIVERY

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Western Forest Products Inc. delivers high quality seed to our forest regeneration programs and makes surplus seed available to other licensees operating in the Maritime Seed Zone of British Columbia. At our Saanich Forestry Centre we manage seed orchards and hedges for five conifer species: western redcedar (*Thuja plicata* Donn ex D. Don), coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Sitka spruce (*Picea sitchensis* (Bong.) Carr.), and yellow-cedar (*Chamaecyparis nootkatensis* (D. Don) Spach). At present, each of our orchards is one of a few serving a specific seed planning unit. Three private industrial orchard complexes and two Ministry of Forests and Range (MoFR) orchard complexes in coastal BC also provide seed to coastal seed planning units.

We employ orchard management techniques to produce seed of greater genetic quality including orchard roguing and ramet replacement. Techniques for improved seed quantity include supplemental mass pollination, insect control and management, and cultural management. Controlled pollination is used in specific cases to improve genetic quality and provide specific insect control where seed quantity is not reduced.

Western Forest Products supports genetics, tree seed, and associated research conducted by Research Branch at the MoFR, the Universities of British Columbia and Victoria researchers, Canadian Forest Service, and associated consultants and contractors. We provide input to seed deployment policy development at the MoFR.

WESTERN REDCEDAR

The second-generation western redcedar orchards delivered a seed crop in 2007 for more than 6 million plantable seedlings with volume gain of 7%. Controlled pollination, abundant background pollen, and very low seed insect damage were instrumental in the delivery of this large crop. The gain, in part, reflects new scores received from the breeding program, roguing to remove clones of lesser genetic worth, and the pollen management techniques applied. Production from our western red-cedar orchards meets current Company seed needs. We will continue to upgrade the orchards as new selections are identified by the breeding program.

COASTAL DOUGLAS-FIR

Our mature coastal Douglas-fir orchard delivered good amounts of seed per ramet in 2007 with volume gain at rotation projected at 11%. This orchard will not meet the current low elevation Douglas-fir seed needs of our Company which have increased more than five-fold due to amalgamation of tenures. Sechelt seed orchards will continue to provide seed towards our needs, but are scheduled for closure. Thus, we are establishing replacement orchards at Saanich Forestry Centre. In the interim, Western Forest Products purchases and deploys seed from other producers, including the Washington State Weyerhaeuser orchards.

Initial third-generation selections are now available from the coastal Douglas-fir breeding program. These will be incorporated into the advanced generation orchards under development at Saanich Forestry Centre. With these selections, seed crops are projected to deliver more than 20% volume gain at rotation.

Grafted stock is on site for the establishment of a second-generation coastal Douglas-fir seed orchard to serve high elevation needs. This orchard replaces the mature first-generation orchard at the Sechelt orchard site. Volume gain at rotation for seedlings deployed from this orchard seed is projected at 10% and will be available in five years' time.

WESTERN HEMLOCK

The low-elevation second-generation western hemlock orchard at Saanich Forestry Centre produces seed with a 14% volume gain at rotation. Small crops from this orchard have been harvested in recent years, but have not covered our current needs. Banked orchard seed from a post-roguing first-generation orchard crop is deployed in the interim. A high-elevation second-generation orchard is under development at this time. It will produce future seedlots of 11% volume gain at rotation.

SITKA SPRUCE

The weevil-resistant Sitka spruce orchard at Saanich Forestry Centre has produced small crops in recent years. Supplemental mass pollination is employed to improve seed set because flowering in this young orchard is not abundant. This orchard seed delivers 85% weevil resistance to regeneration programs. Though volume gain scores have not been developed for this orchard, regeneration from this seed demonstrates very good growth when grown together with non-resistant stock on sites where the weevil hazard is low or nil. New deployment best practices have been published (Heppner and Turner 2006) reflecting confidence of the breeder and entomologist in the resistance of this stock.

YELLOW-CEDAR

Western Forest Products initiated clonal evaluation of yellow-cedar cuttings more than two decades ago. Numerous test sites have been established and data have been collected repeatedly. Dr. J. Russell, MoFR's cedar breeder, has provided analysis of the data and developed genetic worth scores. At present, the Saanich Forestry Centre is delivering yellow-cedar cuttings to our regeneration programs with a projected volume gain at rotation of 20%. However, stool-bed aging has resulted in reduced rootability of many selected clones. Western Forest Products is in the process of assessing selected clones for their rootability and has removed clones with poor rooting ability from the cuttings deployment program in an effort to increase nursery success and thereby avoid increased nursery costs. Because of the rooting challenge, a significant portion of our regeneration program has been served by wild seed in the last two years.

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

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FOREST GENETICS AT THE FACULTY OF FORESTRY, THE UNIVERSITY OF BRITISH COLUMBIA, 2006–08

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GENE CONSERVATION, ECOLOGICAL GENETICS AND CLIMATE CHANGE

S.N. Aitken

Our group in the Centre for Forest Conservation Genetics continues to characterize the genetic structure of forest tree populations in British Columbia (BC) by studying candidate genes, genetic markers, and phenotypic traits, and apply the results in the areas of genetic conservation and forest management. We are increasingly focused on predicting the effects of climate change on native tree species and developing strategies to mitigate these effects. Dr. Tongli Wang has been modeling current and future distributions of native species under a range of climate change scenarios using the machine learning classification tree algorithm Random Forests, as well as developing new analytical methods for using provenance trial data to predict the effects of climate change on populations and to select seed sources for reforestation (Wang et al. submitted). Dr. Pia Smets has been testing the physiological and growth responses of populations of lodgepole pine (*Pinus contorta*) and interior spruce (*Picea glauca, P. engelmannii*, and their hybrids) to temperature, drought, and carbon dioxide. We synthesized literature from a wide range of sources in a review article to evalulate the ability of tree populations to adapt or migrate in the face of rapid climate change (Aitken et al. 2008).

Sitka spruce (*Picea sitchensis*) continues to be our model species for studying evolutionary dynamics across species ranges, including local adaptation, gene flow, and central-peripheral structure (Mimura and Aitken 2007a, b). PhD candidate Jason Holliday has identified close to 200 candidate genes for local adaptation to climate in Sitka spruce based on transcriptional profiles during cold acclimation (Holliday et al. 2008) as part of the Genome Canada and Genome BC-funded Treenomix Project (with Kermit Ritland and Joerg Bohlman). We have now characterized nucleotide diversity within these genes through resequencing individuals from across the species range. PhD student Jill Hamilton is starting a project exploring local adaptation and gene flow across the *P. sitchensis x P. glauca* introgression zone by studying neutral genetic markers (nuclear microsatellite, chloroplast, and mitochondrial markers) and nucleotide variation in candidate genes and phenotypic traits in common gardens. PhD student Amanda de la Torre Cuba is characterizing the genetic structure and degree of introgression in the elevation-dependent hybrid zone between *P. glauca* and *P. engelmannii* in BC. She will also be evaluating the extent to which selection in the breeding program for the interior spruce species complex alters hybrid index, as well as estimating the level of gene flow between conservation populations and planted forests.

We continue to characterize the population, ecological, and conservation genetics of native tree species. Christine Chourmouzis has assessed levels of *in situ* conservation for all 50 or so native tree species in BC and is compiling data on *ex situ* and *inter situ* conservation. Whitebark pine (*Pinus albicaulis*) is a blue-listed species in BC and populations are declining rapidly due to white pine blister rust, caused by *Cronartium ribicola,* as well as mountain pine beetle (*Dendroctonus pseudotsugae*). Group alumnus Andrew Bower characterized the ecological genetics, mating system, and interactions between white pine blister rust and inbreeding in this species (Bower and Aitken 2007, 2008, and submitted). Pacific dogwood (*Cornus nuttallii*) is the provincial flower of BC, yet nothing has been published on the population or ecological genetics of this species. MSc student Karolyn Keir has analyzed genetic diversity across the species range and found relatively low levels of diversity for microsatellite markers and very low diversity for chloroplast markers. Phenotypic traits show some genetic clines associated with climatic gradients; however, differentiation of populations for quantitative traits is weak. MSc student Colin Huebert has established a common garden experiment with Garry oak (*Quercus garryana*) and is analyzing the ecological genetics and potential effects of climate change on western Canada's only oak species. Project descriptions and links to publications are available on our website www.genetics.forestry.ubc.ca/cfcg.

APPLIED FOREST GENETICS AND BIOTECHNOLOGY

Y.A. El-Kassaby

The first wave of the IRC's graduate students' graduation is approaching. Three Masters of Sciences projects are near completion. These include: 1) a study on clonal gametic contribution in a Douglas-fir (*Pseudotsuga menziesii*) seed orchard using SSRs markers by Ben Lai, 2) an investigation of pedigree reconstruction of phenotypically selected yellow-cedar (*Chamaecyparis nootkatensis*) by Nasim Massah, and 3) a GIS-based white spruce genetic deployment over time and space in the Prince George Seed Production Zone by Ding Klaus (co-supervised with M. Meitner). These studies are integrated with the British Columbia Forest Genetics Council's research objectives. Additionally, two PhD students have made significant progress in their respective programs. Mohamed Ismail has completed a study on genetic diversity in black cottonwood (*Populus trichocarpa*) using SSR markers, highlighting the role of ecological differences on gene flow and the distribution of genetic diversity. He is extending his work to investigate a set of defense and adaptive genes' nucleotide diversity using samples covering the species' BC range and collaborating with another graduate student (R. Soolanayakanahally, supervised by R. Guy) to investigate the taxonomical relationship between *P. trichocarpa* and *P. balsamifera*. Tomas Funda is investigating advanced generation seed orchards and has completed an optimization study that allows orchard managers to maximize the genetic gain of an orchard's crop at any pre-set genetic diversity level. He also completed two studies developing SSR markers for western larch (*Larix heterophylla*) and comparing mating system and pedigree reconstruction analyses in seed orchards. Tomas is investigating gametic contribution assessment using DNA markers and comparing his results with those obtained by visual assessment.

My independent research has concentrated on the development of the "Breeding Without Breeding" concept and this work is underway with collaborative efforts with Drs. C. Liewlaksaneeyanawin (PDF), M. Lstibůrek (Czech Republic), and D. Lindgren (Sweden). Additional accomplishments include the development of a user friendly seed orchard design computer program, the development of a mathematical model expressing seed germination for parameters extraction, the development of two new seed orchard design concepts, studying *in situ* wood attribute assessment methods, and factors affecting seed utilization and efficiency.

As a member of two research teams: 1) Mansfield-Guy-El-Kassaby, we secured funds from the Ministry of Sustainable Resource Development, Alberta and the BC Ministry of Forests and Range to study conifer juvenility through functional genomics and 2) Douglas, Mansfield et al. obtained Genome BC funds to investigate the optimization of poplar feedstocks and novel enzyme systems for a BC bioenergy sector.

FORESTRY GENOMICS AND PLANT POPULATION GENETICS

Kermit Ritland

With Joerg Bohlmann (Micheal Smith Laboratory, UBC), I hold a Genome BC/Canada genomics project involving spruce. This involves several collaborations with Sweden, Scotland, and California, as well as with colleagues at UBC (Sally Aitken, Shawn Mansfield, and Paul Wood). Sally Aitken and her student, Jason Holliday, have documented nucleotide variation at many genes putatively involved with adaptation. Shawn Mansfield's post-doc Rebbeca Dauwe has mapped 150 metabolite QTLs and reconstructed pathways based upon these patterns in a 2x3 factorial design. Paul Wood is involved with GELS activities that research public perceptions about forest genomics. Post-doc Ilga Porth is currently finishing a study of 22 800 expression QTLs in the same experiment. In Scotland, our collaborators are developing SNPs for molecular breeding of Sitka spruce and have relied upon our EST-SSRs for initial work; currently we are co-ordinating with the Quebec project to find additional SNPs. At Umeå, Sweden, Rosario Garcia-Gil has been working at developing a molecular marker map for Scots Pine (*Pinus sylvestris*), and visited us for 3 months in early 2008 for this purpose. Further work involving Illumina assays is ongoing. In California, David Neale has collaborated with us in supplying candidate genes for resistance to the white pine weevil (*Pissodes strobi*) in spruce, and also markers that can provide a syntenic map of the pine family. Recently, I was awarded a grant to study genome structure in spruce and pine, through comparisons of spruce and pine. This involves collaborations with both *Arborea* at Laval University in Quebec (John Mackay) and the Mississippi Genome Exploration Laboratory (Daniel Peterson).

Through my NSERC grant, I have been involved with several projects in plant population genomics. In Ramsay et al. (submitted), a MSc student shared with Loren Rieseberg, we identified 45 genes in the terpenoid biosynthetic pathway and inferred patterns of selective constraints in lineages involving *Arabidopsis*, *Populus*, *Ricinus*, and *Vitis*; upstream genes had greater constraint; also a novel "pathway pleiotropy index" better explained patterns. Chen and Ritland (submitted) is the first to incorporate phylogeny into QTL mapping; QTL changes along lineages leading to the evolution of two inbreeding taxa were inferred, and as expected, large effect QTL lay in the ancestor lineage, and smaller effect QTL lay in derived lineages. Ritland and Liewlaksaneeyanawin (submitted) developed a new statistic, the "correlation of gene diversity between linked loci" as a measure of genome structure; two lodgepole pine populations at the periphery of the ranged showed significant positive correlations out to ca. 10 cm, while a population in the middle of the range showed no correlation; theoretical calculations show that population expansion following bottlenecks can create such strong associations over several map units. Albouyeh and Ritland (2008) presented and evaluated an experimental design to estimate heritability of gene expression using parent-offspring regression with two-channel microarrays. Thompson et al. (2008) described estimators for three-gene identity coefficients, and used these to demonstrate that clonal reproduction promotes inbreeding and spatial relatedness in yellow-cedar.

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BC MINISTRY OF FORESTS AND RANGE TREE IMPROVEMENT BRANCH 2006–2008

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POLICY ISSUES

Brian Barber

Since 2006, the BC Ministry of Forests and Range (MFR) has put considerable resources into responding to the mountain pine beetle epidemic and preparing for climate change. In response to these and other drivers the MFR and the Forest Genetics Council of BC also undertook a challenge dialogue with colleagues and stakeholders across BC, Canada, and south of the 49th parallel to develop a collective vision and strategy for Forest Tree Genetic Resource Management (GRM) in BC. The GRM dialogue and its outputs are posted at: http://www.for.gov.bc.ca/hti/grm/grm_dialogue.htm. The Chief Forester also launched the Future Forest Ecosystem Initiative (FFEI), whose objective is to increase the resilience of BC's forests. In support of this FFEI objective,Tree Improvement and Research Branch staff have been working on new climate-based seed transfer standards to assist the migration of seed sources so they are better adapted to the projected climate of a site. Interim measures - changes to elevational seed transfer - were introduced at the end of Nov. 2008.

In November 2007, I completed my Masters of Arts degree in Environment and Management from Royal Roads University. My thesis was titled “Reforestation Strategies for Adapting BC's Managed Forests to Climate Change: Policy Barriers and Opportunities”. I served as Acting Director, Tree Improvement Branch, since September 2007 and was confirmed in the position in November 2008. New staff hired this past year include Lee Charleson, RPF, Seed Policy Officer and Matt LeRoy, RPF, Decision Support Analyst.

SEED POLICY

Lee Charleson

I am very pleased to write my first report as the Seed Policy Officer with the MFR. Moving from Alberta to BC, I started in June 2008 and am enjoying the work in seed information, decision support and policy, with a new team and meeting many foresters from around the province with a great deal of interest and enthusiasm for seed policy and seed deployment issues.

Much of my and my team's time has focussed upon introducing amendments to seed transfer. Also, a new Subprogram was created within the Forest Genetics Council and I am the chair of the Seed Transfer Technical Advisory Committee (TAC). This new TAC takes the lead on identifying and prioritizing knowledge gaps in genecology and seed transfer and addressing the gaps through research. Other activities include a new seed planning reference which is now posted on our website, updates to our Seed Planning and Registry system, spatial analysis of seed transfer amendments, participation in the Future Forest Ecosystem Initiative (Vulnerability Working Group and Tree Species Working Group), and updating the genetic criteria of the State of the Forests report and effectiveness evaluation reporting.

CONE AND SEED ACTIVITIES

Dave Kolotelo

Extension continued to be a very active area over the past two years. In 2007, five Tree Seed Workshops were held throughout BC and in July 2008 a Seed Use Efficiency Meeting was held to commemorate our Tree Seed Centre's 50th Anniversary! Web pages are being developed to provide pdf versions of the PowerPoint presentations and/or abstracts and will be advertised through the Tree Seed Working Group (TSWG) News Bulletin. I continued to chair the TSWG and with Dale Simpson we produced four Newsbulletins over the past 2 years:

#44 December 2006	Seed Dormancy
#45 July 2007	Seed Storage
#46 December 2007	Seed Transfer
#47 August 2008	Seed Use Efficiency

Other extension activities included presentations at the International Plant Propagators Society (Oct/06); Northern Silviculture Committee (Jan/08); Tree Seed Working Group (Aug./08) and other meetings.

I have been chairing our Genetic Conservation Technical Advisory Committee and, together with Sally Aitken and Jack Woods, prepared a Genetic Conservation plan for BC. Noteworthy is our move forward with all 50 BC tree species vs. the past emphasis solely on commercial species. Cataloguing is moving forward through an analysis of protected areas, inter *situ* tests and our ex *situ* seed bank which I am directly involved with managing.

Activities at the Tree Seed Centre have focused on several years of intense cone and seed processing primarily for lodgepole pine (*Pinus contorta*) in response to the Mountain Pine Beetle epidemic. Challenges to conventional processing include collections from dead trees, older cones, lower yielding cones, and the loss of serotiny. A set of bulletins dealing with seed supply and collections can be found at http://www.for.gov.bc.ca/hti/pinebeetle/index.htm. Last, but far from least, in 2008 the Tree Seed Centre (TSC) celebrated 50 years of operation as part of the BC Forest Service with a variety of events including an appreciation event for those contributing to the TSC, a friends and family open house, and a wide variety of tours for various groups.

DECISION SUPPORT

Matt LeRoy

I started as the new Decision Support Analyst for the MFR Tree Improvement Branch shortly after Lee Charleson joined the branch as Seed Policy Officer. Since joining the team my role has largely focused on providing spatial analysis to support interim seed transfer policy amendments and seed planning. In addition to data management and data interpretation I have been busy supplying tree seed related spatial and tabular data to clients engaged in timber supply review and seed planning processes. I am also a new member of the Provincial Forests For Tomorrow seed planning committee.

SEED PRODUCTION

David Reid

Since the 2006 report, activities for our Seed Production section of Tree Improvement Branch have focused on increasing seed orchard production capacity. In the last few years and in the upcoming few years we will have and will be establishing approximately 16 000 additional ramets of lodgepole pine, Douglas-fir (*Pseudotsuga menziesii*), and Ponderosa pine (*Pinus ponderosa*). In some cases, we will be adding additional ramets to existing orchards but we will also be establishing seven new orchards. When this expansion is completed in 2010, the MFR will be operating 44 seed orchards of 10 species comprised of almost 59 000 ramets on 140 ha. The target productive capacity of these orchards is over 1400 kg of seed per year yielding approximately 130 million seedlings.

In 2006 and 2007 our lodgepole pine seed orchards in Prince George (average age 16 years old) and Vernon (average age 20 years), were attacked by the mountain pine beetle. Immediate bole spray applications of Sevin XLR killed the beetles and prevented further attacks. We experienced only minimal tree mortality. It also helped that the orchard trees are kept in good condition with frequent watering and fertilizer applications such that the trees had a good capability of pitching out the beetles as well.

In September 2007, I was fortunate to be able to attend a seed orchard conference in Umea Sweden. Umea, at 64°N latitude (the Arctic Circle is 66°N) is the home of the Swedish University of Agricultural Sciences. It was amazing to see productive seed orchards at this latitude. The conference was organized by Professor Dag Lindgren and was attended by 90 participants from 27 countries.

An important business matter arising from the conference was the re-genesis of the International Union of Forestry Research Organizations (IUFRO) Working Party for Seed Orchards. A business meeting was held and the delegates voted overwhelmingly to re-establish the working party (WP). The WP will be established in Division 2 and the number will be 2.09.01 and will be entitled “Seed Orchards”. Officers were nominated and voted upon at the meeting. The Coordinator is Dr. Kyu-Suk Kang, Korea Forest Research Institute, (kangks@foa.go.kr), while the Deputies are: 1. Dr. Dag Lindgren, Swedish University of Agricultural Sciences, (Dag.Lindgren@genfys.slu.se), 2. Nebi Bilir, Süleyman Demirel Üniversity, ISPARTA, Turkey, (nebilir@orman.sdu.edu.tr), and 3. myself - David Reid, BC Ministry of Forests and Range, (David.Reid@gov.bc.ca).

It was proposed at the business meeting that a formal WP meeting should be held every two years so while the next formal meeting should be in 2009, the IUFRO World Congress will meet in Korea in 2010, and it was voted that the Seed Orchard WP should also convene then. So, if you are interested in this WP, please see the IUFRO website (www.iufro.org/science/divisions/).

It was also voted at the meeting to update the publication “Seed Orchards” that was printed in 1974 and edited by Roy Faulkner of Scotland under the old IUFRO WP No. 2.03.3. The Coordinator and Deputies will be updating the table of contents and then will be looking for volunteers to undertake responsibility for the content of those chapters.

I am also organizing, along with a hardworking committee, our British Columbia Seed Orchard Association biennial meeting for 2009. All are welcome to attend.

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

Michael Carlson, Silvia L'Hirondelle, Barry Jaquish, John N. King,
Greg O'Neill, John Russell, Michael Stoehr, Nicholas Ukrainetz,
Chang-Yi Xie and Alvin Yanchuk

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Keywords: tree breeding, breeding for pests and diseases, wood density, mountain pine beetle, quantitative genetics, adaptation, climate change, assisted migration, physiology

TREE BREEDING

Coastal Douglas-fir (Michael Stoehr)

Subline testing and breeding for third-generation orchards are continuing in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) in seed production unit (SPU) 1. A complimentary testing scheme is used to generate parental breeding values through polycross (GCA) testing and at the same time establishment of full-sib family blocks for selection of the best individual of the best family per subline. Four GCA tests and 2 full-sib block tests are established in four phases (series). Phase 1 GCA seedlings, planted in 1999, were assessed with the Resistograph to determine breeding values for wood density. Final selections from the pre-selected grafts will be made in the fall 2008 after year 11 measurements. Phase 2 sublines (8 sublines) were measured and selections were made in full-sib blocks that were planted in 2003. From each subline, the best three full-sib families and the best three individuals within each family were selected and grafted. These selections will be the basis for final selections at age 11 after a final measurement. Phase 3 was planted in 2006. Control crosses for the last phase were completed spring 2008 concluding the breeding for this generation.

Testing in the coast-interior (submaritime) zone (SPU19) continues in three tests established in 2005. Due to the hot and dry summers, water-use efficiency and summer drought tolerance assessments are part of the testing process in co-operation with the University of Victoria. Plantation establishment and early seedling survival are major challenges in this seed production unit. As a result, summer drought resistance will be incorporated in the final selection criteria.

Seed orchard establishment for high-elevation coastal Douglas-fir (900–1,100 m) (SPU 31) at Western Forest Products (WFP) seed orchard complex in Saanichton is underway with backward selections identified in two progeny tests established on Vancouver Island at 1,000 m. Genetic gains are expected to be around 10% for volume at rotation.

Red alder (Chang-Yi Xie)

Fifty-four forward selections of red alder (*Alnus rubra*) have been made for the northern seed planning zone based on individual breeding values of stem volume and other quality characters including stem form and branch number and size. A clone bank has been established at the Cowichan Lake Research Station (CLRS) with 5 ramets per clone.

Black cottonwood (Chang-Yi Xie)

The third provenance-clonal trial of black cottonwood (*Populus trichocarpa*) was established at Harrison Mills in the spring of 2007.

Data on 3-year height, severity of infection by *Valsa sordida* and *Melampsora occidentalis*, and abnormality of leaf flushing from a common-garden test consisting of 180 provenances from 36 drainages ranging from northern BC to Oregon State, USA have been analyzed. The results demonstrated a discontinuous or ecotypic mode, north-south regional differentiation. Respectively, 40 and 89% of the trees from the northern region were infected by *V. sordida* and *M. occidentalis* while 66% showed various degrees of flushing abnormality. In contrast, only 0.7 and 27% of their southern counterparts were infected by the same diseases, and 1% had abnormal flushing. Trees from the northern region averaged 87% shorter than those from the south. Regional differentiation accounted for the highest amount of variation observed in all traits, with 60% in 3-year height, 34% in *V. sordida*, 76% in *M. occidentalis*, and 50% in abnormal leaf flushing. Regression analyses failed to detect any latitudinal patterns of genetic variation in the southern region and combined analysis revealed geographic patterns that essentially reflected regional differentiation along the ‘no-cottonwood’ belt along the Pacific Coast between the Kitimat River in the north and the Dean-Bella Coola River in the south. Evidence from the species’ distribution biography, ecological characteristics, and life history supported the conclusion that restricted gene migration was the main factor underpinning the processes that resulted in the observed geographic patterns of genetic differentiation.

Big-leaf maple (Chang-Yi Xie)

Four provenance-progeny trials of big-leaf maple (*Acer macrophyllum*) have been established. Each trial includes about 400 families from 43 provenances ranging from the northern limit of its natural distribution in BC to California. Those four sites are located at Sayward (northern Vancouver Island), Skutz Falls (Southern Vancouver Island), Power River, and Carey Island (Chilliwack).

Western Redcedar (John Russell)

Management of the western redcedar (*Thuja plicata*) gene resource, a major component of BC’s coastal mixed-coniferous forests, is in its infancy. The program had it’s beginnings in 1990 focusing on gene conservation and genecology. A tree breeding program was initiated in 1996.

Tree breeding for western redcedar involves the development of three overlapping breeding populations from first-generation selections: 1) volume growth (main population), 2) heartwood durability, and 3) deer resistance.

Volume growth Breeding for first-generation polycross testing, involving approximately 1,000 parents for the maritime low SPU, is complete. Seven annual series of tests have been established totalling 46 sites covering six seed planning units. Parental breeding values for volume at rotation are currently available from all seven series based on 7 year heights (1,000 parents). Rogued and new seed orchards are currently producing seedlots with a genetic worth up to 10%. Approximately 50 parents from the first three series have been selected based on volume growth for the advanced generation population and established into breeding orchards. Assortative mating with selfing is currently ongoing.

Heartwood Durability The original 350 BC parent trees selected in the early 1970’s (also represented in the first three series of polycross testing, described above) were assessed for heartwood durable chemicals using wood cores from grafted ramets established in Cowichan Lake Research Station (CLRS) clonebanks and industrial seed orchards. Fifty selections based on gamma- and beta-thujaplicin concentration have been cloned and established in the advanced generation breeding orchard.

Deer Resistance Selections for a deer resistant population were made from both the Holt Creek family/population study (within-family forward selections) and the CLRS clonebank (parental backward selections). Approximately 100 selections for high needle monoterpene concentration and low deer browse have been cloned and established into a breeding orchard. In addition, approximately 50 genotypes have been selected for low monoterpenes and high deer browse. This population will serve as a control for deployment trials with the resistant population. Assortative mating with selfing is currently ongoing.

Yellow-cedar (John Russell)

Yellow-cedar (*Chamaecyparis nootkatensis*) is a major component of high elevation coastal BC forests. Yellow-cedar propagates vegetatively in nature and, in addition, cone crops are usually infrequent and seed viability and germination, historically poor. The genetic management of this valuable species which includes gene conservation, genecology, and tree breeding has focused on a clonal strategy. Recent advances in pollen and seed management will assist in advanced generation breeding and future rejuvenation of select clones through sexual recombination for operational stock.

Fifteen-year measurements have been completed for the yellow-cedar provenance trials, which are comprised of approximately 50 populations established on 10 sites throughout BC. Preliminary analysis indicates the lack of a correlation between seed-origin climatic and geographical descriptors and mean provenance performance as seen in the 10-year data as well. Current liberal seed transfer guidelines reflect this random provenance genetic variation.

First-generation cloned progeny tests have been established and the focus is currently on maintenance and measurements. The first two series of trials have been measured at eight years in the field and the third series at five years. Clonal values from approximately 5,000 clones, including genetic material from the Western Forest Products (WFP) clonal trials, have been released and selected clones established in operational hedge orchards. Advanced generation selections, based on volume growth from the WFP clonal program and from Ministry of Forests (MoF) genetic trials, have been established in three breeding orchards including one high elevation site for pollen production. Maintenance continued in 2008 in these orchards, as well as monitoring pollen performance and production for advanced generation breeding.

Breeding for Wood Density (Nicholas Ukrainetz)

As wood quality is an important target trait for tree breeding, several tools for measuring wood quality attributes are being tested. One of these tools, the Resistograph, was assessed for various potential sensitivities including temperature, moisture content, battery type, and drill-bit sharpness. The Resistograph provides a good indirect assessment of wood density and has been used in some breeding programs to assess wood quality for selection (coastal Douglas-fir).

Prior to attack by the mountain pine beetle, several lodgepole pine (*Pinus contorta*) provenance tests were sampled for wood density analysis. These data were used to model variation in wood density across the range of lodgepole pine. It was also used to model and map environmental effects on wood density in order to improve deployment strategies of tested seed.

Interior Douglas-fir (Barry Jaquish)

The BC Interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) tree breeding program began in 1982 and is based on phenotypic selection in wild stands, open-pollinated progeny testing, clonal seed orchards established through backward selection on the original parents, and controlled mating to produce pedigree material for second-generation selection. Tree height, diameter, and volume are the major traits considered for improvement. The first-generation of breeding includes 1,466 open-pollinated families in six seed planning units (SPU). Seed orchards were established in the early-mid 1990’s and are now coming into production.

In 2007, 117 controlled crosses were completed in all six SPUs and 215 pollen lots were collected, extracted, and stored for future breeding. Controlled crossing for the highest priority Nelson low elevation SPUs is now about 80% complete. Seven 20-year-old progeny tests in the Nelson high elevation SPU and the Cariboo Transition/Quesnel Lake SPUs were maintained and measured. Data analyses for these zones are in progress. In addition, three sites in the 20-year-old Douglas-fir seed source study in the Nass Skeena Transition zone were maintained, pruned, and measured. This planting includes seedlots from throughout the BC range of Douglas-fir and also includes seedlots from six other non-local species. Trees from western larch (*Larix occidentalis*) seedlots from the Flathead Valley in south-eastern BC were the tallest on all three sites. Of the Douglas-fir seedlots, those from the northern limits of the species' distribution demonstrated superior growth, good survival (>90%), and minimal damage from snow and cold (<5%).

Interior spruce (Barry Jaquish)

The Interior spruce (*Picea glauca* x *engelmannii*) tree breeding program in BC is structured in two phases. Series one began in the mid-1960s and focused on three geographic regions: Prince George, Bulkley Valley, and the East Kootenay. Series two began in the mid-1970s and focused on other geographic regions where Interior spruce is commercially and ecologically important. The program has progressed to the point where much of the current planting stock (more than 80 million seedlings per year) comes from rogued first-generation seed orchards, and full-sib second-generation progeny tests are in place for the three Series 1 SPUs and one Series 2 SPU. In 2008, 65 forward selections from the 10-year-old Series 1 Prince George Selection Unit second-generation progeny tests were established in breeding orchards and clone banks near Vernon.

Controlled crossing to produce full-sib families for second-generation selection is on-going in the Nelson and Thompson Okanagan SPUs. In 2008, 15 first-generation progeny test sites were maintained and measured. In addition, all 18 sites in the three-year-old BC/Alberta spruce climate change study were maintained and measured. This project includes 128 seedlots (22 class A from BC and Alberta (AB); 14 full-sib elite crosses from BC; and 92 wild stand seedlots from BC, AB, Yukon Territory (YT), Northwest Territory (NT), and western US) tested on 18 sites that sample the climatic gradients of spruce in BC and AB. Preliminary results across all 18 sites indicate that BC Class "A" and elite sources have the highest growth potential, while those from YT, NT, and south-western US have the lowest growth potential. The warmest sites (Jordan River and Harrison Hot Springs) had the tallest trees, highest average survival, and minimal damage, while the coldest sites (Mayo, YT and Alexis Ck., BC) had the shortest trees, low average survival and high levels of damage. On warm coastal sites, seedlots from the Coastal Western Hemlock zone and Birch Island performed best, while on the cold continental sites, seedlots from AB and YT performed best.

Western Larch (Barry Jaquish)

Commercial planting of western larch continues to increase in BC with over 6.6 million seedlings planted in 2006/07. Sixty-eight percent (4.5 million) of these seedlings originated from seed orchards located in Vernon. In spring 2008, 97 second-generation crosses were completed and 70 pollen lots were collected and stored for future crossing. Second-generation crossing in the East Kootenay and Nelson SPUs is over 80% complete and we anticipate completion of the crossing program within three years. In 2007, four sites in the Nelson Series 1 open-pollinated progeny test were maintained and measured. Data analyses are on-going.

PEST RESEARCH AND BREEDING FOR RESISTANCE

Resistance to Mountain Pine Beetle (Alvin Yanchuk)

As noted in the 2006 CTIA update, impacts by the mountain pine beetle (MPB) on our lodgepole pine genetic tests, seed orchards, and clone banks continue. Spraying efforts to control MPB on key orchards and gene archives has been effective, but many more field tests were lost; most notably, the large 32-year old provenance-family trial at the Prince George Tree Improvement Station. We are continuing our assessments of various resistance features of provenances and families that had lower attack levels in several key progeny tests. The work by Dan Ott (graduate student at University of Northern British Columbia) is showing that some monoterpenes are associated with less attacked families, but several follow up studies are underway.

Resistance to White Pine Weevil in Sitka and Interior Spruce (John N. King and Barry Jaquish)

The first-generation screening for weevils in Sitka spruce (*Picea sitchensis*) is finished and at present we are starting to screen our F_1 breeding population. Our work in this also involves the University of British Columbia (UBC) genomics project (see http://ubc.ca). Selection and breeding work continues but our focus in the future for the breeding program will be to identify parents for specific traits. A project looking particularly at “constitutive” type of mechanisms such as sclereid cells or constitutive resin cells using embling lines is being initiated at the University of Victoria at Dr. Patrick von Aderkas’s lab. Another activity is evaluating weevil hazard. A series of trials have been established to test resistance over a variety of ecological and potential weevil hazard areas. The main objective is to aid in determining deployment guidelines.

Resistance of Western White Pine to Blister Rust (John N. King and Michael Carlson)

We have constructed pedigreed and F_1 populations from resistant trees from all three of the western white pine (*Pinus monticola*) programs that have screened for resistance (BC, Idaho, and Oregon). In the interior the major emphasis has been on the Idaho selections but local BC screening’s for Dr. Rich Hunt’s Canadian Forest Service (CFS) program will be added. On the Coast we are mainly relying on the CFS screenings but also will be adding much more of the Dorena screenings especially from Washington. Realized gain estimates are available from both the Coast and Interior and substantial gains from our screening effort are shown as ‘Field Resistance’ in these trials. A post-doc working through Dr. Abul Ekrammadhoul’s lab (Dr. David Noshad) will be working closely with us re-evaluating the interaction between rust and host.

SEED TRANSFER, PHYSIOLOGY AND QUANTITATIVE GENETICS

Quantitative Genetics (Alvin Yanchuk)

Dr. Eduardo Cappa (Buenos Aries) recently joined our group as a Post-doctoral Fellow (with Y. El-Kassaby, UBC) working in the area of quantitative genetics. Eduardo's expertise in Bayesian analyses of forest genetics trials, along with traditional REML approaches, is assisting us with analyses of the second-generation western hemlock trials and genetic evaluations with spatial analyses/adjustments of forward selections.

Climate Change (Greg O’Neill)

Research Branch staff (Michael Carlson, Vicky Berger, and Greg O’Neill) initiated a large, long-term field test project (Assisted Migration Adaptation Trial) to better understand productivity and health responses of improved (orchard) populations from BC and neighbouring states of the United States when planted across a wide climatic and latitudinal range. By relating responses to test site climate and latitude, this study will identify which orchard seedlots will be best adapted for any reforestation site under future climate projections.

Assisted migration of populations (planting seedlots adapted to future climates) is potentially one of the most effective strategies to address climate change in forestry. Research Branch staff (Greg O’Neill and Nicholas Ukrainetz) are assessing options for converting BC’s seed deployment system from a geographic-based system to a climate-based system in order to better accommodate assisted migration.

A new tool coined the “Universal Transfer Function” was developed to allow researchers to predict responses of populations from any climate when growing in any climate. The function can be derived from provenance test data and is illustrated using BC’s Illingworth lodgepole pine provenance tests which were re-measured in 2005 at age 32. The function is being used to assess the optimum distance to assist the migration of seed and to assess expected productivity associated with seed deployment systems.

Growth response functions (GRFs) relate the growth of a population to the climate of the sites in which it is tested, and are gaining attention for their ability to predict climate change impacts. However, non-climatic site variation can obscure the climatic tolerance of a population in GRFs. We developed a statistical method of accounting for non-climatic site variation that substantially improved the resolution of GRFs. The method is being used in a project to identify optimum assisted migration distances.

Physiology (Sylvia L’Hirondelle)

The adaptive physiology research program helps forest geneticists and forest nurseries produce stress-resistant trees with high growth potential by testing genetic variation in frost hardiness, photosynthetic potential, and phenology, as well as heat, drought, and other stress tolerances. The current focus is on: 1) studying the relationship between patterns of genetic variation in adaptive traits and site ecology in populations of BC conifers and hardwoods, 2) developing physiology tools to estimate population responses to a variety of climates, in order to assist in seed transfer under potential climate change conditions, and 3) improving the speed and accuracy of tests to predict overwinter storability of seedlings, field performance potential, and balanced seedling growth.

In spring 2008, about 8,000 seedlings of three coastal conifer species (total of nine seedlots) were planted after being lifted for cold storage at a range of dates from October to December. Initial results show that nursery cultural conditions had a large effect on the relationship between frost hardiness and storability. Modifications to storability testing will improve stock handling in the fall and lead to better field performance after spring planting.

Spruce genecology (Nicholas Ukrainetz)

Climate modelling and conventional quantitative analyses have been used to assess patterns of genetic variation for several interior tree species. A genecology study (Barry Jaquish, Greg O’Neill, Alvin Yanchuk, and Sylvia L’Hirondelle) was established at three field sites and one raised-bed nursery site to investigate the genecological variation of interior spruce. A second study was established (Michael Carlson and Vicky Berger) at several field trials which incorporated family and population level variation across the range of paper birch (*Betula papyrifera*) in BC. Using multivariate statistical analyses and climate modelling, patterns of genetic variation for growth, phenology and physiology traits were mapped for each species.

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PART 2

Symposium

ADAPTATION, BREEDING AND CONSERVATION IN THE ERA OF FOREST TREE GENOMICS AND ENVIRONMENTAL CHANGE

Quebec City, Quebec
August 25–28, 2008

Editors
J. Bousquet and J.D. Simpson

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L'ADAPTATION, L'AMÉLIORATION ET LA CONSERVATION À L'ÈRE DE LA GÉNOMIQUE FORESTIÈRE ET DES CHANGEMENTS ENVIRONNEMENTAUX

Québec, Québec
25–28 août 2008

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INVITED SYMPOSIA PAPERS

GENOMIC DISCOVERY FOR APPLICATION IN TREE BREEDING AND FOREST HEALTH MANAGEMENT

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Genomic discovery in forest trees follows paradigms from both agricultural crop and livestock improvement and human medicine. Forest trees in a domesticated state can be improved using genomic-based breeding technologies whereas the health of trees in a natural and undomesticated state might be managed using those same technologies. These applications begin by first dissecting complex traits in trees to their individual gene components and for that the association genetics approach is quite powerful in trees. This is true for several reasons including large, random mating and unstructured populations and the rapid decay of linkage disequilibrium in many tree species. Once marker by trait associations are discovered they can be used in genomic-based breeding and forest health diagnostics. High-throughput DNA sequencing and genotyping technologies have enabled a new generation of research in forest genetics where combined quantitative and population genetic approaches can be used to better understand the relationship between naturally occurring genotypic and phenotypic diversity.

BRIDGING GENOMICS AND BREEDING IN HARDWOOD TREE MODELS

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Forest ecosystems cover approximately 3.9 million hectares or 30% of the world's land surface (FAO, 2001), where trees are the dominant species. Tree species are essential for the survival of these ecosystems and the stability of their composition and diversity. Trees also provide a source of materials for bioenergy and wood, one of the world's most important renewable materials, and contribute an estimated 354 billion US dollars to the world's economy (FAO, 2001). Wood consumption per capita has increased only slightly in the past decades, but world population growth has lead to a significant expansion in demand. In the meantime, wood production and the area dedicated to plantation forestry have remained relatively stable or even declined in parts of Europe and North America. The consequent loss in wood production has been partially offset by a rapid increase in the area of planted forests in Asia and South America and gains in productivity supported by aggressive tree breeding programs and biotechnology.

This presentation will focus on *Eucalyptus* and *Populus*, two woody angiosperms of great economic importance for the forest industry worldwide. *Populus trichocarpa* was the first woody plant genome to be sequenced (Tuskan *et al.*, 2006), and the genome of the *Eucalyptus grandis* genotype Brasuz1 will soon be unveiled by JGI/DOE. Both species are differentiated from conifers in wood morphology, chemistry, and several reproductive, developmental and adaptive traits. As will be discussed below, translating genomics to breeding in these species will present its own challenges. On the other hand, the relative simplicity of their genomes and the availability of a reference sequence may lead to the rapid development and adoption of genomics tools in traditional genetic improvement programs.

Genomics offers a platform to learn about the relationships of genes and phenotypes. For genomic breeders the long term goal is to develop predictive models that will permit estimation of performance and adaptability of genotypes across sites/ecosystems based on genetic data alone. In that scenario, knowledge of the optimal allele combinations necessary for biosynthesis of an ideal product will be essential. The development of genotype-based predictive models is still a long way from reality. However, the first step to develop these models – the discovery of allelic variants that contribute to adaptation and phenotypic diversity – is well under way. Here I will discuss the approaches that we and others are applying in *Populus* and *Eucalyptus* genomic research, aimed at the identification of genes and alleles of value. Current limitations and pitfalls of these methods will also be discussed. Finally, rapidly changing technologies of genomic analyses and their benefits to molecular breeding will also be discussed.

The development of the first genetics maps in the early 1990's created the foundation for quantitative trait loci (QTL) analysis and the characterization of the genetic architecture of wood quality and growth traits in *Eucalyptus*, *Populus* and other tree species. However, major barriers to the effective application of QTL analysis to breeding rapidly became apparent. High levels of genetic heterogeneity and linkage equilibrium of breeding populations meant that marker/trait linkages were of limited application to different families. QTL studies were also limited in resolution and in the genetic variation captured.

Despite these limitations, a QTL approach combined with genome information can be particularly powerful for detection of genes and alleles that confer unique/superior properties to a species. Tree breeders have long recognized that some hardwood species that have strikingly distinct wood quality, growth and adaptive properties can be intercrossed, producing hybrid genotypes that capture the superior characteristics of each species in one individual. This genetic variation has been and will increasingly be a powerful source for detection of major effect genes in experimental hybrid populations. We recently characterized a broad spectrum of phenotypes in a hybrid cross of *P. trichocarpa* and *P. deltoides*, including leaf shape, vessel development and other developmental traits. Analysis of QTLs identified significant genomic regions of interest that, in combination with the *Populus* reference genome sequence and transcriptome information defined genes that are strong candidates to regulate these traits. These alleles of large effect may be relatively easy to detect in a combined QTL/genomic analysis of hybrid segregating populations. For many hardwood species of commercial value, highly significant gains may be achievable through the introgression of these valuable species-specific alleles into the breeding populations through marker-assisted selection or transgenesis.

Some of the limitations of QTL analysis have favored the use of another forward genetic approach, linkage disequilibrium mapping or association genetics, for discovery of genes that regulate quantitative phenotypic variation. Association studies typically rely on the genetic analysis of populations with unknown ancestry and from a diverse genetic background. Success of association mapping depends on the level of nucleotide diversity and linkage disequilibrium in the surveyed population. In the few genes that we and others have analyzed in *Populus* and *Eucalyptus*, significant levels of linkage disequilibrium (LD) only extends to a few hundred base pairs and nucleotide diversity is among the highest recorded in any species. Therefore significant association may readily identify genes that contribute to phenotypes. The limited genetic structure generally observed in these natural populations also minimizes the detection of spurious associations. In fact, the first successful identification of a significant association between a polymorphism and a phenotype, microfibril angle, was reported for *Eucalyptus globulus*. While it demonstrated the feasibility of association genetic studies in long-lived, perennial woody species, several limitations and pitfalls associated with the biology and breeding methods in hardwoods must be recognized.

Breeding of woody angiosperms such as *Eucalyptus* and *Populus* generally explores the vigor of hybrids for creating superior genotypes. While overdominance and epistasis appear to be a significant driving force behind heterosis in these species, it is unclear if association genetics will be able to identify the interspecific allelic combinations that produce these superior phenotypes. It is also unclear if high levels of polymorphism and low LD will be repeated in hardwood species of commercial value but with a narrow natural distribution, or in provenances that may have originated from relatively recent migration events. Selective sweeps around loci that are essential to adaptation will also lead to high levels of LD. Although typically localized, these regions of high LD will in some cases be specifically the ones targeted by breeders. Finally, many of these outcrossing tree species are likely to contain an abundance of high-effect, low-frequency alleles that will be difficult to identify in traditional association studies. Some of these rare polymorphisms may contribute significantly to diversity in the phenotypes most sought by tree breeders. In a recent survey we identified almost 300 rare large-effect SNPs – i.e. polymorphism that lead to the introduction or removal of a STOP codon – in transcribed sequences of a pool of *E. grandis* individuals. The probability of maintaining rare alleles in outcrossing, long-lived perennial species with large effective population sizes is high. It may be possible that the majority of the variance that contributes to superior phenotype detected in some individuals may be due to such uncommon, high effect allelic variants. Therefore, the largest gains from molecular breeding may be achieved not through the continuous incorporation of frequent alleles of small positive effect, but rather through the identification of rare, high-value alleles.

Identifying these rare alleles and increasing their frequency in breeding populations will be challenging. Furthermore, in many hardwood species that utilize a hybrid breeding/clonal deployment approach the challenge will go beyond detecting rare allele of large effects, but rather rare allelic epistatic combinations, which will require analysis of much larger populations. Uncovering them will be unlikely using the current approaches but we can foresee that emerging high-throughput sequencing and genotyping technologies may rapidly change this scenario. While the initial sequencing of the model plant *Arabidopsis thaliana* was a multi-year, multi-million dollar project, several hundred new genotypes have since been sequenced and characterized in a fraction of that cost and time. Similarly, sequencing of *Populus* or *Eucalyptus* genotypes, when supported by the availability of a reference genome, may be achievable now for a cost 2-3 orders of magnitude smaller than that of a decade ago. The consequence of the rapid improvements of genotyping and sequencing methods and major advances in bioinformatics are that it will soon be possible to identify all the sequence variants in very large breeding populations. Already in human genetics, genotyping of several million nucleotide polymorphisms in thousands of individuals is commonly carried out. Unraveling the alleles and interactions of value will provide the foundation for an understanding of all the genetic factors that contribute to the immense phenotypic diversity of tree species, and will be the first step of effectively applying this knowledge to breeding.

BREEDING WITHOUT BREEDING: AN UNORTHODOX CONCEPT

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ABSTRACT

Structuring mating designs for quantitative genetic analyses without making crosses is one of Breeding Without Breeding concept's (BWB: El-Kassaby et al. 2007) four pillars. The application of DNA fingerprinting and pedigree reconstruction is illustrated and methods for forming mating designs using open-pollinated or bulk seed from known or unknown parental population are presented. While the structuring of mating designs is an essential step to BWB scheme, the collective importance of phenotypic or genotypic pre-selection to reducing fingerprinting efforts, spatial analyses to improving the quality of collected phenotypic data and increasing derived genetic parameters' precision, and the application of the state-of-the-art quantitative genetics analyses should not be underestimated.

INTRODUCTION

Tree improvement programs are long-term endeavours requiring specialized expertise as well as organizational and financial commitments for securing their success. These programs follow the classical recurrent selection approach with its repetitive cycles of breeding, testing and selection (Namkoong 1979). After the completion of each cycle, elite genotypes are selected for the following breeding cycle or the establishment of production populations (seed orchards) for the sole purpose of capturing and delivering the attained genetic gain to the forests (Faulkner 1975). While variations from this model exist (Namkoong et al. 1988), without exception, all are anchored around the creation of structured pedigreed material from the selected parents, the establishment of elaborated field trials for testing, and utilization of quantitative genetics to estimate the genetic parameters needed for elite genotype selection and the degree of genetic advance (White and Hodge 1989). The Breeding Without Breeding (BWB) concept of El-Kassaby et al. (2007) offers a drastic deviation from the classical recurrent selection approach and is based on the use of: 1) DNA fingerprinting and pedigree reconstruction (Jones and Ardren 2003) to create mating designs from natural matings among breeding arboreta or seed orchards' parents (Funda et al. 2008), 2) phenotypic or genotypic pre-selection for concentrating the fingerprinting efforts on a reduced set of individuals (El-Kassaby and Lindgren 2007; El-Kassaby and Lstibůrek 2009), 3) spatial statistics to remove unaccounted for patchiness and/or global site variability to improve data quality (Cappa et al. 2009), and 4) the animal model (Lynch and Walsh 1998) for quantitative genetic analyses and the determination of all of the genetic parameters used in the classical approach (El-Kassaby and Lstibůrek 2009).

El-Kassaby and Lstibůrek (2009) presented two approaches to BWB; namely, simplified open-pollinated testing and phenotypic selection. DNA fingerprinting and pedigree reconstruction were used for mating design creation through paternity assignment (i.e., converting the open-pollinated (half-sib) test to full-sib test), and also to control the co-ancestry during elite genotype selection in both approaches. Additionally, El-Kassaby and Lstibůrek (2009) presented the theoretical foundation for their BWB as well as the development of an optimization protocol for maximizing the genetic gain at any desired preset diversity level during the selection phase.

While BWB consists of four pillars (DNA fingerprinting and pedigree reconstruction, phenotypic or genotypic pre-selection, spatial statistics, and the animal model), the DNA fingerprinting and pedigree reconstruction are essential for mating design(s) emergence from natural matings among parents and, in fact, constitute the cornerstone for the whole approach. The following section represents a summary of our efforts to develop a robust pedigree reconstruction and formation of mating designs.

MATING DESIGN FROM OPEN-POLLINATED SEED FROM KNOWN PARENTAL POPULATION

Funda et al. (2008) explored the possibility of using DNA fingerprinting and pedigree reconstruction to construct a mating design from natural matings within a western larch (*Larix occidentalis* Nutt) seed orchard.

Seed from 14 of the orchard's 41 parents were sampled and fingerprinted using 12 microsatellite markers, followed by pedigree reconstruction.

The results produced an incomplete factorial mating design with females sired by an average of 16 males and males mated with an average of 5 females, mating incidences exceeding those from any conventional mating design (Figure 1). It is also noteworthy to highlight the fact that this mating design emerged under high contamination level of 22%, indicating paternity assignment success rate of 78%.

This example resembles the BWB's simplified open-pollinated testing method where the maternal parents of the selected trees and the orchard's parental population are known. In this case, DNA fingerprinting and pedigree reconstruction successfully improved the pedigree relationship of the tested individuals by adding multiple male parents, forming a pedigree matrix mainly consisting of half-sibs with several full-sibs nested within them, thus substantially improving the estimated genetic parameters through the presence of high number of mating bridges needed for the prediction of breeding values using the BLUP analysis (Henderson 1975).

MATING DESIGN FROM BULK SEED FROM KNOWN PARENTAL POPULATION

This scenario resembles the phenotypic selection method of BWB. In this case, the genotype of the parental population is known but the maternal and paternal parents of each individual seed is unknown, a situation similar to selection from plantations produced from bulk seed orchards' crops. Lai et al. (2009) successfully reconstructed a large incomplete diallel mating design from a bulk seed sample from a 49-parent Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seed orchard using a battery of 8 microsatellite markers. Additionally, they tested the success rate of supplemental-mass-pollination (SMP: Wakeley et al. 1966) to verify the possibility of expanding the seed orchard's breeding population through the introduction of new parents as males as proposed by El-Kassaby and Lstibůrek (2009) (Figure 2). The emerged mating design occurred under modest contamination rate of 5% and the SMP success rate was determined to be 5%, an estimate adequate enough to introduce new parents to the orchard's breeding population. This mating design was produced under extreme female and male fertility variation (Lai et al. 2009), indicating that even under these conditions, a mating design could be produced and the impact of this fertility variation on the genetic parameters estimation is negligible as long as random mating exists (Henderson 1988; Kennedy et al. 1988).

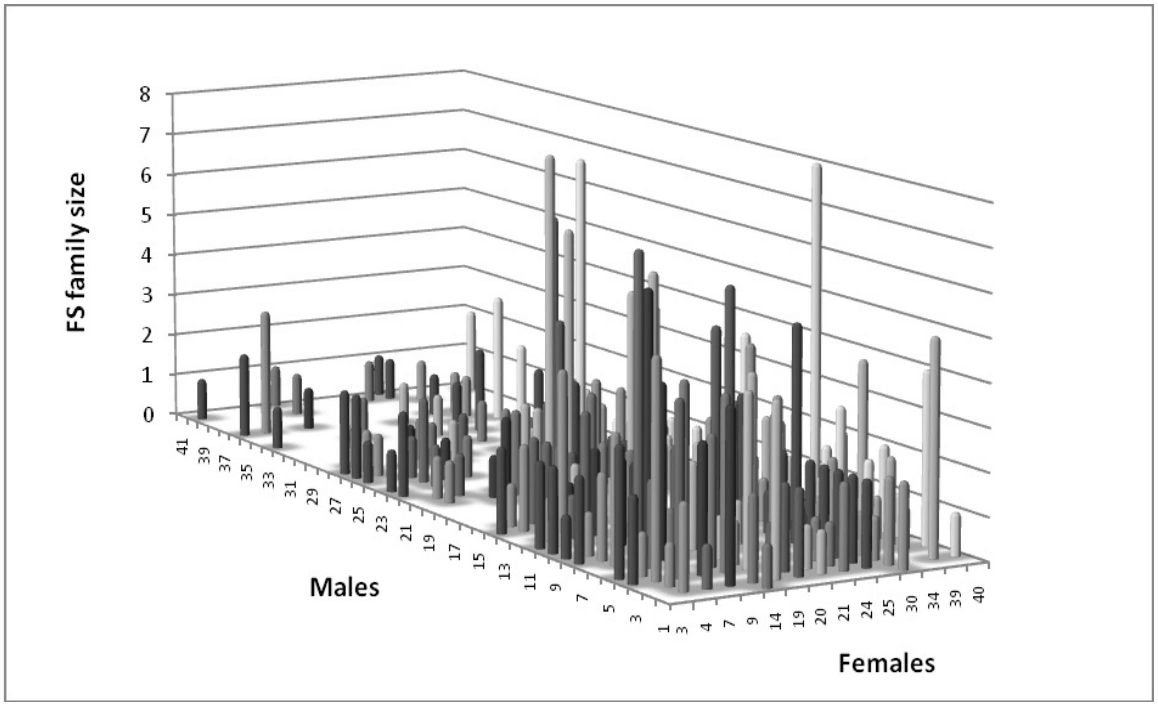

Figure 1. Incomplete factorial mating design emerged from 221 full-sib families nested within 14 wind-pollinated western larch half-sib families.

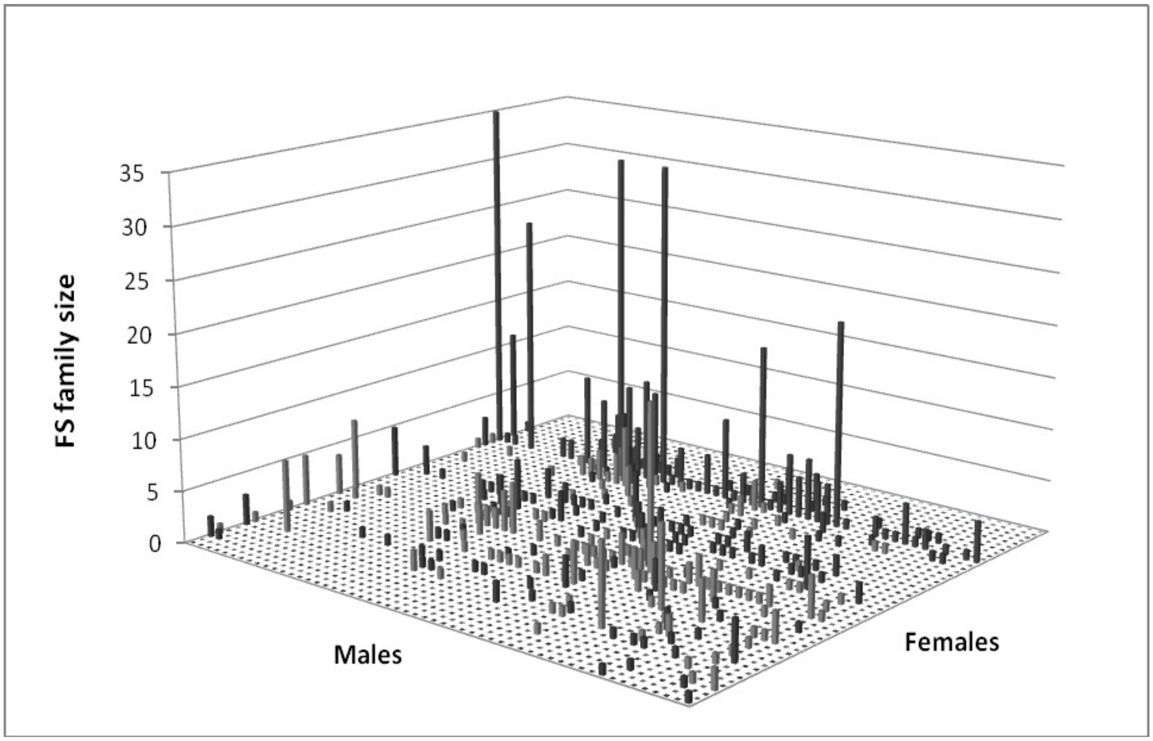

Figure 2. Incomplete diallel mating design emerging from bulk seed collection from a Douglas-fir seed orchard. Matings at the left hand side of the male axis represent SMP success.

MATING DESIGN FROM UNKNOWN PARENTAL POPULATION

Building a mating design from a group of related individuals without known parental population information is among the most challenging tasks. Massah et al. (2009) utilized a two-step analytical approach to deal this problem: Initially, the presence of genetic co-ancestry among individuals is verified using pair-wise relative kinship analysis (Ritland 1996) followed by pedigree reconstruction (Wang 2004)to unravel the present co-ancestry through grouping these individuals into several full-sib families of different sizes nested within multiple half-sib families (Figure 3). The resulting structure allows conducting quantitative genetics analyses to estimate a breeding value for every individual as long as the individual has a full- and half-sib family membership. This was successfully done on a group of yellow-cedar (*Chamaecyparis nootkatensis* (D.Don) Spach) individuals selected from a seedling crop originated from a natural stand bulk seedlot. This analysis assumes that paternal parents exclusively mate with one maternal parent and no parental half-sibs exist across the resulting maternal half-sib families. This situation is possible for yellow-cedar since natural stand seed collections are done using helicopter and seed-cones are collected from multiple donors scattered over a wide geographic location (i.e., seed-cone donors receive pollen from multiple males but these males do not sire any of the other maternal parents).

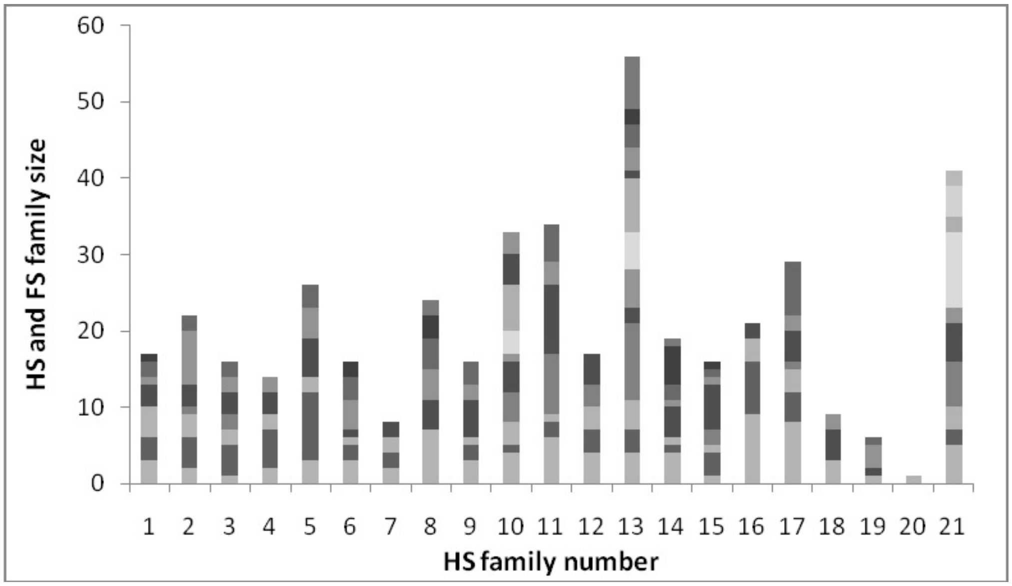

Figure 3. Classification of yellow-cedar individuals into several full-sib families nested within multiple half-sib families.

CONCLUSION

The preceding section provided an illustration of the different methods to structure pedigreed material (half- and full-sib families) to form mating designs for quantitative genetics analyses without the need to conducting even one single controlled cross. The availability of DNA molecular markers and pedigree reconstruction models provided the tools to utilize natural matings among known (breeding arboreta or seed orchard) or unknown (natural stand collections) parents to reconstruct natural matings, thus forfeiting one of the major steps in classical breeding programs. While structuring of mating designs is essential step to BWB scheme, the importance of phenotypic or genotypic pre-selection to reducing fingerprinting efforts, spatial analyses to improving the quality of collected phenotypic data and increasing derived parameters' precision, and the application of the state-of-the-art quantitative genetics analyses should not be underestimated.

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THE PROMISE AND UNRESOLVED CHALLENGES OF MARKER ASSISTED BREEDING IN SOUTHERN PINE TREE BREEDING PROGRAMS

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Molecular markers have revolutionized the science of genetics by directly revealing the variability present in the genetic code and giving clues to the underlying structure of economically important traits. However, molecular techniques and especially forward selection through marker-assisted selection (MAS) has had little impact on applied southern pine tree breeding. Most economically important traits are quantitative, under the control of a number of genes each with small effect. Quantitative trait loci (QTLs) have been difficult to discover and then verify in existing populations. The first generation of QTL experiments was pedigree specific, often revealing significant genotypic, environmental, and temporal interactions. QTLs from these experiments were therefore of limited utility in population improvement programs. Second-generation MAS tools, especially association genetics, holds more promise for population improvement, but will require extensive investments for allele discovery and validation in forest trees. Biological limitations are far from the only problems. When wood is a commodity, quality traits are of little economic importance and the only traits of interest in a breeding program are those that lower the cost of production, i.e. volume growth. This is a composite characteristic that involves many poorly understood and complexly regulated traits. Even in the case of disease resistance genes, MAS may prove less than effective if gene-specific markers for functional variants of resistance alleles are unknown.

Despite these unresolved challenges, the southern pine tree breeding programs should be taking a number of steps to realize the promise of MAS. First and foremost, pedigree verification and quality control should become a regular part of all our programs. Measurements of genetic distance and variation should be obtained to inform population structure, guide gene conservation plans, and to direct possible infusions of new material. Next, because of the long lead time needed and the fact that most existing programs will provide limited types of families with small numbers of seedlings, tree breeders must work more closely with molecular geneticists to design the specialized populations needed for future studies. Better statistical techniques must be developed and adopted by the tree breeding community. Populations should be structured to rapidly fix QTLs of high breeding values in small sub-populations. Finally, economic simulations should be developed and refined to provide a rigorous tool for thinking through MAS breeding plans and to identifying steps with the most economic impact. Many of these needs are currently being addressed through the Conifer Translational Genomic Network which will be briefly described.

INTEGRATING MOLECULAR AND GENETIC DATA FOR A BETTER UNDERSTANDING OF BUD SET IN POPLAR

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The seasonal cycle of growth and dormancy distinguishes perennial plants and represents one of the most basic adaptations of trees to their environment. Because bud set is of prime importance for season length in a changing environment, we dissected this developmental program at the phenotypic, genetic and molecular levels.

Combined metabolite and gene expression studies were used to reconstruct the molecular events during apical bud development in poplar. Bud development could be dissected into bud formation, acclimation to dehydration and cold, and dormancy. To each of these processes, specific sets of regulatory and marker genes and metabolites are associated and provide a reference frame for functional studies. A large set of differentially expressed genes was also expressed during the growth-to-dormancy transitions in poplar cambium and Arabidopsis seeds, suggesting parallels for dormancy regulation in different plant organs.

Bud set in field conditions was evaluated with a new high-resolution scoring system in five breeding pedigrees and a collection of *P. nigra*. QTLs identified in the different genetic maps are investigated for co-localization on the poplar genome sequence. Genomic regions of relevance for bud set are being tested for their adaptive value through association genetics approaches.

WHAT SOURCE AND COMPONENT OF GENETIC VARIATION IS RELEVANT FOR ADAPTATION IN TREES? LESSONS FROM PHYLOGEOGRAPHY AND POPULATION GENOMICS IN EUROPEAN TREES

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Research efforts in population and ecological genetics in trees were driven during recent years by conservation and adaptation issues. Central to the debate is the source and component of genetic variation that will actually contribute to the evolution of trees and allow them to respond to future environmental change. Some of the results obtained in fields that have received major attention during the past ten years in population genetics (ranging from phylogeography to population genomics) were reviewed and how they changed or reinforced our views on genetic variation regarding conservation and adaptation issues were addressed. While traditional approaches allowed the estimation, with reasonable accuracy, of the level of standing genetic variation, findings from phylogeographic studies permitted the elucidation of evolutionary mechanisms that contributed to the extant distribution of variation. In contrast to North American species, European trees exhibit strong phylogeographic structures originating from the genetically isolated refugial zones where trees were confined during the glacial periods. Duration of separation in isolated genepools lasted long enough to allow genetic divergence, not only for neutral markers but also for adaptive traits. However, past differentiation has not persisted in the extant populations in Central and Northern Europe, being erased by geneflow between admixed populations originating from different refugial zones and local selection. There is no footprint left in the todays' distribution of genetic variation across temperate Europe. Hence genetic differentiation for adaptive traits is a consequence of evolutionary trends that lasted rather recently, during the Holocene, and phylogeographic patterns of variation will be of little value for conservation issues. An exception to this is the Mediterranean region, where genetic differentiation was preserved even during recolonisation and local populations may deserve conservation measures.

More recently, genomics has allowed us to investigate the genetic basis of adaptive variation by identifying genes of adaptive significance. While the body of results is still scarce, population surveys of gene frequencies indicate that their level of differentiation is far from reaching the level of differentiation of the adaptive traits measured in provenance tests. Interestingly this discrepancy is expected by theoretical predictions. Indeed the genetic variation of a complex trait is composed of two components: the genetic differences at the gene level (that can be detected by gene sequences and measured as Fst) and the association of alleles at the different genes controlling the trait (that corresponds to disequilibria among genes). Theoretical investigations suggest that for traits depending on many loci and for species with high gene flow such as trees, differentiation of genes will remain at levels close to neutral expectations, while association of alleles will represent the major component of differentiation of the adaptive trait. If these results are confirmed by future experimental data, they will lead to important consequences in conservation issues. As Fst is low, the basis for evolutionary potential can be sampled within a very few populations and future adaptation will proceed by associating alleles differently according to selection pressures.

MAINTAINING OR MOVING THE MOSAIC OF DIVERSITY: GENETIC CONSERVATION AND CLIMATE CHANGE

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The primary goal of genetic conservation is to maintain genetic diversity and population structure to fuel adaptation to new environmental conditions. Most conservation programs in North America rely primarily on *in situ* genetic conservation, where large, natural populations are maintained in parks and protected areas, and allowed to naturally regenerate, evolve and adapt. The extent to which these conservation populations will persist will depend on the extent to which they can adapt to new conditions or migrate to track their climatic niches within or between protected areas. Species distribution models predict wholesale relocation of tree species in the next century, yet migratory responses necessary to track predicted rates of climatic change greatly exceed maximum post-glacial migration rates, and habitat fragmentation between reserves will slow migratory responses.

The extent to which populations will adapt to new climates will depend upon amounts of phenotypic variation for traits involved in local adaptation, strength of selection, fecundity, interspecific competition, and biotic interactions. Populations of temperate and boreal trees show moderate to strong local adaptation to climate, yet exhibit little differentiation for genetic markers, indicating high levels of gene flow via pollen. Peripheral populations have lower levels of genetic diversity and higher levels of inbreeding than central populations, yet isolated peripheral populations may contain phenotypes adapted to conditions at the edge of the species climatic niche. When environments are stable, gene flow limits adaptation; however, as the climate warms, pollen movement carrying pre-adapted alleles from warmer to cooler climates may promote adaptation and migration at the leading edge of species ranges. In contrast to traditional views of managed forests ‘contaminating’ conservation populations through gene flow, facilitated migration via reforestation under climate change-based seed transfer policies may input adaptive diversity into conservation populations. Natural hybridization within genera may also accelerate adaptational responses.

Genomic research indicates traits involved in local adaptation such as phenology and resistance to abiotic stresses appear to be the product of small effects of many genes. The resulting genotypic redundancy of many potential genetic combinations resulting in the same phenotype, combined with high fecundity, may ensure that at least some individuals are adapted to new conditions in each generation, and strong selection could facilitate rapid local adaptation. Widespread species with large populations and high fecundity are likely to persist and adapt over some portion of their ranges, but will likely suffer adaptational lag for a few generations. Interspecific competition may weaken as all tree species experience some degree of reduced fitness, facilitating persistence of populations under suboptimal conditions. Species with small populations, fragmented ranges, low fecundity, or suffering declines due to introduced insects or diseases may be candidates for facilitated migration in a conservation context, although there will be debate around such interventions. Given the extent of uncertainty around climate change, *ex situ* conservation of seeds, tissues, and trees may provide insurance for catastrophic population reductions and thus compliment *in situ* conservation.

EXPRESSION PROFILING, GENOME STRUCTURE, HT SEQUENCING

IDENTIFICATION OF CONSERVED CORE XYLEM GENE SETS: CONIFER cDNA MICROARRAY DEVELOPMENT, TRANSCRIPT PROFILING AND COMPUTATIONAL ANALYSES

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One approach to investigating the molecular basis of wood formation is to integrate microarray profiling datasets and sequence analyses, comparing tree species with model plants like *Arabidopsis*. Conifers may be included in comparative studies thanks to large-scale EST analyses, which enable the development of cDNA microarrays with very significant genome coverage.

We have designed a microarray of 10 400 low redundancy sequences starting from spruce cDNAs. Computational procedures that were developed to ensure broad transcriptome coverage and efficient PCR amplification were used to select cDNA clones, which were resequenced in the microarray manufacture process.

White spruce (*Picea glauca* [Moench] Voss) transcript profiling experiments, that compared secondary xylem to phloem and needles, identified 360 xylem preferential gene sequences. The functional annotations of all differentially expressed sequences were highly consistent with similar analyses carried out in angiosperm trees and herbaceaous plants.

Computational analyses, comparing the spruce microarray sequences and core xylem gene sets from *Arabidopsis*, identified 31 transcripts that were highly conserved in angiosperms and gymnosperms, both in terms of sequence and xylem expression. Several other spruce sequences have not previously been linked to xylem differentiation (including TLPs, *gai*) or were shown to encode proteins of unknown function encompassing diverse conserved DUFs.

AIR TEMPERATURE EXERTS STRONGER CONTROL THAN PHOTOPERIOD OVER THE MOLECULAR REGULATION OF COLD HARDENING IN NEEDLES OF *Pinus banksiana*

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As the climate warms over the coming century, an increased length of the growing season for boreal forest trees is predicted, potentially altering the carbon sink of conifer forests. To understand the role of increased temperature on autumn cold hardening in jack pine (*Pinus banksiana*) we isolated effects of temperature and photoperiod in factorial experiments. Using the Arborea 9k spruce microarray, we studied gene expression in pine needles and assessed changes in photosynthetic gas exchange and energy partitioning. Gene expression data revealed interactive effects of autumn photoperiod and low temperature controlling the cold hardening process. Compared to summer conditions, seedlings acclimated to natural autumn conditions indicated a metabolic switch with significant changes in expression of genes of e.g., photosynthesis, carbon, or lipid metabolism. The affected functional categories had little overlap with the affected categories observed in seedlings acclimated to summer air temperature and autumn photoperiod (warm autumn, representing future climatic conditions). Warm autumn conditions induced expression of genes involved in oxidative stress responses and supressed genes involved in electron transport. This pattern corresponded with changes in rates of CO_2 assimilation under warm autumn conditions, which decreased by 50% compared to summer but were equal to autumn plants. Under warm autumn conditions we further observed dissipation of excess energy not via zeaxanthin, but by an as yet unidentified mechanism which is not detected in pine seedlings grown under summer or autumn conditions. Together, control of the cold hardening process by photoperiod in pine appears to negate any potential for an increased carbon gain associated with higher temperatures during the autumn season.

A NORWAY SPRUCE *FLOWERING LOCUS T* HOMOLOG IS IMPLICATED IN CONTROL OF GROWTH RHYTHM IN CONIFERS

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Norway spruce (*Picea abies*), as many other tree species, shows a strong latitudinal cline in growth cessation and bud set. This variation is to a large extent determined by a genetically controlled response to photoperiod. To identify genes controlling this variation we have adopted a combined approach comprising candidate gene mapping and analysis of DNA variation combined with functional studies to identify and verify those candidate genes. In an effort to identify molecular components of the photoperiodic pathway in Norway spruce, we isolated homologues to photoperiod genes in *Arabidopsis* to study their function. In these studies, a tight correlation between growth rhythm and expression pattern of one Norway spruce FT-like gene (*PaFT4*) was observed over a range of experimental conditions. This suggests that one Norway spruce homolog to the *FT* gene, which controls flowering in angiosperms, is also a key integrator of photoperiodic and thermal signals in the control of growth rhythms in gymnosperms. The data also indicate that the divergent adaptive bud set responses of northern and southern Norway spruce populations, both to photoperiod and light quality, are mediated through *PaFT4*.

EXPRESSION OF *FLOWERING LOCUS T* AND *LEAFY* HOMOLOGUES ARE TEMPORALLY ASSOCIATED WITH ANNUAL FLOWER BUD INITIATION IN *Eucalyptus globulus*

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The transition to flowering in plants is influenced by external signals such as light and temperature, and internal signals. These signals act through a complex genetic pathway mainly studied in the annual plant, *Arabidopsis*. In perennial trees, which are characterised by a juvenile, non-flowering phase lasting years, and an annual cycle between vegetative and reproductive growth, the regulation of flowering time is potentially more complex. *Eucalyptus globulus* is a temperate plantation species and a better understanding of flowering will optimise seed production as well as manage gene flow from plantations into native populations. Our surveys of *E. globulus* clones, grown in a common environment near Hobart, Australia, showed that there is up to a six month genetically-based difference among clones in the timing of flower opening. Surprisingly however, flower bud initiation occurs simultaneously in all clones. The difference in flowering time is therefore mainly due to slower bud development in late flowering genotypes. We used degenerate primers and database searches to isolate the *Eucalyptus* homologues of *FLOWERING LOCUS T* (*FT*) and the floral meristem identity gene *LEAFY* (*ELF1*). Quantitative RT-PCR showed that the expression of these genes in the leaf and apex, respectively, is associated with annual flower bud initiation over a two-year period. Comparison of early and late flowering clones showed no association between the expression of these genes and the timing of flower bud opening. Together, this indicates that these genes could form part of the flower initiation pathway in *Eucalyptus* but do not regulate flower opening time.

WOOD PROPERTY CANDIDATE GENE SELECTION FOR ASSOCIATION STUDIES IN WHITE SPRUCE: A MULTIFACETED APPROACH

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The Arborea project goal is to identify genes that govern naturally occurring phenotypic variation of commercially valuable traits in breeding populations of white spruce (*Picea glauca*) trees. We seek to identify genetic loci, molecular functions, and sequence polymorphisms to enable the development of molecular marker applications for tree breeding. A thorough investigation of the whole genome is currently impossible due to the size of conifer genomes. Therefore, a candidate gene-based scheme was developed. Three different approaches were followed for candidate gene selection. First, a knowledge-based approach used information found in the literature to identify candidate genes in white spruce using similarity searches. For example, Cell Wall Navigator (http://bioweb.ucr.edu/Cellwall/) served to produce a major reference set for data mining the white spruce gene catalogue for putative proteins involved in wood formation. Second, an unbiased approach based on discovery was initiated. We produced several transgenic spruce lines mis-expressing conifer candidate genes related to wood formation. Our main focus was on regulatory genes coding for transcription factors. Four of the transgenes (KN3, MYB1, MYB8, and MYB14) gave phenotypes of interest. Transcript profiling was carried out in these transgenic spruces using a custom cDNA microarray developed by Arborea. A simple procedure was developed to cluster gene expression data in order to identify genes that are most often misregulated in our transgenics. In a third approach, we compared transcript profiles of trees with most extreme wood property phenotypes from a population of nearly 500 trees. The information gathered from all approaches was integrated to assign priorities among 10 000 genes for association analyses. Arborea is a Genome Canada and Genome Québec funded initiative.

MAPPING AND PHYLOGENETIC ANALYSIS OF SPRUCE GENES FOR DECIPHERING THE STRUCTURAL EVOLUTION OF THE CONIFER GENOME

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Gene SNPs were genotyped in white spruce (*Picea glauca*) and black spruce (*Picea mariana*) based on a highly-multiplexed allele-specific primer extension assay (Pavy et al. 2008, BMC Genomics 9:21). They were integrated to the genetic linkage maps previously developed for these two species. Common gene markers between these maps revealed a highly conserved synteny and colinearity. Therefore, we generated a genus-level spruce consensus gene map encompassing 747 genes of known function. The mapped genes were distributed across 65 multigene families represented by at least two members. We analyzed the distribution of multigene families along the spruce chromosomes. We found that 25 clusters of related genes mapped within 2 cM. Phylogenetic analyses of gene families incorporating *Arabidopsis*, rice, and pine sequences indicate that these clusters arose from local duplications all occurring after the Angiosperm-Gymnosperm split (ca. 300 Myr BP) but before the pine-spruce divergence (ca. 140 Myr BP). High sequence homology between spruce paralogs located on different chromosomes also suggests the occurrence of segmental duplications and translocation events. Most of them occurred before the Angiosperm-Gymnosperm split. A few cases of more recent segmental duplications were also observed. For example, conifer *hd-zip* genes were duplicated after the Angiosperm-Gymnosperm split. Two likely ancestral copies were retained on the same chromosome and were separated by more than 30 cM, whereas one copy was translocated to another chromosome. The results indicate that by combining gene maps and gene family phylogenies, powerful phylogenomic inference can be made on the structural evolution of the conifer genome allowing to assess how distinctive its evolution is from that of angiosperm genomes.

THE CONIFER TRANSLATIONAL GENOMICS NETWORK

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The goal of the Conifer Translational Genomics Network (CTGN) is to provide tree breeders across the United States with new tools to enhance and accelerate traditional tree improvement activities. These “knowledge-based” tools derive value from experimentally demonstrated associations between traits of interest, like wood density or disease resistance, and the tree’s genetic code (genetic markers). By adding genetic markers to their tool-chest, breeders will be able to select superior trees more accurately, more rapidly, and at lower cost than using traditional approaches alone. These enhanced approaches will permit greater utilization of the abundant genetic variation inherent in tree populations while simultaneously avoiding controversy associated with other technologies such as genetic engineering. It is anticipated that within five years, cooperatives producing virtually all of the conifer seedlings in the United States (>1.3 billion annually) might benefit from this technology improvement. Methods for implementing the technology in applied tree breeding programs will be thoroughly reviewed and economically evaluated; results of these studies will be delivered directly to tree breeders managing the major tree improvement cooperatives around the country. In addition to validating new genomics-based tools, the project will undertake an assertive and comprehensive education and extension program that will provide: 1) a graduate-level curriculum for teaching molecular tool-based breeding in trees and 2) widespread training for undergraduate and graduate students, tree breeders, managers, lay-people, and other stakeholders through a series of workshops, internships and classes. The CTGN will eventually involve virtually all conifer genomics scientists and tree breeders in the United States.

454 SEQUENCING IN WHITE SPRUCE AS AN ALTERNATIVE SOURCE OF ESTs AND SNPs

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Massively parallel pyrosequencing technologies have created the potential of generating large amounts of sequence data very rapidly. We applied massively parallel EST sequencing to white spruce (*Picea glauca*), for which we used as a reference our GCAT high-quality gene catalogue, produced within the Arborea II program and based on 260 000 Sanger ESTs representing 30 000 genes and 160Mb (5x) of sequence coverage. This case study represents a model for EST-based discovery applied to large genomes for which the complete genome sequence is not available. We studied the use of 454 technology for EST sequencing applied to the following strategies: 1) *de novo* production of gene catalogues, 2) discovery of new genes, 3) discovery of new splicing variants, 4) discovery of SNPs and other genetic variations, and 5) generation of SNP panels adapted to various genotyping populations. We generated 1.1 M ESTs on first- and second-generation instruments (GS20 and FLX), representing several tissues and cDNA libraries and amounting to a total of 225 Mb of sequence (7x). We evaluated sequencing accuracy and EST analysis applied to the discovery of new genes and novel alternative splicing variants. We then applied 454 sequencing for the rapid identification of SNPs adapted to different genetic studies. The SNPs identified from ESTs during the initial phase of the Arborea program have shown that allelic differences between the cDNA libraries and the mapping populations resulted in 60% of SNPs informative for mapping. We tested 454 sequencing for the production of SNPs adapted to genetic mapping. We generated 800 000 ESTs on the 454 FLX from libraries of the two parents of a mapping population and produced a 1536 SNP Illumina GoldenGate genotyping array optimized for genetic mapping. The approach for SNP discovery and validation and the results in terms of genotyping success and mapping informativity will be presented.

QUANTITATIVE GENETICS,
TREE BREEDING

PERFORMANCE OF CANADA RANGE-WIDE WHITE SPRUCE PROVENANCES IN ALBERTA

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We analysed data from one Canada range-wide trial (Canadian Forest Service 410 series) in central Alberta to assess survival, growth, and insect and climatic damages. Average 15- and 24-year survival was 92.1% and 89.3%, respectively. Except for one provenance, provenance survival ranged from 76 to 100%. Average height at 15 (H15) and 24 (H24) years was 2.5m (1.13–3.26m) and 5.52m (2.80–7.10m), respectively. Correlations between H15, H24 and 24-year DBH (D24) were 0.97–0.98, whereas correlation between survival and growth was low (0.31–0.34). Growth (H24) was significantly correlated to latitude (0.45) and longitude (0.76) of seed origin. Best provenances for central Alberta were from southeasten Manitoba ($\leq$ 52° N) and the Ottawa Valley and southwestern Quebec (< 50° N). Provenances with the lowest H24 were from Yukon Territory (YT), Northwest Territories, British Columbia (BC), Newfoundland, and Nova Scotia. Their H24 was similar to that of high elevation provenances from the Rocky Mountains in Alberta.

On average, 28.8% of trees experienced new shoot frost damage at age 15 years; individual provenance means ranged from 0.0 to 63.0%. No regional variation pattern was observed. Average cumulative (age 15–24 years) incidence of white pine weevil and related insect damages was 37.8% (4–100%) with the highest incidence occurring among provenances of low growth potential (BC and YT). More trials have been established in Alberta for further evaluation of these provenances.

GENETIC CONTROL OF WOOD FORMATION AND PLASTICITY IN DOUGLAS-FIR CLONES

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Climate variation within a tree's growing season affects cambium activity creating the earlywood-latewood pattern in the concentric growth rings of most temperate forest trees. Individual reaction norms were estimated for Douglas-fir (*Pseudotsuga menziesii*) clones, synchronizing within-ring microdensity variation with within-growing season weather variation. With this method, special tree rings with false peaks as signals of specific climate events are needed for efficient synchronization. For each growing season an intra-ring density model was constructed, made of successive straight lines determined by breakpoint positions. We assessed the genetic control of density and position of the breakpoints, as well as of simple ring variables. The environment-dependent within-ring phenotypic expression was computed to assess these reaction norms as a measure of plasticity. A moderate to strong genetic control was found for most breakpoint positions and breakpoint densities, as well as for most ring variables. The heritability of plasticity was found to be moderate to high for the different years and sites studied. In the context of global climate change, extreme climate events are predicted to become more frequent and intense. When the environmental shift is rapid, phenotypic plasticity plays an important role in the adaptation process to new climate conditions. If most of the genotypic variation is additive then it is possible to select genotypes with favourable plastic reaction. It is necessary to extend and to validate this approach with other sites and years. However, we are already convinced that wood microdensity profiles have a very high potential to infer individual plasticity.

INVESTIGATING WOOD TO UNDERSTAND ADAPTATION

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Wood supports the tree, stores reserves, and conducts sap from roots to leaves. Species ecological preferences and wood hydraulic properties are related. We investigated the existence of a relationship between wood density and wood hydraulic properties as well as the existence of a genetic control for wood hydraulic properties. Wood hydraulic properties were described using specific conductivity and vulnerability to cavitation. Trees with a high specific conductivity benefit from favorable water conditions, while trees with a low vulnerability to cavitation are resistant to drought.

Change from earlywood to latewood is an individual response to a marked environmental shift and a manifestation of phenotypic plasticity, which is a major component of adaptation in forest trees. The norm of reaction relating ring microdensity to relevant environmental variables was used to measure plasticity. The evolutionary potential, another important component of adaptation, was measured with heritability. We found a significant genetic control for wood hydraulic properties. After a severe drought event, we found significant differences for wood density traits between dead and surviving trees. Finally, significant genetic control was also found for phenotypic plasticity. These results show that up to now, in France, Douglas-fir (*Pseudotsuga menziesii*) seems to adapt to the latest manifestations of global climate change.

But the detection of the effect of climate change is limited in this study by the relatively young age of the genetic tests. The potential of our retrospective approach will be increased in further studies by using natural populations spread over very variable environments.

REFERENCE MATERIALS FOR BUTT LOG SHAPE IN EARLY SELECTION TRIALS OF *Pinus pinaster* Ait.

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An all-round early selection method by shape of butt logs on *Pinus pinaster* is proposed. Parameters for early selection were whorl distance measured in a 2-year-old garden trial, main root re-growth of seedlings in 21-day culture growing in a controlled environment chamber after main root pruning to prevent toppling, and sensitivity to frost injury on buds that could lead to crotch formation. All-round early selection is needed to assure bigger and straighter butt logs for higher value added lumber. Work on identifying reference material for these parameters is presented in this paper. Since whorls in young trees can lead to knots in adult butt logs, whorl distance evaluation was directly used to select 2 short-knot-distance and 2 long-knot-distance reference materials out of 25 known populations. Reference materials for main root re-growth were selected out of 6 different well-known shape butt log adult populations, ranging from straight shapes to warped shapes. Those populations with bigger main root re-growth would be less sensitive to toppling. Finally, buds from 6 different young populations from coast and interior provenance regions, were collected from a provenance trial to conduct a frost resistance test in a programmable freezer. A sample of each population was left at room temperature for damage index evaluation. A study of genetic variation was conducted and reference materials selected on the bases of the three parameters.

CLIMATIC ADAPTATION AND INTRASPECIFIC VARIABILITY IN WATER-USE EFFICIENCY: THE CASE OF TWO IBERIAN FOREST SPECIES (*Juglans regia* L. AND *Pinus halepensis* Mill.)

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One component of adaptedness, relevant to plant capacity to thrive in dry areas, is intrinsic water-use efficiency (WUE_i). Differences in the severity and duration of water stress may act as selective forces shaping the genetic make-up in terms of intraspecific WU strategies potentially linked to climatic features at origin. This work aims at illustrating the adaptive significance of WUE_i for two contrasting species: the xeric *Pinus halepensis* and the more mesic *Juglans regia*. We evaluated $d^{13}C$ (surrogate of WUE_i) and other traits (growth, $d^{18}O$, phenology) for 25 *P. halepensis* populations and 20 *J. regia* progenies in common garden tests. There was intraspecific variability for $d^{13}C$ (higher for *P. halepensis*), with high-WUE_i material having low growth, hence suggesting a trade-off between productivity and adaptedness to drought. The most water-use-efficient material originated from drought-prone sites having high seasonal rainfall and high vapour pressure deficit (VPD) for *P. halepensis*, and low rainfall and high VPD for *J. regia*. This particular behaviour, as compared with other Mediterranean trees, demonstrates different strategies towards reaching a fitness optimum under comparable selective roles of climatic variables.

GENETIC VARIATION IN *Fraxinus excelsior* IN SYMPTOMS OF NOVEL ASH DIEBACK IN DENMARK

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During the last decade a new devastating health problem has appeared on ash (*Fraxinus excelsior* L.) in Poland and the Baltic countries. Recently, the disease has caused severe damage in Sweden and Denmark, and symptoms are being reported from Germany, Austria, Czech Republic, and Slovenia. The disease etiology of the dieback is not yet understood, but evidence points towards a new emerging infectious disease caused by a fungus (*Chalara fraxinea* Kowalski) acting as a necrotrophic pathogen and spreading across the forests and landscapes.

We present results regarding the potential presence of genetic resistance in natural Ash populations based on assessments of existing clonal and progeny trials in Denmark. We found a high frequency of genotypes exhibiting severe symptoms, but also genotypes that exhibited no or a very low level of symptoms. Observation of 40 cloned genotypes (50 ramets/clone randomised at two sites) showed highly significant variation with symptom-free ramets per clone varying from 0 to 94%. An observed bimodal distribution of the tested clones was surprising. This can indicate the action of a few genes because the clones are of putative Danish origin that we expect to be in linkage equilibrium. However, resistance based on a few major genes is unexpected for a necrotrophic pathogen. Results from the genetic analysis of a series of trials are presented and their implication for future research effort discussed. Inoculation experiments to test whether symptom-free genotypes exhibit resistance against infection by *Chalara fraxinea* remain to be done.

LIMITATIONS AND OPPORTUNITIES IN BREEDING AND DEPLOYING MAMMAL-RESISTANT TREES

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Globally, forest productivity losses and mitigation costs due to mammalian damage are staggering. There has been a tremendous amount of research on mammalian resistance mechanisms and genetics. Despite this, there are no breeding programs that have successfully deployed resistant trees in forestry operations that we are aware.

Limitations: The successful deployment of resistant material is expensive and there needs to be a dedicated institutional commitment to research and breeding. Foraging mammals tend to be generalists and display relatively low lethality and intimacy with their hosts. A general result is diffuse coevolution with intraspecific genetic variation in defense mechanisms quantitative rather than major gene resistance. There is a need to understand and manage for relative palatability and mammalian foraging behaviour and genetic variation in these attributes. Adverse genetic correlations between defense and commercial traits can make it difficult to justify resistance breeding. Resistance deployment can be compromised by ontogenetical and environmental variation.

Opportunities: Research results linking mammalian preference and secondary chemicals provide potential selection traits that are highly heritable and can be expressed constitutively in juvenile trees. Secondary compounds can exhibit positive genetic correlations with growth traits, which allows for continued breeding for productivity, in synergy with potential compensatory growth increases following herbivory. Cloning facilitates additional exploitation of resistance variation associated with selection, deployment, and ontogeny. Finally, management to mitigate detrimental impacts of climate change on forests may enable foresters to deploy non-local populations that may have resistance mechanisms not present in local populations.

PERFORMANCE OF BACKCROSSES OF EASTERN WHITE PINE INTERSPECIFIC HYBRIDS IN ONTARIO

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Eastern white pine (*Pinus strobus* L.), once a widespread and predominant forest species, has been dramatically reduced in abundance in Ontario due to historical over harvesting and difficulties in its regeneration. White pine blister rust (caused by *Cronartium ribicola* Fisch.) is often a top contributor to white pine regeneration failure. Despite silvicultural efforts in mitigating blister rust damage for more than half a century, the challenge remains as white pine saplings continue to be decimated by blister rust due to their lack of natural resistance to the introduced pathogen. Ontario's breeding program has attempted to integrate blister rust resistance genes from Eurasian white pine species into eastern white pine. A breeding scheme of interspecific hybridization followed by backcrossing has now produced second-generation backcrosses with up to 87.5% *P. strobus* parentage. Blister rust resistance, growth performance, and wood quality of these hybrid backcrosses are assessed through artificial blister rust inoculation, field trials, and laboratory experiments. Progress to date from this breeding effort will be updated and plans for future development will be discussed.

CLIMATE ENVELOPE MODELLING AND *Salix* IN ONTARIO: AN EVALUATION OF LOGISTIC REGRESSION ANALYSIS AND DISTRIBUTIONAL DATA

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Global warming and concern over biodiversity conservation have prompted numerous investigations of the magnitude of consequences to be expected for terrestrial flora and fauna with respect to climate change. Strategies for predicting species response to climate change have often focused on the characterization of a species' bioclimate envelope whereby areas of suitable habitat are identified through techniques that correlate species occurrence data to environmental characteristics under present-day conditions. Although many statistical methods have been employed to determine significant environmental predictors of species occurrence, results between differing approaches have often contrasted. The degree to which input variables and choice of statistical analysis affect predictions is unclear. An exercise was initiated to test the sensitivity of logistic regression analysis to different distributional data. Envelopes for 30 Ontario *Salix* species were created using data from inferred ranges and collection points. These envelopes were regressed logistically using three methods to determine a selection of predictors that best describe current species distributions.

CLIMATE-BASED SEED TRANSFER AND ASSISTED MIGRATION USED TO MITIGATE CLIMATE CHANGE IMPACTS TO FORESTS

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Assisted migration to maximize tree adaptation over the rotation is recognized as a cornerstone forestry strategy to address climate change. However, geographic-based seed transfer systems do not accommodate assisted migration well and methods to quantify the climatic distance that seed should be moved are lacking.

The objectives of this project are: 1) to compare the merits of competing climate- and ecology-based seed zonation systems with current geographic-based systems and 2) to illustrate a method of quantifying the climatic distance seed must be transferred to maximize adaptation over the rotation.

Six candidate fixed-zone seed transfer systems are developed for British Columbia (BC) based on climate, ecosystem zones, and current seed zones. The merit of each system is evaluated based on average climatic transfer distance, expected productivity, and seed deployability. A zonation system developed by clustering areas of similar climate based on multiple aggregate climate variables resulted in the least climatic transfer of seed, the greatest productivity, and the greatest deployability of seed. Fixed-zone and focal point zone systems are compared and a new hybrid “progressive seed zone” system is proposed to balance trade-offs involving deployability and ease-of-use.

To estimate the optimum distance seed should be transferred to maximize adaptation across the rotation, productivity of populations from a range of climates is estimated for 5 locations in BC by integrating transfer functions from present to rotation age. Results indicate that planting seed adapted to a climate that is slightly warmer than the contemporary climate will result in significantly higher productivity than seed adapted to the current climate.

GENOME SCANS, QTL AND ASSOCIATION MAPPING

EXPRESSION QTL MAPPING FOR WEEVIL RESISTANCE IN WHITE SPRUCE

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Gene expression levels can be regarded as quantitative traits and subject to the same quantitative trait locus (QTL) analysis as traditional morphological traits, but the number of traits can now be enormous and causal associations between genes might be inferred. We studied eQTLs (gene expression) related to weevil resistance using a 21 832-member cDNA array designed from the spruce EST database. A 2x2 factorial with 75 12-year-old progeny per family involving parents of intermediate resistance was used. Progeny were genotyped for 253 informative SNP markers using the Illumina technology. From these data, 92 cy3-cy5 pairs were identified using an algorithm that maximized genetic distance between segregating sib pairs (this increased our power by ca. 25% over random pairings). Multi-year scores for weevil attack were transformed into four principle components, and two principle components summarized growth. About 1% of spots showed strong QTL co-location with attack or growth QTLs (sharing 40% or more of their QTLs); from these we will derive candidate ESTs for SNP association studies. Clusters of co-segregating eQTLs are being identified to characterize regulatory networks and potential master genes. The covariance of QTL effects between parents along the genome was unexpectedly zero, probably indicating a bias in the mapping procedure (QTL in one parent masks another). This and other difficulties, as well as the novel prospects of eQTL mapping, will be discussed.

IDENTIFICATION OF QTL INFLUENCING ADAPTIVE TRAITS IN WHITE SPRUCE

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The timing of bud set in white spruce (*Picea glauca*) is an important component of cold hardiness and is also highly correlated with growth performance. Discovering the genes that control this trait is a challenging task, as the genetic control of bud set is polygenic with interactions between genetic and environmental factors. In the Arborea genome project, several approaches are being used to discover these genes, including genome-wide linkage analysis. Over the last year, a composite linkage map has been assembled from two crosses of white spruce; the map spans more than 1000 loci, two-thirds of which are expressed genes (803 SNPs and 33 ESTPs). For each cross, "macro-phenotype" data for growth and budset were collected for two years by conventional methods under outdoor and indoor conditions (across clonal replicates) through declining day length. "Micro-phenotyping" (metabolite profiling, expression profiling) is underway. Currently, we have identified two, six, and seven significant QTLs for bud flush, bud set, and growth, respectively. Phenotypic explained variances (PEV) ranged from 5.2 to 29.2%. Out of these QTLs, 11 were found to be common between experimental years, 11 were identified simultaneously across the two different environmental conditions (indoor, outdoor), and six were observed in both crosses. These results, in combination with other approaches (e.g. expression profiling experiments and genome scans), will help determine a priority list of candidate genes for large-scale association studies in white spruce.

A GENOME SCAN OF GENE SNPs FOR THE SEARCH OF POLYMORPHISMS INVOLVED IN ADAPTIVE POPULATION DIFFERENTIATION IN BLACK SPRUCE

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In North American boreal black spruce (*Picea mariana*), significant variation in quantitative traits related to growth and phenology has been observed and shown to be related to photoperiod and temperature. Different approaches involving association and QTL studies are currently deployed to help decipher the gene architecture underlying this variation. We have also been studying molecular variation of adaptive nature among natural black spruce populations using a genome scan of gene SNPs covering all linkage groups. Using a set of 583 SNPs spread in 320 genes, trees from various populations shown to be differentiated for quantitative characters (Q_{ST}) were genotyped using a highly multiplexed platform. Populations were regrouped to maximize environmental variance between them according to temperature, photoperiod, and precipitation. To detect SNPs and genes possibly involved in adaptation, an outlier approach (F_{ST}) was applied to each scenario of population grouping. A number of outlier loci representative of different gene families and spread on diverse linkage groups were detected for each of the three environmental conditions tested. A few outliers were common to two environmental conditions. Although small, the number of positive cases suggests that some of the SNPs tested are likely to explain part of the adaptive variation observed in black spruce. The identification of outlier gene SNPs in genome scans of natural populations appears as a valuable exploratory approach to provide new candidate gene loci for association studies in black spruce and other conifers.

ASSOCIATION MAPPING FOR WOOD CHARACTERS IN WHITE SPRUCE IMPLICATING HUNDREDS OF GENES AND COMMON SNPs

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Assessing wood fibre characters is expensive and can only be achieved when trees are old enough to collect wood samples that are representative of the whole trees. Tree breeders are looking for innovative approaches to eliminate the need to resort to these time-consuming and expensive assessment methods, to speed up breeding cycles, and to make selections with higher precision. Association studies are expected to provide tree breeders with the tools they need. In this project, we collected wood cores from 500 trees in a 30-year-old white spruce (*Picea glauca*) provenance-progeny test. Physical and chemical wood properties of each sample were assessed using SilviScan technology, as well as wet chemistry and spectroscopy determinations. Around 550 candidate genes for wood formation were identified based on e-Northern analyses, microarray gene expression studies, and gain-of-function experiments as well as data from relevant scientific literature, Over 2000 common SNPs were identified in these candidate genes by resequencing or inferred from overlapping ESTs in contig assemblies. A set of 969 SNPs representative of 522 genes was successfully genotyped for each of 469 trees using the highly multiplexed Illumina GoldenGate assay. Associations between genotypic and phenotypic data were searched using regression and mixed-model analyses and for both additive and dominant effect models. After corrections for multiple testing, a small number of candidate gene SNPs was found to explain a small but significant portion of the variation observed in some of the wood characters.

DISCOVERY OF GENES FOR IMPROVED CELLULOSE AND CELLULOSE-EXTRACTABILITY FROM POPLAR SECONDARY XYLEM

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The Department of Energy's "Breaking the Biological Barriers to Cellulosic Ethanol" report identifies poplar as one of the key feedstock species for cellulosic ethanol production in many regions of the country. The goal was to perform SNP discovery using high throughput DNA sequencing (Agencourt Biosciences) and SNP genotyping (Illumina) to associate genetic variation in genes involved in cellulose and lignin biosynthesis with phenotypic variation in cellulose quantity, quality, and extractability in a large clonal black cottonwood (*Populus trichocarpa*) genetic test plantation belonging to GreenWood Resources. A set of 40 genes, known to be highly expressed and associated with the desired phenotypes, was sequenced using a panel of 15 unrelated poplar clones. Genomic sequences of ~179 000 bp covering the entire protein-coding regions, including introns, and 1000bp upstream and 300bp downstream were retrieved from JGI. Two hundred non-overlapping amplicons were selected to cover the length of the genes and were sequenced by Agencourt in both directions and submitted to an automated pipeline developed in-house for sequence alignment and SNP discovery. Utilizing Illumina's Golden Gate Assay, 456 poplar clones were genotyped for ~1536 SNPs. Wood samples were collected from 1100 trees from the 456 poplar clones. High-throughput phenotyping has been performed on the cores using pyrolysis molecular beam mass spectrometry to analyze wood chemistry components such as lignin, cellulose, and hemicellulose. Association genetics analysis will be used to identify genes controlling cellulose quantity and quality phenotypic variation in poplar.

PHYLOGENY, PHYLOGEOGRAPHY
HYBRID ZONES

CROSS-SPECIES SPATIAL AND TAXONOMIC DIFFERENTIATION PATTERNS IN OAK – AN APPROACH WITH MICROSATELLITE MARKERS

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Oaks are characterized by high adaptability and genetic plasticity and have served, in several cases, as model organisms for studying evolutionary processes. Interspecific gene-flow and introgressive hybridization have been described as important evolutionary mechanisms within the genus.

In our study we carried out genetic analysis at eleven nuclear microsatellite loci in five oak species in order to provide insights into differentiation patterns. Diversity profiles of markers varied among species and provenances. One variable locus provided phylogenetically relevant information distinguishing oaks of section *Robur* from those of section *Cerris*. A number of markers displayed selective sweeps in certain species, supporting ecological barriers between interfertile species. Another set of loci showed geographic structures, with interspecific differentiation in neighbouring stands being lower than overall intraspecific differentiation, indicating gene-flow and genetic introgression between species.

In conclusion, our results support the view that selective forces act at certain genome regions, while the rest of the genome remains subjected to gene-flow from related species (genic view of speciation). Furthermore, it is shown that the distribution of these regions differs from one species to the other. Finally, our results provide novel knowledge about diversity and differentiation patterns in three Mediterranean oak species.

MOLECULAR EVOLUTION IN COMPLEX FOREST TREE GENE POOLS: THE CASE OF *Eucalyptus globulus* IN SOUTHEASTERN AUSTRALIA

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Eucalyptus globulus is the most important plantation eucalypt in temperate regions of the world. It is part of a species complex consisting of four taxa variously described as species or subspecies (*bicostata*, *globulus*, *maidenii*, and *pseudoglobulus*). The cores of these taxa are morphologically and geographically distinct, but are linked by intergrade populations that are intermediate in morphology. The evolutionary origins of the intergrade populations are unknown; some could be the result of divergence within a continuous series of populations while others may result from hybridization and introgression between previously isolated gene pools. To assess the molecular affinities within the complex, we used nine microsatellite DNA markers to genotype 1198 trees representing 33 morphological core and intergrade populations from across the natural range of the species complex in southeastern Australia. In some geographically or ecologically isolated populations, genetic drift and inbreeding have resulted in reduced genetic diversity and marked molecular divergence from the rest of the gene pool. The molecular affinities of some populations do not reflect their morphological affinities, which has implications for conservation planning, as well as for seed collections for native forest regeneration, reforestation, and breeding programs. Furthermore, the analysis suggests that some intergrade populations have arisen through primary differentiation while others are the result of hybridisation and introgression, and also provides insights into the possible geographic origin of the species complex.

MOLECULAR BIOGEOGRAPHY OF MESOAMERICAN FIRS: FROM THE PLIOCENE ANCESTORS TO THE REMNANTS OF THE HOLOCENE COLLAPSE

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The montane forest ecosystems of Mesoamerica (i.e., Mexico and northern Central America) are among the most complex and biodiverse habitats of the world, as they sit at the northern limit of a suture zone between species migrating from both North and South America. Conifers are among the most widely represented taxa in these forest communities. They entered the region by the north at end of the Tertiary, and then underwent recurrent cycles of latitudinal and altitudinal migrations which are held responsible for the high number of species observed nowadays. We surveyed the evolutionary consequences of these cycles in eight Mesoamerican firs (*Abies* Miller), a predominant component of the Mesoamerican montane forests, by studying their variation in different cytoplasmic DNA regions with contrasted inheritance. The phylogenetic analysis of informative mtDNA and cpDNA regions showed that the Mesoamerican firs form a monophyletic group closely related to the species from southwestern North America. None of the inferred phylogenies matched the current taxonomical classifications of Mesoamerican firs. The distribution of the interspecific variants observed at highly polymorphic mtDNA and cpDNA-SSR markers was geographically and taxonomically structured. The five northernmost taxa (*A. flinckii*, *A. vejari*, *A. durangensis*, *A. duragensis* var. *coahuilensis*, and *A. concolor*) formed different divergent groups, which were more related to the species from southwestern North America than to the three southernmost Mesoamerican firs. These three southern taxa (*A. guatemalensis*, *A. hickelii*, and *A. religiosa*) formed a relatively homogeneous group, which included a few divergent populations located at the limits of their respective ranges or in the Transverse Volcanic Belt in central Mexico. Altogether, these trends suggest that Mesoamerican *Abies* share a common ancestor and they would be the result of at least two different migratory waves, followed by various episodes of local divergence and speciation.

MOLECULAR PHYLOGENY AND BIOGEOGRAPHY OF *Pseudotsuga* (Pinaceae): INSIGHTS INTO THE FLORISTIC RELATIONSHIP BETWEEN TAIWAN AND ITS ADJACENT AREAS

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Intercontinental disjunction and island biogeography are particularly active research fields in recent decades, especially with the development of DNA technology. In this study, using sequences of five cpDNA regions and one single-copy nuclear gene (*LEAFY*), we investigated the biogeography and evolutionary history of *Pseudotsuga* (Pinaceae), a small genus with two species (*P. menziesii* and *P. macrocarpa*) distributed in western North America, one species each in Japan (*P. japonica*) and Taiwan (*P. wilsoniana*), and four species (*P. brevifolia*, *P. forrestii*, *P. sinensis*, and *P. gaussenii*) in mainland China. Both cp DNA and nuclear gene phylogenies strongly support two monophyletic and sister clades, East Asia and North America. Fossil records and molecular clock estimation suggest that *Pseudotsuga* might have originated from North America and then migrated to East Asia by the Bering land bridge during the early Miocene. Within the East Asian clade, it is very interesting that two copies of the *LEAFY* gene were found in *P. wilsoniana*. The phylogenetic topology and molecular dating, together with biogeographic distribution, indicate that one copy was very likely obtained from an ancient population of *P. brevifolia* at about 12 ±1.22 my BP while the other might be inherited from the ancestor of an East China species at ~5 my BP (Pliocene). This result seems to imply that Taiwan is closely related to both southwest and east China in its flora and could be more southwards than its present geographical position before the mid-Miocene. *P. japonica* has a very close relationship with the two East China species, *P. sinensis* and *P. gaussenii* in the *LEAFY* phylogeny while it is a sister group to all the other Chinese species in the cpDNA tree. The discordant position of *P. japonica* might result from the difference in the inheritance pathway between nuclear and chloroplast genomes or chloroplast capture.

GENE FLOW, MATING SYSTEM, PEDIGREE RECONSTRUCTION

AN APPLIED POPULATION GENOMIC APPROACH TO ASSESS THE RISKS POSED BY TREES WITH NOVEL TRAITS

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Populus species and hybrids are favoured for emerging applications in biofuels, carbon sequestration, and environmental remediation, yet the intentional planting of trees with novel traits can affect the integrity of native species. We adopt an integrative approach to risk assessment that teams theoretical modelling with marker-based studies of gene flow in natural populations of native poplars, using exotic genes from introduced species as a proxy for any genomic invasion through sexual introgression. Previous work showed that exotic poplars, in plantations, sired hybrid seed with adjacent native poplars at significant frequencies (3–62%). But would the gene stop there? By genotyping trees from natural populations, we show that F1s are indeed fertile, that 3% of trees are of hybrid origin, that disturbed habitats are more apt to contain hybrids, and that introgression is consistently biased toward native *P. balsamifera*. As the genome of balsam poplar appears most susceptible to infiltration, we are genotyping 1500 trees throughout the species' range to study the genetic structure of natural populations and evaluate molecular evidence for selective sweeps. These features will dictate the extent to which introgressed DNA could spread throughout the entire range. The risks posed by trees with novel traits need to be considered in light of the extensive contamination that has already occurred by exotic poplars, as well as the fitness effects of the traits in question.

POLLEN FLOW BETWEEN LOMBARDY POPLAR AND NATURAL POPULATIONS OF BLACK POPLAR

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In French landscapes, black poplar (*Populus nigra* L.) can be found in three interconnected ecosystems. *P. nigra*, a major species of the riparian forest, is widely used in monoclonal plantations mostly through *P.* x *euramericana* interspecific hybrids. *P. nigra* is also widely represented in rural landscapes by Lombardy poplar. As a first step to assess the impact of cultivated or ornamental poplar genetic pools on genetic diversity of wild populations, we quantified pollen flow from Lombardy poplar to wild stands and identified biological and physical factors controlling reproductive success through modelling. Pollen flow was measured through *in situ* paternity analysis using 10 SSR markers. Located along the Loire River, the study site (11.5 ha) hosts 484 wild *P. nigra* trees and 13 Lombardy poplars. Flowering phenology data collected in 2006 and 2007 showed that Lombardy poplar trees are among the earliest flowering trees. In 2006, 1350 seeds were collected from 24 females distributed over the site and for their flowering date. The average percentage of seeds pollinated by Lombardy poplar reached 1.9%. Pollen dispersal kernels were estimated from a mating model based on all available genotypic and spatial data. We detected a high immigration rate (51.3%) and a fat-tailed dispersal kernel. Importance of biological factors (tree size, flowering synchronism, quantity and viability of pollen, and pollen competition) and physical factors (distance between males and females, wind) have been tested. Phenological assortative mating and short distance pollen dispersal were identified as the most important factors influencing hybridization between Lombardy and wild poplars.

THE INTERPLAY BETWEEN GENE FLOW AND PHENOLOGY: DOES GENE FLOW AFFECT LOCAL ADAPTATION IN FOREST TREES?

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Phenology is an important adaptive trait in forest trees. However, due to its quantitative character, mating between phenologically different individuals (long distance pollen-mediated gene flow) may cause such offspring to have their phenology shifted as compared to local matings. We investigated bud burst phenology of 1230 two-year-old seedlings grown in nursery conditions, originating from a mixed-oak stand. Local adults (421) and all seedlings were genotyped using five SSR loci, and 856 seedlings were assigned to one of 15 mothers. We found that 0.451 ± 0.019 of the seedlings resulted from pollen immigration from outside of the stand (ca 5 ha) and the seedlings due to detectable pollen immigration were identified. ANOVA indicated that the day of bud burst was significantly variable among the 15 half-sibs ($F = 13.8$; $P < 0.001$), but also the seedlings sired by nonlocal fathers were delayed ($F = 8.02$; $p = 0.005$) as compared to those from local matings. Using a simplified additive model, we approximated the phenology of the mother trees and then the mean phenology of fathers responsible for pollen immigration within each half-sib. Pollen immigration was significantly (nonlinearly) related to bud burst phenology of a given mother tree, with higher immigration observed among early and late mothers. However, the phenology of immigrant seedlings suggested that for early mothers, immigrating pollen came from populations of earlier phenology, while for late mothers from populations of similar phenology. We develop and discuss the theory that relates long distance gene flow and adaptation in forest trees.

ISOLATION BY DISTANCE WITHIN NATURALLY ESTABLISHED PURE BEECH STANDS

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Isolation by distance (IBD) plays a central role in shaping genetic structure of plant populations. However, IBD is often rarely detected in forest trees because they are long-living organisms subjected to intensive silvicultural management. European beech (*Fagus sylvatica*), because of its biology, makes a good counter-example.

We investigated whether the effects of IBD take place in pure beech populations. For this purpose, two stands (518 trees) were sampled and genotyped at 9 SSRs. Using the Markov Chain method we detected 2 and 4% inbreeding within each respective population. One tree possibly originated from self-fertilization; hence inbreeding was mainly due to mating between relatives. Spatial autocorrelation analysis revealed clustering of relatives in both populations. Correlograms were fitted with a curve expected under IBD. The neighbourhood size equaled 61–93 and 78–101, depending on the stand.

Additionally, we assessed contemporary gene dispersal, based on 384 seedlings sampled in one stand. Using a modeling approach we found that average seed dispersal distance was 8.5 m and seed dispersal followed a fat-tailed dispersal curve. Seed immigration from a background population reached 1%. On the other hand, pollen immigration equaled 66%, whereas pollen dispersal followed a fat-tailed distribution (36.1 m on average). No seedlings resulted from self-fertilization. The mode of dispersal corresponded to a neighbourhood of 185 individuals.

We conclude that dense populations of European beech are subjected to IBD. The spatial genetic structure is primarily due to restricted nut dispersal. However, because pollen dispersal is also limited bi-parental inbreeding takes place to some degree.

MAGNITUDE OF EARLY INBREEDING DEPRESSION OF AN ISOLATED, RELICT POPULATION OF SAKHALIN SPRUCE (*Picea glehnii* Mast.)

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Inbreeding depression is crucial to conservation and evolutionary genetics of conifers. We intended to quantify the magnitude of early inbreeding depression by applying the hierarchical Bayes model to genetic and ecological data sets. The study was conducted at an isolated, relict population of Sakhalin spruce (*Picea glehnii* Mast.) in northern Japan. Leaf tissue from all adult trees (59 trees) and 96 seeds from each of 15 mother trees was sampled and genotyped at 11 microsatellite loci. We extended the likelihood-based method to estimate male reproductive success (e.g., Burczyk et al. 1996) to incorporate the impact of inbreeding depression on self-pollinated embryos and individual reproductive success. For an accurate estimate, the proportion of filled seeds was also considered as the factor related to early inbreeding depression. Posterior of parameters were obtained by Malkov-chain Monte Carlo iterations. Our results showed that the magnitude of early inbreeding depression was high ($\vartriangle = 0.91$) despite a high self-pollination rate ($s = 0.48$). In addition, the magnitude of inbreeding depression varied among mother trees ($\vartriangle = 0.78$–1.00) which indicates that the frequency and distribution of lethal alleles varied among mothers.

ESTIMATION OF POLLEN DISPERSAL CURVES AND HETEROGENEITY IN MALE FECUNDITY FROM GENETIC DATA IN PLANTS

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The availability of highly polymorphic markers has yielded the possibility of directly estimating contemporaneous gene flow in plant species. This project focussed, in particular, on pollen flow. The methods used as an input were genotypic data of mothers, potential fathers, and seeds collected from the mothers. The methods are either direct, based mostly on categorical or fractional paternity analyses, or indirect (e.g., TwoGener or Kindist) based on the comparison between the allelic frequencies in the pollen cloud of the mothers. Both methods aimed at estimating both the dispersal curve and the heterogeneity in male fecundities. We have now applied these methods to a substantial set of plant species, including several forest tree species. We have shown that pollen dispersal is leptokurtic in many cases, with many events of reproduction at short distances but also a small but non-negligible part of long-distance dispersal events. We have also detected, in several cases, a heterogeneity of male fertilities that is generally connected with differences in some phenotypic traits. These results are important in the context of conservation biology for threatened fragmented species, or more generally for the question of the spreading of advantageous and deleterious mutations over the landscape.

ESTIMATION OF THE SEED DISPERSAL KERNAL FROM EXACT IDENTIFICATION OF SOURCE TREES

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The exact identification of individual seed sources through genetic analysis of maternal origin seed tissue has recently brought the full analytical potential of parentage analysis to the study of seed dispersal. No specific statistical methodology has been described so far, however, the dispersal kernel function can be estimated from categorical maternity assignment. We present here a maximum-likelihood procedure to estimate the seed dispersal kernel from exact identification of seed sources. Using numerical simulations, we show that the proposed method, unlike other approaches, is independent of seed fecundity variation, yielding accurate estimates of the shape and range of the seed dispersal kernel under varied sampling and dispersal conditions, even when seed rain densities are unknown. We also demonstrate how an obvious estimator of the dispersal kernel, the maximum-likelihood fit of the observed distribution of dispersal distances to seed traps, can be strongly biased due to the spatial arrangement of seed traps relative to source plants. Finally, we illustrate the use of the proposed method with a previously published empirical example for the animal-dispersed tree *Prunus mahaleb*.

INTEGRATING FUNCTIONAL GROWTH CURVES AND FAMILY GENETIC STRUCTURE INTO PLUS TREE SELECTION

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Pedigrees of two natural red spruce (*Picea rubens*) stands, containing previously selected plus trees, were reconstructed with DNA markers using an explicit/group likelihood approach. Increment cores from pedigreed trees were used to model individual growth trajectories using b-splines in a mixed model framework. This 'random regression' animal model permits partitioning of fixed environmental sources of variation from the proposed random genetic and non-genetic sources of variation among individual trees. Full models incorporating reconstructed pedigrees of trees within natural stands were compared against reduced models without any pedigree. Potential natural/artificial selection response of phenotypic versus genotypic selection of plus trees is discussed in relation to breeding programs and conservation.

MORE EFFICIENT BREEDING OF NORDMANN FIR USING MICROSATELLITES?

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This presentation presents results from a study of whether microsatellites can be used to make breeding of Nordmann fir (*Abies nordmanniana*) more efficient.

One example is the conversion of half-sib trials to full-sib trials by use of paternity analysis. By this approach some benefits, compared to the present ongoing half-sib testing, are potentially attained: 1) more precise breeding values of the parent trees, not dependent on the assumption of equal contributions from an infinite pollen cloud, 2) the possibility to analyze full-sib families and thereby estimate the proportion of non-additive genetic variance originating from dominance and epistasis effects, and 3) possible forward selection based on full pedigree information. Another new use of microsatellites in breeding of Nordmann fir is the establishment of quasi field trials in Christmas tree production stands. By quasi field trials is meant trials where trees were not originally planted in an experimental design, but where the pedigree information is found subsequently. In practice, genotypes of trees from stands made from bulk seed harvest of a clonal seed orchard may retrospectively identify both mothers and fathers. This will save time and in particular the administrative cost of establishing and maintaining new field trials.

MATERNAL ANALYSIS OF VALLEY OAK (*Quercus lobata* Née) RECRUITS USING MIXED PERICARP AND SEEDLING ASSAY

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The spatial pattern of parentage for already dispersed natural recruits is becoming an issue of increasing importance. Separate designation of maternity and paternity for seedlings or saplings typically requires an assumption of which parent is the closer, but that supposition is too strong, given the fat tailed dispersal curves of many species. For germinating seedlings, typically still attached to the seed, it is possible to determine the maternal genotype from the seed coat, but it is not unusual for seed coat genotyping to provide only partial information on maternal genotype, particularly where the seeds have been *in situ* for some time, pre-germination. We developed a combination analysis of maternity, using both seed coat and seedling genotypes, and deploy it for an analysis of natural recruits of California Valley oak (*Quercus lobata* Née). This novel analysis is a special case of standard single-parent parentage analysis (neither parent known); it improves the power of maternity analysis markedly, relative to what can be done with partial genotypes of seed coats alone. Of 397 recruits in 21 maternal seed shadows, most with fairly complete seed coat data, we resolved maternity of 350 seedlings exactly and ~20 more with high likelihood. Of 317 recruits in five open patches, most with at least two missing loci, we resolved maternity of 280 seedlings exactly and ~20 others with high likelihood. Paternal inference follows by subtraction and the improvement in parental inference is valuable for spatial pattern analyses.

NUCLEOTIDE DIVERSITY, LD
SELECTION SIGNATURES

THE GENETIC BASIS OF ADAPTATIONS IN BUD PHENOLOGY ACROSS A LATITUDINAL GRADIENT IN EUROPEAN ASPEN (*Populus tremula*)

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The initiation of growth and dormancy represents critical ecological and evolutionary trade-offs in perennial plants and latitudinal clines in important phenological traits common in many plants. In European aspen (*Populus tremula*), the most important environmental cue regulating dormancy initiation is a shortening of the photoperiod. QTL mapping has implicated genes, in the photoperiodic pathway, that control growth cessation. Here we present data from a study on the genetic basis of variation in phenology in European aspen across a latitudinal gradient. We show that genetic differentiation of neutral markers is low despite strong differentiation in phenology traits, demonstrating local adaptation to the photoperiodic regime. Sequence data from about 25 genes from the photoperiodic pathway were used to show that mutations, found in a few of these genes, are associated with natural variation in phenology. There is also evidence suggesting that patterns of nucleotide polymorphism, at several of these genes, have been shaped by natural selection.

DIVERSITY AND SELECTION IN A SUITE OF COLD HARDINESS-RELATED CANDIDATE GENES IN SITKA SPRUCE (*Picea sitchensis*)

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Cold acclimation in temperate and boreal conifer trees is a complex process that involves extensive metabolic and transcriptional remodeling, the timing and extent of which varies widely within species along latitudinal and elevational gradients. We are combining transcriptional profiling with association mapping to better understand the genomic architecture of cold hardiness in conifers, using Sitka spruce (*Picea sitchensis*) as a model. A microarray study, conducted during the fall hardening period, revealed wholesale remodeling of the transcriptome within-populations, and substantial differential gene expression among populations. Based on these data, a suite of candidate genes was selected for population genomic dissection of cold hardiness. To identify single nucleotide polymorphisms (SNPs) we have, to date, successfully re-sequenced the coding regions of 145 of these candidate genes in a panel of 24 individuals from across the species range. Analysis of the first 107 of these genes shows low linkage disequilibrium and an average level of nucleotide diversity for a conifer ($\pi = 0.0042$). Tests of selective neutrality suggest many candidate genes are under purifying selection, while a few appear to be under positive or balancing selection. In addition, F_{ST} outlier approaches reveal the action of diversifying selection. Although mean F_{ST} for Sitka spruce is ~0.1, values for some SNPs were as high as 0.7. The ultimate goal of this study is to identify SNPs that are associated with phenotypic variation. As such, the Illumina 'GoldenGate' platform is being employed to genotype ~500 SNPs in an initial mapping population of 490 individuals. Significant associations will be verified in an independent population of 1000 individuals.

ANALYSIS OF THE CAD2 GENE POLYMORPHISM IN *Eucalyptus urophylla* POPULATIONS ALONG AN ALTITUDINAL GRADIENT ON TIMOR ISLAND

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Our studies describe nucleotide variability within the Cinnamyl Alcohol Dehydrogenase (CAD) gene, a structural gene of the lignin biosynthesis pathway, within a representative sample of *Eucalyptus urophylla* distributed at various altitudes on the island of Timor. We sequenced 100% of the CAD2 gene from 10 individuals by direct sequencing of the PCR product. The gene includes 5 exons and 4 introns with a total length of 5395 bp and showed 93% similarity with the *E.gunnii* CAD2 gene. The nucleotide variability analysis on 10 individuals consisted of 160 SNPs and 16 INDELs. A heterogeneous distribution of the polymorphism was noticed in the gene with 1 SNP per 74 bp in the exonic regions and 1 SNP per 21 bp in the intronic regions. Four non-synonymous SNPs and 1 SNP translated in polar/non-polar aminoacids were identified. Preliminary analyses were conducted with the DNAsp software. The diversity in the silent regions is higher ($\pi=10.8\times10^{-3}$) than in the non synonymous sites ($\pi=0.74\times10^{-3}$). The sliding window approach detected variation of the polymorphism along the gene with 5 peaks showing high π values.The *D* Tajima test for the whole gene is negative but not significant showing no impact of natural selection. However, the sliding windows approach detected variation within the gene and showed two regions (exon 3 and exon 5) with a negative significant Tajima and Fu and Li's *D* and *F* tests. Divergence among both populations (superior to 1000 m and inferior to 1000 m) also varied along the gene and some peaks corresponded to positive Tajima *D* values and high polymorphism. A detailed analysis of variation in exons 3 and 5 was undertaken with 85 individuals distributed along the altitudinal gradient.

A SURVEY OF NUCLEOTIDE DIVERSITY ACROSS EXPRESSED GENES IN WHITE SPRUCE

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Baseline data about nucleotide diversity, haplotype structure, and the strength of linkage disequilibrium are highly useful to increase the efficiency of association studies. To obtain an overall picture of these parameters at the genome level, resequencing of 100 expressed genes distributed along the 12 linkage groups of white spruce (*Picea glauca*) was undertaken for a set of 48 haploid megagametophytes. On average, 7.8 non-synonymous SNPs and 30.8 silent SNPs were found per 1000 bp, for an average of 0.025 substitutions per site and a nucleotide diversity value of $\pi = 0.005$ for a first subset of 70 genes. The number of haplotypes per gene was highly variable and ranged from 3 to 39, with an average haplotype diversity of $H = 0.78$. Disparities were observed within and among gene families. For instance, genes encoding xyloglucan endotransglycosylases showed very high nucleotide diversity with 98 to 124 silent SNPs per 1000 silent positions. Linkage disequilibrium (LD) was generally weak with an average r^2 of 0.17. The distance for a half-decrease of LD was within 100 bp. For most of the genes, Tajima's *D* statistics were negative but none of them was significant ($P \leq 0.01$). Fu's *Fs* statistics were negative for most of the genes but significant for only 2 genes ($P \leq 0.01$). Altogether, these trends suggest that demography rather than selection is the main factor shaping sequence diversity in white spruce.

PATTERNS OF NUCLEOTIDE DIVERSITY AND SELECTIVE NEUTRALITY ACROSS 125 CANDIDATE GENES PUTATIVELY ASSOCIATED WITH COLD TOLERANCE IN DOUGLAS-FIR (*Pseudotsuga menziesii*, Pinaceae)

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Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) ranges from the Pacific coast to the eastern slopes of the Rocky Mountains and from Canada to Mexico, occurring from sea level to over 3000 m in elevation. Douglas fir populations are adapted to the diverse environments that occur across this immense region and express remarkable genetic variation in adaptive traits such as cold tolerance, drought tolerance, and vegetative bud phenology. These traits are critically important fitness components of Douglas-fir, influencing survival, growth, and stem quality, particularly at early ages in natural stands. Here, we focus on resequencing a set of candidate genes (n=125) derived from three express sequence tag (EST) libraries putatively affecting cold tolerance. Resequencing was conducted within a diversity panel comprised of 24 range-wide samples. Sequence manipulation, assembly, alignment, and statistical analyses were all carried out using the *PineSAP* and *DnaSAM* bioinformatic pipelines developed at UC Davis. Patterns of diversity across all genes were similar to previous estimates in conifers, with an average nucleotide diversity (π) of 0.00478 ± 0.00475. Standard neutrality tests revealed 10 genes putatively affected by natural selection. Incorporation of demographic scenarios as part of those hypothesis tests did not drastically change the results. Moreover, estimated selection coefficients using a Poisson random field approach illustrated that most of the non-neutral variation was consistent with purifying selection suggesting strong selective constraint. These results are extended to the importance of single species and comparative resequencing projects within conifers.

DISSECTING THE ROLE OF DEMOGRAPHY AND NATURAL SELECTION IN SHAPING POPULATION GENETIC STRUCTURE OF TWO MEDITERRANEAN CONIFERS: *Pinus pinaster* AND *Pinus halepensis*

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Deciphering the molecular basis of adaptation in non-model tree species still represents a daunting task, not only because of limited access to all areas of the genome but also because factors other than natural selection – such as demography – can shape the genetic structure of populations, therefore mimicking natural selection. In this study, the main objective was to dissect the respective role of demography and natural selection in shaping population genetic structure of two Mediterranean conifers, *Pinus pinaster* Ait. and *Pinus halepensis* Mill., which differ in levels and distribution of genetic variation and width of ecological niche. We examined natural populations at the species distribution scale, with two main objectives: 1) Characterize the evolutionary and demographic history of the populations. First, insights on demography and population structure were gained by screening supposedly neutral markers (chloroplast and nuclear microsatellites) in the same populations. Second, coalescence simulations were built considering neutral marker information and using only DNA sequence silent polymorphisms. 2) Identify candidate genes associated to important traits that are under natural selection. For this objective some functional genes putatively associated with drought stress tolerance and phenology were used, in a comparative (among-species) framework. The nucleotide variation detected within these genes allowed us to detect loci subject to selection (for instance, some dehydrins) by combining various neutrality tests, in particular compound tests, and to infer likely demographic scenarios in both species.

STRONG LINKAGE DISEQUILIBRIUM AND BALANCED SELECTION IN *Pinus pinaster* TRANSCRIPTION FACTORS PUTATIVELY INVOLVED IN WOOD FORMATION

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DNA polymorphism of 9 transcription factors, putatively involved in wood formation, was studied in 6 *Pinus pinaster* natural populations from the French Atlantic coast. No differentiation was detected between populations, in line with the hypothesis of a large, unstructured meta-population. We tested whether patterns of diversity at each gene departed from the neutral model expectations. *HDZ31*, *LIM2*, and *Myb1* transcription factors showed a significant excess of intermediate frequency alleles (positive Tajima's *D* and Fu's *Fs*) and low haplotypic diversities, suggesting balanced selection. The diversity pattern in *Myb14* was more ambiguous but also suggested that this locus may be subjected to balancing selection. *Myb4*, *Myb5*, and *Myb8* showed no or low polymorphism, which could be consistent with purifying selection. *Myb2* and *SCL1* did not depart from neutrality using the same tests. These contrasting results across 9 different loci were more consistent with signatures of selection than with strong demographic events, a hypothesis that also fits with the high diversity observed for neutral markers in this metapopulation. Within-gene linkage disequilibrium was found to be higher than previously described in other conifer species and did not decay with distance, except for *Myb2*, contrasting with most published results so far in the Pinaceae. These results underline the putative role of these transcription factors in adaptation, making them good candidates for an association mapping study.

ESTIMATING PARAMETERS OF A SPECIATION MODEL IN SPRUCE SPECIES

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In the present project nucleotide polymorphism was estimated at 10 to 13 nuclear genes (around 10 000 bp) in different spruce species with contrasting distribution ranges and post-glacial histories. Data are currently available for 5 species, three North American ones (*Picea breweriana*, *P. glauca*, and *P. mariana*), one European (*P. abies*), and one Asian species (*P. likiangensis*) and efforts are under way to gather sequence information in additional species from the Qinghai-Tibetan Plateau. Preliminary results indicate large differences among species in levels of nucleotide diversity, with *P. breweriana* being the less variable, *P. likiangensis* the most variable and, the boreal species falling in between in spite of their extensive distribution range. These data are currently used to estimate time of divergence, current and ancestral effective population sizes, as well as migration rate among these species using an isolation-with migration model.

WHAT ACCOUNTS FOR HIGH PROTEIN HETEROZYGOSITY AND LOW NUCLEOTIDE DIVERSITY IN SCOTS PINE (*Pinus sylvestris*)?

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Among plants, the highest levels of allozyme diversity have been observed in outcrossing woody plants. However, nucleotide diversity estimates for many trees are lower or similar as in other plants. We examined the possible causes of discrepancy between allozyme diversity and nucleotide variation in *P. sylvestris*. By studying the nucleotide diversity directly in allozyme coding loci we examined the roles of mutation, recombination, and selection in creating high allozyme heterozygosity. We first identified six allozyme coding genes by associating the electrophoretic mobility changes with the sequence level changes in individual haploid megagametophytes. These loci were then sequenced in a population sample (n=35). We found levels of nucleotide variation slightly, but not significantly, higher than at other loci. Individual protein heterozygosities did not correlate with the nucleotide diversities. Recombination rates were surprisingly low when compared to other loci and several of the allozyme genes had unusual clusters of closely related haplotypes. Haplotype clustering might indicate action of selection. Our results suggest that allozyme heterozygosities might not always accurately predict the level of neutral genetic variation.

GENOGEOGRAPHY, POPULATION STRUCTURE, CONSERVATION

SPATIAL GENETIC STRUCTURE OF NATURAL POPULATIONS OF WHITE POPLAR (*Populus alba* L.) AT REGIONAL AND LOCAL SCALES

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Poplar is a riparian tree in which spatial genetic structure is conditioned by a dependence on phreatic soils and a reproduction system that combines sexual and clonal reproduction. Genetic differentiation among main river-basin populations and clones within populations was studied for Iberian populations of white poplar (*Populus alba* L.) based on cpDNA (2 polymorphic cpSSRs out of 10 tested and 2 gene fragments) and nuSSRs (5 loci screened in 5 populations out of the 40 used in the regional study). We found a total number of 27 haplotypes, most of them unique to a single river-basin, and notable differences in haplotype frequency for shared-haplotypes, resulting in high among-basin genetic differentiation. Comparison with reference to sympatric populations of black (*P. nigra*) and grey (*P.* x *canescens*) poplars showed that no haplotype was shared among species. Sequencing of gene fragments produced cpSSRs scores that indicated high rates of homoplasy. Finally, within-population analyses based on nuSSRs showed a high level of clonality in all populations, with exceptional clonal assemblies extending tens of kilometres. High variability among basins reinforces the idea that these areas must be used as basic conservation management units.

PURSUING GLACIER RETREAT: GENETIC CHALLENGES FOR THE SPREAD OF *Larix decidua*

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There is considerable evidence that climate warming promotes an upward shift of plant populations in Alpine regions. Because climate is changing faster than in recent history, it is important to understand the colonization pattern of individual species to reveal if plant migration can follow this pace without losing genetic diversity.

Successional gradients along glacier forefields and moraines are perfect natural experiments to study movement of propagules, the establishment of sub-populations, and associated genetic challenges due to possible founder effects. How genetic diversity and structure is built up over time in mature trees was studied using SSR-markers for *Larix decidua* along a 5 km successional transect. Additionally, extensive sampling of different aged individuals in a recently established sub-population that lacks mature trees and in a mature sub-population revealed how spatial genetic structure changes over time at a given site. Moreover, this gives insights into seed-pool diversity in relation to the age of sub-populations.

This study elucidates the strength of founder events and counteracting gene flow and will lead to management strategies for maintaining genetic diversity and supporting long-term species persistence.

INCORPORATING GEOGRAPHICAL GENETICS INTO CLIMATE CHANGE MODELS OF CALIFORNIA VALLEY OAK (*Quercus lobata*)

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Conservation biologists have emphasized the need to prioritize areas for preservation. In some cases, the motivation is species-specific and, in others, ecosystem-based. In California, oaks are important tree species and key determinants of major ecosystems, including oak woodland, oak savanna, and riparian oak forest. Given the widespread distribution of oaks among the largely unprotected private lands and the high risk of landscape transformation, significant public and private funds are being directed towards oak habitat protection and restoration. In this paper, we examine geographic patterns of genetic variation in populations of Valley oak (*Quercus lobata* Née), an endemic whose distribution has been more reduced by habitat conversion than any other major tree oak species in California, to inform regional conservation planning. We use the Maximum Entropy (MAXENT) method to model the habitat distribution of Valley oak genotypes based on climate and soil variables. We then identify regions that might support Valley oak populations under predicted future climate regimes. The genetic data are based on nuclear microsatellite genotypes for ~ 200 individuals sampled from over 50 sites distributed throughout the species' range. These analyses are the first steps to analyzing the ability of Valley oak to adapt to predicted climate change. Eventually, we plan to sample additional populations and to incorporate information from genes under selection into climate change models.

BEAUTIFUL, CHARISMATIC BUT LACKING DIVERSITY: THE STORY OF PACIFIC DOGWOOD (*Cornus nuttallii*) AS TOLD THROUGH MICROSATELLITE AND CHLOROPLAST SEQUENCE DATA

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In the past, conifers have been the primary focus of population and conservation genetic studies in Pacific Northwest trees. These studies have provided tremendous insight as to how genetic diversity varies across species ranges and how historic range contractions and expansions have affected the genetic structure of these wind-pollinated and mostly wind-dispersed species. The addition of studies focused on broadleaved species, such as Pacific dogwood (*Cornus nuttallii* Audubon ex Torr. & A. Gray), which share similar distributions, but possess biological vectors for pollination and seed dispersal will broaden our understanding of tree evolutionary dynamics. Results from both microsatellite and chloroplast DNA sequence data reveal low diversity in both genomes throughout the range of Pacific dogwood. Of the eight microsatellite markers tested, three were monomorphic and five were found to have an average of 4.4 alleles/locus. Furthermore, only a single base pair substitution was found after sequencing 2262 non-coding base pairs of the chloroplast genome in 100 individuals. Such uniformly low diversity raises the issue of how genetic conservation efforts should proceed with this species and others sharing a similar degree of genetic depauperateness. In addition, genetic theory and supporting empirical evidence predict certain phylogenetic patterns to emerge in range-wide studies of Pacific Northwest species. Pacific dogwood appears to contravene these expectations. Such a violation begs the inference of current evolutionary forces acting on this species, whose range has been historically fragmented by human development, has suffered from an introduced fungal parasite, and is currently being forced to adapt to a changing climate.

POSTGLACIAL HISTORY OF THE MARITIMES IN EASTERN CANADA: INFERENCES FROM JACK PINE (*Pinus banksiana*), A BOREAL TREE SPECIES

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The current range of jack pine (*Pinus banksiana* Lamb.), a nearly transcontinental conifer in the North American boreal forest, was covered by the Laurentian ice sheet during the last glacial maximum 18 000 BP. A previous genogeographic survey based on mtDNA polymorphisms indicated a clear genetic differentiation of the western portion of the range, suggesting a genetically distinct refugium for the region. Genetic diversity was higher in the East, indicative of a more complex scenario potentially involving more than one genetically distinct refugium. Intensive population sampling was conducted in the Maritimes at the eastern end of the natural range and the genetic structure was assessed using minisatellite-like polymorphisms detected at the mtDNA locus *nad7* intron 1. Most populations from Prince Edward Island, New Brunswick, and Nova Scotia were fixed for a single haplotype typical of the region. While most of the region was glaciated at LGM, a plausible explanation would be that these populations descended from a glacial refugium located on the exposed and unglaciated continental shelf in the region. A few populations with mixed haplotypes were also indicative of a possible zone of contact between genetically distinct migration fronts from the northeast and the south. These findings have repercussions on the interpretation of the biogeography of the Maritimes, where the natural distribution of several plant species appears to be disjunct.

THE POSTGLACIAL AND DEMOGRAPHIC HISTORY OF BLACK SPRUCE INFERRED FROM CHLOROPLAST DNA MICROSATELLITE DATA

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The genogeographic structure and postglacial history of black spruce (*Picea mariana*), a transcontinental North American boreal conifer, was inferred using chloroplast DNA SSRs. Samples were collected from 30 natural populations covering most of its range. Two cluster analyses using Bayesian and genetic distances approaches resulted in the delineation of at least three genetically distinct cpDNA lineages partially congruent with those inferred from mtDNA data (Jaramillo-Correa et al. 2004). Some of the geographical areas occupied by cpDNA lineages were substantially expanded eastward compared to mtDNA lineages, presumably reflecting that pollen is dispersed longer distances than seeds. The recent demographic history of black spruce was also assessed using a mismatch distribution estimated at both the population and cluster levels. The results suggested that western Canada stands (British Columbia, Alberta, and Yukon) were more at demographic equilibrium than Alaskan and eastern stands. The spatial distribution of the allelic richness across populations seemed to follow the same trend, as most of the genetic diversity was observed in the populations that experienced recent demographic expansion. Based on cpDNA evidence and palynology, two glacial lineages are thought to have persisted south of the ice sheets, in the Great Lakes region and near Western Oregon or Washington States, respectively. A third glacial lineage would have survived east of the Appalachian Mountains in the Northeast. Additional evidence from mtDNA will be presented that suggests the existence of a genetically distinct glacial lineage in Alaska.

ASSESSMENT OF GENETIC DIVERSITY AND POPULATION STRUCTURE IN LATVIAN *Pinus sylvestris* POPULATIONS USING NUCLEAR, CHLOROPLAST AND MITOCHONDRIAL SSR MARKERS

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Scots pine (*Pinus sylvestris* L.) is the most important tree species in Latvia, both ecologically and economically. Scots pine covers 40% of the total forest area of Latvia. Breeding programs have been in place since 1958, and logging and forest husbandry have been carried out for a much longer time. Many small-scale introductions of Scots pine have been made over time and most of these introductions have not been recorded. Latvian Scots pine accessions were so distinctive that they were designated as a separate sub-species (*Pinus sylvestris* L. var. *rigensis* (Desf.) Asch. & Graebn.), however, this designation has not been commonly used in recent times.

Our aim was to survey the genetic diversity and population structure of Latvian Scots pine populations and to compare these with other regions. We utilised nuclear, chloroplast, and mitochondrial SSR markers. As expected, the nuclear SSR markers showed high genetic diversity and low population differentiation. However, the chloroplast and mitochondrial markers found showed an increased population structure, particularly differentiating the introduced accessions from the Darmstadt region in Germany. Latvia is in the centre of the natural range of Scots pine and this region has been characterised as having optimum growing conditions for pine. This would tend to increase the genetic diversity of local populations and allow for the selection of phenotypically superior trees. Our results show that mitochondrial markers seem to be the most promising in identifying population differentiation and investigating the fine-scale genetic structure of Latvian *P. sylvestris* populations.

A GENOME SCAN OF GENE SNPs TO ASSESS THE IMPACT OF FIRST-GENERATION ARTIFICIAL SELECTION ON WHITE SPRUCE GENETIC DIVERSITY

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We used a genome scan to assess whether genetic diversity in white spruce (*Picea glauca*) was affected by domestication. For this purpose, two populations were assembled. The first one was composed of trees belonging to the first-generation white spruce breeding population in Quebec, which was created by selecting the best families in provenance/progeny tests based on their estimated breeding values for 15-year height, combined with within-family mass selection. Genetic gain has been estimated at 20%. The second population was assembled by random selection of trees from non-selected families from the same provenances. Genetic diversity and differentiation between the two populations were estimated by genotyping 142 trees for 1256 single nucleotide polymorphisms (SNPs) distributed among 584 expressed gene loci of various GO classes, and spanning the 12 linkage groups of white spruce. The amplitude and significance of genetic differentiation between the two populations were estimated by F_{ST} using FDIST2. Average within-population genetic diversity parameters and fixation indices were not significantly different between the two populations. The average F_{ST} value was small and not significantly different from 0. Only one SNP involving a gene for lignin synthesis showed significant differentiation between the two populations. These results indicate that despite moderate selection for height growth, genetic diversity was maintained in the genetically improved population.

TREE SEED WORKING GROUP WORKSHOP

Workshop Theme

"Preserve and Multiply Forest Genetic Resources"

MEETING PROVINCIAL SEED PRODUCTION OBJECTIVES IN BRITISH COLUMBIA: SYSTEMS FOR RANKING PRIORITIES AND DETERMINING ORCHARD SIZE

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Provincial objectives, set by the multi-stakeholder Forest Genetics Council of British Columbia (FGC), call for 75% select seed[1] use by 2013 and for an average genetic worth for growth of 20% by 2020. With annual provincial planting averaging about 225 million seedlings per year across over 14 species, priority setting and seed planning are significant tasks.

The planning process involves a series of steps. Initially, there is a process for setting priorities among various seed planning units[2] (SPU). For SPUs that meet criteria for breeding and seed orchard investments, seed needs within each zone are forecast. Historic orchard production curves are developed and, based on these, total orchard size needs are predicted. Seed use and orchard production figures are tracked and presented annually to aid orchard managers in the development of specific orchards and to help broader planning to ensure provincial objectives are met. Breeding investments are guided by SPU priorities and by orchard development timing. Orchard roguing decisions are made by individual owners, but cooperative planning and discussions provide them with the information they need to make informed decisions. Provincial financial support for boosting seed orchard quality (seedlot genetic worth) and for adjustments to meet FGC objectives provide added incentive for orchard operaters to undertake management that will ultimately support FGC objectives.

In the multi-stakeholder system in BC, orchards are owned and operated by both the public and private sectors. Providing good information on seed needs, on annual production, and on forecast production is a key element to ensuring open discussion and cooperation among participants and to the development of a comprehensive seed-production system that meets broader stakeholder objectives.

This presentation will overview the planning and reporting system used in BC for seed orchard and seed supply planning.

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[1] Select seed is seed from trees that have been genetically selected from natural populations, including orchard seed from parents with known and positive breeding values and seed from provenances with known traits. No seed used in BC is genetically modified.

[2] A seed planning unit is a unique combination of species, seed zone, and elevation band which is used for seed planning and which has specific breeding and seed orchard populations. An example is Douglas-fir in the maritime seed zone under 700 m elevation.

ZOOM IN ON SEED PRODUCTION IN QUEBEC

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The Ministère des Ressources naturelles et de la Faune du Québec (MRNF) is responsible for seed production in Quebec. It is coordinated by the Direction générale des pépinières et des stations piscicoles (DGPSP) with the collaboration of the MRNF's local offices (established within the 10 regions), public nurseries, the Centre de semences forestières de Berthier (CSFB), and the Recherche forestière. In the early 1980s, Quebec started a tree improvement program for the 3 major species (black spruce (*Picea mariana*), white spruce (*Picea glauca*), and jack pine (*Pinus banksiana*)). The first seed orchard was established in 1978. Over time the proportion of improved seeds has risen steadily to satisfy up to 85% of our needs. Seed is produced in 141 seed production facilities, consisting of approximately 500 000 seed trees. Our priority is to increase the level of genetic gain of seedlings at the lowest cost by using, among other techniques, controlled pollination. The harvesting techniques used for the annual collection of 2,000 hL of cones and hardwood seed are adapted for use in the seed orchards ensuring safety and using productive equipment. The CSFB annually processes an average of 550 million seeds and the extraction methods have been improved leading to significantly higher germination in most species. Since 2001, we have used a computerized system (SEMENCES) to support the management of all activities from plus-tree selection to seedlot delivery to public or private nurseries.

FRENCH FORESTRY PATTERN: FROM SEED TO PLANT

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Forest tree seed and stock plant sales have been monitored in France since 2003 under the new European Union Directive. It sets very strict traceability requirements from the original parent trees to the young plants in the field and classifies the seed sources according to four different categories: Source-identified, Selected, Qualified, and Tested. Seed certified in any of these categories is what we call Forest Reproductive Material. Most forest seeds are collected in selected stands chosen on the basis of phenotypic selection, but the best genetic improvement is obtained from the creation of seed orchards; the Qualified and Tested categories. Genetic improvement is carried out by governmental research institutes. This work is based on criteria partly defined by the final users, the nurserymen, and foresters. Finally, the design, creation, and management of the French seed orchards are the result of valuable cooperation between private and public partners. Through an example, we will follow the course of a seedlot, from harvest to plantation, via factory processing. It will show how, throughout its life, a certificate of provenance attests to the origin of the seeds.

THE *ex-situ* GENETIC CONSERVATION PLAN FOR BRITISH COLUMBIA TREE SPECIES

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The BC Ministry of Forests and Range's Tree Seed Centre has been safeguarding seed samples from most seedlots that have been registered for use in BC. Historically, the seed bank sample was obtained from seed remaining from the submitted sample provided to the lab for initial seedlot testing and then stored separately from the operational seedlot. The current seed bank emphasis is on: 1) estimating the number of viable seeds per sample, 2) allocating the samples to a land classification matrix, 3) identifying gaps in the matrix, and 4) planning activities to populate gaps in the matrix.

The initial criteria that we have defined for a primary seed bank sample is that the sample should contain 1000 or more viable seeds and that a germination capacity estimate used to calculate number of viable seeds should have been performed within the last ten years. For seedlots which are still active (being used operationally) our seedlot retesting program meets these needs, but for expired seedlots (no remaining operational balance) the sample will either be retested if considered large enough or considered a secondary sample not meeting our minimum requirements, but still retained.

The seed bank samples are being summarized by Species and Biogeoclimatic zones (BEC - the BC land classification system[1]) and this same matrix process is also occurring for *in situ* and "inter situ" reserves. The current objective for the seed bank is to have at least five primary samples of seed for each species x BEC zone combination in which the species occurs.

Moving forward the plan is to increase the coverage of our seed bank through the following: 1) Germination testing the seed samples from expired seedlots to estimate number of viable seeds. Samples can then be allocated to the primary or secondary category. 2) Adding seed from operational seedlots to existing seed samples or initiating new samples to ensure that at least 1000 viable seeds are represented per sample. 3) Performing specific collections to address gaps in the species x BEC matrix. This method will probably be most common for our non-commercial tree species.

The plan for our seed bank is continuing to develop and evolve.

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[1] Further information on the Biogeoclimatic Ecosystem Classification system can be found at http://www.for.gov.bc.ca/hre/becweb/

CONSERVATION OF GENETIC DIVERSITY BY TREE SEED CENTRES

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Storage of seed is an effective and efficient means of conserving genetic diversity of woody plant species providing that seed maintain their viability for many decades. There are ambitious international programs such as the one led by Kew Gardens in Great Britain to bank seed from 10% of the world's wild plant species by 2010 but there is an opportunity for programs to be conducted at the national level in Canada. One must first determine (decide) how many trees seed must be collected from in populations in order to conserve a significant proportion (95%+) of the genes. This is dependent on the genetic variation pattern of the species, the mating system (open-pollination, self-pollination, insect pollination), population size, whether populations are isolated, etc. Will seed be bulked or kept separate by individual trees and how much seed should be stored? Seed collections made by the National Tree Seed Centre are primarily from individual trees in populations. Seed is collected from a minimum of 15 trees in a population and when possible up to 25 trees. A sample of seed from each collection is placed in the gene conservation collection for storage at -20°C. Generally, at least 2,000 viable seed per individual-tree collection and 5,000 viable seed per bulk collection are stored for genetic conservation. Seed are stored using a variety of containers of different sizes.

WATER ACTIVITY: A NEW TOOL FOR MOISTURE MANAGEMENT OF SEEDLOTS IN TREE SEED CENTRES

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Measurement of water activity (Aw) is a concept developed, and mainly used, by the food processing and pharmaceutical industries. Contrary to gravimetric moisture content (MC) that quantifies the total amount of water in a product, Aw qualifies the intensity of the connections between water and other molecules (such as lipids, carbohydrates or proteins) and therefore illustrates water availability and mobility in the substance. Aw has three main operational advantages: 1) rapid measurement (less than 20 minutes per sample), 2) non-destructive testing, making it suitable for use with rare or valuable samples such as pollen or seeds from specific crossings or *ex-situ* genetic resource conservation, and 3) ease of use, requiring little training.

In 2007, the DRF (MRNF, Quebec) decided to adopt the method developed by Cemagref (France). The work began with black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*), the two most important reforestation species in Quebec. To date, our results follow the same pattern as those obtained in France. An optimal Aw is determined for each species which will be used to optimize seed processing in tree seed centres. Depending on the seedlot, a given Aw may result in different MC because of the variability in seedlot traits like maturity, origin, or crop year. Managing seedlots with Aw results in moisture management not being sensitive to genetic, and consequently phenotypic, diversity which is important for conservation purposes.

AN INTEGRATED APPROACH TO PRESERVE AND MULTIPLY IMPROVED MATERIAL OF WHITE SPRUCE BY SOMATIC EMBRYOGENESIS

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Somatic embryogenesis (SE) is a vegetative propagation method that presents several advantages in tree improvement strategies. In fact, SE offers an opportunity to select the best-tested clones and deploy them in clonal forestry. This technology, combined with cryopreservation (storage in liquid nitrogen), has the potential to become a valuable tool in tree breeding programs. Cryopreservation can be used for preserving the germplasm and maintaining the embryogenic capacity of the tissue. In Québec, the ministère des Ressources naturelles et de la Faune (MRNF) decided to integrate SE into breeding programs. The emphasis has been placed on white spruce (*Picea glauca*) because its improvement program is the most advanced. However, the MRNF is scaling up this technology. Efficient collaboration between the Saint-Modeste nursery and the Direction de la recherche forestière (DRF) permits development and optimization of this technique under laboratory and forest nursery conditions, as well as its use in seed orchard management. Protocols have been refined for several coniferous species such as white spruce, black spruce (*Picea mariana*) and hybrid larch to improve the SE stages. For example, between 2004 and 2007, over 1,200 clones, originating from 41 selected white spruce families (controlled crosses, MRNF), were produced in a forest nursery and cryopreserved. SE will be a key element in the long term deployment of improved varieties.

SEED SIZE AND FAMILY EFFECTS ON VEGETATIVE PROPAGATION RATES OF STOCK PLANTS FOR THE MASS CUTTING PROPAGATION OF WHITE SPRUCE (*Picea glauca*) IN QUEBEC

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Seeds used to carry out mass propagation of white spruce (*Picea glauca*) by cuttings come from selected controlled crosses. Large variation in terms of germination kinetics, growth, crown architecture, and cutting yield is observed within stock plant populations, compromising production objectives for cutting propagation. Nursery managers must characterize each controlled cross for each phase of cutting propagation and optimize their cultural practices. In 2005 and 2006, we compared the growth of white spruce seedlings from seven recommended controlled crosses, classified by seed size (**A**: 1.5 ≤ diameter < 1.75 mm and **B**: diameter ≥ 1.75 mm). Smaller seeds produced significantly smaller stock plants and growth varied greatly among crosses. Differences between the two seed sizes also significantly affected cutting yield and quality : stock plants from size **A** produced fewer cuttings than those from size **B** (8 vs. 14/stock plant), but cuttings from **A** showed greater dry mass (203 vs. 184 mg) and lower cutting water content (71.5 vs. 74.3%) than those from **B**. In comparison, differences were small among crosses. In a rooting experiment conducted in 2006 under nursery conditions, all cuttings showed excellent rooting success (> 80% rooting percentage). Variations were small between seed sizes and crosses. These results suggest that white spruce stock plants should be sown separately by seed size. With an adapted irrigation and fertilization regime for smaller seeds, growth will be more uniform and all stock plants will attain the specifications for cutting propagation programs.

GENETIC PARAMETERS OF SEEDLING JUVENILE CHARACTERS OF 75 OPEN-POLLINATED WHITE SPRUCE (*Picea glauca* [Moench] Voss) FAMILIES

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In Quebec, 28 million white spruce (*Picea glauca*) seedlings are planted annually. Previous studies have shown that several characteristics of white spruce seedlings exhibit significant between-family variability. Such characteristics have been considered as the basis for early selection of older tree traits, although not as characteristics to be improved. However, the use of this variability, through selection and breeding, may help improve seedling quality. Given the importance of a high-quality root system to seedling establishment, it is of interest to estimate genetic parameters for rooting characteristics from a large number of families. Seedlings from 75 half-sib families were grown under nursery conditions. At the end of the first two growing seasons, significant differences among families were found for all seedling traits with the exception of photosynthetic capacity. Family differences explained up to 37% of total phenotypic variation. Family variation of morphological traits was associated with family variation of nitrogen absorption rate and nitrogen utilization. Genetic correlation estimates showed a strong link between root dry mass and diameter at the end of the first and second (r = 0.68, 0.56) growing seasons, suggesting a non-destructive way of identifying families with heavier root systems.

UTILIZATION OF FAMILY GENETIC VARIABILITY OF WHITE SPRUCE (*Picea glauca*) TO IMPROVE ROOTING OF PLANT CUTTING ROOT SYSTEMS

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Different cultural techniques are used at an operational scale to improve rooting of white spruce (*Picea glauca*) by mass cutting propagation. This technique could be enhanced by the evaluation and utilization of families that perform well, in terms of rooting and cutting growth, while respecting genetic diversity. Cuttings were taken from 3-year stock plants, representing 75 open-pollinated white spruce families. For each family, 405 cuttings were propagated under operational conditions in double-walled enclosures. After transplanting, 180 cuttings were available from each family. Evaluation of rooting ability and root architecture at the end of the rooting period demonstrated the existence of large genetic variability, notably a high family heritability for root length (0.64) and dry weight (0.63). Family heritability at the end of the first growing season was calculated and estimates for height (0.76), number of branches per cutting (0.74), and root dry weight (0.74) indicate the potential for increasing rooting efficiency by selecting families with the best performance.

Fraxinus GERMPLASM COLLECTIONS IN THE UNITED STATES

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Fraxinus species are wide spread and invasive in the northeastern United States. They are prized for many cultural, ecological, and economic reasons that include basket making material, baseball bats, furniture, and urban shade. Since 2002 the exotic insect *Agrilus planipennis* has been threatening the status of all *Fraxinus* species that it has come in contact with. Loss of ash trees has been rapid and extensive in infested areas. Therefore, the USDA Forest Service and the USDA Agricultural Research Service, National Plant Germplasm System, have identified *Fraxinus* as a genus to be given top priority for *ex situ* germplasm conservation. The focus of this effort has been on seed collection. The poster describes the process used to define seed collection areas, the methods that will be used to refine and evaluate collection efforts, progress to date, and the intended deployment of the collected material to restore *Fraxinus* to a prominent and sustainable position in the hardwood forest ecosystem of the eastern United States.

EFFECTS OF CONE NUMBER AND THINNING ON NUTRIENT CONTENT IN NEEDLES OF KOREAN PINE (*Pinus koraiensis*)

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We investigated the distribution of nutrient content (N, P, and K) in current and one-year old needles of Korean pine (*Pinus koraiensis*) to overcome the influence of alternate bearing. One experiment was designed with three numbers of cones (one, three, and five) under natural conditions and cone number of another experiment was controlled by thinning. Test samples were separated into three fractions: current needles on the shoot and one-year old needles above and under the cone. On branches without cones, N, P, and K concentrations in needles were higher than those in needles of one-year old branches. Under natural conditions, P concentration was the lowest in needles on five-cone branches in August. Macronutrient concentrations were lowest in needles above the cone, with K concentrations being substantially lower. Under cone thinning, N, P, and K concentrations were highest in needles on one-cone branches and lowest in needles on five-cone branches. Needle nutrient concentrations were lowest in needles above the cone, with the lowest concentrations found in needles on five-cone branches. In conclusion, the current needles of shoots show that cones are a strong sink of nutrients such as N, P, and K and the increase of cone number influences nutrient removal from needles.

CLONAL VARIATION in *Picea abies* STOCK PLANTS PRODUCED BY SOMATIC EMBRYOGENESIS AND THEIR USE IN CUTTING PROPAGATION PROGRAMS

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Quebec promotes a gradual integration of somatic embryogenesis (SE) in clonal forestry, seed orchard management, and cutting propagation programs (CPP). However, before SE can be introduced into CPP, we need to determine the physiological and morphological attributes of cuttings produced by somatic stock plants.

Somatic plants of *Picea abies*, representing 15 unrelated clones, were grown in a greenhouse. Stock plant yield (lateral cuttings $\geq$ 5 cm) showed a significant clone x year interaction ($p = 0.0123$) with increasing differences among clones over the two sampling years.

A rooting experiment was conducted in 2004, using a randomized block design within a mist enclosure. After 12 weeks, all rooting variables showed important clonal effects ($p < 0.0033$) including rooting percentage (clonal means ranging from 5 to 71%), root abundance (2.5 to 5.8 main roots/cutting and 5 to 22 mg root biomass), root diameter (0.53 to 1.03 mm), and number of root apices (46 to 436/cutting). Since clonal ranks were similar between variables, clones could be classified as good, intermediate, or poor-rooting. These results show that both stock plant yield and rooting ability are under genetic control and should be considered in clonal selection and CPP.

WOOD QUALITY WORKING GROUP WORKSHOP

Workshop Theme

"Managing for Wood Quality Attributes and Economic Value"

OVERCOMING ADVERSE GENETIC CORRELATIONS BETWEEN WOOD QUALITY AND QUANTITY TRAITS THROUGH DEVELOPMENT OF BREEDING OBJECTIVES IN ADVANCED BREEDING GENERATIONS OF RADIATA PINE

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Radiata pine (*Pinus radiata*) has been bred for three generations in Australia since the 1950s with realized genetic gain up to 33% for volume from the first-generation and more than 10% predicted gain from the second-generation. The focus of third-generation breeding in the Australian national breeding program has shifted to wood quality traits with: 1) development of economic breeding objectives, 2) integration of quantitative genetics, molecular genetics and wood science, and 3) application of Best Linear Unbiased Prediction (BLUP) and a Web-based interactive database for customized delivery of breeding values. To further increase genetic gain, development of strategies dealing with adverse genetic correlations between wood volume and quality traits, infusion of new genetic material from the range-wide collections, increase of recombination rate and selection intensity, purging of inbreeding depression, and deployment by clonal forestry will be critical. In this paper, adverse genetic correlations between wood quality and quantity traits are summarized for radiata pine and optimal strategies to overcome such adverse genetic correlations are explored.

We surveyed 55 genetic studies published from 1960 to 2007 involving 11 growth, form, and wood quality traits. Estimated heritability for wood quality traits was higher than for growth and form traits. Among six wood quality traits, wood density had the highest heritability (0.63), followed by microfibril angle (0.61), spiral grain (0.55), fibre (tracheid) length (0.54), stiffness (0.50), and shrinkage (0.20). It was found that growth rate (DBH) was adversely correlated with all wood quality traits (for both density and mean stiffness r_g= -0.48). This indicates breeding for overcoming or at least coping with adverse genetic correlations is the most challenging issue in the advanced generation breeding for radiata pine.

We are developing two solutions to overcome these adverse genetic correlations. One short-term solution is to develop an economic breeding objective for various end products. The economic breeding objective (economic weights) has been developed for the production of structural products and risk traits of radiata pine in Australia. A bio-economic model was constructed to link the radiata pine production system with breeding objective traits. The economic weights for breeding objective traits for structural products and risk traits were derived and used for the development of a selection index. Optimal selection strategies for coping with adverse genetic correlations were studied using genetic parameters estimated in radiata pine and the economic breeding objective. Results indicate that the optimal strategy is index selection using economic weights for breeding objective traits. A long-term solution is to dissect the genetic base of the adverse correlations between wood quantity and quality traits and develop a locus-based model to explore the best breeding strategies to overcome or breed out the adverse genetic correlations.

DEVELOPMENT OF AN ECONIMIC WEIGHT-BASED SELECTION STRATEGY FOR MAXIMUM ECONOMIC VALUE

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White spruce (*Picea glauca*) is one of the most important commercial and reforestation tree species in Canada and is highly valued for a variety of primary and secondary products. Knowledge of various fibre quality traits of white spruce wood and wood products has recently been acquired through several studies. Building on the knowledge generated from the previous studies, this project focuses on developing an economic weight-based selection system for alternative breeding objectives such as mean annual increment, stem straightness, reduced average branch size, and wood stiffness in order to achieve the maximum economic value and profit in a white spruce breeding program. To estimate the economic weight of each growth and wood fibre quality trait, a complete vertically integrated enterprise is simulated in which product flows and cost/income flows are followed from plantation establishment to lumber/pulp shipping. This project will likely stimulate similar studies on other species and influence other breeding programs in Canada toward an economic weight- and end use-based selection strategy.

EFFECT OF CYCLOPHYSIS ON INTRA-RING COMPONENTS OF GROWTH AND WOOD DENSITY OF JACK PINE

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The longitudinal gradient of selected wood quality parameters was examined ring by ring from base to top of the merchantable stem of jack pine (*Pinus banksiana*), one of the most important commercial tree species in eastern North America. Previous common rootstock experiments as well as wood quality studies on longitudinal variation showed that the maturation process of the apical meristem (cyclophysis) is generally related to growth decline. Its impact on wood quality has, however, not been clearly demonstrated and even often found to be controversial. A total of 59 dominant trees, up to 75 years old, were collected from five mature stands in the Abitibi region, Quebec. Wood density was measured using x-ray densitometry from the pith to the bark for each cross-section taken systematically along the merchantable stem. Annual ring density (ARD), earlywood density (EWD), latewood density (LWD), transitionwood density (TWD), annual ring width (ARW), earlywood width (EWW), latewood width (LWW), and latewood percentage (LWP) were analyzed. A high degree of correlation between vertical and radial patterns was found in the juvenile wood, especially for annual ring and earlywood parameters, indicating a similar maturation pattern by the apical meristem and cambium. With advance of tree age, however, such a high correlation disappeared rapidly, indicating a complex adaptation mechanism of trees to intrinsic as well as extrinsic changes, as they become older and bigger. In our sample trees, the transition occurred around age 10 years throughout the stem. As well, a possible implementation of these findings for wood categorization will be discussed.

GENETIC CONTROL OF WOOD PROPERTIES OF WHITE SPRUCE: TRENDS WITH CAMBIAL AGE

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There is current interest for genetic selection of *Picea glauca* (Moench) Voss to improve the quality of wood and logs harvested in the future. To be able to integrate wood properties into breeding programs, genetic parameters must be considered and correlations between different mechanical, anatomical, as well as growth parameters have to be estimated. We determined wood properties with SilviScan technology using 12 mm increment cores taken at breast height from 375 trees from three provenance-progeny tests. Anatomical wood properties such as cellular dimensions, cell wall thickness and mechanical properties, like wood density and the modulus of elasticity were calculated for each growth-ring. Variance analyses were used to estimate variance components in order to derive parameters such as heritability and genetic correlations between characteristics. First analyses show that heritability is not stable from ring to ring and follows trends with cambial age. For example, estimates of heritability for cell wall thickness were low near the pith, but increased toward the bark and levelled out ($h^2 \approx 0.5$) at the 7th ring. Heritability of wood density increased until growth ring 8 ($h^2 \approx 0.65$), and then decreased until ring 13 ($h^2 \approx 0.3$). These trends and the amount of the genetic control over traits of interest represent valuable information for selection of individuals for the next breeding generation of white spruce.

GENETIC VARIATION IN PHYSICAL AND CHEMICAL WOOD QUALITY TRAITS IN *Pinus taeda*

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We examined genetic variation and inheritance of physical and chemical juvenile wood properties of loblolly pine (*Pinus taeda* L.). Significant variation among families and clones was observed for most traits. Heritabilities were high for microfibril angle, modulus of elasticity, and density and were moderate for alpha cellulose yield and coarseness. We did not find noticeable genetic variation for lignin content. Juvenile wood (ring 3 from the pith) and transition wood (ring 8 from the pith) were significantly different for all the micro wood traits. Cellulose yield was higher, lignin content was lower, and fibers were longer in transition wood than in juvenile wood. Microarray analysis of cDNAs associated with xylem development suggested that some specific genes have transcript levels that are positively or negatively correlated with specific wood properties. Major advances in genetic improvement of wood properties in tree breeding programs can be enhanced by integration of diverse disciplines that include high-throughput phenotyping methods and integrated research on wood properties, tree breeding, propagation, and genomics.

In situ WOOD QUALITY ASSESSMENT IN DOUGLAS-FIR

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The extent of genetic control and estimates of phenotypic and genotypic correlations for two *in situ* wood quality assessment methods (acoustics velocity - Director 2600® and drilling resistance - Resistograph®), X-ray densitometry wood density, and growth traits were evaluated on 967 30-year-old Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) trees representing 20 unrelated, full-sib families growing on four comparable progeny test sites (two of which were spaced and pruned) in coastal British Columbia. In general, no significant differences were observed between treatments, indicating that spacing and pruning did not affect the studied variables at the time of assessment. Family effect did not differ for the growth and yield traits. However, highly significant ($p < 0.01$) differences were observed for wood density as well as both *in situ* assessment tools. Growth traits and wood quality attributes, individually, produced high and positive phenotypic and genetic correlations. In contrast, high and negative correlations were observed between growth traits and wood quality attributes. Family heritability estimates were moderate (0.43–0.49) for growth traits and high (0.88–0.95) for wood quality attributes, while individual tree heritability estimates were low (0.04–0.08) for growth traits and moderate to high (0.14–0.68) for wood quality attributes. The observed high heritability estimates and the phenotypic and genotypic correlations are indicative of modest to strong genetic control over the quantified variables. However, they highlight their opposing genetic mechanisms. The high and consistent genetic correlations observed between the *in situ* techniques and X-ray wood density support their use as non-destructive, rapid, and low cost assessment methods. Although the reliability of the *in situ* assessment methods was verified through cumulative pith-to-bark genetic and phenotypic correlations with wood density, they proved to be very inconsistent in early growth (growth rings corresponding to years 1–8) suggesting these tools are only appropriate for larger, older trees and are not sufficiently sensitive for early assessment strategies.

GENETIC PARAMETERS FOR TROPOLONES ASSOCIATED WITH *Thuja plicata* HEARTWOOD DURABILITY

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Western redcedar (*Thuja plicata*), with its natural heartwood durability, is used in exterior applications such as fencing, roofing, decking, and siding. Enhancing the durability of second-growth western redcedar is an important component of the breeding program in BC. Heartwood tropolones including ß thujaplicin, γ thujaplicin, and ß thujaplicinol are excellent natural fungicides and are believed to be responsible for much of the woods' resistance to fungal attack. In this study, 4 mm increment cores were taken at breast height from two to four ramets from each of 328 trees at age 20 years. These parents represent a subset of the approximate 1,000 parents whose polymixed progeny are currently being field-tested for growth and deer resistance (needle monoterpenes). Two samples were taken from the heartwood of each core for chemical analyses: outer (rings 1–5 after sapwood) and inner (rings 6–pith). Analyses were performed on a Thermo HPLC consisting of a P4000 pump, AS3500 autosampler, and a UV6000LP photodiode array detector (Thermo Electron Corporation). Repeatabilities and genetic correlations were moderate to high for the individual heartwood tropolones. Best linear unbiased predictions were developed for the thujaplicins and parents selected for inclusion in the breeding program. Currently, tropolone parental selections are being treated as a separate breeding population, however, low to moderately positive correlations among height, needle monoterpenes, and tropolone concentrations may allow for the development of a growth and durable breed.

TREES: STRUCTURAL MARVELS BUT VERY IMPERFECT WOOD FACTORIES

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Despite rapid success with breeding programs, forest trees are still in a very early stage of domestication. Indeed, intensified crop management, aimed at reducing effective growing costs, has exposed a lack of genetic domestication in the form of a need for genetic improvement of wood properties. Such improvement of wood properties is being widely pursued, but this needs to be done in a broad context of genetic domestication. In nature, trees have evolved various physical architectures that confer ecological fitness. Such architectures typically serve to get the crowns and reproductive structures clear of competitors while producing the minimum of wood needed to achieve that. Apart from any inefficiency that this may entail in sheer wood production, it typically entails within-tree variations in properties that, while mechanically very efficient in an intact stem, are very undesirable for processing and converting into end-products. Worse than that, there is reason to suspect that the adaptive behaviour of wood properties that serves trees well in the wild can become even counterproductive when soil fertility is boosted in order to raise production. There have been calls for trees with shorter boles. More radically, however, there is a case for seeking to produce stems that are mechanically inefficient, in the sense of being ‘overdesigned’.

In this connection, it is instructive to consider cereal breeding, with the dwarf wheats of the ‘Green Revolution’. The very features that make such cultivars efficient grain-producing 'factories', notably the short stems, also make them very poor competitors. Such cultivars are genetically uniform, through using or developing an inbreeding system, so if they are grown as pure crops, solely in their own company, the weak competitive ability does not matter. As such, these cultivars represent a ‘crop ideotype’. Historically, much of crop plant breeding has succeeded by boosting the harvest index, namely the proportion of primary biomass production that can be utilised. The potential for such gains in forest trees is much less, in that the bole already represents a relatively high proportion of primary biomass. Nevertheless, there is probably appreciable scope in the longer term for exploiting divergences between competitive ability and crop-ideotype features in approaching the biological limits for wood production, with a potential bonus in reducing unwanted variation in wood properties.

In forest trees, clonal forestry, using monoclonal blocks, offers the prospect of beating the exigencies of competitive ability in a manner similar to that of inbred agricultural cultivars. For achieving this, however, various big challenges remain in the long term, notably: 1) 'designing' and breeding trees that are good at both achieving quick site occupation and producing wood almost gratuitously thereafter and 2) avoiding, in that process, sacrificing the responses that are essential to field survival and mechanical stability.

EVALUATION OF THE RESISTOGRAPH FOR WOOD DENSITY ESTIMATE AND THE USE OF MULTI-TRAIT SELECTION INDEX FOR GENETIC SELECTION IN JACK PINE

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A 15-year-old jack pine seedling seed orchard was used to evaluate: 1) the potential of the Resistograph for estimating wood density in standing trees and 2) the use of multi-trait selection index to guide selection in the seed orchard. Traits assessed included tree height, diameter at breast height, stem volume, wood density (by x-ray densitometry on increment cores), stem biomass, resistance (resistograph), stem straightness, branch angle, and branch diameter. Genetic parameters including heritability and correlations (genetic and phenotypic) were determined for all traits. In addition, genetic gains were estimated for different selection index scenarios. There was a positive and weak correlation between the estimated relative wood density by Resistograph and actual wood density as measured by x-ray densitometry on increment core samples. The implications of the results on potential use of the Resistograph were discussed. There was a positive but weak genetic correlation between wood density and growth traits. Different selection index scenarios were reviewed and their impact on genetic gain will be presented.

ARBOREA–TREENOMIX
JOINT WORKSHOP

Workshop Theme

"Canadian Conifer Genomics"

THE ANALYSIS OF GYMNOSPERM GENOMES: IS THEIR STRUCTURE AND HISTORY MAKING THEM EASIER TO SEQUENCE?

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The emerging model for genome evolution in flowering plants proposes that: 1) the main factor underlying the increase of genome size is represented by the mobilization of a few families of LTR-retrotransposons that tend to be species-specific, 2) most detectable LTR-retrotransposons appear to have inserted within the last 0–4 million years while older elements cannot be fully detected due to progressive rearrangements that made them unavailable for dating, and 3) repetitive DNA, particularly LTR-retrotransposons, undergo a relatively rapid turnover due to specific processes of DNA removal. With this model in mind we approached the analysis of two gymnosperm genomes, Norway spruce (*Picea abies*) and Ginkgo (*Ginko biloba*), to investigate the age and distribution of their repetitive components with particular emphasis on the features of LTR-retrotransposons. Surprisingly, our data revealed that gymnosperm genomes do not fit the angiosperm model and rather showed that the increase of genome size was caused by the proliferation of several but non-abundant retrotransposon families, whose insertions, in spruce, can be dated back to more than 100 million years ago. On the contrary, Ginkgo, which is regarded as a botanic fossil, may have experienced a more recent burst of retrotransposon mobilization. The slow mutation rate of gymnosperms was not sufficient to explain the extraordinary degree of sequence conservation between repetitive elements that mobilized in the same time span in both Ginkgo and conifers at least 100 million years after their divergence. We propose a model for gymnosperm genome evolution that combines a low mutation rate with a slow but steady retroelement proliferation and a low rate of DNA removal.

We will discuss the implications of these findings for possible strategies for sequencing conifer genomes by considering both traditional as well as new sequencing technologies.

TERPENOID SYNTHESES IN CONIFER DEFENSE

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Much of the ecological and evolutionary success of conifer trees is thought to be due to their diverse terpenoid-based chemical defense systems. Conifer terpenoids also provide a rich source for traditional and modern biomaterials. The many monoterpenes, sesquiterpenes, and diterpene resin acids of conifer oleoresins provide a well characterized example of chemical diversification in plant secondary metabolism. In a single conifer tree, several hundred terpenoid compounds are produced from only three intermediates of the central isoprenoid pathway. Large families of terpenoid synthases (TPS members of the TPSd family) and cytochrome P450 monooxygenases (P450 members CYP720B family) are the key enzymes for terpenoid chemical diversity in conifer defense. Members of the multigene TPSd family of conifer terpenoid secondary metabolism share common ancestors with single-copy TPS genes of gibberellic acid phytohormone biosynthesis. Our ongoing functional characterization of the conifer TPSd gene family is based on a resource of 437 000 ESTs and a set of 10 500 non-redundant, high-quality, full-length cDNAs from spruce (*Picea*), transcriptome and proteome data, and enzyme assays with recombinant proteins. Results from this work provide insights into the genomics and molecular mechanisms of terpenoid chemical diversity in conifer defense.

GCAT: AN EST-BASED GENE CATALOGUE FOR WHITE SPRUCE

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We developed a novel gene catalogue assembly tool (GCAT) to produce a high-quality EST-based white spruce (*Picea glauca*) gene catalogue supporting the Arborea II program and featuring improved accuracy and usability over currently available tools such as UniGene and TIGR-like assemblers. The GCAT pipeline is composed of multiple steps such as sequence filtering, clone analysis, gene-oriented clustering, splicing variants analysis, and automated annotation. We designed GCAT to produce entities fitting a correct biological model comprised of genes, splicing variants, ESTs, and cDNA clones. We studied over 50 000 clones having both 5' and 3' ESTs to develop a sequencing error model and clustering metrics as well as quality control methods for the detection of sequencing and cloning artifacts. In terms of usability, we developed GCAT to enable the iterative addition of sequencing data, to integrate manual data curation with automated processing, to produce gene identifiers that are stable over time, and to enable tracking of changes in gene identification.

The white spruce GCAT gene catalogue combines data from the Arborea and Treenomix programs and contains 260 000 Sanger ESTs from 200 000 cDNA clones and represents 30 000 genes with 160Mb (5x) of sequence coverage. We also included into GCAT 1.1 M ESTs recently produced on GS20 and FLX 454 sequencers and representing 225MB (7.5x) of sequence coverage.

The GCAT white spruce catalogue is at the core of Arborea II functional genomics activities. We will present its use for the integration of functional genomics data into a candidate gene selection tool, as well as its outcomes on discovery activities such as production of a full-length gene catalogue, high-throughput SNP discovery, and the design of a multi-species gene expression microarray for spruce.

COMPARATIVE MAPPING BETWEEN WHITE SPRUCE AND LOBLOLLY PINE

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A genetic map based on SNP and SSR markers was constructed for white spruce (*Picea glauca*). The map consists of 12 major linkage groups, contains 505 markers (347 in silico SNPs, 130 COSs, 9 ESTPS, and 19 SSRs), and spans 1,835 cM. Previous comparison of spruce and loblolly pine (*Pinus taeda*) maps revealed a high level of synteny and gene order in Pinaceae using ESTPs. In this study, we mapped an additional 139 and 78 conserved markers using the CJE mapping approach in white spruce and loblolly pine, respectively. Comparison of the newly mapped COS markers revealed conserved synteny across 12 linkage groups for the 57 orthologous loci, together with 26 orthologous markers (only 3 markers in common) mapped previously by the Quebec Arborea project, resulted in 80 orthologous markers mapped in both species. On average, 77% of orthologous markers arranged in a linear order between white spruce and loblolly pine. Our findings represent the most detailed comparative assessment of synteny between the two genera of Pinaceae to date, and indicate a high level of conserved synteny between white spruce and loblolly pine, which diverged ~ 140 million years ago.

THE EFFICIENCY OF A HIGHLY MULTIPLEXED SNP GENOTYPING ASSAY FOR THE CONIFER *Picea*

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The genotyping efficiency of custom highly multiplexed SNP arrays based on the GoldenGate allele-specific primer extension assay (Illumina) was tested in species with poorly known genomes to accelerate the mapping of expressed genes onto conifer linkage maps. Two highly multiplexed Illumina bead arrays were designed for gene mapping in white spruce (*Picea glauca*). A major resequencing effort was undertaken for 1515 expressed candidate genes for growth, adaptation, and wood formation. Nearly 4000 orthologous SNPs were detected in 967 polymorphic genes. Around 72% of the identified SNPs were from exons and 18% were non-synonymous polymorphisms. We selected subsets of 768 and 1536 resequenced SNPs for two SNP arrays by discarding SNPs with lowest functionality scores for the GoldenGate assay. In excess of 70% of SNPs had high quality GoldenGate assay scores and segregated in the mapping populations, with little missing data for SNPs successfully genotyped. A third array of 768 SNPs for the GoldenGate assay was also constructed for gene mapping in black spruce (*Picea mariana*) after resequencing and the success rate was comparable. These success rates are slightly lower than those reported for newly discovered SNPs in organisms with completely sequenced genomes. Thus, these results are viewed as highly positive considering that the target species is a conifer, a group of taxa with essentially unsequenced genomes, and reputedly known to contain large gene families and large tracks of noncoding repetitive elements. This highly multiplexed genotyping assay is currently used in various genome scan applications in the sub-mentioned species, including QTL, association and comparative mapping, as well as for outlier identification in natural populations. White spruce SNPs were also tested blindly in other spruce species directly at the level of the GoldenGate genotyping assay. Several hundreds SNPs could be recovered for various species.

FUNCTIONAL GENOMICS IN CONIFER DEFENSE: CYTOCHROMES P450 IN RESIN ACID FORMATION

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Diterpene resin acids (DRAs) are major defense chemicals in the conifer oleoresin. The large structural diversity of resin diterpenoids, formed initially by diterpene synthases, is further increased by cytochrome P450 dependent oxidations. Previously characterized P450 enzymes in conifer diterpenoid metabolism are involved in DRA formation in loblolly pine (*Pinus taeda*) and in the formation of taxol and related taxoids in yew trees (*Taxus* spp.). EST mining of nearly one million spruce and pine ESTs as well as full-length cDNA cloning identified genes broadly representing terpenoid phytohormone metabolism in the multifamily CYP85 clan. A deep CYP85 clan inventory revealed three apparently conifer-specific P450 families, CYP716B, CYP720B and CYP725. The CYP720B family contains at least 36 homologs, which makes it the largest known multigene family of the CYP85 clan. Members of the CYP720B family are involved in the consecutive oxidation of diterpenoids en route to the different DRAs of conifer oleoresin. We will present new results from functional genomics approaches in Sitka spruce (*Picea sitchensis*). We demonstrate that individual multi-substrate and multi-functional members of the CYP720B family catalyze all three steps from diterpene olefins to the resin acids, each with a specific substrate pattern. The results of our work provide insights into the genomic basis and molecular mechanisms of diterpenoid chemical diversity in conifer defense.

COMBINING GLOBAL METABOLITE PROFILING, QTL ANALYSIS AND NETWORK ANALYSIS TO ELUCIDATE WEEVIL RESISTANCE IN INTERIOR SPRUCE

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Shoot feeding weevils are among the most devastating pests of conifer forests, imparting severe consequences to forest health and significantly influencing plant biomass accumulation. A complex combination of molecular, morphological, and biochemical traits are believed to confer the innate variation in susceptibility to weevil attack between individual trees. These complex interactions create significant challenges to characterizing the specific relationships between tree susceptibility and defense mechanisms, whether morphological or biochemical. As measured by mass spectrometry, the abundance of specific metabolites can be regarded as quantitative traits. We characterized stem tissue of 314 segregating Interior spruce (*Picea glauca*) trees derived from a factorial cross design involving 3 resistant females and 2 susceptible males and identified metabolite quantitative trait loci (mQTL) for 147 compounds in the xylem and 166 compounds derived from bark tissue. To elucidate genetic links between phenotypic as well as biochemical traits, the co-localization (physical clustering) of QTLs for all pairs of traits (314 metabolites, as well as 6 traits related to weevil resistance and tree growth) and all traits were subjected to cluster analysis to infer groups of co-regulated genes. Several clusters were found and members of clusters were often in the same metabolic pathway. Metabolite network analysis, based on QTL co-localizations, demonstrated strong links between growth, weevil resistance, and a set of metabolite quantitative traits. Visualization of the networks of the significant QTL co-localizations clearly separates xylem metabolites from bark metabolites and demonstrates that a distinct cluster of metabolites is strongly connected to all growth and weevil resistance traits. Furthermore, the extent of QTL co-localization between pairs of traits was highly correlated with their phenotypic correlation, indicating relatively high heritability of metabolite abundance in xylem and in bark.

MYB14, A MEMBER OF A NEW SUBGROUP OF R2R3 *MYB* SEQUENCES, IS IDENTIFIED AS A PUTATIVE ACTOR IN WOUND RESPONSE IN CONIFERS

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R2R3-MYB transcription factors are regulatory genes implicated in key aspects of plant growth and development. Several angiosperm genes from the subgroup 4 of R2R3-MYBs have been shown to regulate phenylpropanoid metabolism, which led us to investigate the *Pinus taeda* (*Pt* MYB14) which also belongs to this subgroup. A phylogenetical tree based on the partial amino acid sequences from pine, spruce, and several angiosperms indicated that *Pt* MYB14 and several related sequences define a sub-clade (Sg4-C) that is specific to conifers. *Pt* MYB14 was further investigated by ectopic overexpression (OE) in white spruce (*Picea glauca*) used as a heterologous conifer expression system. *Pt* MYB14-OE was driven by using either *ubiquitin* or *cinammyl alcohol dehydrogenase* promoter to compare constitutive and tissue-preferential OE, respectively. As reflected by histological analysis, chemical determinations, and transcript profiling (microarray and RT-quantitative PCR), overlapping results were observed between both constructs. Together, the results indicate that MYB14 is most likely involved in wound response mechanisms in conifers because both the ubiquitous and tissue-preferential overexpression lead to the misregulation of genes related to mevalonate (MVA), non-mevalonate (MEP), and jasmonic acid pathways as well as downstream pathways leading to sesquiterpenes. This putative role is highly consistent with the rapid induction of *MYB14* transcript accumulation upon wounding and application of jasmonate in pine and spruce plantlets.

SNP DISCOVERY AND ASSOCIATION MAPPING IN THE TREENOMIX PROJECT

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In the Treenomix project, single-nucleotide polymorphisms in white spruce (*Picea glauca*) are being discovered with both in-silico methods and wet-lab methods. In the wet-lab, we have developed and implemented a pipeline for SNP discovery which involves identification of candidate genes for weevil resistance, design of primers, amplification of a 12-member panel, and detecting SNPs in heterozygous sequence traces. Currently we have identified 766 non-synonymous SNPs among 800 candidate genes.

Additional candidate genes have been recently identified by co-location of expression QTLs with weevil resistant QTLs. In the in-silico approach, we find that deep single-tree EST databases are particularly interesting to analyze not just for SNPs, but for gene expression bias between the two homologous copies of a gene and for the extent of near-identical paralogues. The extent of these two phenomena will be discussed. Also, the heterozygosity found in these is compared to that found with the wet-lab pipeline. Finally we briefly describe the design of the association study which is being conducted with these SNPs.

ASSESSMENT AND DATABASE INTEGRATION OF WOOD PROPERTIES FOR ASSOCIATION STUDIES OF WHITE SPRUCE

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In the Arborea project, we aim at developing marker-assisted selection for physical and chemical wood traits, in white spruce (*Picea glauca* [Moench] Voss), through association studies. Related to this goal, a vast effort has been undertaken to assess diverse wood characters in a group of 695 trees representing discovery and validation populations. The SilviScan technology was used for the assessment of wood physical and anatomical properties. Wood density, micro-fibril angle (MFA), modulus of elasticity (MOE), wall thickness, and more were measured or estimated. Chemical properties were assessed by Raman and Near Infra Red spectroscopy for the whole population of 695 trees. For this purpose, prediction models were developed by using a set of samples from 40 unrelated trees which were submitted to wet chemistry analysis, as well as Raman and Near Infra Red spectroscopy. In order to facilitate association studies, these phenotypic data were submitted to extensive quality controls and then integrated into a relational database along with metadata. This database of phenotypes contains millions of entries and uses the tree number as a key in order to create rapid links with genotyping data from the TreeSNPs database (presented elsewhere). In addition to supporting research which could lead to molecular breeding applications, this collection of intensively characterized trees represents a valuable resource for future investigations related to wood properties and tree growth.

ASSESSING THE ECONOMIC IMPACT OF INTRODUCING WEEVIL RESISTANT SPRUCE IN BRITISH COLUMBIA

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This paper develops a spatial agent-based forest sector model to assess the economic impacts of introducing weevil resistant spruce planting stock to the forest sector in British Columbia. Long-term effects are modeled by simulating harvest activities and determining the value of avoided merchantable volume losses over time. While weevil damages were substantial (14 million m^3/year), only a relatively small proportion of these damages can be avoided by planting resistant stock. Losses began to decline after approximately 150 years and the maximum impact reduction of 30% was achieved after approximately 230 years. Results from this type of analysis can be used as background information for policy decisions related to the economic returns and uncertainties associated with implementing these types of large-scale pest management activities.

FOREST GENOMICS: TOOL OF SUSTAINABLE DEVELOPMENT

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A balance between economic benefits, increasing timber demand, and environmental and social issues is one of the modern forestry challenges. The different techniques of tree genetic improvement seem to be an interesting tool to address such issues. Economic benefits of two white spruce genetic improvement approaches, seed orchards and multiclonal varieties, are defined for a functional zoning management context. Stochastic prices, resulting from a Markov model, are used to estimate these benefits. Biodiversity constraints are also incorporated to observe the trade-off between economic and environment dimensions. According to the results, improved white spruce plantations could contribute between 20 to 40% of the total profits, depending on the technique used. The inclusion of biodiversity constraints could reduce the profits by an average of 15%. On the other hand, the use of white spruce plants coming from multiclonal varieties planted on high quality sites (SI 12 m at 25 years) will allow us to preserve an average area of 250 ha in a forest landscape of 6600 ha (on a horizon of 150 years) for conservation purposes, thereby increasing protected area in the province of Quebec.

AN *in vitro* APPROACH FOR STUDYING GENE REGULATION IN SPRUCE: THE TANGO OF TRANSCRIPTION FACTORS AND GENE PROMOTORS

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Gene transcription is one of the major control points for regulating gene expression and phenotype. By their binding to specific target sequences within promoters, transcription factors (TF) specifically control the expression of inducible, tissue specific, and developmentally modulated genes. Our goal is to conduct a functional study in white spruce assessing the interaction between different TFs and the promoter regions of genes related to seasonal growth and wood quality.

The functional interactions are to be determined in embryogenic tissue using an *Agrobacterium* based transient expression *in vitro* assay, with white spruce (*Picea glauca*) promoter sequences and GUS as the reporter gene. Full length white spruce TF sequences were obtained from the large scale cDNA sequencing project of the Arborea project while the promoters were isolated by chromosome walking. The effects of the over expression of up to 200 transcription factors will be evaluated on the expression of 20 different promoters. We hope using this high throughput approach will reveal global patterns of gene regulation related to growth and wood quality.

CUTTING TREES WITH LASERS: CHARACTERIZING CONIFER DEFENCE IN SINGLE CELL TYPES

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Terpene-rich resin plays an important role in the defence of spruce trees against insect and fungal attack. Large axial resin ducts in the primary phloem form part of the first lines of constitutive defence. The induced defence response is marked by *de novo* formation of additional traumatic resin ducts in secondary xylem. Tracheid mother cells in the vascular cambium are recruited to produce traumatic resin ducts over a period of 2–4 weeks before resuming tracheid production. These developmental changes are associated with increased terpene synthase activity, increased resin accumulation, and the acquisition of resistance. Previous research on conifer defence genomics has been done on whole phloem and xylem tissue. This research uses laser microdissection (LMD) to isolate specific cells from heterogenous tissues to study the spruce defence response with single cell type resolution. High quality, undegraded total RNA has been extracted from single cell types including cambium and resin duct epithelia. These templates are being used for cell-type specific gene expression analysis using quantitative real time PCR. RNA yields are sufficient to construct cell type specific cDNA libraries from a single seedling without pooling samples or RNA amplification. Results will lead to a better resolution of or understanding of the mechanisms of conifer defence.

GENOMIC ANALYSES OF BUD FORMATION IN WHITE SPRUCE

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Bud formation represents one of a suite of adaptations that enables perennial plants such as white spruce (*Picea glauca* [Moench] Voss) to survive winter and resume growth the following spring. Bud formation is one factor that influences the duration of the growing season, which in turn is a determinant of growth and yield on one hand, and risk of cold injury on the other. The aim of this project is to dissect the molecular events that occur during terminal bud formation in white spruce. To this end, we are carrying out microarray transcript profiling through a time course of bud formation, focussing on bud formation induced by short photoperiods. Statistical analyses of these data reveal approximately 4500 genes that are differentially expressed during at least one time point during bud development relative to Day 0 shoot tips. Differentially expressed genes were classified into functional categories using FunCat to provide a reference frame for further analyses of molecular processes that occur during bud formation. Inspection of these functionally-categorized genes reveals groups of genes, representing defined processes, that are coordinately expressed at distinct time points during bud formation. These waves of gene expression coincide with morphological changes in bud formation observed using light microscopy, allowing us to begin to correlate molecular and cellular events. These analyses have been used to identify candidate genes that are being used in QTL and association mapping of the time-to-budset trait, with the goal of discovering genes that exert genetic control over phenotypic variation of this complex adaptive trait.

FORMATION OF 3-CARENE IN SITKA SPRUCE THAT ARE RESISTANT OR SUSCEPTIBLE TO ATTACK BY WHITE PINE WEEVIL

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White pine weevil (*Pissodes strobi*) is an endemic insect in British Columbia that attacks Sitka spruce (*Picea sitchensis*), a commercially and ecologically important tree in coastal BC. Sitka spruce shows widely varying levels of resistance to attack by weevil. Terpene content and composition of oleoresin may possibly contribute to resistance. We are therefore investigating the differential production of 3-carene and other monoterpenes, in trees from two extreme chemotypes: one highly resistant tree genotype (898) that produces 3-carene versus one highly susceptible tree genotype (903) that produces no 3-carene. Mining of ESTs, full length cDNA cloning, and biochemical characterizations indicate that there may be more than one closely related 3-carene synthase and/or 3-carene synthase-like genes occurring in both chemotypes. Genomic DNA sequencing suggests even greater diversity. Thus, it is likely that the already diverse terpene synthase gene family has yet another level of complexity than previously supposed. We are dissecting this complexity at the levels of genomic DNA, transcript expression, enzyme activities, and terpenoid chemistry.

CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED PROTEINS DURING DORMANCY ACQUISITION IN WHITE SPRUCE (*Picea glauca*)

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White spruce (*Picea glauca*) is a forest tree of economic importance in Canada, providing a steady source of lumber and pulp for the forest industry. Perennial plants, like forest trees, undergo dramatic physiological and developmental changes as they become dormant for overwintering. To understand the molecular changes that contribute to white spruce's ability to survive winter, we examined the changes in the proteome of woody stems from two year old white spruce seedlings undergoing active growth (Day 0 treatment) under long day conditions, to that of seedlings that had been placed in short days for 10 weeks (8-hour photoperiod; Week 10 treatment). Differential expression analysis using two dimensional gel electrophoresis (isoelectric focusing in the first dimension followed by SDS PAGE in the second dimension) revealed that 32 polypeptides were upregulated in Day 0 relative to Week 10 stems, while 184 polypeptides were upregulated at Week 10 compared to Day 0. Polypeptides excised from the gels were digested using trypsin and analyzed using liquid chromatography – mass spectrometry in tandem (LC-MS/MS). To annotate the polypeptides, the resulting peptide profiles were compared to virtual profiles from the NCBInr and ESTothers databases using the MASCOT algorithm. Amongst the differentially expressed proteins, we see major changes in proteins involved in cell rescue and defense (chitinases, pathogenesis related proteins, peroxidases) and carbon metabolism (enolases, pentose phosphate pathway enzymes). Since chitinases are upregulated prominently after 10 weeks of short day photoperiod, phylogenetic analyses and further data mining are underway to examine them in more detail.

THE ARGININE METABOLIC NETWORK IN WHITE SPRUCE

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A plant's nitrogen economy is important for its survival and development, especially in perennial plants such as conifers. Conifers have acquired efficient mechanisms for uptake, storage, and redistribution of nitrogen in various forms including free amino acids, amines, and storage proteins to overcome nutrient-poor forest soil, seasonal variation in nitrogen availability, and adverse weather conditions. However, the large genome size and long lifespan of conifers has made it difficult thus far to understand the specific mechanisms of nitrogen utilisation, particularly with respect to seasonal nitrogen cycling, i.e., how they store nitrogen during dormancy acquisition and utilize nitrogen at the start of the subsequent growing season. Arginine, a protein amino acid high in nitrogen content, is an important form of organic nitrogen in conifers. Arginine levels increase dramatically during the latter part of the growing season in white spruce (*Picea glauca* [Moench] Voss) (C. Dhont et al., unpublished data) indicating a role for arginine in seasonal nitrogen cycling. From microarray data (El Kayal et al., unpublished data) we have determined that a subset of these putative arginine metabolism genes are differentially regulated during dormancy acquisition in white spruce. The expression level of arginine-related genes was, therefore, analysed by qRT-PCR in actively growing trees and trees undergoing dormancy acquisition. Here we suggest the candidates for a larger arginine metabolic network than what has once been believed, which likely plays an important role in nitrogen utilisation during the annual growth cycle in white spruce.

TRANSCRIPTIONAL PROFILING OF BUD FORMATION IN WHITE SPRUCE REVEALS GENES IMPLICATED IN THE REGULATION OF BUD DEVELOPMENT

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Perennial plants have a unique ability to suspend and recommence growth in response to seasonal environmental conditions. The timing of growth and rest cycles involves an adaptive balance between maximizing growth and minimizing frost damage. The survival of at least one bud meristem throughout the winter is required for growth in the following season. To identify genes and processes associated with terminal bud formation, we subjected two-year-old white spruce (*Picea glauca* [Moench] Voss) seedlings growing in long photoperiods to short periods in order to induce rapid and synchronous bud set. Plants were harvested over a 10-week time course and used for microarray analyses carried out with an 11k white spruce low-redundancy cDNA microarray. Statistical analyses of these data have revealed that more than 4,500 genes are differentially expressed in the shoot tip over the course of bud formation. Several of these genes within this dataset represent putative orthologues of genes from other species known to play roles in meristem maintenance and/or development of new organs. These genes include homeobox genes, regulators that have known roles in the control of development. For example, genes belonging to the KNOX family show concerted upregulation during the mid to latter stages of bud formation. Quantitative PCR validation is in progress and will be presented in concert with microarray analyses.

TREE SNPs: A DATABASE DEDICATED TO SNP DISCOVERY IN TREES

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The TreeSNPs database was designed to track the process of large-scale SNP discovery through the different steps of gene resequencing, in the context of the Arborea white spruce (*Picea glauca*) genomics project (www.arborea.ulaval.ca). It allows the whole process to be coordinated and sequentially done by different project teams, using an intuitive Web interface, and integrates user-input data generated from the following steps: 1) the establishment of lists of candidate genes; for example, genes involved in wood formation or growth, 2) identification of homologs, 3) generation of primer sets covering different genic regions, 4) PCR results, 5) sequencing results, and 6) SNP identification. In addition, a number of tables store and make easy the retrieval of related information inherent to LIMS (PCR primers and protocols) and also to nucleotide databases (cDNA and genomic sequences). As of today, TreeSNPs allowed the Arborea project to keep track of nearly 5500 identified gene sequences distributed among 133 gene families and to integrate 150 000 nucleotide sequences, 4100 primers, and nearly 6000 SNPs or indels. Although being primarily developed for white spruce genomics, TreeSNPs could easily accommodate any other plant or tree genomic project given its gene-centric data model. In addition, the system is based on open-source components and is platform-independent.

AN *in silico* APPROACH OF GENE EXPRESSION PROFILING FOR DORMANCY DEVELOPMENT IN WHITE SPRUCE

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Microarray technology allows gene expression profiling by measuring relative transcript abundance for genes that correspond to probes represented on an array. However, many microarrays do not contain every gene in the genome, meaning that expression of missing genes is not measured. Another method, referred to as a digital Northern, can be used to compare the numbers of expressed sequence tags (ESTs) between cDNA libraries, thus allowing an alternative means of comparing the relative transcript abundance. We have used this approach to identify genes involved in dormancy acquisition in white spruce (*Picea glauca* [Moench] Voss). For these analyses, we selected six standard, full-length libraries representing needles, stems, or buds sampled either during active growth or dormancy. Approximately 6500–7000 clones were sequenced from each library. Analyses were facilitated by the availability of a white spruce EST assembly that includes these six libraries. Digital profiling revealed that using a P-value of 0.05, there are 192, 98, and 214 differentially expressed genes between actively growing and dormant needles, buds, and stems, respectively. At a P-value of 0.14, there are 911, 765, and 950 differentially expressed genes in needles, buds, and stems, respectively. A considerable number of these genes have no known function. These *in silico* analyses complement cDNA microarray analyses conducted on the same tissues.

SOMATIC EMBRYOGENESIS
WORKSHOP

INDUSTRIAL APPLICATION OF SOMATIC EMBRYOGENESIS

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J.D. Irving, Limited has worked with somatic embryogenesis of conifers since 1994, including extensive collaboration with Dr. Y.S Park of the Canadian Wood Fibre Centre. The major species are white spruce (*Picea glauca*), Norway spruce (*P. abies*), and white pine (*Pinus strobus*), although tests of black spruce (*P. mariana*) and red spruce (*P. rubens*) have also been established. These efforts are aimed at supporting company goals of increasing high quality future timber supplies.

Initial efforts were focused on production of many varieties across diverse selected families for field testing. Over 2,000 varieties have been established in field tests across the company's landholdings since 1999. Increasingly, emphasis has shifted to operational large-scale production efficiency and quantifying potential gains in productivity and value through this approach relative to traditional seed orchard production methods.

USE OF SOMATIC EMBRYOGENESIS IN MASS PROPAGATION OF HYBRID VARIETIES OF WHITE PINE AND WHITE SPRUCE

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Vegetative propagation is perceived to generate significant benefits in producing eastern white pine (*Pinus strobus*) interspecific hybrids for blister rust (*Cronartium ribicola*) resistance. Gene loci responsible for blister rust resistance are largely heterozygous among the hybrid genotypes currently bred in Ontario. Sexual propagation through seeds will inevitably produce non-resistant seedlings in the mixture. Furthermore, it is observed that hybrid progenies tend to have larger variability in growth, cold hardiness, and tree form than those of pure eastern white pine, with exceptional individuals often showing substantially higher growth rates. Individuals with most desirable economic attributes can only be fully utilized through asexual propagation.

Somatic embryogenesis (SE) is most desirable compared with other forms of vegetative propagation for white pine. Although eastern white pine and its interspecific hybrids are relatively easy to root at juvenile ages, their rooting rates, nevertheless, decrease as donor trees mature. Low multiplication numbers and higher costs in maintaining hedges can potentially reduce the economic attraction of rooted-cuttings. The lack of a taproot for rooted-cuttings can potentially be a serious problem for white pine to adapt and stabilize on drought prone uplands with sandy soil.

White spruce (*Picea glauca*) is increasingly recognized for its higher wood productivity at rotation age than black spruce (*P. mariana*). Ontario has yet to enhance its tree improvement activities for this species. The maturing SE technology in white spruce provides Ontario with a new opportunity to develop multi-varietal forests as white spruce varies considerably in growth rate, tree form and, possibly, pest resistance.

For operational and research purposes, the Ontario Forest Research Institute has established an SE applications laboratory with the assistance of Dr. Y.S. Park, Canadian Wood Fibre Centre, Fredericton.

SOMATIC EMBRYOGENESIS IN WHITEBARK AND LIMBER PINES AND ITS APPLICATION IN SPECIES CONSERVATION AND RESTORATION

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Whitebark pine (*Pinus albicaulis*) and limber pine (*P. flexilis*) are soft pines growing in high elevation forests of western North America. They are ecologically important keystone species that are an integral part of the ecosystem. However, over much of their range including British Columbia and Alberta, the species are seriously threatened due to susceptibility to white pine blister rust (*Cronartium ribicola*), attack by mountain pine beetle (*Dentroctonus ponderosae*), and prolonged fire suppression preventing natural regeneration. Also, projected global climate change casts further uncertainty. Therefore, protection, genetic resource conservation, and restoration efforts are urgently needed.

Somatic embryogenesis (SE) in whitebark pine has been obtained for the first time. Embryogenic lines were successfully cryopreserved and thawed, and plants were generated. Somatic embryogenesis, in combination with other conservation measures, provides another tool for developing rust-resistant genotypes as well as long-term storage of valuable genotypes in liquid nitrogen without changing genetic make up or the loss of juvenility. The application of SE in ecologically important and threatened species provides a new dimension for genetic resource conservation and species rescue. SE in limber pine was also obtained. Plant regeneration experiments are currently in progress.

APPLICATIONS OF SOMATIC EMBRYOGENESIS IN FOREST TREE RESEARCH

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Somatic embryogenesis in conifer species holds many unexploited possibilities in biotechnology, metabolomics, genomics, and proteomics. Not only can embryos be produced in abundant quantities for different types of analyses, but embryo formation can be studied directly, free of interfering layers that are normally found in surrounding seed tissues. Precision in sampling makes metabolomic, genomic, and proteomic studies relatively straightforward. The controlled synchronicity of embryo cultures allows studies of phenotypic variation in clones, whether on morphology, physiology or development.

One of the recent possibilities to arise is that some aspects of phenotype, such as cold tolerance, can be regulated during embryogenesis. Interdisciplinary studies also become possible. Embryogenesis is the first moment of lateral organ formation in a plant’s life. Mathematical and chemical modeling of initiation on a hemisphere and spacing of cotyledons are more easily done with conifer somatic embryos than any other plant system. Even though the lack of a published genome is a current impediment, researchers should be encouraged to investigate these gymnosperms for fundamental answers to long-standing questions in embryogenesis in seed plants.

REJUVENATION OF 7-YEAR-OLD WHITE SPRUCE SOMATIC TREES THROUGH SOMATIC EMBRYOGENESIS

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Somatic embryogenesis (SE) has revolutionized conifer tissue culture and has become a method not only for mass propagation but also a stepping stone for development of other biotechnologies leading to new products in forestry. In many conifer species, SE, based on immature seed explants, has been routinely used for development of clonal lines, however explants taken from mature trees proved to be recalcitrant. The ability to propagate mature conifers through SE is still in the future realm although some progress has been made recently. True rejuvenation of mature trees and production of somatic seedlings representing a specific and proven genotype, could be compared to a “playback” of the tree’s ontogeny to the seedling stage with the unprecedented benefit of having achieved multiplication at the same time. Without a doubt such a technology would also have application in species and/or population conservation and would add an additional tool to other means of conservation and restoration. Results towards achieving rejuvenation will be presented using 7-year-old white spruce somatic trees, which provide a unique opportunity to study the critical factors involved in triggering SE from differentiated somatic cells.

POSTERS

PHENOLICS DEFENSE UNDER NATURAL SELECTION

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A central goal of the Treenomix Conifer Health program is to apply systems biology to the study of conifer defense in response to insect herbivory. However, detailed dissection of the mechanisms of defense and resistance in conifers is hampered by the large genome size and the lack of physical maps. Here we present a phylogeny-driven method to support the process of candidate gene selection and annotation of gene function. The specific objectives of this phase of our study are: 1) to describe the modes of selection working on the transcriptome of different tissues and 2) to categorize the transcripts, especially those involved in defense response, in accordance to their mode of evolution. Our results from a transcriptome analysis involving five species of spruce using the Treenomix 21.8 cDNA microarray indicate different trends in the evolution of bark/phloem versus needles. Cross-species comparison of the pattern of variation of neutral genetic markers reveals the congruence of the needle transcriptome to the neutral genetic divergence, suggesting that the transcriptome of bark/phloem is evolving under specific selective pressures. Comparison of the transcripts associated with defense response between the tissues indicates an over-representation of phenolics biosynthesis transcripts in bark phloem. Of the total 340 transcripts of phenolics pathway spotted on our chip, 150 show differential expression in phloem and 94 in needle tissues ($Q<0.05$), fitting the concept of increased fitness against pressures from bark borer pests. Ongoing work includes extending the comparisons over the xylem tissue and other functional categories.

GENETIC DIVERSITY AND RELATIONSHIPS OF WILD AND CULTIVATED CHERRY (*Prunus avium* L.) IN GREECE

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The genetic diversity and relationships of wild and cultivated cherry (*Prunus avium* L.) in northern Greece are reported. The plant material investigated, included a collection of selected plus trees from wild populations stemming from a breeding program dedicated to wood production and a collection of the most widely used sweet cherry cultivars in northern Greece. All selected trees are present in gene conservation plantations. Genetic diversity was investigated based on 12 microsatellite (SSR) loci. Notable amounts of genetic diversity were observed. Percent polymorphic loci were 91.6. Allelic numbers ranged from two to seven, while most private alleles occurred in the wild cherry individuals. Expected heterozygosity and Shannon information index presented generally high values, which were higher in the wild cherry material. An analysis of molecular variance indicated that a significant amount (32%) of the total variation resided among populations. Results from a principal coordinate analysis indicated the diverse origin of this material. Sweet cherry cultivars formed a short independent cluster which was conspicuously differentiated from all wild cherry individuals that formed a large widely dispersed group in a graph of the first two principal coordinates. It appears that the widely cultivated sweet cherry trees in northern Greece are genetically well differentiated from the wild populations of the greater geographic area and probably do not share the same origin.

WATER ACTIVITY IN SEED AND POLLEN BANKS: AN EFFICIENT TOOL TO IMPROVE CONSERVATION OF FOREST GENETIC RESOURCES

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Genetic diversity of forest reproductive materials, in combination with environmental effects, induces significant intra-specific phenotypic diversity of materials like pollen and seedlots. This makes it difficult to predict the moisture behaviour of forest reproductive material and consequently complicates management procedures. We built and analysed numerous sorption isotherms to describe the moisture behaviour of forest tree seed and pollen lots. As expected, we obtained moisture behaviour models and, more advantageously, optimal equilibrium relative humidity (eRH) values for seed and pollen storage. Water activity (a_w) measurement is a non destructive, portable, and rapid technique to assess moisture content. Both a_w and eRH are reliable indicators of the status of water in compounds like seeds or pollen because they are a function of the water potential (Ψ), or energy status of water in hygroscopic matrixes. Gravimetric moisture content (MC) of a given sample is not a factor but a consequence of the combination of water potential and the variable amounts of hygroscopic (starch, proteins) and non hygroscopic (lipids) components. Moreover, sorption isotherms reveal a significant intra-specific variability of resulting MC for a given a_w, seriously weakening the operational prediction and use of gravimetric moisture content from eRH. Water activity assessment appears to be a reliable moisture monitoring technique for materials having high phenotypic variability; it results, for a given species, in reproducible moisture management procedures based on single recommended a_w values. Therefore, a_w will be a useful tool for improving the conservation of diversity.

EFFECT OF SAMPLE SIZE ON POPULATION GENETIC STRUCTURE ESTIMATES IN FOREST TREE SPECIES

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Sample size is one of the critical factors determining the accuracy of estimation of population genetic structure parameters. Most of the population genetic studies so far have employed sample sizes of 15 to 35 individuals per population. Such sample sizes have little power to detect rare alleles, which contribute to the majority of the inter-population genetic differences in most forest tree species. This, in turn, may result in biased estimates of the population genetic structure. A number of simulation studies have examined this phenomenon. However, the distribution of allele frequencies in natural populations is often different from that implied by simulation algorithm and there is little empirical information on this subject. Based on the actual multilocus data set for 5 populations of red spruce (*Picea rubens*), with 180 individuals sampled per population, we created a simulated population data set of 10 000 individuals. We applied random sampling and estimated common population genetic diversity and genetic structure parameters at various sample sizes. Sample sizes of 35 and below demonstrated lack of power to detect rare allelic variants, thus leading to biased genetic differentiation estimates. More detailed results and possible implications of sample size for population genetic studies in forest trees were presented.

Copaifera GENOMICS: NEW TOOLS FOR BIOFUEL RESEARCH?

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Copaiba trees are a group of rainforest trees in the genus *Copaifera*. These trees are found in the West Indies, Puerto Rico, Brazil and in the Amazon valleys. Nobel laureate Dr. Melvin Calvin referred to these copaiba trees as “diesel” trees (Calvin 1980). Some copaiba trees, such as *Copaifera langsdorfii*, yield terpene hydrocarbons. Oleoresin is a mixture of turpentine (85% monoterpenes or C_{10} and 15% sesquiterpenes or C_{15}) and rosin (diterpene resin acids of C_{20}) (Steele et al. 1998). In particular, the ‘diesel’ nature of copaiba oil is due to the presence of oleoresin in the oil. It has been reported that this type of oleoresin (or diesel fuel) could be directly used as fuels for automobiles without any further filtration (Calvin 1980). Copaiba oil (also known as ‘balsam’) has a wide array of medicinal uses. The purpose of the proposed research is to understand the molecular mechanisms of diesel-like fuel (oleoresin) production from Copaiba trees. Our first goal for this project is cloning oleoresin producing genes. Our second goal is development of an EST (expressed sequenced tags) library from various tissues and at various developmental stages of copaiba trees. The final goal is expression of oleoresin synthase genes in non food crops as potential sources of biofuel. Comparative genomics between model tree species (e.g., poplar, pine), map based cloning, whole genome sequencing will help us to understand the pathway of oleoresin synthesis in copaiba.

ECOLOGICAL GENETIC VARIATION AND ADAPTIVE CLINES IN PACIFIC DOGWOOD

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As climate change progresses, tree populations may become maladapted to their new local climate regimes. Conservation and restoration efforts must therefore be guided by an understanding of the extent to which populations are locally adapted and how adaptation relates to geographic and climatic gradients. Although the genecology of wind-pollinated and wind-dispersed widespread, economically important conifer species has been well studied, little is known about tree species with other traits. One such species, that may be of conservation interest in the future, is Pacific dogwood (*Cornus nuttallii* Aud.), an insect-pollinated broadleaf found along the Pacific coast of North America. A common garden experiment for this species was established in Vancouver, BC, using populations represented by open-pollinated families representing the entire species range. Significant linear regressions ($p<0.05$) were found relating mean population height to provenance mean frost-free period ($r^2=0.52$) and mean coldest month temperature ($r^2=0.54$), while mean population budburst score was significantly related to provenance latitude ($r^2=0.59$), longitude ($r^2=0.47$), mean annual temperature ($r^2=0.66$), and mean summer precipitation ($r^2=0.46$). For both height and budburst score, families within populations explained more of the overall variance than populations. Population differentiation (Q_{ST}) estimates obtained for first year height and budburst score were 0.088 and 0.113, respectively, compared to an estimated neutral marker differentiation (F_{ST}) value of 0.071. As adaptive variation in these two traits is significantly related to climatic gradients, yet Q_{ST} values are only slightly greater than the F_{ST} estimate based on microsatellite markers which reflects selectively neutral evolutionary forces, Pacific dogwood appears to be more weakly locally adapted than many widespread, common tree species.

COASTAL DOUGLAS-FIR (*Pseudotsuga menziesii* var. *menziesii*) HABITAT SUITABILITY IN NORTH AMERICA AND EUROPE UNDER GLOBAL CHANGE

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Coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) has been broadly planted all over the temperate areas of the world since the 19th Century. In the last years, foresters have observed from French populations problems from pests and diseases and failures in uplands. Current adaptation of Douglas-fir to local conditions has been questioned and current populations are expected to be poorly adapted to future climates.

In this study we used several coastal Douglas-fir European plantations in order to predict the phenology timing as well as current and future habitat suitability of the species under climate change. French plantations coming from Washington and Oregon provenances were sampled.

We analyzed differences in the timing of budburst, flowering, and ripening using thermal phenological models for each location. Finally, we applied the process-based Phenofit model in order to predict Douglas-fir distribution under current and future conditions in North America and also in Europe. Phenofit is a process-based model that uses phenological events to predict species distributions. It joins a phenology model and a survival model to predict area suitability of species under different climate scenarios. Previous versions of Phenofit have been already applied to predict several North American tree species distributions under climate change.

GENOME SCAN TO DETECT ADAPTIVE POLYMORPHISM IN AN ENDEMIC FOREST TREE, *Eucalyptus gomphocephala*, IN WESTERN AUSTRALIA

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Eucalyptus gomphocephala (or "Tuart") is an endemic forest tree species that occurs along an environmental gradient (particularly for water availability and soil type) on the Swan Coastal Plain in south-western Australia. *E. gomphocephala* communities harbor significant floral and faunal biodiversity, but some natural populations are currently suffering severe decline, the causes of which are currently unknown. Further, there is little genetic information available to guide conservation and restoration decisions for this iconic forest tree species. In this PhD research project a genome scan is being conducted to detect outlier loci among natural populations of *E. gomphocephala*. This will allow us to search for the genetic basis of local adaptation directly at the genome level without *a priori* knowledge of genome sequence, candidate genes, or selected traits. Outlier loci may be important for fitness, local adaptation, or speciation, giving an insight into natural patterns of both neutral and putatively adaptive population genetic variation, which can better inform decisions for conservation and seed-sourcing for ecological restoration.

Three hundred individuals from 10 natural populations of *E. gomphocephala* will be genotyped using 12 putatively neutral simple sequence repeat (SSR, or microsatellite) markers and also using approximately 30 SSR markers derived from expressed sequence tags (ESTs) that show significant homology to genes of known function. We have identified 528 *Eucalyptus* ESTs downloaded from GenBank that contain SSRs, and over 100 of these ESTs show significant homology, including genes whose functions are associated with heat and water stress responses, salt tolerance, and defence responses in other species, each of which could potentially contribute to local adaptation in *E. gomphocephala.* Reciprocal growth trials in the field and a common garden experiment in a glasshouse will also be established to measure local adaptation and to assess the predictive ability of EST marker variation to detect adaptively significant provenances.

GENETIC PARAMETERS AND GENOTYPE-ENVIRONMENT INTERACTIONS FOR TOTAL HEIGHT IN WESTERN HEMLOCK: PRELIMINARY RESULTS

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Genetic parameters for age10 height were estimated using second-generation tests of western hemlock (*Tsuga heterophylla*) (149 parents, 498 families, and 38 948 progeny) with two different mating structures: 1) ‘Local Diallels’ (LD) series, representing five geographic populations and 2) ‘Elites’ (E) series (30 ‘best’ parents), planted on six and four sites, respectively. Using ASREML2 (Gilmour et al. 2006), a univariate individual tree model was used to estimate variance components and heritabilities (h^2), dominance to additive variance ratios (σ^2_D/σ^2_A), type-b genetic correlations ($\sigma^2_f/\sigma^2_f+\sigma^2_{fe}$), as well as a multi-site tree and family model fit with heterogeneous error variances among sites. Individual tree h^2's were low, varied among sites, but averaged ~0.062. The ratio of σ^2_D/σ^2_A variances ranged from 0 to 1.56 among the 10 sites. The h^2 from the combined sites analysis was lower than the individual site estimates (LD and E crosses, 0.048 and 0.057, respectively), largely due to genotype by site interaction. The type-b genetic correlations were high at 0.66 for the LD series and 0.80 for the E series. Rank correlations between least-square family means indicated that ranking of families changed considerably for the LD series (0.10–0.38); however, rank changes were moderate for the E series (0.29–0.50). Adjustment with first-order separable autoregressive residuals and cubic smoothing splines for columns and/or rows, to model the spatial heterogeneity, reduced the residual variance from 16% to 56%; implications of spatial analyses will be discussed with respect to western hemlock genetic improvement.

COMPARATIVE ANALYSIS OF MEDITERRANEAN PINE GENOME STRUCTURE: STUDY OF THE MOLECULAR ORIGIN OF THEIR EVOLUTION AND POTENTIAL APPLICATIONS IN BREEDING AND CONSERVATION

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Pine genomes are characterized by their highly conserved structure. Comparative mapping among pine species has revealed a high degree of co-linearity. However, nuclear DNA content among pine species as well as intraspecific genetic diversity and phenotypic plasticity shows a remarkable degree of variation. It has been suggested that the large genome size and its variation are adapted responses to the environment. We have analyzed the genome of three autochthonous Mediterranean pine species, (*P. pinea*, *P. pinaster*, and *P. sylvestris*), which show different levels of intraspecific genetic variability. Four to six populations of each species were chosen covering the natural distribution range of the species and therefore growing in divergent environments. Nucleotide variation of candidate genes, as well as high throughput markers targeting different elements such as microsatellites and retrotransposons versus anonymous AFLPs, have been analyzed to obtain insights about the mechanisms of pine genome evolution.

SPATIAL GENETIC STRUCTURE IN A NATURAL POPULATION OF BLACK POPLAR (*Populus nigra* L.) ALONG THE LOIRE RIVER

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Black poplar (*Populus nigra* L.) is a riparian pioneer species which has a great ability to vegetatively propagate. Despite its economical and ecological importance, it's endangered by the fragmentation of its natural habitat and by introgression from cultivated poplars.

We investigated the patterns of spatial distribution and genetic structure of a natural population along the Loire Rriver by exhaustive characterization of 414 georeferenced adult individuals for sex, flowering phenology and their genotypes at 10 SSR markers.

Among these individuals, 202 distinct genotypes were identified with 78 groups of identical genotypes (2 to 18 individuals per group). The genetic designation of these groups was coherent with the sex of the individuals within a group, their spatial proximity, and their flowering synchronism. In spite of the considerable proportion of asexual reproduction, tested loci were highly polymorphic with a mean number of alleles of 11.8 per locus. The high values of observed and expected heterozygosity (0.74 and 0.75, respectively) show a high genetic diversity in the population. These values were not significantly different and the inbreeding coefficient doesn't indicate a deviation from Hardy-Weinberg equilibrium (F_{IS}= 0.008).

All the individuals belonging to the clonal groups are currently genotyped with AFLP markers to verify the power of discrimination of the set of SSR used. Results of a spatial analysis of neutral (SSR) and non-neutral diversity (sex, flowering phenology) was presented.

GENETIC VARIATION OF *Pinus sylvestris* IN THE EASTERN CARPATHIANS ASSESSED BY cpSSR MARKERS

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Scots pine (*Pinus sylvestris* L.) has the widest distribution of the pine species, covering much of Eurasia. The populations are locally adapted to very different environmental conditions. There is very limited information regarding genetic marker variation of Scots pine growing in the mountains. In this study, the genetic structure of *Pinus sylvestris* was investigated using ten chloroplast, paternally inherited simple-sequence repeat markers for eight isolated populations in the Ukrainian Eastern Carpathians. Among 238 individuals analyzed, the 51 size variants defined 117 different haplotypes. The majority of haplotypes (65%) were detected only once. The average number of haplotypes per population was 18.25. Our results indicate a very high level of within-population haplotypic diversity (He = 0.981). The AMOVA showed low, but significant, differentiation (PhiST = 0.07, P = 0.001) with most of the total variation attributable to within-populations. The average mean distance among individuals among populations, according to SMM, was 3.89. The average within-population genetic diversity observed in this study, is one of the highest reported for Scots pine.

GENE FLOW BY POLLEN IN THE CERRADO TREE SPECIES, *Tabebuia aurea* (Bignoniaceae)

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The Brazilian Cerrado is one of the most endangered ecosystems in Brazil due to fragmentation caused by the expansion of agricultural lands. *Tabebuia aurea* (Bignoniaceae) is a widespread Brazilian Cerrado tree species pollinated by large-sized bees and wind. We are interested in understanding the mating structure and gene flow of this species, understanding the fragmentation effect on population dynamics, and generating information for conservation strategies. At the Ecological Station of Aguas Emendadas, Brasília, leaves from adult individuals from one population (almost 250) were sampled. At least four fruits from 24 adults were sampled and ten seeds per fruit were germinated. All adults and seeds were genotyped using 10 microsatellite loci developed for *T. aurea*. Our results showed a high and significant inbreeding coefficient (f = 0.115) that indicates an excess of homozygotes in the population that resulted from mating between closely relatives. The analysis of half-sib families indicated a high outcrossing rate (t_m = 1.02), but with high biparental inbreeding (0.502) Although many events of long distance gene flow were observed, mating between nearest neighbours was also found. Additionally, multiple paternity was ubiquitous and many seeds were sired by self-pollination. These results may be due to the big-bang flowering pattern that favors low distance gene flow. Nevertheless the maintenance of a large population may be important for long term species viability, providing that there is pollen flow from unrelated individuals thereby preventing inbreeding depression that may lead to loss of evolutionary potential.

INTEGRATING CLONAL TEST RESULTS IN THE QUEBEC BLACK SPRUCE IMPROVEMENT PROGRAM

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Testing selected individuals with clonal vegetative copies (ramets) could provide more efficient and precise genetic information for further tree evaluation than progeny selection (ortet). The integration of clonal field trials in advance tree improvement programs can increase the efficiency of breeding strategies and potentially genetic gains. For the black spruce (*Picea mariana*) second-generation program in Quebec, superior individuals selected in progeny tests have been reproduced by cuttings to constitute new breeding populations and to establish clonal field trials. Furthermore, this material allowed us to plant clonal seed orchards in all regions. For each breeding zone, approximately 100 individuals from 60–70 families were selected in 5–6 progeny tests. Two clonal tests were planted in each zone. Trees were ranked from the 5-year-old data and the best ones were identified for each family. These unrelated parents were then combined into new elite candidate breeding populations. Moreover, ranking clones allowed a special seed collection from a group of 30 superior individuals in the clonal seed orchards, giving an additional gain of 4–5% in height growth, estimated at 5 years. Eight- and ten-year-old measurements of these tests should give more precise yield estimates. These tests may also contribute in the future for other genetic related studies, in particular for wood quality.

AN INTEGRATED ANNOTATED MICROSATELLITE MAP FOR LOBLOLLY PINE

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Microsatellite (short simple repeat, SSR) markers were developed for loblolly pine (*Pinus taeda* L.) SSR primer pairs were derived from *P. taeda* and *P. radiata* genomic libraries, from pine EST databases, and from the literature. A total of 1509 primer pairs was evaluated for quality of amplification and polymorphism in *P. taeda*. Population diversity measures will be reported for about 130 selected SSR loci. Segregating mapping populations from the *P. taeda* Base and Qtl pedigrees were genotyped for 241 SSRs. Mapping data for 233 SSR loci were integrated with data from published RFLP and ESTP loci to obtain a 449 marker-map of 12 linkage groups. Of the 311 cDNA-derived markers, 82% could be associated with NCBI *P. taeda* UniGenes or PGI pine TC contigs. Reference protein annotations of the UniGenes and TCs were used to assign Gene Ontology (GO) terms to the mapped loci. Putative duplicate and paralogous loci were identified based on their map positions, GO terms, and UniGene or TC memberships. Of 131 non-SSR EST loci, 86% had GO terms of known function, while only 47% of the 85 EST-SSR loci could be assigned functional GO terms. This difference is being investigated and the results will be reported.

SIMPLE METHOD FOR SNP TYPING OF DROUGHT STRESS RELATED GENES IN EUROPEAN OAK (*Q. robur*, *Q. petrea*)

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Forest populations of long-living trees like oak will face considerable environmental challenges because of predicted global warming. So far the genetic diversity of these populations was assessed based mostly on neutral markers like microsatellites or AFLP. In the present work the genetic diversity of drought/osmotic stress related genes with putative importance in response of oak populations to foreseen environmental changes will be assessed. Austrian populations growing under different climatic conditions will be compared. The diversity assessment will be based on ten selected drought/osmotic stress related genes originating partly from previous work at ARC and partly from a running investigation, where oak microarrays will be used for identifying differentially expressed genes. These latter genes need new SNP discovery by sequencing alleles originating from different climatic regions and from geographically distant locations. Allelic diversity will be established by three independent SNP loci per gene allowing the theoretical identification of eight alleles per gene locus provided that each SNP yields two variants. A novel and easy to apply technique for SNP detection is being developed, where SNP-variant specific primers and a common real time interface for end point detection are used. The provided approach will present a high throughput, small scale and low cost method for identifying genotypes. The obtained diversity data will be correlated to geographic locations and heat/moisture index during the vegetative period of the selected populations.

TANDEM REPEATS IN A GROUP II INTRON PROVIDE RESOLUTION IN PHYLOGENETIC AND PHYLOGEOGRAPHIC STUDIES OF THE GENUS *Populus*

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We detected a variable region in a chloroplast group II intron. A large portion of this variation is made up by complex tandem repeat structures, thus the region proved useful in inter- and intraspecific phylogenetic analysis. We used indel/gap coding to establish a phylogeny of 46 poplar accessions.

Additionally we investigated the secondary structure of the intron. Particularly interesting is the finding that one of the tandem repeats is not forming a hairpin itself, nor in a repeated state. Rather the folding suggests that repeats are involved in attracting inversed structures along the 5' direction of the sequence, thus shortening the distance between conserved regions of certain domains. Furthermore the variable region is found in domain IV. On the one hand this domain has lost its maturase function, thus it evolves fast and nucleotide composition is no longer important. On the other hand, domain IV is reported to be highly specific due to high variability regarding additional splicing factors. In the folding of the rpl16 intron in poplars a structure of two hairpins might be involved in attracting the splicing enzyme. Without one 16bp repeat those hairpins fail to be formed, whereas a 2-5 fold repetition does not influence the hairpin structure.

TWO MITOCHONDRIAL GROUP II INTRONS IN *Picea omorika*: NEW INSIGHTS INTO EVOLUTIONARY RELATIONS AMONG SPRUCES

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Evolutionary relations of *Picea omorika*, a Tertiary relict species endemic to the northern Balkans, and other *Picea* species were tested using published data on two mitochondrial group II introns. Both introns - nad1i477, characterized by pronounced intra- and interspecific variability in spruces, and less variable nad5i230, revealed a close evolutionary relationship of *P. omorika*, *P. abies*, and some Asian spruces as well as evolutionary relations among other species which were not concordant with recently published molecular phylogenies. Nad1i477 enabled division of all spruces into two groups with essentially different organization and secondary structure of the domain IV. Group A spruces (*P. omorika*, *P. abies*, and some Asian spruces) harbored a variable region characterized by the presence of short fragments (c.a. 30 bp) repeated in tandem and organized into long hairpin(s). This variable region was absent in other Asian spruces and all American spruces, designated group B. Instead, those spruces harbored a long stretch (c.a. 1300 bp) organized into a large stem-loop structure. Five haplotypes, previously described at nad5i230, corresponded well to the geographical distribution of all species and to the described grouping - group A spruces harboring haplotypes D and E are confined to Europe and Asia, while group B spruces harboring haplotypes E, C, A, and B are confined to the Himalayan-Hengduan region and America. The only spruce which did not follow the described pattern was *P. breweriana* which was characterized as an isolated lineage. The results are interpreted in relation to 1) the possible occurrence of hypervariability at nad1i477, 2) evolutionary relations of *P. omorika* and the American spruces, *P. mariana* and *P. rubens*, and 3) the origin of spruces.

TO POOL OR NOT TO POOL

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Resequencing projects within the *Pinaceae* generally benefit from using the maternal haploid megagametophyte to estimate haplotype frequencies in the populations. In most other plants, including trees, diploid DNA is the starting material. Single nucleotide polymorphisms can be confidently scored from "diploid chromatograms", but insertion-deletion polymorphisms cause reading-frame shifts that usually ruin the reads. Yet, small size amplicons can be sequenced from both ends and then even frame shifts can be dealt with. However, the only general method that is able to deal with long indel-prone amplicons (such as promoter sequences) is the cloning of the diploid PCR products into plasmid vectors, followed by bacterial transformation and colony isolation, which will provide haploid copies of the targeted amplicons. After success in other applications, pooling genomic DNAs or PCR products could alleviate this expensive and time consuming process. Because pooling can potentially introduce sampling biases, it becomes necessary to test its effects before it can be applied on a daily basis.

In this work, we have measured the biases introduced by different pooling strategies into the haplotypic frequencies of several *P. radiata* amplicons. We discuss the effects of these differences on the most common nucleotide polymorphism descriptors as well as on various neutrality tests that depend upon them.

MOSAIC STRUCTURE AND HETEROGENEOUS PATTERNS OF NUCLEAR, CHLOROPLAST DNA, AND PHENOTYPIC VARIATION IN THE HYBRID ZONE BETWEEN *Quercus affinis* AND *Q. laurina*

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The structure of hybrid zones reflects a combination of dispersal and selection, either intrinsic (endogeneous) or environmental (exogenous). The genetic and geographic structure of the hybrid zone between two Mexican red oaks, *Quercus affinis* and *Q. laurina*, was examined by analyzing variation at 14 diagnostic RAPD loci, 9 phenotypic traits, and chloroplast DNA (cpDNA) in 11 populations sampled along a 500 km transect intersecting the hybrid zone. The framework of hybrid zone theory was used to analyze the data. Positions and/or shapes of fitted clines for six out of fourteen RAPD markers deviated significantly from a pattern of coincidence and concordance among all clines, while the remaining eight had similar centers and widths. In addition, cline models could not account for the abrupt frequency fluctuations exhibited by eight markers in some populations. Morphological characters and cpDNA did not follow a pattern of smooth clinal variation either. Linkage disequilibria among species-specific markers were in general absent in the populations. Three morphological characters and five RAPD markers showed an association with temperature-related climatic variables. Therefore, results revealed that the geographic structure of the hybrid zone between *Q. affinis* and *Q. laurina* resembles a mosaic rather than a tension zone and that patterns of variation are heterogeneous among phenotypic and molecular characters, suggesting that locus-specific forces are contributing to the structure of this hybrid zone and that environmental selection has played a significant role.

HOW TO WIN A RACE WHEN YOU CANNOT MOVE: GENE FAMILIES FOR THE DIVERSIFICATION OF CONIFER CHEMICAL DEFENSE

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Species of spruce are the most widely planted forest trees in Canada. As very long-lived and sessile organisms spruce trees require both diverse and lasting defence systems for protection against faster evolving, mobile insect pests and pathogens. The diterpene resin acids (DRAs) present in conifer oleoresins provide a major chemical defence system. The formation of a suite of diverse DRAs in conifers involves gene families of diterpene synthases (diTPS) and cytochrome P450 dependent mono-oxygenases (P450s). Related diTPS and P450s also form the essential gibberellin phytohormones. With around 10–40 Gb the genomes of conifer trees are estimated to be 40 to 100 times larger than those of poplar or Arabidopsis. However, despite these differences in genome size, conifer diTPS and P450s essential in primary phytohormone metabolism are mostly conserved as single copy genes. In contrast, phylogenetically related families of conifer specific diTPS and P450 genes involved in the secondary metabolism of DRA formation and chemical defence have substantially expanded forming large and diverse multigene families. We are presenting new results from a genomic and functional analysis of the diTPS and P450 gene families of phytohormone and DRA biosynthesis in species of spruce. Our results provide a conceptual framework to explain the diversity of conifer chemical defence.

EXTREMELY HIGH CHLOROPLAST DNA HAPLOTYPE DIVERSITY IN REMNANT POPULATIONS OF *Quercus castanea* IN THE CUITZEO BASIN, STATE OF MICHOACÁN, MÉXICO

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Within the last four decades, *Quercus* forests of the Cuitzeo Basin in Mexico have been dramatically reduced to a large number of small patches of variable size (<1 km^2–12 km^2). The aim of this research was to evaluate levels of chloroplast DNA (cpDNA) haplotype diversity in adults and seedlings from fragments of different sizes to infer if this recent habitat fragmentation has begun to alter the genetic structure of this oak species. We sampled 10 adult trees and 10 seedlings from 33 populations of *Quercus castanea* distributed throughout 4026 km^2 of the basin and screened polymorphism at 7 cpDNA microsatellite loci. Remarkably high cpDNA variation was found, with 70 haplotypes (the highest number of haplotypes so far found in any oak species) present in this relatively small area and high levels of genetic diversity per population for both the adult and seedling cohorts. Levels of haplotype diversity did not differ between adults and seedlings and neither varied according to fragment size, however we found less exclusive haplotypes in populations from small fragments. A hierarchical analysis of molecular variance (AMOVA) did not detect genetic differentiation between populations of adults and seedlings. The level of cpDNA differentiation among all populations was R_{ST}= 0.32. We conclude that *Quercus castanea* is a species with high haplotypic diversity and that so far there are no evident effects of habitat fragmentation on its genetic structure within the region examined.

CONSERVATION GENETICS OF GARRY OAK (*Quercus garryana*)

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Garry oak (*Quercus garryana*) is a deciduous tree endemic to western North America. It is confined within Canada to only a few isolated locations in southwestern British Columbia (BC). Although accounting for less than 0.3% of BC's entire land coverage, Garry oak-associated ecosystems support tremendous biodiversity and are home to a large number of rare species. Populations have, however, been declining since European settlement. It is estimated that only 1–5% of pre-European Garry oak ecosystems remain uncompromised in BC today. However, species distribution models predict the area climatically appropriate for Garry oak to triple in BC by the 2080's. Using a common garden experimental design, data regarding growth and partitioning, bud phenology and cold hardiness are being collected from 1700 individuals (15 populations) representing the species' entire range. Estimates of quantitative genetic variation (Qst) will be compared to published estimates of neutral molecular variation (Gst) in order to evaluate the extent to which diversifying selection has resulted in local adaptation. Results will be used to recommend seed transfer guidelines and conservation strategies for current and future climates in BC and elsewhere.

MORPHOLOGICAL VARIATION IN LEAF AND SEED CHARACTERISTICS AMONG EIGHT PROVENANCES OF *Zelkova serrata* (Thunb.) Makino IN KOREA

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Ten morphological traits of leaves and seeds of *Zelkova serrata* were examined in eight selected provenances throughout Korea. Nested ANOVA revealed that there were statistically significant differences among provenances as well as among individuals within provenances in most leaf morphological traits but not in seed length and width. Significant amounts of variation were explained by a linear combination of temperature and latitude but not by longitude or altitude of the provenance. The results of principal component analysis (PCA), for measured traits, showed that the first component accounted for about 46% of the total observed variation while the second accounted for 17%. A dendrogram, reconstructed by cluster analysis, might infer that the decrease in population size caused by high demand for the wood and continual seed transfer and planting of *Z. serrata* for centuries has resulted in genetic disturbances responsible for the discrepancy between genetic relationships and geographical distribution among provenances of the species at present. The information on the genetic status of *Z. serrata* from this study will be helpful to prepare a more reasonable strategy for provenance testing and breeding programs of this species in Korea.

TEMPORAL CHANGE OF RELATIVE HEIGHT GROWTH AND SELECTION OF SUPERIOR FAMILIES IN PROGENY TESTS OF *Pinus densiflora*

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This study was conducted to select superior families based on open-pollinated (OP) progeny tests of *P. densiflora*. A total of 232 OP families were analyzed for relative height growth. The OP progeny test trials were established at 1 to 4 sites from 1975 to 1987. To minimize temporal and spatial variation, we applied the standardization method for family selection. In each progeny test, superior and inferior families were selected at ages of 10, 20, and 30 years. Relative height growth rate (RHGR), grow speed at a given time unit, was comparatively high at age of 10 ranging from 0.2–0.6 and showed a large variation among families. However, after age 15, the RHGR was low (0.04–0.09) and also the variation was not significantly different among families. To reduce selection errors due to age differences (from age 23 to 35) of tests, we made family selection after age 15 when the RHGR values were stable. Height growth at each age was transformed to height growth at age 35 based on the RHGR. Families KW85, KB49, KW71 and CN4 were selected as superior families and KW40, KW49, KB21 and GG1 were considered as inferior ones. Rank correlations (r) between test ages and selection age 35 were high and statistically significant; r = 0.870 between age 30 and 35, 0.792 between age 20 and 35, and 0.503 between age 10 and 35. Ranks of inferior families were more stable at the early ages than that of superior families.

TRADE-OFF BETWEEN GENE DIVERSITY AND SEEDLING UTILIZATION FOR GENE CONSERVATION: *Cornus kousa* AS A MODEL

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Theory on the trade-off between gene diversity and seedling utilization was applied to Korean *Cornus kousa* populations under a gene conservation program. Constraints on seedling utilization among families could be made on the parental proportion as both lower and upper bounds. The lower bound means that seedlings are not used from families with low contribution. The upper bound describes balancing, meaning that equal numbers of seedlings are utilized from families with high seedling production to avoid over-representation of the most fertile. For gene conservation of *C. kousa*, a total of 40 families were collected from natural distribution areas and propagated to produce 505 individuals (on average 12.6 trees per family). For *ex situ* gene conservation of *C. kousa*, the status number was estimated to be 20.3 if all seedlings were used. Higher status numbers could be obtained by balancing the number of seedlings utilized per family. But, this high status number was associated with a loss of seedling utilization. If it was decided to truncate the number of progeny at 32 trees (lower bound = 0.0, upper bound = 0.8) and the contribution of each family was restricted to less than 6.0%, this increased the status number from 20.3 to 22.6 while 87% of the seedlings were used. In conclusion, a trade off between equalizing parental contribution and obtaining an acceptable number of seedlings was achieved by controlling parental contribution and genetic diversity.

ASSESSMENT OF GENETIC AND WOOD DENSITY VARIATION OF *Liriodendron tulipifera* L. POPULATIONS IN UNMANAGED FORESTS OF THE SOUTHEASTERN UNITED STATES

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Liriodendron tulipifera L. is prolific throughout the Southeastern United States and has increasingly important roles in forestry and wood products in this region. The relatively low density and intermediate strength of the wood makes *L. tulipifera* versatile for use in wood products. This study utilizes a molecular marker technique, amplified fragment length polymorphism (AFLP), to determine the degree of genetic variation of *L. tulipifera* and potential correlations of genetic polymorphisms with variations in wood density. Wood density was determined using an X-ray densitometer. AFLP was performed using five primer combinations on samples from six study sites in the mountains and coastal plain of the Southeastern United States. The range of average wood density is greater in the mountains than in the coastal plain. Significant differences in wood density varied between sites. The highest levels of genetic differences are expected between the mountains and coastal plain populations, with the Virginia Coastal Plain site having a Dice coefficient of 0.9087.

MOLECULAR CHARACTERIZATION OF HYBRID *Castanea* CLONES FOR FOREST REPRODUCTIVE MATERIAL CERTIFICATION

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TRAGSA applied the RAPD methodology for the molecular characterization of clones of its hybrid *Castanea* collection. Currently, basic materials of all forest Galician nurseries are being tested using this same method for its approval or refusal. Plant DNA extraction was accomplished using the DNeasy Plant Mini Kit (Qiagen) following manufacturer instructions. For RAPD genotyping, we tested 3 different 10-mer primers (coded as OPA2, OPA4, and OPS11, Operon Technologies) in order to have properly identified the whole hybrid collection. PCR products were separated in an agarose gel and scored for polymorphisms with the aid of the GeneTools software. Our results were reproducible, being able to distinguish between clones. OPA2 displayed different band patterns for all clones except for clones HS and 7810. These two clones were distinguished using OPA4 primer but on the contrary this primer did not allow us to distinguish clones 1483 and 90025. OPS11 did not allow us to distinguish between clones 3 and 88 or 19 and 7521, but it allowed us to make a clear differentiation for the rest of the clones. Therefore, we think that this method could be useful for the molecular identification of basic materials of the hybrid *Castanea* clones recently included in the Spanish Catalogue. In addition, we are starting to use microsatellite (SSRs) markers for the molecular characterization of new selected clones because of their high reproducibility, high discriminating power, cost effective, and less time consuming. In order to distinguish the species *Castanea sativa* from putative hybrids, we selected 3 different SSRs that have been used in the identification of different *Castanea* species.

SELECTION OF *Castanea sativa* Mill. FOR RESISTANCE TO *Phytophthora cinnamomi*: TESTING OF SELECTED CLONES

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Between 2000 and 2005, TRAGSA conducted an exhaustive prospecting of *Castanea sativa* trees in Galicia. Third Forest National Inventory and Forest Map were used, localising ink disease severely affected areas, where chestnut was the primary or secondary species. Two hundred and six trees were selected throughout 18,808.84 ha in 1 and 2 Bouhier's regression areas. These clones were established for *in vitro* propagation (Cuenca et al. 2005) as well as tested for resistance by inoculating excised shoots of adult material (Robin et al. 2006). Results of these tests were not repetitive due to the high dependence on the physiology of material coming from the field. Therefore, root inoculation was conducted on acclimated vitroplants and an *in vitro* test (Rial et al. 2007) was carried out to determine a possible correlation between both types of tests and therefore the possibility of performing the *in vitro* test on the remainder of the collection. Physiology of acclimated vitroplants had an important influence on *Phytophthora* proliferation, as all the clones tested during spring or early summer died at a higher percentage than those tested at the end of the summer. From the 18 clones tested from May to July, 3 of them survived at a percentage higher than 75% and one of them presented no ink disease symptoms. All explants tested *in vitro* died but there seems to be some relationship between resistance and time to express the symptoms *in vitro*. The more resistant the clone is the longer it takes to die. Molecular characterization by SSRs (Yamamoto et al. 2003) for those clones presenting resistance features is in progress to determine if they are pure *C. sativa* genotypes or resistance is due to the presence of Asiatic alleles.

ASSOCIATION STUDIES OF WHITE SPRUCE WOOD TRAITS: ASSESSMENT OF PHYSICAL AND CHEMICAL PROPERTIES

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In the Arborea project, we aim at developing marker-assisted selection for physical and chemical wood traits in white spruce (*Picea glauca* [Moench] Voss) through association studies. Related to this goal, a vast effort has been employed to assess diverse wood characters in a group of 695 trees representing discovery and validation populations. SilviScan technology was used for the assessment of wood physical and anatomical properties. Wood density, micro-fibril angle (MFA), modulus of elasticity (MOE), wall thickness and more were measured or estimated using x-ray densitometry, x-ray diffraction patterns, and image analysis. Chemical properties were assessed by Raman and Near Infra Red spectroscopy for the whole population of 695 trees. For this purpose, prediction models were developed by using a set of samples from 40 unrelated trees which were submitted to wet chemistry analysis, as well as Raman and Near Infra Red spectroscopy. These phenotypic data are being integrated with large-scale genotyping data to enable association studies. This collection of intensively characterized trees also represents valuable resources for future investigations related to wood properties and tree growth.

ASSOCIATION MAPPING FOR WOOD QUALITY IN *Pinus pinaster* AQUITAINE BREEDING POPULATION

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Improvement of wood quality related traits is currently hampered by costly chemical and technological assays and the necessity to wait until the trees are nearly mature to evaluate wood properties. The availability of a vast quantity of genomic data opens a new avenue to identify early selection criteria based on molecular information and therefore increase selection efficiency. Association mapping is becoming a method of choice to identify QTN (quantitative trait nucleotide) that contribute to complex trait variation. The implementation of this approach requires on the one hand knowledge of the molecular mechanisms underlying trait variation and polymorphisms within candidate genes, and on the other hand the availability of phenotypically well characterized genetic material. We are developing this strategy for the French maritime pine breeding program, an economically important forest tree species in southwestern Europe. About 500 trees from the breeding population were evaluated for wood physical and chemical properties through the analysis of 2800 half-sib progenies and 1500 clones in 8 field tests. These same trees are being genotyped at 185 SNPs obtained from the sequencing of 41 candidate genes, and an additional set of 200 eSNPs detected in 147 EST-contigs (to be used as a control). Statistical association between the breeding values of the 500 trees and their respective genotypes will be tested using mixed models accounting for relatedness among individuals of the breeding population.

A TRANSCRIPTOMIC APPROACH IN MARITIME PINE TO REVEAL THE MOLECULAR MECHANISMS INVOLVED IN WOOD FORMATION

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Gene assisted selection for wood quality and end-use properties requires a better knowledge of the molecular mechanisms involved in the formation of secondary xylem (wood). We characterized wood forming samples of maritime pine (*Pinus pinaster*) collected during a growing season and along a cambial age gradient at the anatomical and chemical levels. The variability found within a single individual led us to hypothesize that this plasticity can result from the differential expression of genes/proteins during wood formation. SSH libraries were first generated to identify the most representative genes of each type of wood (early, late, juvenile, mature wood) as well as those that are specific to differentiating xylem. Second, a microarray was established based on 11 000 unigenes derived from 27 000 ESTs. In this poster we reviewed the main results of this transcriptomic study aiming at providing expressional candidate genes that are being validated by reverse and forward genetic approaches.

CHITINASE GENE POLYMORPHISM CHARACTERIZATION IN WESTERN WHITE PINE FAMILIES WITH QUANTITATIVE RESISTANCE

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Western white pine (*Pinus monticola*) possesses major gene resistance and quantitative resistance mechanisms against rust infection by *Cronartium ribicola*. The phenotypic variation of quantitative resistance is controlled by interaction of environmental factors and polymorphic genome structures in individuals. The candidate gene approach is generally considered an effective strategy for identifying loci involved in complex and economically important traits. Candidate gene markers, based on resistance or defense-related genes, provide useful tools for molecular characterization of plant disease resistance. In the present study, western white pine chitinase genes were investigated as functional candidates involved in quantitative resistance. We cloned and sequenced chitinase genes in various western white pine families. DNA polymorphisms, including single nucleotide polymorphisms (SNPs) and indel polymorphisms, were identified and evaluated in western white pine chitinase genes for association with phenotypes of quantitative resistance. In a further study, a diagnostic tool would be developed from this kind candidate-based marker for polygenic resistance to *C. ribicola* in white pine breeding populations. SNP and other markers derived from functional candidate genes will be useful for exploring the biological roles of defense-related genes in quantitative resistance to white pine blister rust.

ANALYSES OF GENE EXPRESSION CHANGES IN *Pinus* SPECIES USING A LOBLOLLY PINE cDNA MICROARRAY

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Microarray techniques have transformed gene expression analysis by facilitating the interrogation of thousands of genes in a single experiment, thereby contributing valuable insights into growth, development, regulatory mechanisms, and disease processes in every species where it has been applied. Based on previous gene discovery projects in our laboratory that produced a set of ca. 177 000 3' and 5' single-pass loblolly pine (*Pinus taeda*) ESTs, we recently completed the manufacture of a *P. taeda* cDNA spotted array consisting of 26 496 features from ca. 25 000 unique genes. In this poster we discussed results from two separate studies where labeled targets derived from either loblolly or maritime pine (*P. pinaster*) tissues were used to assess differential gene expression patterns resulting from drought stress or embryonic developmental transitions, respectively. The results were discussed in comparison with previous studies.

In vitro REGENERATION OF *Pinus pinaster* ADULT TREES

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The regeneration of adult conifer trees by means of *in vitro* culture has been the subject of intense study during the last 20 years. Propagation by tissue culture is the best method to obtain multiple clonal trees and to conserve the genetic gain in breeding programs. However, this methodology has several problems related to phase changes (juvenile-adult) that limit its practical applications. In this study, carried out from November 2006 to March 2007, shoot buds were collected from 20 *Pinus pinaster* adult trees (>20 years old). Buds were transversely cut into 0.5-1 cm slices and cultured (4 explants/Petri dish) on different media [DCR, WP, and modified LP] supplemented with cytokinins [6-benzyladenine (BA), meta-topolin (mT), or zeatin (Z)], at two concentrations (25 or 50 mmol/L). Highest organogenic response, i.e., axillary shoot formation ability, was obtained on DCR medium supplemented with 25 mmol/L Z and mT, and 25 or 50 mmol/L BA. All the shoots that were cultured on DCR with 25 mmol/L BA developed as healthy and well rooted plantlets. The ability to regenerate adult trees represents a significant progress in the application of biotechnology to forest tree improvement programs and it opens the possibility of using micropropagated selected trees in agro-forestry areas under biotic or abiotic stress.

SPATIAL DISTRIBUTION OF OAK CHLOROTYPES IN Cyprus

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Due to complex geology and climate stability, the Mediterranean Basin exhibits a unique biodiversity. Species' long term persistence has led to high genetic diversity which is strongly spatially structured. Phylogenetic studies based on organelle DNA markers could provide useful information about the distribution of genetic variation and the underlying evolutionary mechanisms. In our study, chlorotype diversity at five variable cpDNA microsatellite loci and its spatial distribution were analyzed in 22 natural populations of the three oak species represented in *Cyprus*: golden oak (*Q. alnifolia* Poech), kermes oak (*Q. coccifera* L. ssp. *calliprinos* Webb.), and gall oak (*Q. infectoria* ssp. *veneris* A. Kern). Gall oak stands possess the same chlorotype in all study areas, which is distinct from golden and kermes oak. This confirms that gall oak (section *Robur*) does not hybridize with the latter species, which belong to a separate taxonomical unit (section *Cerris*). Our data reveal geographic structures and a high proportion of shared haplotypes between golden and kermes oak, possibly caused by genetic introgression. Additionally, the chlorotype distribution in a mixed stand supports unidirectional introgressive hybridization. Finally, kermes oak displays higher cpDNA richness in pure populations when compared with golden oak, which may be caused by differential seed dispersal strategies. In summary, our results support accumulation of genetic richness in relic Mediterranean oak populations. Cytoplasmic exchanges and introgression have already been described in Mediterranean evergreen oak species and seem to be confirmed in our case as well.

GENOMIC DIVERSITY OF THE SHELTERBELT POPLAR ASSOCIATION MAPPING POPULATION

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The Shelterbelt Garden is being developed in Indian Head, Saskatchewan by the AAFC-PFRA to represent genetic variation from throughout the range of *Populus balsamifera*. The Garden contains over 500 genotypes from more than 35 populations. Future uses of the garden include association mapping and breeding stock. We present a genome-wide assessment of nucleotide variation represented in a subset of 15 individuals within this Garden. Sequence data from >100 randomly selected gene-associated regions will be presented and summarized. Nucleotide diversity is low (average Pi=0.0016). Preliminary analyses indicate that mean Tajima's D does not differ significantly from neutral expectations. These data will be interpreted with respect to the utility of the Garden for future association mapping endeavors.

MORPHOLOGICAL AND GENETIC GEOGRAPHIC DIVERSITY IN *Pinus pinaster* Ait. PROVENANCES

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Maritime pine (*Pinus pinaster* Ait.) is a widely planted conifer of major economical and ecological value. Possible scenarios of climate change predict increasing water stress in areas of the western Iberian Peninsula. Consequently, an ongoing study is investigating geographical drought adaptation patterns among Atlantic populations, focusing on evaluation of morphological, physiological, and molecular traits related with water deficits.

This study evaluated the variability of morphological traits of cones from two pine stands with contrasting climate (Bragança and Leiria, Portugal). This study also tested genetic diversity among 18 maritime pine populations with nuclear microsatellite primers designed for *Pinus halepensis* Mill., *Pinus taeda* L., and *Pinus pinaster* Ait.

Significant differences were found between Bragança and Leiria for cone length and diameter, average number of seeds per cone, and 100-seed weight. SSR results obtained until now suggest the selection of five primers whose amplification protocols were already optimised for loci (A5B10; A5B01; RpTEST11; Ptctg275; Itph4516). These primers were tested in five populations (Cuenca, Galicia, Landes, Corsega, and one Portuguese) and polymorphism was observed among them.

GENETIC STRUCTURE AND HYBRIDIZATION BETWEEN THREE SPECIES OF RED OAKS (Lobateae) *Quercus* (Fagaceae) IN THE SIERRA TARAHUMARA, MEXICO

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The evolutionary implications of hybridization between more than two parental species involve not only the presence of hybrids but also the complexity of the hybrid system, which might include a wide range of genotypes, depending on physiological, genetic, and environmental traits such as soil, temperature, and altitude among others. A complex of three not closely related Mexican red oaks, *Quercus hypoleucoides*, *Q. scytophylla*, and *Q. sideroxyla* shows considerable evidence of morphological intergradation in some locations in the Sierra Tarahumara, in northwest Mexico. In order to understand the evolutionary process of hybrid formation between these three oak species, we characterize and differentiate between species and intermediate individuals in the intergradation zone using morphological and molecular markers. We analyzed morphometric leaf shape variation using 1150 digital images of leaves from the three species and intermediate forms, and the genetic structure among the same groups using seven nuclear microsatellites. Moderate levels of genetic differentiation between species were in general consistent with the morphological discrimination between species. Nevertheless, morphological traits did not always reflect the genetic admixture of individuals. The Bayesian method revealed a gradient in the genetic composition of populations and species, which was markedly related to altitude. These findings suggest a secondary contact found in the intergradation zone, where a high proportion of backcrossed individuals between species were found.

INVESTIGATING NITROGEN USE EFFICIENCY IN SRC WILLOWS AND ITS EFFECT ON BIOMASS YIELD

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Fast growing species like SRC willows recycle nutrients and thus require lower amounts of fertiliser than annual crops. Consequently, higher greenhouse gas reductions and energy gains are associated with biofuel chains from willows and other perennial crops. In breeding for higher biomass yields, it is important that these favourable balances are maintained and that requirements for nitrogen are not increased. However, little is known regarding the physiological and molecular components important for effective nutrient use by willows. This work focuses on the K8 segregating population maintained at Rothamsted Research which comprises 947 full-sib *Salix viminalis* x *S. schwerinii* hybrid individuals. This population is well characterised and has been extensively used for mapping and Quantitative Trait Locus (QTL) studies. To investigate the possible link between biomass production (i.e., yield) and nitrogen use efficiency (NUE), a subset of 12 genotypes, including the yield extremes, was selected for a greenhouse experiment designed to follow the seasonal cycling of nutrients at the whole plant level. The carbon and nitrogen budget is being documented as well as the allocation and partition of compounds using stable isotopes (13C and 15N). At the molecular level, gene expression studies (microarrays and quantitative PCR) using the selected genotypes will be employed to identify candidate genes involved in NUE. Candidates will be mapped and co-location with known willow yield QTLs determined. The results will generate further understanding of the relationship between NUE and biomass yield and provide a route towards molecular selection strategies for use within willow breeding programs.

THE FUEL IN THE WILLOWS – DEFINING AND IMPROVING SUGAR YIELDS IN SRC WILLOW

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In the production of biofuels from 'second-generation' lignocellulosic feedstocks, enzymatic hydrolysis (saccharification) can be used as a method to release energy from the most naturally abundant compound on earth, cellulose. However, properties of lignocellulose cell walls tend to make them highly recalcitrant to saccharification and so a pre-treatment step is usually required. This is often the most energy expensive step in the production of lignocellulose-derived biofuels. Increasing the accessibility of cellulose to cellulase enzymes, whilst not compromising whole plant integrity, would greatly decrease the energy input needed for saccharification. Furthermore, although future developments in bioprocessing beyond ethanol may well offer advantages they will still require sustainable biomass feedstocks optimised for their content of accessible high energy carbon sources such as glucose. SRC willows are fast growing woody plants with good potential for high biomass yields over short time periods which represent a source of virtually carbon-neutral energy. Preliminary saccharification of different willow species has identified naturally occurring variation in sugar yields. To study this further, a QTL experiment, based on 144 genotypes of the K8 willow mapping population, is underway to identify loci affecting sugar yield. Compositional and growth trait data are also being collected to examine the relationship of sugar yield to biomass yield and tree morphology. The data generated could then be employed in breeding programs for the selection of willows having improved sugar yields, with the potential for such traits to be transferable to other fuel-dedicated lignocellulosic crops.

DEVELOPMENT OF A VERTICALLY INTEGRATED ENTERPRISE MODEL FOR THE DETERMINATION OF ECONOMIC WEIGHT-BASED BREEDING OBJECTIVES FOR WHITE SPRUCE

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Breeding objectives to achieve further genetic gains in white spruce (*Picea glauca* (Moench) Voss) should be defined on the basis of economic weights of selection traits. To estimate these economic weights, an enterprise model must be developed to simulate the existing context. In this project, we develop a vertically integrated enterprise model. In this model, pulp and structural lumber are produced in the eastern Canada context, and product flows and cost/income flows are followed from plantation establishment to lumber/pulp shipping. The model also considers all interrelated stochastic processes found in lumber and pulp manufacturing. Several biological and production parameters such as wood chemistry and physical properties, labour costs and productivity, tree spacing, and many other parameters can influence production step costs and wood/products flows. Plantation production scenarios, which are chosen using yield tables, are automatically optimized using net present value (NPV). Intensive scenarios of thinning operations are simulated in light of the province of Quebec's legislation and are applied on precise stem diameters, depending on the types of thinning operation chosen. Stem diameter distributions are generated combining a Weibull's distribution percentile recovery method and volume production tables. Harvesting costs and productivity are calculated using customizable models. Recovery models are used to determinate which part of each stem should produce lumber, pulp or sawdust, and the quantity of material fashioned by product. Economic weight-based breeding objectives are determined using Monte Carlo sensitivity analysis. This analysis monitored incremental variation of NPV and profitability index depending upon an incremental parameter variation. Specific distributions are applied on entry parameters and thousands of random simulations are produced. The complete enterprise model was developed using spreadsheet software and sensitivity analysis were performed using the @RISK for EXCEL software v5.0 (Palisade 2008).

DEVELOPMENT OF SPECIES-SPECIFIC DNA MARKERS FOR IDENTIFICATION OF *Alnus* HYBRIDS

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Two *Alnus* species (*A. glutinosa* and *A. incana*) are found in natural stands throughout Latvia. *Alnus* species are not commercially grown; however, there is interest in utilising these species for commercial plantations due to their rapid growth rate and ability to improve poor quality soils. Naturally occurring hybrids have been reported to be faster-growing and larger than the parent species.

Molecular markers have not been widely applied or developed in *Alnus* species. We investigated the utility of birch SSR markers as well as developing new, *Alnus* specific markers to identify species-specific markers. Cross-species amplification success of the birch SSR markers was high; however, the rate of null alleles was also considerable. We identified one SSR marker that clearly differentiated the two species. Sequencing of this locus determined that no major insertions or deletions were found outside the repeat region. We designed PCR primers from the *Alnus* sequences available in GenBank and amplified fragments from *A. incana* and *A. glutinosa*. These fragments were hybridised to create artificial “hybrids” which were then digested using CJE (CEL-I) to identify species-specific SNPs. These SNPs were confirmed and characterised by sequencing and SNP specific genotyping assays developed.

The extent of natural hybridisation is not known and has not been investigated using DNA markers. The identification of hybrids by morphological features is difficult, as these characteristics are found in a continuum between pure *A. incana* and *A. glutinosa* types. Development of multiple species-specific SNPs will enable us to determine the extent of natural hybridization.

ADAPTIVE GENETIC VARIATION IN SCOTS PINE (*Pinus sylvestris*) IN SCOTLAND

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In Scotland, seed zones of Scots pine (*Pinus sylvestris*) are based on molecular variation which might not reflect fine-scale patterns of local adaptation. It is likely that divergent selection maintains adaptively differentiated populations across the range. Therefore, revision of seed zones is required. We are studying patterns of adaptive variation in 21 populations, each of which is represented by four mother trees, in two pairs at different altitudes. From each mother tree we have collected 40 open-pollinated progeny. These 3360 seedlings are growing in a randomized block design in a common-garden environment, and variation within and among populations will be studied in traits related to phenology, growth, and physiology. Family structure will allow estimation of additive genetic variances and narrow-sense heritabilities for these traits. Effects of local selection can be recognized by comparing levels of differentiation in quantitative traits (Q_{ST}) and at neutral markers (F_{ST}) to each other. Observed patterns of variation will also be linked to various geographic and climatic factors such as temperature, rainfall, altitude, and soil type. The results will be used to revise the existing seed transfer guidelines for pines in Scotland.

INTRODUCTION BETWEEN NATIVE AND EXOTIC POPLARS ON THE CANADIAN PRAIRIES

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Since the 18th century, many European poplars have been brought to Canada by settlers, and these trees still enjoy active use in forestry and shelterbelt breeding programs. Given the facility by which poplar species intercross, exotics may impact the genetic integrity of natural populations. The objective of this study is to determine the potential gene flow between cultivated poplars and their natural congener, *Populus balsamifera*, on the fragmented landscape of the Canadian prairies. We first determined the parentage of 30 common shelterbelt hybrids as many were derived through open-pollination. DNA sequencing of eight unlinked nuclear gene regions and a chloroplast intron confirmed the presence of exotic alleles from *P. laurifolia* and *P. nigra* with variable prevalence, in addition to *P. deltoides* alleles. Second, we sampled four different sites where mature natural populations of *P. balsamifera* grew adjacent to mature poplar shelterbelts. Foliage DNA was analysed to confirm the genetic identity of maternal trees and seeds were collected to detect and quantify spontaneous hybridization between these trees and exotics established in the surrounding landscape. Seeds are being genotyped for forty-two species-specific markers. Preliminary analysis of the foliage revealed the presence of exotic alleles in the natural populations of *P. balsamifera* suggesting that gene flow between exotic and native poplars may have already occurred. This will be confirmed through genotyping.

REPRODUCTIVE AND MOLECULAR ECOLOGY OF *Salix reticulata*

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Willows (genus *Salix*) are abundant plant taxa in alpine and subarctic ecosystems across the northern hemisphere. *Salix* are known to facilitate succession on barren substrate and provide shelter and food for many bird, insect, and mammal species. With the onset of proposed global climate change, alpine regions are forecasted to experience reduced habitat as a result of increasing tree-line competition on alpine flora, particularly dwarf willows. While many studies have addressed the reproductive dynamics of willows, few have addressed their population genetic structure and diversity in relation to reproductive ecology. Preliminary analysis of microsatellite markers of *Salix reticulata* has revealed high levels of polymorphism within localized alpine regions of the Canadian Rocky Mountains. Analysis of pollination strategy and levels on colonality are currently underway. Knowledge of reproductive ecology with respect to the increasing need for a genetic understanding of populations is an integral aspect to any future management and restoration projects.

EVALUATION OF GENETIC POLYMORPHISM OF ALL LITHUANIAN *Populus tremula* SELECTED TREES, TAKING INTO ACCOUNT THEIR CONTAGION WITH STEM ROTTING FUNGI

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Populus species have acquired an important place in basic and applied research of woody plants. The practical role of *Populus* species in world forestry and their importance to research as a woody-plant model have led to increasing interest in this genus (Tzfira et al. 1997). *Populus tremula* is the only natural *Populus* species in Lithuania. Naturally occurring *Populus tremula* individuals are often affected by stem rotting fungi, especially *Phellinus tremuloides*.

The aim of this study was to assess the genetic polymorphism of selected *P. tremula* in accordance to their contagion to *Phellinus tremulae*. In our research we have evaluated the phenotypic appearance of all selected (plus) trees in Lithuania as well as the surrounding tree stands. Selected tree leaf and stem samples were taken for further investigation and genetic polymorphisms of all 137 Lithuanian selected trees were assessed. As well, we have assessed their contagion with stem rotting fungi.

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PHOTOS

André Rainville extols the virtues of tree improvement while Paul Charrette makes sure that Kathie Brosemer is paying attention.

Martin Perron describes the larch clonal test while Penguin Lu looks at it from a different angle.

Marie-Josée Mottet tells it all to a group of interested people.

Brian Barber and André Deshaies are serenaded by La famille Painchaud.