PROCEEDINGS OF THE THIRTY-THIRD MEETING OF THE

CANADIAN FOREST GENETICS ASSOCIATION

PART 1Minutes and Members' ReportsPART 2Symposium



COMPTES RENDUS DU TRENTE-TROISIÈME CONGRÈS

DE

L'ASSOCIATION CANADIENNE DE GÉNÉTIQUE FORESTIÈRE

1^{ere} PARTIE Procès-verbaux et rapports des membres

2^e PARTIE Colloque

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PROCEEDINGS

OF THE

THIRTY-THIRD MEETING

OF THE

CANADIAN FOREST GENETICS ASSOCIATION

PART 1

Minutes and members' reports

Whistler, British Columbia July 22–25, 2013

> Editor J.D. Simpson

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

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Alberta Innovates Bio Solutions BC Ministry of Forests, Lands and Natural Resource Operations Brookfield Timberlands Management Centre for Forest Conservation Genetics Forest Genetics Council of British Columbia Genome British Columbia Genome Canada Natural Resources Canada SelectSeed Ltd. TimberWest University of British Columbia Vernon Seed Orchard Company

COMPTES RENDUS

DU

TRENTE-TROISIÈME CONGRÈS

DE

L'ASSOCIATION CANADIENNE GÉNÉTIQUE FORESTIÈRE

1^{re} PARTIE

Procès-verbaux et rapports des membres

Whistler, Colombie-Britannique 22–25 juillet 2013

> Rédacteur J.D. Simpson

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Sous le patronage de

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IN MEMORIUM

E. Kristian Morgenstern 1928 - 2008

Kris Morgenstern may have been short in stature but he was a giant in the world of forest genetics.

Kris was born in Königsberg, East Prussia (present-day Kaliningrad Oblast located between Poland and Lithuania). He immigrated to Canada in 1953 from Germany where he had graduated from forest ranger school. He worked at forestry jobs in the Upper Ottawa Valley, including the former Petawawa Forest Experiment Station, for several years before attending the University of New Brunswick where he graduated in 1959 with a BScF. In 1960 Kris was hired as a Research Scientist at Petawawa. He moved on to the University of Toronto and graduated in 1961 with a MSc in forest genetics and returned to Petawawa where he initiated and coordinated range-wide seed collections of black spruce that led to the establishment of 33 provenance trials across the country and in the United States. Kris pursued further education at the University of Hamburg and received his PhD in 1966. He returned to Petawawa and continued black and red spruce genetics research. He and three other Petawawa colleagues, including Kit Yeatman, coauthored a publication on plus tree selection based on data and experience from genetics tests he had established. In 1981 Kris returned to the University of New Brunswick as a professor in an industry supported Chair for forest genetics and tree improvement. He taught undergraduate and graduate courses in forest genetics and worked closely with the New Brunswick Tree Improvement Council. Many graduate students conducted research under his guidance. He retired in 1994 and returned to the Ottawa Valley near Pembroke where he became a consultant and worked in his woodlot.

Kris published widely in scientific journals and conference proceedings. He was quiet and mild mannered but was not afraid to interject when he felt that a point needed to be made or somebody's logic needed to be corrected. He published a book in 1996 entitled *Geographic Variation of Forest Trees: Genetic Basis and Application of Knowledge in Silviculture.* He also served for a couple of years as Secretary of the Committee of Forest Tree Breeding in Canada, a former body of the Canadian Forest Genetics Association.



Christopher W. Yeatman 1927 - 2013

Kit, as most everybody called him, was well spoken and passionate about forest genetics, tree improvement and forestry in general. His most notable advice was "Seed origin – first, last and always".

Kit was born in 1927 in Port Pirie, South Australia. In 1950 he graduated with a Bachelor of Science in Forestry and a Diploma in Forestry from Adelaide University and the Australian Forestry School. He traveled to England in 1951 and worked for the Forestry Commission in Edinburgh, Scotland. Kit immigrated to Canada in 1953. In fact he and Kris Morgenstern met on the ship that brought them to Canada. Kit began his forestry career in the Upper Ottawa Valley working for the summer of 1953 at Petawawa Forest Experiment Station and later that year accepted a position in the tree breeding program lead by Mark Holst. He continued his studies in forestry at the Yale School of Forestry and received Masters and PhD degrees in 1957 and 1966. His career interests were the genetics and breeding of jack pine and gene conservation. He coordinated the establishment of a series of range-wide jack pine provenance trials in the mid 1960's which are located throughout Ontario, in Quebec and New Brunswick and the Lake States. Much of what we know today about genetic variation in jack pine came from these experiments. Another of Kit's passions was genetic conservation locally, nationally, and internationally. In his younger days he did much of the breeding and cone collection in crowns of mature trees. This led to the publication of a tree climbing manual and the hosting of tree climbing courses at Petawawa. Kit retired from Petawawa in 1989 and spent two years in Thailand working with the Canadian International Development Agency's ASEAN-Canada Forest Tree Seed Centre at Muak-Lek, Saraburi, Thailand. He posthumously received the Queen's Diamond Jubilee Medal in 2013.

Kit was an enthusiastic supporter of the Committee of Forest Tree Breeding in Canada and Canadian Tree Improvement Association (the precursors of the Canadian Forest Genetics Association) where he was Executive Secretary for two years, Treasurer for six years, and Editor for 15 years. He published widely and was widely respected in the forestry community for his insights and friendly advice.



Dale Simpson Natural Resources Canada

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Chang-yi Xie Ministry of Forests, Lands and NRO Victoria, BC Tongli Wang University of British Columbia Vancouver, BC

Dr. Jack H. Woods Forest Genetics Council of BC Vancouver, BC

Dr. Alvin Yanchuk Ministry of Forests, Lands and NRO Victoria, BC

BUSINESS MEETING MINUTES

CFGA/ACGF 33rd Business Meeting Minutes

Sally Aitken chaired the 33rd Business Meeting of the CFGA/ACGF held at Whistler, British Columbia on Tuesday July 23, 2013. Seventeen Active Members were present.

384 Minutes of the 32nd Meeting

(as printed in the proceedings from the 32nd meeting, Part I)

Motion:That the minutes of the 32nd Business Meeting be approved as published.Moved by:Om RajoraSeconded by:Dave KoloteloCarried.Carried.

385 Membership

385.1 Active Membership

The names of four nominees were presented as follows:

Andy Benowic	z Alberta Environment and Sustainable Resource Development Edmonton, AB
Laura Gray	University of Alberta Edmonton, AB
Pia Smets	University of British Columbia Vancouver, BC
Barb Thomas	University of Alberta Edmonton, AB
Motion: Moved by: Seconded by: Carried.	That the nominated Active Members be duly elected. Jack Woods Yill Sung Park

386 Chair's Report

The 33rd meeting of the Association was held at Whistler, British Columbia. The meeting was jointly hosted by the University of British Columbia and BC Ministry of Forestry, Lands and Natural Resource Operations. Hence the two co-chairs.

The conference was joint with four organizations: Canadian Forest Genetics Association (CFGA), Western Forest Genetics Association (WFGA), International Union of Forest Research Organizations (IUFRO) Population, Ecological and Conservation Genetics (Working Group 2.04.01), and IUFRO Breeding and Genetic Resources of Pacific Northwest Conifers (Working Group 2.02.05). The success of this conference can be attributed to the organizing committee and the registrants who numbered 138.

The Organising Committee consisted of the following individuals: Logistics - Diane Douglas, Tongli Wang, Pia Smets, Sally Aitken, and Lee Charleson; Registration - Diane Douglas, Pia Smets, Jack Woods, Sally Aitken, and Lee Charleson; Finances - Jack Woods, Sally Aitken, and Diane Douglas; CFGA student sponsorship - Dave Kolotelo; Field trip - Michael Stoehr and Greg O'Neill; Scientific: CFGA - Alvin Yanchuk; WFGA - Andreas Hamman and Glenn Howe; IUFRO 2.04.01 - Wickneswari Ratnam, Reiner Finkelday, and Om Rajora; IUFRO 2.02.05 -Keith Jayawickrama, Michael Stoehr, and Tom Blush.

The support provided by the 12 sponsoring organizations was critical for meeting the financial obligations required to host a conference of this size at this venue.

We wish the organisers for the next meeting the best of luck.

Sally Aitken and Lee Charleson Co-Chairs

387 Treasurer's Report

Michele Fullarton was unable to attend but provided a financial statement for the period of June 2011 to June 2013 which was tabled for information and acceptance by the membership (see Attachment #1). A significant debit was \$ 15,000 provided to the conference organizing committee for initial operating funds. Jack Woods anticipated that the organizing committee would be able to return these funds to the Association. The statement shows a balance of \$ 6,146.23.

Motion:That the financial statement be accepted as presented.Moved by:Alvin YanchukSeconded by:Michael StoehrCarried.Carried.

388 Financial Contributions

Alberta Innovates Bio Solutions BC Ministry of Forests, Lands and Natural Resource Operations Brookfield Timberlands Management Centre for Forest Conservation Genetics Forest Genetics Council of British Columbia Genome British Columbia Genome Canada Natural Resources Canada SelectSeed Ltd. TimberWest University of British Columbia Vernon Seed Orchard Company

Motion:	That the	CFGA/ACGF	executive	of	the	33 rd	meeting	express	our sincere
	appreciati	on to these contr	ributors.						
Moved by:	Dave Kolotelo								
Seconded by:	Om Rajora								
Carried.									

389 Editor's Report

The proceedings of the 32^{nd} meeting were uploaded to the web site in August 2012. An announcement was sent to all Active members, Honorary members, registrants, and International addresses that the proceedings were available on the web site. A copy of the proceedings on DVD was sent to Canadian universities and other libraries.

Membership consists of: 19 Honorary, 86 Active, and 61 Canadian libraries.

Dale Simpson

390 Education Committee

Requests to nominate a student for sponsorship to the Forest Genetics 2013 meeting were forwarded to all seven Canadian universities with accredited undergraduate forestry programs. Student nominations were received from five universities with sponsored students and affiliations indicated below.

Kathleen Janz	University of Northern British Columbia
Jonathon Degner	University of British Columbia
Chen Ding	University of Alberta
Samuel Nsiah	Lakehead University
Mebarek Lamara	Laval University

In a review of past CFGA/CTIA student sponsorships it was determined that at least 30% of sponsored students were still involved in the general areas of forest genetics or tree improvement. This program is considered a success and although it is costly and its sustainability was raised during our business meeting the consensus was to continue to deliver the program in its current format. I'd like to thank Pia Smets for arranging flights, transportation, and accommodations for the students.

At this year's conference, both the CFGA and Western Forest Genetics Association (WFGA) each provided an award for best poster and best presentation. There was a joint evaluation committee consisting of Alvin Yanchuk, Sally John, Dave Kolotelo, Andy Bower, and Brad St. Clair. All students were eligible for all awards, but no student could win more than one award were criteria we used.

The CFGA Gene Namkoong award (\$300) is presented to a student for the best poster. The winner was Mebarek Lamara from Laval University for his poster "Coexpression network analysis of candidate genes related to wood physical traits in white spruce". The WFGA equivalent award went to David Montwé from the University of Alberta for his poster "Tree ring analysis indicates genetic component in drought response of Doudglas-fir".

The CFGA Carl Heimburger Award (\$500) is presented to a student for the best oral presentation. The winner was Adriana Suarez-Gonzalez from the University of British Columbia for her presentation "Population genomics in *Populus trichocarpa* (black cottonwood) reveals a candidate gene for local adaptation". The equivalent WFGA award went to Chen Ding from the University of Alberta for his presentation "Sub-optimal populations: Postglacial refugia-induced melting-pot of *Populus tremuloides*".

Dave Kolotelo

391 Working Group Reports

391.1 Tree Seed Working Group

The Tree Seed Working Group continues to publish a biannual Newsbulletin and both Dale Simpson and I graciously thank all that contribute articles. Since our last meeting we produced editions 54, 55 and 56 with number 57 in preparation and primarily representative of Abstracts from the TSWG workshop at the BC provincial Tree Seed Centre on July 22, 2013. Past editions of the Newsbulletin dating back to 1983 can be found here: <u>http://www2.gov.bc.ca/gov/topic.page?id=4E4651B3A01448FAB6F39ACAD1C348C1</u>. We try to encourage Newsbulletin participation by having a lottery at each workshop for those who have contributed articles since the last TSWG workshop. This year's grand prize winner was Fabienne Colas who won a copy of "Conifer Reproductive Biology" by Claire Williams. Other winners included Al Foley, Michele Fullarton, and Michael Stoehr.

The 2013 workshop focused on 'Reproductive Biology" and included a few more general presentations, but its focus was on North Okanagan lodgepole pine seed set issues which is BC's largest seed production problem. Thank you to all of the presenters for their time and effort. The venue was interesting as we converted part of our cone processing area to a theatre which created an appropriate atmosphere for the event. The Abstracts, author contacts and the pdf versions of Powerpoint presentations are available at: http://www.for.gov.bc.ca/hti/treeseedcentre/tsc/workshops&presentations/tswg 2013/index.htm

The program was followed by a tour of the BC provincial Tree Seed Centre.

I'd like to thank the unsolicited sponsorship received from the Vernon Seed Orchard Company and SelectSeed Ltd. The workshop was successful with 48 people attending, although a sombre element was present with the recent passing of Tim Lee who managed the Vernon Seed Orchard Company. Tim was a strong tree improvement advocate, friend to many, and one who could always be counted on to do the right thing. The workshop was dedicated to the memory of Tim Lee.

Dave Kolotelo Chair

392.2 Wood Quality Working Group

Alvin Yanchuk, Chair, reported that the Working Group has been inactive for a number of years. He pointed out that at past conferences it has contributed to promoting wood quality by organizing workshops. He suggested that with no strong leadership it may be appropriate to discontinue the Working Group.

Motion:That the CFGA/ACGF Wood Quality Working Group be terminated.Moved by:Alvin YanchukSeconded by:Om RajoraCarriedCarried

393 Old Business

393.1 New Logo (former business meeting items 367.2 and 379.2)

Dale Simpson reported that at the 2008 meeting in Quebec City that a motion was passed to change the Association's name from Canadian Tree Improvement Association / L'association canadienne pour l'amélioration des arbres to Canadian Forest Genetics Association /

L'association canadienne génétique forestière. A suggestion was made to revise the Association's logo to reflect this. At the 2011 meeting in Thunder Bay, Paul Charrette presented two versions for consideration. One consisted of the Association's name in bilingual format inserted within the maple leaf of the current logo. The second version contained the Association's name in bilingual format in a circular format around the outside of the maple leaf. It was agreed that the Active and Honorary Members be polled as to which version was preferred. The poll resulted in 93% preferring the second version which was used for this conference. It was suggested that the logo be made available on the web site.

 Motion:
 That the logo of the CFGA/ACGF be placed on the web site as a downloadable file.

 Moved by:
 Jean Bousquet

 Seconded by:
 Sally John

 Carried
 Carried

394 New Business

394.1 Student Sponsorship Program

When the organizing committee began developing a budget for the conference they were concerned about the cost of sponsoring students to attend, particularly owing to the cost of the venue and concern about attendance. As well there was concern with regard to non-Canadian delegates subsidizing this program through their registration fees. Several in attendance at the business meeting who were involved in organizing previous conferences felt that the program is a success (over 30% of sponsored students have continued in a genetics/tree improvement career), it was part of the conference, and the cost was built into the registration fee. Delegates should not feel that they are being gouged to cover the cost of this program as it is really just another expense item for the organizing committee. The consensus was that the program is successful and should continue.

395 Future Meetings

395.1 Location of the 2015 Meeting

Yuhui Weng confirmed that the meeting will be in Fredericton, NB.

395.2 Location of the 2017 Meeting

Edmonton, AB.

395.3 Location of the 2019 Meeting

Quebec, possibly.

396 Election of New Executive

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

Chairperson:	Michele Fullarton NB Department of Natural Resources	
Vice-Chairpersons: Symposium	Yill Sung Park Canadian Forest Service	
	Yuhui Weng NB Department of Natural Resources	
Vice-Chairpersons: Local arrangements	Kathy Tosh and Tom Ng NB Department of Natural Resources	
Treasurer:	Michele Fullarton NB Department of Natural Resources	
Editor:	Dale Simpson Canadian Forest Service	
Executive Secretary:	Dale Simpson Canadian Forest Service	

397 Adjournment

Motion:	That the 33 rd business meeting of the CFGA/ACGF be adjourned.
Moved by:	Annette van Niejenhuis

Attachment # 1

CFGA/ACGF Financial Statement June 2011-June 2013

Holdings – June 10, 2011	
Cash balance	\$ 22,374.94
Total Holdings	\$ 22,374.94
Credits (June 2011-June 2013)	
Interest (on chequing account balance)	\$ 3.02
Total credits	\$ 3.02
Debits	
Student awards (2011 CFGA) Seed money for CFGA 2013 Mailing (Proceedings and CFGA documents) Website (hosting and annual fees for domain) Draft fee	\$ 800.00 \$ 15,000.00 \$ 150.12 \$ 274.11 \$ 7.50
Total debits	\$ 16,231.73
Holdings – June 17, 2013	
Cash balance	\$ 6 146 23
Total Holdings	¢ 0,140.23
	φ 0,140.23

ACTIVITY REPORTS FROM ACTIVE MEMBERS

NEW BRUNSWICK TREE IMPROVEMENT UPDATE

Michele Fullarton and Yuhui Weng

NB Department of Natural Resources Kingsclear Provincial Forest Nursery 3732 Route 102 Island View, NB E-mail: Michele.Fullarton@gnb.ca

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

The New Brunswick Tree Improvement Council (NBTIC) was formed in 1976. It is comprised of industrial, scientific, and government agencies. The objective of the Council is to provide well adapted, genetically improved seed for the reforestation of Crown land. The Department of Natural Resources (DNR) coordinates the NBTIC activities and is responsible for all breeding and growing of tests. NBTIC industrial members plant, maintain, and measure genetic tests. DNR is responsible for growing all seedlings for reforestation of Crown land. New Brunswick has a total area of 7.3 million hectares (ha), with about 3 million ha of Crown forest land. The Seedling Production section of DNR is currently growing 25 million seedlings annually for planting, with all the seed coming from seed orchards. This equates to about 11,700 ha planted annually for 2009–2011. The tree improvement program continues to focus on the main reforestation species: white spruce (*Picea glauca*), black spruce (*Picea mariana*), red spruce (*Picea tubens*), Norway spruce (*Picea abies*), and white pine (*Pinus strobus*). Other species include balsam fir (*Abies balsamea*), jack pine (*Pinus banksiana*), and tamarack (*Larix laricina*).

SEED ORCHARDS AND SEED PRODUCTION

Orchard establishment is now complete for the main reforestation species, with a total of 304 ha comprising seven species (Fig. 1).

These orchards are meeting the reforestation demands for Crown land. Seed production in 2011 was fairly good, with almost 140 million seeds collected, the majority of that from first- and second-generation white spruce orchards. In 2012, however, there was a poor crop, with fewer than 43 million seeds collected. The seed collected from DNR's black spruce second-generation orchards was collected by clone. This will give us the opportunity to provide a better clonal mix to the nursery, increasing volume gains in plantations and maintaining an acceptable diversity level. In 2013, indications in the orchards are that there will be high cone production in our high value second-generation white spruce orchards. Trees in these orchards were injected with $GA_{4/7}$ in 2012. In 2013, the DNR nursery seeded over 4 million white spruce using second-generation orchard seed.





TREE BREEDING, TESTING AND SELECTION

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

In the NBTIC program, 349 tests have been planted on 400 ha located throughout the province. In 2012 and 2013, there were no new tests established. The current focus is on completing second-generation breeding and testing.

NBTIC DATA ANALYSIS

The data analyst position has continued to be funded by the NBTIC. A total of 8 NBTIC test series were analyzed in 2011 and 2012 which included 2 progeny tests, 4 full-sib progeny tests, and 2 gain tests. In addition, 3 papers published in 2011 and another 4 papers were written and published in 2012 by the data analyst, in collaboration with colleagues at CFS-Atlantic and the University of New Brunswick.

Other than data analysis and research, the data analyst led the modification of the white spruce breeding strategy. The NBTIC has the most advanced breeding programs across Canada (i.e., completion of second-generation black spruce and jack pine and initiation of second-generation white spruce). Thanks to the scientific leadership provided by Canadian Forest Service, various breeding plans (such as sub-line, unbalanced mating design, and others) have been applied to the NBTIC program to maximize gains. Now, with the availability of data, the benefits of applying these plans have been re-evaluated based on the concept of balancing gain and diversity. Results of these analyses, paired with a literature review and NBTIC panel discussion, guided the modification of the white spruce breeding strategy. Important modifications include: 1) sub-line is kept, not as a unit for inbreeding control but for managing thebreeding work, 2) a double pair-mating design is used to replace the unbalanced, positive assortative mating, 3) more focus on with-in family selection, and 4) multi-varietal forestry is incorporated into the

strategy. It is important to note that these changes may not be optimal for other species and circumstances.

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NATIONAL TREE SEED CENTRE

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The mission of the National Tree Seed Centre is to safeguard Canada's forest genetic resources in the face of climate change and other threats by acquiring, evaluating, preserving, and providing a national collection of forest genetic resources to assist in securing the forest biological diversity that underpins the sustainable development of Canada's forests. The Centre aims to store seed of tree and shrub species from throughout their ranges in Canada. On-going activities include collecting and processing seed; conducting tests such as germination, moisture content, and 1000-seed weight; and setting up and evaluating research trials. This report covers activities during 2011 and 2012.

In order to expand the geographic sampling for species and to add new species to the inventory seed is obtained by collecting, donation, and purchase. Collecting is focused on obtaining seed from natural populations thereby capturing the natural genetic variation that is present within species. Most of the collection effort is focused on collecting seed from a minimum of 15 trees in a population and keeping the collections separate by tree. Many of the accessions donated or purchased are bulk collections from natural populations. Seed is primarily stored in two categories: Seed Bank where seed is available for research and Gene Conservation. During the two-year period over 105 seedlots from 14 species were collected and 324 seedlots from 37 species were donated or purchased. Almost 6,700 seedlots from 148 species exist in Seed Bank. The Gene Conservation collections from species or populations that are unique or threatened as well as samples from seedlots that were collected for Seed Bank. The USDA National Centre for Genetic Resources Preservation donated 94 ash seedlots for back-up storage as per a storage agreement between the USDA and the Seed Centre. As well 22 *Pinus albicaulis* seedlots from three National Parks in Alberta and British Columbia were donated for back-up storage. About 4,800 seedlots from 50 species are stored in Gene Conservation.

Germination testing of seedlots in storage is an integral part of the Seed Centre's annual activities. For most species, germination tests are conducted every ten years. Over 1,950 tests were completed during the two-year period. The data not only provide an indication of the quality of each seedlot but when combined with previous test data they provide an indication of the long-term storage potential for a species. This is particularly important when seed is being stored long term for genetic conservation purposes. There are some seedlots that have been in storage for over 60 years. Seed storage potential was evaluated for 34 species. Seedlots of *Picea mariana* and *Pinus resinosa*, in storage for over 40 years, had the highest germination of 96.5% and 98.0%, respectively. *Betula alleghaniensis* seed stored for almost 40 years germinated at 83%.

Operational research is an important activity. Evaluating germination test data is one aspect as discussed above. Another activity is setting up storage trials to evaluate storage potential of seed stored at various temperatures. Four-year results from a *Thuja occidentalis* storage experiment revealed that seed stored at 4° C exhibited a steady decline in germination and an increasing proportion of low vigor germinants. In contrast seed stored at -20°C only had a slight decline in germination. Willow seed do not have a seed coat and are sensitive to their environment. Thirteen-year results from a storage experiment where *Salix bebbiana*, *S. discolor*, and *S. eriocephala* seed were stored at -20°C and at -145°C in the vapor phase of liquid nitrogen demonstrated that seed stored best in liquid nitrogen vapor.
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RECENT ADVANCES IN TREE REPRODUCTION, SEED AND SEEDLING PRODUCTION IN QUEBEC

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The use of forest plantations with high morpho-physiological quality plants produced from genetically improved seeds can increase the productivity of Quebec's forests. Each year, more than 120 million seedlings are produced in the 19 forest nurseries in Quebec (6 public and 13 private). For environmental and financial purposes, Quebec's forest nurseries must constantly innovate to develop new techniques that will optimize irrigation and fertilization according to seedlings' stages of growth. These techniques allow not only to meet the standards and criteria of morpho-physiological quality of seedlings grown in Quebec, but also to reduce the leaching of minerals, thereby helping to preserve groundwater quality and reduce costs related to the use of fertilizers.

Continuous technology transfer to Quebec forest nurseries and technical support associated with it are among the priority activities of our team. Whether in technical committees or visits, this transfer allows forest nurseries to incorporate the latest technological innovations. These improvements are the result of a synergy between our team and other researchers and practitioners from different national and international organizations.

SEEDS

Larix Indoor Seed Orchard

The production of hybrid larch (HL) seedlings faces a problem of height uniformity in the production of stecklings, namely the production of small and bushy seedlings where it is difficult to collect cuttings. Currently, all HL seedlings are produced from cuttings due to the lack of seeds. The seeds are produced by mass pollination, in an indoor orchard, using a polymix of pollen whose composition is variable from year to year. For seed extraction, cones are mixed, making it impossible to know exactly the parents of the seeds. Much work has been done to better understand the production of stunted plants. Two irrigation regimes were tested but they did not have a significant effect on the variability of plant height; but seedlot had. In the different lots, we find unfavorable genetic combinations resulting in the production of stunted plants. Cones were harvested by maternal genotype and seedlings were produced. Some maternal clones produced high proportions of stunted plants, so they were eliminated from the orchard. To solve the problem of the stunted plants, it would be necessary to make controlled crosses and to characterize the seeds and the seedlings produced.

Water Activity

Water activity (a_w) measurement is now part of routine quality tests conducted at Berthier Tree Seed Centre. Water activity helps to improve management and control of the water status of seedlots while greatly reducing the energy costs associated with seed extraction, mainly the final drying. See Colas et al. 2012 for more details.

The non-destructive character of a_w measurement allows for dynamic monitoring of the water status of seedlots in conservation banks (short or long term). When a change of a_w is detected, a germination test will be conducted. Then, the lot will have its water activity adjusted using a dryer managed by a_w , and reintroduced into the bank with the updated data. If a_w is unchanged, it is considered that the lot will not have undergone ageing and germination will not be changed, making a germination test unnecessary.

Work continues to develop a new container in collaboration with the Centre for Mineral Technology and Plastics. A first prototype was designed, but adjustments to its composition were needed. New tests will begin in summer 2013.

SEEDLING PRODUCTION

Root Growth, Plug Cohesion, Mineral Nutrition, and Carbohydrate Content of 1+0 *Picea mariana* Seedlings in Response to a Short-Day Treatment

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

Adaptation to Climate Change: The Case of Extreme Winter in Forest Nurseries in Quebec

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

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

If in mid-December snow accumulation is insufficient, the nursery could apply artificial snow to protect tree seedlings from freezing temperatures. Our results show that the presence of a snow cover (5-10 cm) is an excellent insulator that protects tree seedlings against low temperature extremes. See Lamhamedi 2012 and Lamhamedi et al. 2012 for more details.

Relationships Between Cold Hardiness, Root Growth Potential and Photosynthesis of Nursery-Grown White Spruce Seedlings

White spruce (*Picea glauca*) is the third species used in Quebec reforestation programs. The acquisition of cold hardiness at the beginning of autumn is a key factor to develop frost resistance during winter. At the end of their second growing season under standard cultural practices in a forest nursery, seedlings were hardened under natural conditions of photoperiod and temperature. After being subjected to artificial freezing tests on four sampling dates during the fall, the seedlings were compared for cold tolerance and recovery of gas exchange.

The effect of artificial freezing treatments on seedling photosynthesis varied with frost intensity and degree of needle hardening. The mass of new roots formed over a 21-day cultivation test period following the freeze tests was positively related to the photosynthetic capacity of the seedlings and negatively related to the proportion of damaged needles.

These results imply that autumnal cold damage to needles directly affects seedling root growth and emphasizes the importance of maintaining seedlings in an environment favourable for photosynthesis during the fall to promote root growth. See Carles et al. 2011c for more details.

An Operational Method for Estimating Cold Tolerance Thresholds of White Spruce Seedlings in Forest Nurseries

Cold damage leads to the rejection of millions of seedlings each year in northern forest nurseries. Nursery managers need a procedure to estimate the degree of hardening at a specific time in order to make decisions to avoid seedling loss. Estimates based on variables that are quick and easy to measure such as thermal time, a variable quantifying the cumulative effect over time of temperatures below a given threshold, and apical dry mass ratio (DM/FM) hold particular promise. In this study, two-year-old white spruce containerized seedlings were subjected to artificial freezing tests. The progression of DM/FM during the fall was determined and the thermal time was quantified by calculating hardening degree days (HDD) based on air temperature and a specific threshold for white spruce. Results established that cold tolerance thresholds are associated with easily identifiable specific DM/FM and HDD values for nursery x species x seed source combinations. See Carles et al. 2012 for more details.

Differences in Growth and Mineral Nutrition of Seedlings Produced From Ten White Spruce Seed Orchards

To meet the needs for improved white spruce seeds in the province of Quebec, 17 first-generation seed orchards (SO) were established. These SOs are located in different bioclimatic regions and contain seed trees originating from geographically and genetically distinct sources. To evaluate the influence of SO on seedling growth and morphology, seedlings produced from seeds originating from the ten most commonly used first-generation SOs were raised under similar nursery conditions. Tissue nutrient concentrations of seedlings changed similarly for all the different seed sources during the second growing season. At the end of the second growing season, only shoot phosphorus concentrations were significantly different. When modeled with a logistic function, there was a significant difference between height and diameter growth curve parameters of seedlings from distinct SOs during the second growing

season. These differences led to significant differences in the height and shoot dry mass of the seedlings at the end of their second growing season, but not in their diameter or root dry mass. The ten SOs were clustered in two groups according to the above-ground characteristics of their progeny. This limited amount of morphological diversity suggests that expanding the size of the present seed zones may be an appropriate course of action for white spruce seed production in Quebec. See Carles et al. 2011a for more details.

Genetic Parameters of Morphological and Physiological Characteristics of Containerized White Spruce Seedlings

The root systems of containerized seedlings must be sufficiently developed and have adequate root plug cohesion to permit handling and the planting of the seedlings with minimal root damage. Genetic variability in morphological and physiological seedling characteristics of 75 open-pollinated white spruce families was estimated to determine whether genetic selection for improved seedling root systems is possible. Seedlings were grown for 2 years under standard cultural practices in a forest nursery. Gas exchange measurements and seedling morphological characteristics (height, diameter, shoot and root dry mass, and root to shoot ratio) were measured at the end of the two growing seasons whereas seedling mineral (N, P, and K) status was assessed at the end of the first growing season. Genetic parameters (heritabilities (h²) and genetic correlations) were estimated for every seedling characteristic and a strong genetic control associated with large genetic variation was observed at both family $(0.20 \le h_f^2 \le 0.88)$ and individual (0.21 \leq h²_i \leq 0.97) levels. A single, late season measurement of physiological characteristics did not reveal a physiological basis for family variability in seedling root growth. Nevertheless, family variation was large enough to permit genetic improvement of two-year-old seedling juvenile morphological characteristics. Strong, positive genetic correlations enable us to foresee that using root collar diameter is an effective method for indirectly selecting white spruce families with heavier root systems. See Carles et al. 2011b for more details.

SOMATIC EMBRYOGENESIS

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

Priorities for research and development in the field of SE are focussed on the morpho-physiological characterization of somatic clones (SC) in the nursery, the integration of stock somatic plants in the propagation chain, the correlation between the performance of nursery clones and field-testing clones, assessing the quality of a new generation of seed produced from SC, and simulating the productivity of clones.

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

The characterization of controlled crosses between black spruce somatic clones was completed. The seedlings produced have the same morpho physiological quality as seedlings whose seeds are from the natural forest or seed orchards. These results show that, after the selection of the best individuals, clonal tests can be converted into seed orchards for the production of high genetic quality seeds. Furthermore, these results will allow for an acceleration of the breeding programs with the rapid introduction of new materials of high quality.

Genetic Parameters and Clonal Variation in Growth and Nutritional Traits of Containerized White Spruce Somatic Seedlings

Clonal forestry can significantly increase forest productivity and its establishment requires a high level of clonal variation to maximize genetic gain and diversity. An evaluation of the genetic parameters of somatic clones at a juvenile stage is necessary to better understand the amplitude of clonal variability and the degree of genetic control during two growing seasons (1+0 and 2+0) under forest nursery conditions.

The analysis of variance of white spruce clones showed a highly significant clonal effect for the majority of the growth characters at the end of both growing seasons and for the mineral status at the 2+0 stage. Our results reveal that height exhibits high clonal heritability which remained stable over the two growing seasons ($H_c^2 = 0.60$). Strong genotypic and phenotypic correlations were observed between height and diameter at the end of the first growing season and between height and the rest of the growth characters at the end of the second growing season. The strong clonal variation, genetic control, and genetic correlation particularly of height found in this study indicate that selection of the best performing clones is possible for intensive forest management. These encouraging results suggest that it is possible to make an early selection of SCs according to several specific morpho-physiological criteria in the nursery. This will help to significantly reduce management costs and clonal test installation in reforestation sites. See Wahid et al. (2012 a,b) for more details.

CONCLUSION

The production of high quality seedlings is the result of the acquisition and development of new knowledge and its continuous transfer and implementation at an operational scale. In close collaboration with researchers and graduate students from different research centres, the seed and plant production research group has emphasized the transfer of expertise and knowledge and the integration of research results in Québec forest nurseries. This transfer has been realized through scientific and technical publications, visits and conferences, as well as workshops (especially Carrefour Forêt Innovations 2011) and training sessions on particular themes. During these events, 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 Québec, as well as managers who work in the field of artificial regeneration.

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TREE IMPROVEMENT PROGRESS AT THE DIRECTION DE LA RECHERCHE FORESTIÈRE

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WHITE SPRUCE TREE IMPROVEMENT André Rainville

During the past two years, efforts in clonal forestry, initiated in 2007, continued while measurements at 2 years of age in the nursery and 4 years in two clonal tests were collected. Analysis revealed that the relative performance of clones seems to be stable over time. Observations taken in the spring of 2013 have shed light on the necessity to evaluate clonal variability in terms of nutrition needs, mainly boron concentrations in soils and foliage since symptoms of deficiency were detected.

Wood cores from trees in older genetic tests were used to evaluate wood properties of selected trees and the efficiency of early evaluation methods using the Hitman ST300, which measures the acoustic circulation speed. Since both showed a strong correlation with microfibril angle and stiffness at an individual level, we expect to rank trees for these two characteristics in the future.

CLIMATE CHANGE ADAPTATION André Rainville

In the spring of 2013, a preliminary "assisted migration" project was initiated at a small scale and only for white spruce (*Picea glauca*). The project was created in collaboration with ecophysiologists (Mohammed S. Lamhamedi from Ministère des Forêts, de la Faune et des Parcs (MFFP) and Hank Margolis from Laval University) and geneticists (Jean Beaulieu from Canadian Forest Service (CFS) and Jean Bousquet from Laval University). It aims to evaluate the adaptation capacity of white spruce provenances (seed orchards), first during their production phase in the nursery (work conducted by a student, Lahcen Benomar) and second after having been transferred to 9 sites (3 south-north transects of 3 tests each), some representing extreme conditions. Phenology measures (growth, bud flush burst, bud set, flowering, etc.) will be used to validate the new transfer model.

LARCH TREE IMPROVEMENT Martin Perron

In spring 2012 and 2013, two progeny trials of full-sib families (*Larix decidua* (E), *L. kaempferi* (J), ExJ, and JxE) were established in order to better understand the genetic cause of heterosis and to identify bestadapted hybrid types for the sugar maple-yellow birch and balsam fir-white birch bioclimatic regions.

For *Larix xmarchlinsii*, an estimated genetic gain of 85 m^3 ha⁻¹ in merchantable volume is expected 30 years after plantating with our production population (20 Japanese larch and 20 European larch) in comparison with the original trials (Perron 2011).

JACK PINE TREE IMPROVEMENT Mireille Desponts

In 2011, the last *Pinus banksiana* clonal seed orchard of the network was established to supply the Eastern Quebec seed zone. Those clonal seed orchards, were created using selections made in seedling orchards and produced an additional genetic gain of 5% for height. Second-generation full-sib crossing is still being conducted in a second breeding population.

BLACK SPRUCE TREE IMPROVEMENT Mireille Desponts

In spring 2011, two full-sib *Picea mariana* second-generation progeny trials were established in Gaspe. Controlled crosses for another population for the north-east region were completed in 2012. Furthermore, sampling and laboratory measurements were completed in order to estimate genetic parameters of wood properties in our breeding populations. Selection for density and modulus of elasticity in clonal trials has been conducted since 2013.

NORWAY SPRUCE TREE IMPROVEMENT Marie-Josée Mottet

Recent studies from 10-year-old tests of open-pollinated and full-sib *Picea abies* families show strong genetic effects for white pine weevil resistance (*Pissodes strobi*). General combining ability (GCA) was the main genetic effect, but specific combining ability (SCA) represents a large proportion of genetic variation. These results confirm that breeding Norway spruce for weevil resistance could be successful, and emphasize the need for an optimal selection procedure to exploit both GCA and SCA. Two clonal tests were established in 2013 with trees originating from controlled crosses made between weevil-resistant parents selected by CFS, and replicated by J.D.Irving, Limited using somatic embryogenesis techniques. Finally, since planned Norway spruce reforestation in Quebec will be mainly concentrated in the Lac-Saint-Jean area, future breeding work will also involve the identification of seed sources for northern regions.

HYBRID POPLAR IMPROVEMENT PROGRAM Pierre Périnet and Marie-Josée Mottet

For the 2011 to 2013 period, the Quebec hybrid poplar program focused on the selection of advancedgeneration parents of *Populus trichocarpa* (T), *P. maximowiczii* (M), and *P. deltoides* (D). Parental trees, from our species collections and trials, were selected and successfully propagated through cuttings. Fieldcollected branches with staminate inflorescences were forced in a greenhouse for pollen production. Pollen was stored for long-term conservation. In 2012, 55 families of TxT and MxT were obtained for progeny testing. Seeds from these progenies were stored in the seed bank for further evaluation, particularly, to achieve clonal selection for biomass and bio-energy production.

In 2012, three experimental plantations were established in the Bas-Saint-Laurent and Lac-Saint-Jean areas to promote and demonstrate the short-rotation-intensive-culture (SRIC) hybrid poplar crop concept for biomass production. The general objective of this study was to compare coppicing ability and biomass yield among different vigorous poplar clones already available in Quebec.

Several hybrid poplar populations from the 1996, 1998, and 2004 breeding programs are still under evaluation through many clonal tests established in different regions for local adaptation, vigor, and *Septoria* resistance. Promising clones of MxT, MxB, MxD, and MxN are expected to be released soon from these programs.

Over the last few years, five *P. maximowiczii* x *P.nigra* (MN) clones were released for commercial planting in sugar maple regions 1, 2, and, 3 of southern Quebec. Seven more clones, hybrids of MxT, RxM, MxB, DxM, and MxN, were also released for balsam fir-yellow birch and balsam fir-paper birch regions.

A *Septoria* canker study was undertaken to develop a management strategy reducing primary infection in nurseries and plantations. In another experiment, inoculation techniques with *Septoria musiva* on poplar shoots were compared in greenhouse conditions.

OPTIMIZING TREE IMPROVEMENT METHODS Martin Perron and Mireille Desponts

Accurate assessment of the transferability of a character from one generation to another is crucial for advanced generation breeding programs. It contributes to the choice of the adopted improvement strategy and the estimation of predicted genetic gains. Genetic improvement of wood characteristics of forest trees is increasingly considered for commercial species in plantations around the world. However, the evaluation of these traits requires considerable resources. Our simulation study with tamarack (*Larix laricina*) and black spruce data sets identified an optimized method to reduce sampling and accurately estimate genetic parameters of wood properties (Perron et al. 2013.)

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PROGRESS IN FUNCTIONAL, ECOLOGICAL AND EVOLUTIONARY FORESTRY GENOMICS RESEARCH AT UNIVERSITÉ LAVAL

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INTRODUCTION

This report summarizes our efforts and results obtained in forest and environmental genomics over the 2011–2013 period. The second phase of our major spruce genomics program *Arborea* (lead by J. Mackay and J. Bousquet) was completed in 2011 and has been followed by the undertaking of the SMarTForests project - Spruce Marker Technologies for Sustainable Forestry under the Applied Genomics Program of Genome Canada and the additional financial support of Génome Québec and Genome BC. The project is lead by J. Mackay, J. Bohlmann, J. Bousquet, and A. Yanchuck and it also involves other research scientists from Université Laval (N. Gélinas), the University of British Columbia (K. Ritland, S. Jones, and G. Bull), the University of Alberta (J. Cooke), the Canadian Wood Fibre Centre (CWFC) (J. Beaulieu), the Canadian Forest Service (CFS) (N. Isabel, A. Séguin), the Ministère des Ressources naturelles du Québec (MRNQ) (M. Perron, A. Rainville, and M. Desponts), J.D. Irving, Limited (G. Adams), and several international collaborators. Emphasis has been placed on expanding spruce genomic resources by sequencing the genome of *Picea glauca* and developing genomic prediction systems for assisting breeding operations including transcriptome-wide association studies and genomic selection proof-of-concept demonstrations in *P. glauca* and *Picea mariana*. The characters considered are diverse and include pest resistance, wood quality, growth, and phenology.

Under the European Union Framework Program and the leadership of several European research organizations and the leadership of M.T. Cervera (INIA, Spain), the ProCoGen large-scale project was also launched in 2012 where J. Mackay and J. Bousquet are involved in collaborative research including comparative genomics and genome sequencing and mapping of European conifers. In collaboration with C. Plomion (INRA-Bordeaux, France), a textbook presenting the state-of-the-art research conducted worldwide on conifer genomics and related genetics and tree breeding aspects was also published (Plomion et al. 2011).

During the last period, the group included N. Pavy as senior research associate and several postdoctoral researchers: S. Hornoy, J. Prunier, G. De Lafontaine, E. Campagnac, A. Azaiez, A. Atangana, and B. Pelgas, P. Lenz and T. Doerksen based at CFS. Many graduate students have been involved in the diverse research projects, including at the MSc level: J. Pickford, G. Sarasin (now manager of the B-Adapt project funded by CIDA for the African Model Network in Cameroon), P. Mutonkole, R. Serra, M.B. Nadeau, E. Yusufu Kachaka, A. Bernard, É. Warren, G. Tessier, É. Fortin, G. Germanos, M. Lemieux (now at Nova Scotia Department of Natural Resources), P. Talbot (now at the National Capital Commission); and at the PhD level: S. Koné (now assistant professor at the University of Bamako), N. Rasool (now assistant professor at the Univ. of Kashmir), C. Rochon (now research scientist at CFS-Québec), M. Da Silva Folli (now postdoc at the Univ. State of Espírito Santo), G. Wani Ahmad, J. Umba, T. Lubalega, E. Kimpembe Ndinga, A. Ntumba Tshinyama, G. Sene, G. Adoumandjali, F. Bedon, J.

Godbout (now postdoc at CFS), J. Prunier (now with SMaRTForests), B. Cinget, B. Dufils, E. Raherison, J. Sena, M. Lamara, A. Sahli and J.P. Verta. Several of these students were or are currently co-supervised by adjunct professors J. Beaulieu, N. Isabel, and A. Séguin from CFS.

PHYLOGEOGRAPHY AND GENETIC DIVERSITY AT THE GENOME LEVEL Jean Bousquet

Over the last two years, as part of our ongoing work of the phylogeographical atlas of conifers, several range-wide phylogeographical studies have been completed, including the cpDNA phylogeography of Tsuga canadensis (Lemieux et al. 2011), the mtDNA and cpDNA phylogeography of Pseudostuga menziesii (Wei et al. 2011), that of several mesoamerican Abies species (Aguirre-Planter et al. 2012), and the phylogeography of Picea mariana based on gene SNPs (Prunier et al. 2012). For widely distributed species, these studies have revealed the existence of pronounced large-scale geographical structures including suture zones, with more prononced geographic differentiation for mtDNA than for cpDNA or nuclear gene SNPs. Current range-wide studies are targeting Abies balsamifera and Larix laricina. At the interspecific level, large-scale natural introgression beyond the current zone of contact between Pinus banksiana and Pinus contorta has also been revealed (Godbout et al. 2012). The spontaneous hybridization between exotic poplars and natural populations in the agro-forestry landscape of the Canadian Prairies has also been estimated, though rates were typically low due to the efficient buffering of the pollen cloud by local indigenous stands (Talbot et al. 2012). Phylogenies, including all species worldwide, have been reconstructed for the genus Picea (Bouillé et al. 2011) and the genus Abies (Aguirre-Planter et al. 2012) with interesting implications for speciation processes in conifers. Simple protocols proposed for barcoding of plants have also been tested genus-wide in Fraxinus, further indicating their limited success for tree species (Arca et al. 2012). These studies have involved various collaborators including the teams of J.P. Jaramillo-Correa and L. Eguiarte (Univ. Autonomous of Mexico), D. Hinsinger and N. Frascaria-Lacoste (Univ. of Paris at Orsay), J. Vargas-Hernández and J. Lopez-Upton (Programa Forestal – Campus Montecillo, Mexico), F.C. Yeh (Univ. of Alberta), J. Beaulieu and N. Isabel (CFS), B. Jaquish (BC Ministry of Forests), and W. Schroeder (Agric-Can, Saskatoon).

Under the Arborea and SMarTForests projects, various genome scan approaches based on the highthroughput genotyping of a large number of SNPs from genes distributed over the genome of spruce taxa have been deployed over the last two years to test research hypotheses in relation to association and population genomics. A large-scale association genetics study and the investigation of relationships with gene expression have been conducted for wood characters in P. glauca (Beaulieu et al. 2011). The current work implicating the genotyping for many thousand genes aims at testing multi-gene prediction approaches such as genomic selection for growth and wood characters in breeding populations of half-sib families and also, full-sib families from advanced generation breeding in P. glauca and P. mariana, in collaboration with CWFC (team of J. Beaulieu) and MRNF (M. Desponts, M. Perron, and A. Rainville). An association genetics study for growth and phenology characters based on a candidate gene SNP approach has also been conducted for *P. mariana* (Prunier et al. 2013). The study indicates that the approach resulted in a much higher rate of positives that the traditional approach solely based on candidate genes. It also showed that cohorts of positive SNPs overlapped partially with the age of the material and their site location, indicating genotype x environment interactions and partially different gene sets being involved from age to age. A genome scan approach based on gene SNPs has also been used to monitor genetic diversity after the application of different selection intensities for growth characters in P. glauca, indicating little significant effect at the gene SNP level, which agrees with traditional views on the genetic control of quantitative characters (Namroud et al. 2012). Other scans of gene SNPs were aimed at identifying natural variation related to climate and of possible adaptive value in P. mariana (Prunier et al. 2011, 2012) or in Picea abies (Chen et al. 2012). These studies also represent pionneer work aimed at disentangling the recent effects of natural selection from more long-term historical effects on the extant large-scale geographical population structure. These studies implicated again various collaborators including M. Lascoux (Uppsala Univ.).

Other studies conducted in collaboration with N. Isabel and J. Beaulieu (CFS) aimed at developing basic knowledge and genomic resources regarding SNP diversity in conifer species, with implications for association studies and other genome scan applications. Linkage disequilibrium (LD) has been investigated for 100 genes in *P. glauca* and in a number of other conifers, showing quite variable levels

of LD between species and genes (Pavy et al. 2012a). Genotyping chips containing many thousands of gene SNPs have also been developed for *P. glauca*, including the estimation of SNP sharing frequency among spruce species (Pavy et al. 2013). Sharing was significant and varied according to phylogenetic distance, minor allele frequency in *P. glauca* natural populations, and putative bottlenecks in endangered species. One such useful application of SNP transfer from *P. glauca* for a population genomics study in *P. abies* has recently been shown (Chen et al. 2012). The transferable SNPs are currently being used in a number of population genomics and gene/QTL mapping studies for various spruce species worldwide. These efforts implicate several teams including J.P. Jaramillo-Correa (Univ. Autonomous of Mexico), M. Lascoux and M. Fladung (Uppsala Univ.), P.K. Ingvarsson (Univ. of Umea), S.A'Hara and J. Cottrell (British Forestry Commission), and K. Ritland and S. Aitken (Univ. of British Columbia),

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

FUNCTIONAL GENOMICS OF WOOD TRAITS AND INSECT RESISTANCE John Mackay

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

Our activities in the area of large-scale gene discovery and transcriptome profiling have led to the development of a catalogue of 27,720 *P. glauca* genes, which includes 23,589 different full-length complementary DNA isolates (Rigault et al. 2011). From this uniquely comprehensive resource, an oligonucleotide microarray (MA) was developed representing 23,853 genes (Raherison et al. 2012). With this microarray, we developed a database of reference gene expression levels for diverse tissues (PiceaGenExpress) and are completing a thorough characterization of the wood transcriptome in several conifers. This reference resource may be used to study any subset of genes; for example, it served to define co-expression groups among sequences that are unique to conifers.

We have used the unique biology of the conifer megagametophyte to develop a novel experimental approach with which we are characterizing heritable variation in gene expression within individual trees (Verta et al. 2013). The megagametophyte is a haploid seed tissue derived from the same maternal gamete as the embryo. Expression alleles that segregate according to Mendelian ratios were found in 796 to 918 genes within individual *P. glauca* trees by microarray (MA) profiling. The study showed higher levels of gene expression diversity in genes involved in adaptation, such as genes related to responses to abiotic stresses. A co-segregation analysis of the expression data was used to efficiently reconstruct regulatory networks without requiring controlled-crosses. A network linking 180 different genes was related to the regulation of protein synthesis and identified putative regulators previously uncharacterized in plants.

Transcriptome profiling has also been very efficient in the recent discovery of a gene with a major effect in natural resistance to spruce budworm (*Choristoneura fumiferana*). A naturally-occuring resistance phenotype was described in *P. glauca* by a research collaborator (É. Bauce, Univ. Laval) and was linked to high concentrations of phenolic compounds (acetophenones), piceol, and pungenol in the foliage. We used microarray profiling to show that a sequence encoding a glycosyl hydrolase (PgbetaGlu-1) was very strongly expressed in the resistant trees and close to background in the non-resistant trees. Molecular analysis of the RNA transcripts and genomic DNA sequences indicate that the differences in expression may be caused by a heritable variation. These findings were presented at the Plant and Animal Genome Conference in San Diego and a manuscript is in preparation. This discovery may impact on integrated pest management including tree breeding and the detection of susceptible trees in forest plantations or natural stands. Comparing groups of trees that are of different genetic backgrounds or have contrasted phenotypes is also producing promising results in other areas of investigation. One such study compared trees that transition from juvenile to mature wood at different ages. Transcriptional signatures in the cambium/xylem of late maturing trees involved many stress related transcripts, whereas cell wall biosynthesis transcripts were linked to early maturation. We are also investigating developmental changes that modify the ability of vegetative tissues to undergo a transition toward somatic embryogenesis. The experiments compared different genotypes and were carried out in collaboration with K. Klimaszewska and R.G. Rutledge from CFS. Results indicate that stress responses may play a key role in the embryogenic transition.

Regulation of wood formation and secondary metabolism has been investigated through functional analysis of xylem-preferential R2R3-MYB genes (MYB1 and 8) which we showed have overlapping roles in the regulation of secondary cell wall biosynthesis in conifers. We have also uncovered other R2R3-MYB genes (MYB14 and 15) that regulate isoprenoid metabolism and the accumulation of mono and sesquiterpenes in a plant. The two sets of MYB genes were shown to regulate some of the same target genes in monolignol and shikimate pathways, and have opposite effects on metabolism through activation (MYB1, 8) and repression (MYB14, 15) (C3). Interestingly, the putative target genes of these MYBs overlap with the sets genes associated we link to early and late cambial maturation.

GENOME SEQUENCING AND ANALYSIS John Mackay

As part of the SMarTForests project, we recently contributed to a report describing the sequence of the entire genome of white spruce (Birol et al. 2013). Sequencing and assembling the large and highly repetitive spruce genome has recently become possible with new sequencing methods but conifers represent the largest genomes ever sequenced and thus push the boundaries of the current technology. A whole genome shotgun sequencing strategy was followed by using two different Illumina sequencing platforms and an assembly approach using ABySS. We obtained a 20.8 giga base-pairs (Gb) draft genome in 4.9 million scaffolds. We demonstrated how recent improvements in the sequencing technology, especially increasing read lengths and paired end reads from longer fragments have a major impact on the assembly contiguity. Scalable bioinformatics tools are instrumental in providing rapid draft assemblies. The *P. glauca* genome sequencing and assembly data are available through NCBI (accession #: ALWZ0100000000 PID: PRJNA83435 at http://www.ncbi.nlm.nih.gov/bioproject/83435). We also participated in an effort to sequence and analyse the *P. abies* genome through an international consortium led by Swedish scientists (Nystedt et al. 2013).

We also carried out a fine-scale analysis of the evolution of gene structure in *P. glauca*. The study entailed a comparative analysis of intron size between conifer trees and angiosperm plants. The gene structures of the 35 genes were compared with both the angiospermes *Arabidopsis thaliana* and *Zea mays*. The number and length of exons was well conserved but on average *P. glauca* genes had four times more intronic sequence than *A. thaliana* and 1.8 times more than maize, due to the presence of a few long introns. We found that longer introns do not seem to hinder or otherwise impact levels of gene expression despite the higher energetic cost for transcribing genes with long introns (J. Sena, in prep.)

LINKAGE MAPPING AND STRUCTURAL GENOMICS Jean Bousquet

In collaboration with N. Isabel (CFS) and as part of the *Arborea* and SMarTForest projects, three major studies were completed and published during the last period regarding gene and QTL (Quantitative Trait Loci) mapping to better understand the architecture of the spruce genome. For *P. glauca*, two composite linkage maps implicating respectively 800 and nearly 2000 mapped genes were published (Pelgas et al. 2011, Pavy et al. 2012b). QTLs for growth and phenology characters were also mapped using clonal material from different pedigrees, measured at different ages, and tested in different environmental conditions (Pelgas et al. 2011). Given the large number of progeny analysed, many QTLs were detected but most were of small effect. A large proportion of them were repeated across pedigrees, years, and environmental conditions. Co-localizing QTLs between growth and phenology were also noted,

confirming expected pleitropic effects. QTLs for growth and phenology were also mapped in *P. mariana*, again indicating small effects (Prunier et al. 2013).

The macrostructure of the spruce/conifer genome was also found to be highly conserved with little evidence for polyploidy or chromosome duplication during the last 300 Myr (Pavy et al. 2012b). Evidence for this was obtained for the phylogenomic analysis of 157 gene families in spruce, pine, rice, and *Arabidopsis* and from comparing gene maps between spruce and pine. Phylogenetic trees and the positioning of gene duplicates on the spruce genome indicated that relatively few genomic rearrangements have occurred in the conifer lineage since the split of angiosperms from gymnospems around 300 Myr ago. As well, the comparison of the gene maps between spruce and pine, based on 212 shared homologous genes, showed very high synteny and collinearity of gene positions, indicating that the macrostructure of the conifer genome has become paralysed despite the 120 to 140 Myr separating the two lineages.

This relative stasis is not without paralleling the archaic morphological nature of conifers and modest diversification rate, which are likely associated with the maintenance of large population sizes and archaic reproductive isolation mechanisms. For comparison, in the same amount of time, the angiosperms have completely reshuffled their genomes and witnessed morphological innovation and species diversification at a rapid pace. We hypothetize that the large size of conifer chromosomes resulting from the accumulation of repeat elements (Nystedt et al. 2013) may be involved in this paralysis of the genome macrostructure, leading to a syndrome of genome aging perhaps unique to conifers and not seen in model angiosperms.

Efforts are currently devoted to complete the next-generation linkage map of the *P. glauca* genome which will position 9000 genes, which should be helpful for scaffolding the *P. glauca* genome sequence (see above), to further map QTLs and eQTLs related to adaptation and wood formation (in progress), and to identify co-localizing genes. Preliminary analysis indicates that spruce chromosomes are quite comparable in terms of gene number and that the distribution of genes is quite homogenous over recombination distance, with significant deviations around putative centromeric regions. Numerous clusters of conifer-specific duplicated genes have also been noted (Pavy et al. in prep).

MOLECULAR ECOLOGY AND ENVIRONMENTAL GENETICS – INTERNATIONAL LEVEL Damase P. Khasa

At the international level, the work led by PhD student A. Atangana (now posdoc at Dept. of Wood and Forest Sciences Univ. Laval and Dept. of Renewable Resources, Univ. of Alberta) on phenotypic diversity in fruit and seed traits in Allanblackia floribunda or tallow tree has shown tree-to-tree variation in stearic and oleic acid content in seed fat (Atangana et al. 2011a). Repeatabilities were estimated for measured characters, and relationships between these investigated characters. Repeatability estimates of mean seed mass per fruit, fruit length, fruit mass, and fruit diameter were moderate, whereas repeatability in total seed mass per fruit and number of seeds per fruit was low. An overview on the physiology of rooted leafy stem cuttings of tropical tree species with particulir emphasis on the role of auxins was completed (Atangana et al. 2011b). Recommendations were made for further investigations on: 1) how post-severance stress affects the hydrolysis of auxin conjugates, 2) the effects of interacting factors (genes, endogenous auxin level, type of auxin conjugates, presence of inhibitors in cuttings, substrate composition, and exposure to light) on auxin efficiency, 3) how the variation of auxin levels in leafy cuttings affect cell enlargement, and 4) the effects of carbohydrate allocation between leaf and stem cuttings on cutting's response to auxin application to better understand the mechanisms involved in auxin biosynthesis and function in plants. In collaboration with L. Bernier (Univ. Laval) and with the Training Project in Natural Resources Management in the Congo Basin supported by the Canadian International Development Agency (CIDA) and led by D. Khasa. PhD student J. Djeugap Fovo recently completed his thesis on germination constraints and interspecific diversity of pathogenic fungi associated with Ricinodendron heudelotii in Cameroon. A review paper from this thesis has highlighted the constraints and opportunities of tree domestication of this agroforestry species in Cameroon (Djeugap et al. 2013).

With the support of the Agence universitaire de la francophonie (AUF) and Natural Sciences and Engineering Research Council (NSERC), we completed a study on the impacts of monospecific exotic and native tree species plantations on the diversity and abundance of mycorhizobial communities and the

physico-chemical soil properties in the Sahelian area of Burkina Faso (Sene et al. 2012a,b, 2013). The purpose of this work was to define the potential role of man-made forest systems on the soil physicochemical characteristics and the soil microbial communities (soil legume nodulating rhizobia and arbuscular mycorrhizal fungi communities). The work provided information about the natural legume nodulating hizobia and arbuscular mycorrhizal fungi communities associated with a range of old tree plantations and some aspects of their relationships to soil factors, which may facilitate the management of man-made forest systems that target ecosystem rehabilitation and preservation of soil biota. In the Sahelian zone of Burkina Faso, we have also shown that the use of ecologically adapted specific rhizobial and arbuscular mycorrhizal inoculants is a promising biotechnology for revegetation of gold mine tailings (Vézina et al. 2012). Work on biohydrometallurgy has focused on investigating potential microbes and methods that can be developed into full biobeneficiation processing of iron ore minerals, including the possible relationship that exists between ectomycorrhizal weathering and bioleaching processes (Adeleke et al. 2011, 2012). An affordable method was also developed for reduction of K and P levels of iron ore minerals, using fermented spoiled grapefruits. This method is being prepared for filing a South African patent that should have a significant impact on the iron ore industry. The work for identification of traits conferring invasiveness to alien plant species using populations from native and invaded regions is in progress, with the support of NSERC and Department of Foreign Affairs and International Trade through CBIE under the framework of GSEP. As well, we have shown the efficacy of non-aerated sheep compost tea to control foliar fungal pathogens such as grey mould (Botrytis cinerea) of yomato (Solanum lycopersicum) (Koné et al. 2011).

MOLECULAR ECOLOGY AND ENVIRONMENTAL GENETICS – CANADIAN LEVEL Damase P. Khasa

For our work at the Canadian level, we pursued the development and use of molecular markers in ecological studies of mycorrhizal fungi. We are currently optimizing new microsatellite markers identified from *Rhizophagus irregularis* (formerly *Glomus irregulare*). We are also using simple and cost effective PCR-RFLP followed by sequencing to study the molecular diversity of mycorrhizal communities. The technique is now being applied to assess the ecto mycorrhizal communities in *Eucalyptus urograndis* monocultural and silvopastoral plantation systems in Brazil (Pickford, Bousquet, Kasuya and Khasa, in prep.).

During the last two years, we completed the *in vitro* selection of salt-tolerant arbuscular mycorrhizal (AM) fungal isolates that could, in the future, be used to pre-inoculate plants used in reclamation of saline substrates produced by the oil sands industry (Campagnac and Khasa 2013). A clear genetic variability was observed. Indeed, fungal development of *R. irregularis* isolates differed in saline media. Significant progress has also been made at the field level for enhanced revegetation and reclamation of oil sands disturbed sites using actinorhizal and mycorrhizal biotechnology (Greer *et al.* 2011). Actinorhizal alders (*Alnus viridis* ssp. *crispa* and *A. incana* ssp. *rugosa*) and ectomycorrhizal (ECM) fungi-inoculated *P. banksiana* and *P. glauca* seedlings outplanted on the Suncor MD5 reclaimed overburden site in Alberta have shown promising results in the use of pre-inoculated seedlings in enhancing growth and establishment of alders and conifers on oil sands reclamation sites. In addition, alder rhizosphere bacterial populations showed increases in diversity and catabolic activity.

The BIOFILTREE project jointly funded by l'Agence nationale de la recherche of France (ANR) and the NSERC international strategy has also made good progress over the last two years. This large-scale research project involves the isolation, identification, and selection of Frankia, ectomycorrhizal, and endophytic fungi for reclamation of oil sand tailings. Several ectomycorrhizal and endophytic fungal isolates have been isolated from the mining and natural sites and characterized using molecular approaches. Three field trials (2011, 2012, 2013) have also been established in gold mine tailings in Val d'Or in the Abitibi-Témiscamingue region of Quebec, by exploiting actinorhizal and mycorrhizal symbioses and amendment treatments. Metagenomics of the microbial communities over time and space as well as the physiological and gene expression studies of the micro- and macrocosm experiments are also being conducted. Three MSc students enrolled at the Univ. of Sherbrooke, one MSc student at McGill Univ., and one MSc student and one postdoc at Univ. Laval are involved in these studies.

Other efforts on ectomycorrhizal fungi have focused on the ecology and sporocap productivity of *Cantharellus cibarius* var. *roseocanus* in an eastern Canadian *P. banksiana* stand (Rochon et al. 2011). This work showed that preferred microhabitats of this species were characterized by high stand density,

high C:N ratio, and frequent presence of moss. The Solidago puberula – Comptonia peregrina – Pinus banksiana association, the presence of lichen, and as much clay and silt content as can possibly be found on moderately acidic sandy soil favoured the productivity of this chanterelle, whereas the presence of ericaceous species was negatively correlated with chanterelle productivity. Positive correlations were found between total rainfall one week prior to fructification, air temperature two weeks prior to fructification, and sporocarp productivity. DNA of this species was detected in organic and mineral soil horizons. It is anticipated that this project and others aimed at better understanding the genetic and environmental conditions maximizing fungi productivity will help develop forest bioproducts in a sustainable manner.

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

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This report summarizes the progress made by the Canadian Forest Service (CFS) - Canadian Wood Fibre Centre (CWFC) in forest genetics, genomics, molecular ecology, and biodiversity for the 2011–2013 period. Our research activities were mainly focused on spruces. This work was made possible through grants obtained from the NRCan–Forest Innovation Program, the CFS–Genomics R&D Initiative, and through our participation in the SMarTForests project (J. Mackay, J. Bohlmann, J. Bousquet, K. Ritland, and collaborators), a major initiative funded by Genome Canada, Genome Quebec, and Genome BC to develop molecular breeding in softwood trees. This research was also partly supported by the Fonds de recherche du Québec - Nature et Technologies (FRQ-NT), thanks to a grant awarded to J. MacKay, J. Beaulieu, and collaborators. We also continued investing considerable time and effort in population genetics studies of forest tree species as part of our participation in the research activities on the genetics and physiology of white spruce (*Picea glauca*) seedlings and clones were also conducted in collaboration with Hank Margolis of Université Laval and Mohammed Lamhamedi of the Ministère des Ressources naturelles (MRN), with funding received from FRQ-NT.

Research accomplishments were made possible thanks to the generous contribution of dedicated staff, graduate students, postdoctoral fellows, and collaborators who deserve our recognition. These are among others: Julien Prunier, Alain René Atangana, Sylvie Carles, Nadya Wahid, Brian Boyle, Cuauhtémoc S.-Romero, Sylvie Blais, France Gagnon, Daniel Plourde, Éric Dussault, Philippe Labrie, Esther Pouliot, Isabelle Lamarre, and Pamela Cheers.

SPRUCE GENOMICS

During the last two years, grants were obtained from the CFS-Genomics Research & Development Initiative (GRDI) and the NRCan-Forest Innovation Program to carry out association studies and genomic selection of wood traits in white spruce. These grants were used in conjunction with funds awarded to the SMarTForests project, a major initiative funded by Genome Canada, Genome Quebec, and Genome BC to develop molecular breeding in softwood trees and co-led by J. Mackay and J. Bohlmann (U. Laval and UBC, respectively).

A discovery population of about 1,700 white spruce was assembled by sampling trees in a 30-year-old provenance-progeny test. Increment cores were collected on these trees and phenotypic values were assessed for a variety of wood traits and properties at FPInnovations–Paprican's facilities in Vancouver. Radial profiles of over 25 wood physical properties were obtained for each of the 1,700 trees. A high-density SNP genotyping array was built to genotype the population using the Illumina Infinium iSelect genotyping bead chip (Pavy et al. 2013). Thus, trees were genotyped for more than 6,300 SNPs mined in

2,660 gene sequences. Association mapping as well as genomic selection studies were carried out to estimate genomic estimated breeding values (GEBV) and estimate the gains expected from selection based on DNA marker information. The number of SNPs associated with wood traits was rather limited when a false discovery rate correction was applied to control for multiple testing despite the large population size and large number of SNPs involved. Moreover, as expected for quantitative traits following Fisher's theory, the percentage of variation in wood traits explained by each significant SNP was very low (less than 5%). While association mapping makes it possible to identify genes that are involved in the control of economically important traits, the information obtained cannot be easily used by breeders to help them develop more efficient breeding strategies. Genomic selection, on the other hand, rather than seeking to identify loci significantly associated with a trait, uses all marker data to predict the performance of individuals or their breeding value. Selection can then be based on genomic selection predictions. We thus carried out genomic selection studies on the white spruce discovery population assembled using all 6,300 SNPs or subsets of SNPs identified after association studies, but without applying a correction for multiple testing. The discovery population was divided into training and validation datasets in order to estimate the accuracy of predicted GEBV. The models showing the most accurate estimates were those built with subsets of SNPs having the largest effects on wood and growth traits. The accuracy of GEBV was over 0.5, and was slightly higher than that achieved through traditionally estimated breeding values. The accuracy of GEBV obtained after cross-validation with unrelated individuals was also in the same range, which was unexpected and which suggests the presence of shared ancestry in extant white spruce populations. Genetic gains per unit of time obtained with genomic selection were up to twice those expected using traditional approaches. These results indicate that genomic selection could likely be successfully implemented in this economically important species.

In collaboration with the Canada Research Chair in Forest and Environmental Genomics (CRC-FEG) (J. Bousquet), a scan involving over 1,100 SNPs from about 700 expressed genes was used to assess the potential impact of artificial selection for height growth on the genetic diversity of white spruce. Two case populations of different sizes simulating different family selection intensities (K = 13% and 5%, respectively) were delineated from the Quebec breeding population. Their genetic diversity and allele frequencies were compared with those of control populations of the same size and geographical origin to assess the effect of increasing the selection intensity. The two control populations were also compared to assess the effect of reducing the sample size. As a result of all population pairwise comparisons, we found that genetic parameters were comparable and no alleles were lost in the case populations compared with the control ones. The only exception was for few rare alleles in the large case population. The distribution of allele frequencies did not change significantly between populations, except for about 10 SNPs showing allele frequency changes between case and control populations of the same size. Globally, the results indicate that neither the reduction in sampling size nor the increase in selection intensity was sufficient to induce any significant change in the genetic diversity of the selected populations. Apparently, no loci were under strong selection pressure, confirming that the genetic control of height growth in white spruce involves many genes with small effects (Namroud et al., 2012).

The genomic architecture of adaptive traits in black spruce (*Picea mariana*) was also examined using SNPs developed in the context of other projects carried out with the CRC-FEG as well as provenanceprogeny tests comprised of seed sources from the province of Quebec and a backcross family clonally replicated on two sites. Outlier detection methods as well as association studies and QTL mapping were used to identify genome regions and gene SNPs associated with these adaptive traits. QTL mapping made it possible to identify 22 unique genomic regions that were related to budset timing and tree height assessed over multiple years and sites. Similarly, outlier detection and bulk segregant analyses made it possible to identify 52 candidate SNPs associated with the same traits. These SNPs were subsequently tested in genetic association studies. This research highlighted the fact that some genomic regions as well as some significant SNPs were shared between budset timing and tree height in black spruce. This is likely an indication of the presence of pleitropic effects of some genes (Prunier et al. 2013). Another study addressing the issue of adaptation to climate in boreal black spruce as well as the potential effects of historical events on the geographical distribution of adaptive genetic polymorphisms had also been carried out previously using 41 populations distributed across its natural range. Forty-seven candidate gene SNPs were tested for relationships with climatic factors using an outlier method and regressions between allele frequencies and climatic variations. It was possible to identify 23 adaptive genetic polymorphisms that were related to temperature and/or precipitation. Two main intraspecific lineages related to glacial vicariance were also detected at the transcontinental scale. It was possible to conclude that the standing genetic variation in the potentially adaptive nature of black spruce was modified by historical events, leading to different adaptive routes between both intraspecific lineages.

Thanks to collaboration with the Canadian Space Agency (CSA-ASC) and the NASA, we carried out a study on the effect of weightlessness on gene expression in white spruce. Hence, 18 seedlings produced through somatic embryogenesis (SE) at J.D. Irving, Limited facilities in New Brunswick were grown both at the Space Life Sciences Laboratory (SLSL) of the Kennedy Space Center and at the Destiny Laboratory of the International Space Station (ISS) for 30 days. Plants were placed in closed environment incubators under controlled light, temperature, humidity, and CO₂. At the end of this period, the leading shoot from three plantlets of each of the three lines tested were sampled and pooled in Kennedy Space Center Fixation Tubes containing a RNA stabilization solution. Samples grown at the ISS were brought back to Earth to the Kennedy Space Center by space shuttles and then transported to Quebec City along with samples collected on plants raised at the SLSL. Transcript levels were determined by quantitative real-time polymerase chain reaction for 27 candidate genes and three reference genes on the nine seedlings grown in each environment. About two-thirds of the 27 genes produced a larger number of transcript molecules in microgravity conditions. However, only three genes showed significant differences between the two environments, and all of them were upregulated in microgravity. These genes appear to be involved in important processes such as cell propagation, plant development and response to stress, and their upregulation likely contributed to influencing seedling growth patterns (Beaulieu et al. 2013).

QUANTITATIVE GENETICS

Quantitative Genetics of Wood Properties

In collaboration with J. Mackay (U. Laval), we assessed acoustic velocity as a selection trait for the improvement of mechanical properties in two 15- and 32-year-old white spruce genetic tests (Lenz et al. 2013). We found that individual heritability of acoustic velocity was moderate and of the same magnitude as that of wood density (≈ 0.40), and that considerable genetic gain could be expected when selecting for this trait or for a combination of wood density and acoustic velocity. The relationship between the latter trait and cellulose microfibril angle (MFA) was strong at the genetic level and selection for acoustic velocity could effectively improve MFA, which is an important determinant of wood mechanical properties. The relationship between acoustic velocity and tree height, although positive, was weak. Both traits could nevertheless be simultaneously improved. Overall, juvenile acoustic velocity appears to be an appropriate trait to select for wood quality in a white spruce breeding program.

Other Quantitative Genetics Studies

In collaboration with H.A. Margolis (U. Laval) and M.S. Lamhamedi (MRN), and with funding obtained from FRQ-NT, genetic variability of morphological and physiological characteristics of containerized white spruce seedlings of 75 open-pollinated families was investigated to determine whether genetic selection for improved seedling root systems was possible. Seedlings were grown for two years in a forest nursery and various traits, including height, diameter, shoot and root dry mass, and root to shoot ratio, were measured at the end of the second growing season. Genetic control of these various traits varied from 0. 21 to 0.97 at the individual level and from 0.34 to 0.88 at the family level (Carles et al. 2012). The strong and positive genetic correlation between root collar diameter and root dry mass (0.68) suggests that indirect selection for a stronger root system could be made using root collar diameter.

To evaluate the influence of white spruce seed orchard material on seedling growth and morphology, seedlings produced from seeds originating from the 10 most commonly used first-generation white spruce seed orchards in Quebec were raised under similar nursery conditions. Seedlings from each seed orchard were destructively sampled at two-week intervals during the second growing season. Height and root collar diameter of each seedling was measured, and the above- and below-ground portions of the seedlings were oven-dried to determinate shoot and root dry masses. Growth curves for height and diameter for each seedling were fit to a logistic model and were used to compare the growth rhythm of the seedlings from the 10 seed orchards. Results made it possible to show that two-year-old white spruce seedlings produced with seed from geographically and genetically distinct seed orchards and raised under similar nursery conditions differed in height and shoot dry mass, but not in diameter or root dry mass. Two groups of seed orchards could be distinguished based on above-ground seedling traits, namely those located in the sugar maple bioclimatic domain, which produced taller seedlings, and those located in the balsam fir bioclimatic domain. These results support the idea that the present seed zones could be

broadened and aligned with the two breeding zones delineated for white spruce in Quebec (Carles et al. 2011).

There is renewed interest for clonal forestry in Eastern Canada because it can lead to significant increases in forest productivity and help respond to the demand of the global market that is continuously increasing. It is in that context that Quebec's MRN has recently built new facilities to produce several million white spruce SE-derived seedlings every year. The use of SE in clonal forestry requires an accurate assessment of genetic parameters and of the performance stability of clones in plantations. Studies were thus carried out to estimate genetic parameters and clonal variation in growth and nutritional traits of containerized white spruce somatic seedlings in the nursery and performance stability in clonal tests using seed from selected full-sib families (Wahid et al. 2012a,b, 2013). Results showed highly significant clonal effects for most growth traits analyzed at the end of two growing seasons in the nursery as well as for the seedlings' mineral status at the end of the second growing season. The broadsense heritability of seedling height was 0.60 at both age 1 and 2. At the end of the nursery experiment, the two-year-old containerized somatic seedlings were planted in two clonal tests in abandoned bare-root beds at two provincial forest nurseries. Four years after establishment of the clonal tests, ramets were measured for several growth and morphological traits. The results showed that the SE clones exhibited large variability in growth and branch characteristics, particularly for branch length and diameter. Heritability estimates were lower than those observed in the nursery, presumably because a planting shock effect was still present. Although there was a significant site effect for most traits, the genotype x site interaction was low. Consequently, the correlation between the two sites for the same characteristic was high. There is a clear indication that the performance of SE seedlings is stable between sites and that there is some opportunity for the selection of clones that adapt and perform well over different ecological regions in Quebec.

Quantitative genetics studies were also carried out in collaboration with Professor Cuauhtémoc Sáenz-Romero during his sabbatical stay in Quebec City. Fourteen *Pinus patula* populations were sampled in the state of Oaxaca, Mexico, along an altitudinal gradient (2,400 m to 3,050 m). Seedlings were grown in growth chambers at the Laurentian Forestry Centre under two temperature treatments aiming to simulate current temperature (average of 17°C) and a future global warming scenario (average of 21°C). Seedlings were evaluated after a second growing season (between 8 and 12 months of age). It was possible to show that the populations differed significantly for seedling height and aerial dry weight, and that they exhibited a significant although weak altitudinal clinal pattern, with populations from lower altitudes having on average taller and heavier seedlings than populations from higher altitudes (Sáenz-Romero et al. 2011). However, despite the fact that the mean height and dry weight of the aerial part of the seedlings growing under simulated climate change temperatures was, on average, 4.6% higher than that of seedlings growing under current temperature, the differences were not statistically significant.

OTHER RESEARCH

Assisted migration or colonization is a strategy envisioned by forest geneticists to mitigate the negative effect of climate change on tree species. In a paper published in The Forestry Chronicle (Pedlar et al. 2011), we outlined what we consider to be the major steps that should be involved in the implementation of assisted migration and assessed the capacity to carry out each step in Canadian forests. In a second paper, we discussed the concept of assisted migration, showing that implications clearly diverge when it is applied to species rescue than when it is applied to the maintenance of forest productivity in the context of forest operations (Pedlar et al. 2012).

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NORTHEAST SEED MANAGEMENT ASSOCIATION (NeSMA) SUMMARY OF FIELD OPERATIONS

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NeSMA is a partnership of 8 forest companies and the Ontario Ministry of Natural Resources (MNR) operating in north-eastern Ontario. The company has tree improvement programs for black spruce (*Picea mariana*), jack pine (*Pinus banksiana*), and white pine (*Pinus strobus*). This report summarizes the main operational activities of the company during 2011 and 2012. Due to the downturn in the forestry sector the field operations continue to be considerably reduced compared with previous years.

FIRST-GENERATION PROGRAMS

Most of the seed orchard work consisted of crown management and cone collection in 5 black spruce and 2 jack pine seed orchards. The total volume of cones collected from the black spruce orchards was 44 hectoliters (hL) and 112 hL from the jack pine orchards. The company partners funded this work.

Orchard seed is preferentially used to produce planting stock for the 11 forest areas managed by the company partners. The amount of improved stock planted each year varies by forest; in 2011 74% of the 10.8 million jack pine seedlings planted were grown from improved seed and 51% of the 9.4 million black spruce trees planted. The numbers were 46% and 80%, respectively, for the two species in 2012 with the planting totals by species within about one million seedlings of the 2011 totals.

Two jack pine seed orchards at the Island Lake Tree Improvement Area were thinned. Approximately 4,000 trees were removed from the 32 hectare area to reduce crowding and to extend the productive life of the seed orchards. The trees were removed from the orchard and topped in designated areas. The stems of the trees were converted to fuel wood for local use.

All trees in the Aidie Creek and Ramore jack pine seed orchards received a flower induction treatment in anticipation of a larger cone crop in 2013.

The Ontario Rangers and Ontario Stewardship Rangers completed necessary maintenance work at the Ingram and Eby Township white pine genetic tests and at the Ramore and Aidie Creek jack pine and black spruce seed orchards.

Forest Health Technicians visited some genetic tests and seed orchards. White pine blister rust (*Cronartium ribicola*) control measures were undertaken in 2 of the white pine genetic tests planted in 2002; the lower branches on each tree were removed. The trees at one of these tests were assessed. The data collected were analyzed as well as data collected earlier from another test established in 2002. Data collected from the 3 tests established in 2006 were also analyzed.

SECOND-GENERATION PROGRAMS

Maintenance work was completed in the black spruce archive to control insects and competition thanks to funding from Forest Genetics Ontario. The trees in the archive were inventoried and the rootstock pruned.

The genetic tests and clonal archive were monitored to track their health and condition. Insect control in two black spruce genetic tests was avoided due to a crash in the insect population. Data previously collected from two Elite and two Infusion tests were analyzed. Ontario Stewardship Rangers completed maintenance work at the black spruce archive.

In 2011 work began to establish a jack pine archive supported by funding from Forest Genetics Ontario. The rootstock was acquired, transplanted into larger pots, grown, and later put in cold storage. Site preparation was completed and scion collection was initiated. In 2012 work continued to establish a jack pine archive. While there were difficulties with scion collection due to warm weather collections were made from 163 selections. There were 1,156 scions grafted over a five day period. Unfortunately there were no successful grafts. An investigation of the problem revealed that it was likely an issue with the rootstock. On a better note, Hearst Forest Management began establishment of its jack pine clonal seed orchard by planting about 200 grafts in Casgrain Township near Hearst.

OTHER ITEMS

A project was completed which reviewed all of the administrative and forest genetics program information in order to ensure that all paper and electronic files were up-to-date, organized, and secure. In addition, information which was no longer needed was disposed of in an appropriate manner.

An internal program review resulted in a document outlining the status and management options for individual programs and seed orchards.

SUPERIOR-WOODS TREE IMPROVEMENT ASSOCIATION

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The Superior-Woods Tree Improvement Association (SWTIA) is a cooperative among forest industry based in northwestern Ontario and the Ministry of Natural Resources. The members work cooperatively on applied tree improvement programs in jack pine (*Pinus banksiana*), black spruce (*Picea mariana*), and white spruce (*Picea glauca*). The Associaton is a member of Forest Genetics Ontario (FGO). The SWTIA members continued to make progress over the past two years in their first- and second-generation tree improvement programs that provide improved seed for use in Crown land reforestation programs. In addition, the members supported a number of forest genetics research projects.

Association members continued to manage first-generation black spruce and jack pine seedling seed orchards and white spruce clonal orchards to produce improved seed. Final roguing was completed in one jack pine orchard and started in another. Overall, 360 hl of cones were collected from the first-generation seed orchards for all species over the past two years. In addition, members continued to make progress wth second-generation programs in black spruce and jack pine. The second-generation clonal orchards started to produce cones and a number of small cone collections were made with one larger collection (23 hl). It is anticipated that the second-generation clonal orchards will soon be able to provide a large portion of the seed requirements for planting programs.

The Association supported a number of forest genetics-related research initiatives and continued to provide support to forestry students studying forest genetics at Lakehead University. The SWTIA members assisted Dr. Bill Parker, Lakehead University and graduate student Samuel Nsiah with the establishment and assessment of their project titled "Seed Source Portfolio Selections in Black Spruce to Compensate for Predicted Climate Change in Ontario". The project received financial support from FGO and considerable in-kind support from Dr. Pengxin Lu, Ontario Forest Research Institute.

FOREST GENETICS AT THE ONTARIO FOREST RESEARCH INSTITUTE (OFRI) 2012 – 2013

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Keywords: White pine blister rust, genetic resistance, hybrid backcross, climate change, adaptation strategy, assisted migration, provenance, white spruce, somatic embryogenesis, Dooks needle blight

BREEDING EASTERN WHITE PINE FOR BLISTER RUST RESISTANCE

The breeding of eastern white pine (*Pinus strobus*) for blister rust (caused by *Cronartium ribicola*) resistance continued but at a relatively smaller scale during this reporting period, compared with the previous years. This is mainly because breeding has now focused on elite lines which confer strong major gene resistance. Third-generation backcrosses of eastern white pine hybrids have been bred with Himalayan blue pine (*P. wallichiana*), with about 94% parentage of eastern white pine. Two rounds of artificial blister rust inoculation experiments with seedlings were completed. Progeny of the elite families demonstrated a high level of resistance with above 50% post-inoculation seedling survival, compared with close to 0% survival of the controls.

A package of technology, which comprises blister rust resistant eastern white pine hybrid backcrosses and somatic embryogenesis (SE) propagation, seems to provide a promising option to help white pine regenerate in areas where high blister rust hazard causes regeneration failures with non-resistant seedlings. This is being demonstrated at a field site about 40 km north of Sault Ste. Marie, ON, where previously planted eastern white pines were decimated by blister rust (above 90% infection rate) 10 years after establishment. At the same site, we established a trial containing eastern white pine controls, openpollinated hybrid families, and rooted cuttings of hybrid seedlings which survived blister rust infection in artificial inoculation experiments. Four years after planting, the rates of stem infection by blister rust were 13.3%, 3.7%, and 0%, respectively, for eastern white pine control, elite hybrid families, and rooted cuttings. As we now can use SE to effectively propagate hybrid white pine vegetatively, an operational deployment of resistant stock is technically feasible in the near future when it becomes desirable.

Several new field trials are close to 8 years of age for assessing adaptation and growth as well as blister rust resistance. The plan for the coming years is to generate a package of data and information about blister rust resistance, adaptation, growth, wood quality, and potential environmental impacts of the hybrid seedlings to support science-based policy development to guide the future use of blister rust resistant eastern white pine hybrid stock.

FOREST ADAPTATION TO CLIMATE CHANGE WITH ASSISTED MIGRATION

Working with OMNR researchers (Drs. Bill Parker and Steve Colombo) and Dr. Bill Parker (Professor at Lakehead University), a project was initiated to assess the benefits and risks associated with assisted migration as an adaptive approach to climate change in Ontario. The project started with the use of data from historical provenance trials established in Ontario 30–40 years ago led by pioneer forest geneticists of the Canadian Forest Service. The 410-series range-wide provenance tests of white spruce (*Picea glauca*) are being remeasured to generate time series data, which may be used to examine provenance responses to climatic variables at different time intervals. Currently, data from the 2001 measurement are being analyzed to reveal continental scale variation among provenances in adaptability and productivity

under the climatic conditions at 16 Ontario sites. Preliminary results indicated that although trees of white spruce from all provenances could survive and grow across Ontario sites, the best performers are mostly those originating from south-central Ontario and southwest Quebec. Provenances from west of Ontario, which are generally associated with higher latitudes and elevation, had significantly inferior survival and growth to those of Ontario origins. Within Ontario, transferring white spruce seeds from south-central Ontario to more northerly sites is seemingly with low risk. Increasing susceptibility of southern provenances to frost damage at northern sites was not evident from these long-term provenance trials. A northward seed transfer within safe distances would result in appreciable genetic gain in forest productivity at no or minimal costs, compared with ongoing tree improvement programs with local breeding populations. The results also indicated the high ecological and economic values of conserving the gene pool of white spruce in south-central Ontario.

FIVE-NEEDLE PINE DOOKS NEEDLE BLIGHT

We recently reported the differential susceptibility of five-needle pine species to Dooks needle blight (Lophophacidium dooksii). Dooks needle blight was first reported on eastern white pine needle samples from Ontario and New Brunswick in 1984. More recently, increasing incidents of needle browning of eastern white pine has been observed in eastern Canada and the northeastern USA. An outbreak of the disease in a five-needle pine genetic archive containing nine five-needle pine species and their interspecific hybrids provided an opportunity to observe the differential susceptibility of these pine species to the disease. Survey results indicated that four of the nine species in the archive were susceptible, including new hosts western white pine (*Pinus monticola*) and Himalayan blue pine, as well as previously reported hosts eastern white pine and Macedonian pine (P. peuce). Japanese white pine (P. parviflora) and Korean pine (P. koraiensis) were seemingly not affected by the pathogen. While P. peuce and its interspecific hybrids showed the highest susceptibility and the heaviest needle damage, P. parviflora and its interspecific hybrids, including those with P. peuce, were rarely infected, indicating potential strong and dominant genetic resistance to the pathogen. Pinus monticola and P. strobus showed similar levels of susceptibility and needle damage. The take-home message was that Macedonian pine, which is often planted as an ornamental tree in North America, should be removed as much as possible as the species easily hybridizes with eastern and western white pines and seems to carry the most susceptible genes to Dooks needle blight. While pure Himalayan blue pine was susceptible, its hybrids with eastern white pine bred by OFRI for blister rust resistance were almost all disease free. It was unknown whether genes resistant to blister rust also conferred resistance to Dooks needle blight.

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FOREST GENETICS AND TREE IMPROVEMENT ACTIVITIES FOR ALBERTA ENVIRONMENT AND SUSTAINABLE RESOURCE DEVELOPMENT 2011-2013

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This report is a summary of selected activities the Alberta Tree Improvement and Seed Centre (ATISC) has engaged in since June 2011. They are either new or are considered a significant addition to the regular activities of the tree improvement programs in Alberta. These activities fall into four categories: 1) Alberta Environment and Sustainable Resource Development (ESRD) and private company tree improvement programs being jointly implemented through a three-year climate change adaptation project called "Tree Species Adaptation Risk Management". This project is extending the climatic range of provenance trials, assessing the risk climate change poses to Alberta tree improvement programs, and developing cost efficient techniques for producing aspen (Populus tremuloides) rootlings. 2) Floral induction using gibberellic acid ($GA_{4/7}$) in white spruce (*Picea glauca*) seed orchards. This is in response to increased seed demand for reclamation in the oilsands area in northeastern Alberta and a need to explore methods for increasing seed production in existing orchards. From experimental induction, it was determined that a concentration of 100 mg $GA_{4/7}$ per ml alcohol is sufficient to induce cone production without a need for girdling. 3) ATISC seed experiments to: (i) identify appropriate containers for longterm cold storage, (ii) improve germination of whitebark (Pinus albicaulis) and limber pine (P. flexilis), and (iii) identify an appropriate time for collecting limber pine cones. 4) Shrub seed registration and storage. ATISC is increasingly handling and storing shrub seed used for reclamation in areas disturbed by mineral and energy extraction. This adds to the range of seed biology and technology challenges ATISC has to tackle.

FLORAL INDUCTION WITH GIBERELLIC ACID

In the spring of 2011, the ATISC in collaboration with Dr. Richard Pharis (University of Calgary), used gibberellic acid ($GA_{4/7}$) and girdling to induce cone production in two white spruce seed orchards at the ATISC site in Smoky Lake. This was an experimental trial to determine the appropriate dose of $GA_{4/7}$ and whether or not $GA_{4/7}$ has to be combined with non-hormonal treatments to be successful.

The $GA_{4/7}$ doses were 160, 80, and 40 mg per ml of 95% ethanol. Girdling was applied alone and in conjunction with the three $GA_{4/7}$ doses. Ten clones were included in the trial with the age of trees averaging 21 years from grafting and diameter at the point of $GA_{4/7}$ injection averaging 12 cm. Gibberellin injection and girdling were applied concurrently shortly after vegetative bud burst and the accumulation of 177 growing degree days. The $GA_{4/7}$ dose per tree depended on tree diameter at the point of injection and it ranged from 1.0 to 2.0 ml. The volume of solution per injection hole was kept constant at 0.5 ml.

The average number of cones per tree in the 2012 season was 9 (control); 38 (girdling alone); 1368, 1664, 1371 (high, medium, and low $GA_{4/7}$ dose, respectively); and 1521, 1850, 1236 (high, medium, and low $GA_{4/7}$ dose combined with girdling, respectively). The differences in cone production between the control and all $G_{4/7}$ doses were statistically significant. However, the differences were not statistically significant between the control and girdling, among the three $GA_{4/7}$ doses, and all $GA_{4/7}$ doses with all girdling- $GA_{4/7}$ dose combinations.

Although clones differed in cone production, $GA_{4/7}$ substantially increased cone production in all clones. In addition, $GA_{4/7}$ increased cone production by 58 fold compared to three fold in pollen strobili production over untreated trees. Gibberellic acid induced reproduction did not appear to have adverse effects to trees. In the year following the treatment, trees maintained normal health and vigor, and the seeds produced through induction had normal viability.

Based on this trial, the recommended procedure for white spruce floral induction at ATISC would be: 1) use a concentration of 100 mg $GA_{4/7}$ per ml alcohol, 2) not use girdling as an adjunct treatment, 3) withhold watering for the month following injection unless soil moisture is too low to jeopardize the health of the trees, 4) inject $GA_{4/7}$ on a three-year cycle, 5) treat one-third of the orchard in any given year, and 6) apply $GA_{4/7}$ only in years when an abundant crop is not expected (since $GA_{4/7}$ is applied after reproductive buds have flushed, the size of the current year's crop can be assessed prior to treatment). Additional experiments are planned in the near future to evaluate the efficacy of $GA_{4/7}$ treatment over the longer term.

CLIMATE CHANGE ADAPTATION

In April 2012, Alberta Environment and Sustainable Resource Development (ESRD) and private companies involved in tree improvement programs initiated a project titled "Tree Species Adaptation Risk Management (TSARM)". The project's objective is to integrate climate change adaptation into Alberta genetics and tree improvement programs. The Climate Change and Emissions Management (CCEMC) Corporation, which is an independent organization rooted from the Alberta Climate Change Strategy of 2008 is funding the TSARM project. This three-year project has four major components that are briefly described below.

- 1) Provenance testing to extend conifer provenance trials into dry and short growing season regions of the province where no provenance or progeny trials currently exist. In particular, this will enable identification of drought tolerant populations, families, and clones of major commercial forest tree species for deployment under a warmer and drier climate projected for the prairies. Four large experimental sites are being developed in Brooks (driest part of Alberta), northern Alberta, and the subalpine.
- 2) Climate change risk assessment of all government, private company and cooperative tree improvement programs to determine and correct areas of vulnerability in terms of program design; parental genotypic composition in the breeding population; seed orchard capacity and biological data collection; seed and clone banking; progeny testing and assessment and many other risk criteria. The objective of the TSARM project is to ensure that all areas of program weakness that would be aggravated by climate change are identified and fixed.
- 3) Data analysis and climatic modeling, to combine progeny and provenance trial data accumulated over a period of 30 years across all regional tree improvement programs. This will identify (i) similarities among currently separated programs, (ii) potential for sharing parent trees, seed orchards, and seed or clones, (iii) opportunities for joint testing among programs, and (iv) potential for including climatic stress tolerance as one of the seed orchard roguing criteria and other adaptation-related questions.
- 4) Developing techniques for efficient vegetative propagation of aspen to enable operational planting of the species. The current aspen forest is composed of clones, many of which may date back to the last glacial retreat and have been sustained by coppicing from the root system. If climate change renders these clones maladapted, better-adapted clones will have to be artificially planted. Currently, the cost of producing a single plantable aspen rootling is too high to allow operational planting. The TSARM project is working with forest nurseries in Alberta to find propagation techniques that reduce this cost significantly.

The results from the TSARM project will enable the government of Alberta to develop interim seed and clonal transfer guidelines for both wild and genetically improved material that considers among other things, adaptation to climate change, especially drought tolerance.

SHRUB SPECIES SEED COLLECTION AND STORAGE

The Alberta Forest Genetic Resource Management and Conservation Standards (FGRMS) set the policy framework for reforestation and reclamation of public lands. While the standards were implemented primarily for trees, they were also developed with the broad understanding that reclamation of public land by non-timber entities would utilize plant materials other than seedlings of traditional reforestation tree species. FGRMS are currently being used to guide collection and use of materials of all woody species used on public land reclamation to ensure that local adaptation and minimum genetic diversity requirements are met. As part of extension of FGRMS to shrubs, ATISC organized a two-day seed conservation workshop to provide practitioners and scientists with seed procurement and handling knowledge. It is expected that ATISC will organize this workshop annually with topics covering crop assessment, seed collection, post-harvest handling, and germination problem solving techniques for shrub seeds. To date, 362 seedlots, representing 24 shrub species, have been collected and registered for public land use. Advice and information is provided as needed to local nurseries and collectors.

SEED STORAGE CONTAINER TESTING

Over the years, ATISC has used a variety of glass and plastic containers to store seed. Because some of these containers were not designed for cold storage, they may not provide a hermetic (airtight) seal required for long-term storage. To address this problem, ATISC conducted a trial to test the seal effectiveness of containers being considered for the long-term storage of research and conservation seedlots.

The test evaluated 16 containers: 1) ten containers currently used, 2) two types of static shield plastic bags, 3) three types of foil bags, and 4) the 'zip-loc' type bags used for seed transportation. The testing method was that used by the Millennium Seed Bank at the Royal Botanic Gardens, Kew (Manger et al. 2003), which was originally adapted from a technique developed by Gómez-Campo (2002). It involves sealing the containers with self-indicating silica gel inside and placing them in high humidity and low temperature conditions.

Eleven of the 16 container types tested failed, including some of those currently in use at ATISC. Of the 5 container types that passed, the tri-laminated foil bags from a European supplier were chosen. In addition to providing a hermetic seal, they can eliminate much of the air stored with the seeds thereby limiting oxidation, provide protection from sharp-edged or pointed seeds that perforate other types of bags, do not have gussets impairing seed removal, and are available in sizes ranging from approximately 70 ml to 4 L. Consequently, ATISC has adopted these types of bags, and all of its more than 7,000 research and conservation seedlots have been re-bagged in tri-laminated foil bags. This test ended after 70 days but new replicates of the chosen Baltimore foil bags have been set up and will be monitored at 12 and 24 months.

COLLECTION AND CONSERVATION OF LIMBER AND WHITEBARK PINE

The Alberta Tree Improvement and Seed Centre continued to make seed collections for whitebark and limber pine both listed as endangered species in Alberta. The population decline in these species in Alberta is due to white pine blister rust, mountain pine beetle and limited natural regeneration. In the last two years, ATISC has collected 28.3 kg and 10.1 kg of limber and whitebark pine respectively. In total, ATISC has 70.1 kg and 38.0 kg of limber and whitebark pine, respectively.

COLLECTION AND EX SITU MATURATION OF LIMBER PINE CONES

White pine blister rust caused by *Cronartium ribicola* and mountain pine beetle (*Dendroctonus ponderosae*) have caused a significant decline of limber pine in Alberta. The draft recovery plan for limber pine in Alberta includes seed collection and storage, and possibly genetic resistance screening and restoration with resistant stock in the future. However, the short growing season in the Rocky Mountains where the species is confined and Clark's nutcracker (*Nucifraga columbiana*) which is a predator, impedes seed maturation and collection necessary for implementation of the recovery plan. In practice, only pine seeds with embryos occupying over 90% of the corrosion cavity are considered fully mature

and ready for collection. In addition, past tests of limber pine seedlots yielded low and inconsistent germination, which points to the possibility of inadequate treatment. However, germination trials conducted in 2012 at ATISC have provided a better treatment method that produces 90 - 100% germination of filled, viable seeds.

To determine the appropriate stage for cone collection, ATISC did a study on limber pine seeds collected from two distinct sites in Alberta. Cone collections were made every two weeks from mid-August to October 2012 when the cones had naturally opened on the trees. Seed embryo lengths, dormancy, desiccation tolerance, and longevity were measured at the time of collection and then every two weeks while cones were 'matured' in a chamber at 90% RH and 20°C. At the writing of this report, the study is ongoing but some obvious trends have emerged. The *ex situ* cone maturation appears to increase greatly the germination and desiccation tolerance of early harvested seeds. Overall, limber pine seeds appear to be short-lived and maturation efforts may have actually aged later harvested seeds. We found that embryo length was not a reliable indicator of seed maturity when assessing maturity in the field, because some of the seed collected with embryos occupying over 90% of the corrosion cavity had not yet gained desiccation tolerance or maximum longevity. When the tests are complete and the data analysed, this study will shed light on some obstacles that have posed significant problems for limber pine *ex situ* conservation efforts in Alberta.

DOTHISTROMA NEEDLE BLIGHT ON LODGEPOLE PINE

There has been a recent outbreak of Dothistroma needle blight in the lodgepole pine (*Pinus contorta*) clone bank at the ATISC, which has been confirmed by Natural Resources Canada (NRCan). Further taxonomic work to determine the species is ongoing. To prevent further spread and mortality, the clone bank has been spaced and sprayed with Bordeaux Mixture to control the disease. The origin of the infection is not known, however, ATISC will inspect all pine seed orchards and field trials that have received grafts and seedlings from the nursery and greenhouses at Smoky Lake to ensure that the disease is not being spread through ATISC's plant propagation program.

The origin of the disease in the ATISC pine clone bank is unknown but ATISC is working with ESRD's Forest Health Section and NRCan to identify whether this is an isolated case or the problem may be more widespread and under reported in the province. ESRD Forest Health Section and NRCan are also assisting ATISC in determining another needle blight problem occurring on interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) in a provenance test and clone bank west of Rocky Mountain House, which has been tentatively identified as Rhabdocline and only rarely reported in Alberta. This occurrence is beginning to cause mortality in some provenances, which appear more susceptible.

TREE IMPROVEMENT ALBERTA

To coordinate the Tree Species Adaptation Risk Management (TSARM) project described earlier and other tree improvement activities in Alberta, private forest companies and ESRD have created a working consortium called Tree Improvement Alberta (TIA). Members are ESRD, Ainsworth Engineered, Alberta Newsprint Company, Alberta Pacific Forest Industries, Blue Ridge Lumber (West Fraser), Canadian Forest Products, Daishowa Marubeni International, Hinton Wood Products (West Fraser), Manning Diversified Forest Products, Miller Western Forest Products, Northlands Forest Products, Sundre Forest Products (West Fraser), Tolko Industries, Weverhaeuser Pembina, and Weverhaeuser Grande Prairie. Although the current major undertaking of TIA is to coordinate the implementation of the TSARM project among the climate change adaptation activities being implemented by project participants, TIA is considering a range of issues to expand its operations beyond climate change. TIA has galvanized cooperation between government and industry tree improvement programs by focusing on common challenges such as climate change, having a common understanding and appreciation of provincial tree improvement and seed transfer standards, and sharing information in a cordial and timely manner. In April 2013, TIA organized a two-day field tour of ESRD and industry species provenance and progeny trials in southwestern Alberta. The 27 participants included scientists, foresters, and silviculturists representing TIA members, Alberta Innovates Bio Solutions and Alberta Innovates Technology Features, and ESRD area foresters. After visually observing provenance differences in growth and susceptibility to rusts and needle blights, forest practitioners expressed their appreciation of the need to observe provincial seed transfer guidelines more than they were before the field tour.

NURSERY AND PLANT PROPAGATION

Between June 2011 and June 2013, ATISC produced over 37,000 seedlings for research and conservation purposes. Almost 10,000 seedlings were grown for a black spruce (*Picea mariana*) progeny trial that has been established at three sites in northeast Alberta. Over 5,000 limber pine and whitebark pine seedlings were salvaged from germination trials based on seed harvest date and maturation. A portion of these seedlings was inoculated with mycorrhyzae and planted in Waterton Lakes National Park.

In the same period, new incidences of pests and diseases have occurred on stock grown within the greenhouses. They include *Pythium* spp. (water-born) and *Fusarium* spp. (soil-born). Nevertheless, these diseases have not substantially affected the quality of seedlings grown in ATISC greenhouses. Management of scions and stock infected with scale and white pine blister rust, and potentially Dothistroma and Rhabdocline needle blight have also presented a challenge for the ATISC plant propagation program.

ATISC produced over 2,000 grafts during the reporting period. Most of these grafts were part of the conservation program for pines funded by the mountain pine beetle initiative. Additionally previously grafted trees currently affected by Dothistroma in the ATISC pine clone bank were re-grafted in order to preserve those clones.

RETIREMENTS AND NEW STAFF

After 26 years at the ATISC, Christine Hansen retired from the Alberta Public Service effective February 28, 2013. Christine joined the Alberta tree improvement program in 1987 and was very involved in its core activities, especially field trials, data analysis, seed orchard establishment and monitoring, and development of Alberta tree improvement standards. ATISC and ESRD thank Christine for her long and productive career and wish her well in her future endeavours.

In September of 2011, the ATISC welcomed Lindsay Robb as their Provincial Seed Specialist. Lindsay was born in Kingston, Ontario and completed a M.Sc. at Laurentian University on botanical restoration and nitrogen cycling. She then moved to the United Kingdom where she lived for 10 years and worked as a Germination and Longevity Specialist at the Millennium Seed Bank (MSB), part of the Royal Botanic Gardens, Kew. Lindsay researched and trained others around the world on germination problem-solving methods and tools, and developed standard operating procedures for initial germination tests, a retesting program, and chemical viability testing. She then moved into seed longevity, analysing 3,000+ species with 40 years of test data in an effort to use taxonomy, climate, seed morphology, and/or habitat to determine which new seed collections may be shorter-lived under seed bank conditions and whether alternative storage measures (e.g., cryo-storage, ultra-dry storage, low oxygen storage) should be considered in an effort to improve longevity.

Lindsay is continuing her love of teaching at ATISC by running an annual seed conservation workshop for the past two years. She has eagerly plunged into work on Alberta's two endangered tree species (limber and whitebark pine) and has been providing practical propagation advice to local nurseries experiencing issues of growing woody shrubs for oilsands reclamation. She has also been testing and revising extraction and germination procedures used for Alberta's tree species, as well as updating lab equipment and techniques.

In February 2013, Andy Benowicz joined ATISC as a Forest Genetics Specialist. Andy comes with a range of backgrounds from quantitative genetics and tree breeding to tissue culture. He graduated from the University of British Columbia (UBC) with a M.Sc. in Forestry specializing in forest genetics and plant physiology. After graduation, he worked as a Research Scientist in UBC on a number of projects related to genetic diversity of commercial forest tree species. Subsequently, CellFor, a forest biotechnology company, hired Andy where he worked for over 10 years as a Tree Improvement Manager. CellFor produced, through tissue culture (somatic embryogenesis), commercial quantities of varietal (i.e., clonal) coniferous seedlings for forest plantations in southern US, Canada, New Zealand, Australia, Chile, and Brazil. Andy was responsible for field performance testing of thousands of genotypes and selection of the best varieties for production based on tree growth, disease resistance, tree form, wood properties, and tissue culture propensity. He is a co-inventor on several plant varietal patents.

Just prior to joining ATISC, Andy worked as a Forest Genetics Consultant with the BC Ministry of Forests, Lands and Natural Resource Operations.

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WESTERN FOREST PRODUCTS INC. SAANICH FORESTRY CENTRE ORCHARDS

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Western Forest Products (WFP) delivers high quality seed to all forest regeneration programs in the operations in coastal British Columbia. Surplus seed is available to other licensees operating in the Maritime Seed Zone. At the Saanich Forestry Centre, seed orchards are managed for four conifer species: coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*), western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), and Sitka spruce (*Picea sitchensis*). Yellow-cedar (*Callitropsis nootkatensis*) hedges are managed as well. Seed needs average 7.5 million annually for land tenures on Vancouver Island and the mainland coast from Squamish to the North Coast.

We employ orchard management techniques including orchard rouguing, ramet replacement, and supplemental mass pollination to produce high quantities of quality seed. Techniques for improved seed quantity include induction, supplemental mass pollination, insect control and management, and cultural management.

Western Forest Products supports research into genetics, tree seed production, and related studies conducted by researchers at the Ministry of Forests, Lands and Natural Resource Operations, the Universities of British Columbia (UBC) and Victoria, Canadian Forest Service, and associated consultants and contractors. We continue to be active in the BC Forest Genetics Council and its committees.

COASTAL DOUGLAS-FIR

We are upgrading and increasing capacity of our coastal Douglas-fir orchards to meet our needs. Our low-elevation orchards include selections with average genetic worth for volume of 19 and maintain wood density. These selections include third-generation parents identified in the province's coastal Douglas-fir breeding program. As the orchards are not at capacity yet, WFP presently purchases and deploys seed from other producers, together with the seed from our orchards. Our 2012 crop produced seed for more than 1 million plantable seedlings, with genetic worth for volume of 12, and for wood density of 0.

We established a second-generation high elevation Douglas-fir orchard replacing the mature firstgeneration orchard at the Sechelt orchard site at the Saanich Forestry Centre. The breeding value of the stock in this orchard is 13. No crops have been managed in this young orchard yet. Low and high elevation coastal Douglas-fir comprises about 35% of our annual planting program.

We continue to provide seed for studies into parental contributions that UBC's Dr. El Kassaby and his research team are conducting.

WESTERN REDCEDAR

As the provincial western redcedar breeding program identifies additional selections for orchards, WFP incorporates those selections in our orchards. Roguing and ramet replacement balanced with maintaining

production keep these orchards at the leading edge. Our western redcedar orchards delivered a seed crop in 2012 for more than 1.6 million seedlings with volume gain of 17%. We also manage a western redcedar orchard comprised of selections from Haida Gwaii. This low-gain seed is preferred to wild seed by most licensees operating there. Western redcedar comprises about 35% of our Company's annual planting program.

Our operations have established browse resistance operational trials using the stock developed by Ministry breeder Dr. Russell and his group.

WESTERN HEMLOCK

Our attention has returned to western hemlock as markets improve. In 2012 we rogued the low elevation orchard to raise the potential quality of seed crops to a genetic worth of 17. We anticipate managing a crop in 2013 to replenish our banked supply and replace the deployment of lower gain seed with this high gain crop. Our high-elevation second-generation western hemlock orchard produces a small amount seed. The breeding value of the stock in this orchard is 11. Western hemlock seedling requests average 10% of our annual planting program.

SITKA SPRUCE

The weevil-resistant (*Pissodes strobi*) Sitka spruce orchard at Saanich Forestry Centre produced a good crop in 2010, with seed for 3.8 million seedlings. This orchard includes selections identified by the Ministry's Sitka spruce breeding program and its seed delivers 85% weevil resistance to regeneration programs. Demand for this seed is low; WFP requests for Sitka spruce are between 2 and 5% of our annual planting program. No further crops will be managed until volume gain is delivered with the weevil resistance or demand reduces the banked supply. Regeneration from this seed demonstrates very good growth when grown together with non-resistant stock on sites where weevil hazard is low or nil. We also manage a Sitka spruce orchard with selections from Haida Gwaii, where the weevil is not present. Test scores for growth provided the basis for roguing this orchard in 2012. The breeding value of this orchard is now +5 and further selections may be added if demand warrants.

YELLOW-CEDAR

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

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

BRITISH COLUMBIA MINISTRY OF FORESTS, LANDS AND NATURAL RESOURCE OPERATIONS TREE IMPROVEMENT BRANCH

Brian Barber, Charlie Cartwright, Lee Charleson, Jim Corrigan, Diane Douglas, Barry Jaquish, Stephen Joyce, Dave Kolotelo, Sylvia L'Hirondelle, Leslie McAuley, Greg O'Neill, John Russell, Michael Stoehr, Ward Strong, Nick Ukrainetz, Darrell Wood, Chang-Yi Xie, Alvin Yanchuk and Susan Zedel

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BRITISH COLUMBIA'S FOREST GENETICS CONSERVATION AND MANAGEMENT PROGRAM Brian Barber

In 2012, the BC Forest Service celebrated its 100th anniversary. In conjunction with this milestone, Gerry Burch, former Chief Forester, BC Forest Products and Dr. Mike Meagher, former BC tree breeder, wrote a biography of Dr. Alan Lindsay Orr-Ewing: The Father of Forest Genetics in British Columbia. This book can be purchased through the Forest History Association of BC. Since the last CFGA report, the Ministry's organizational structure and governance model for forest genetic conservation and management has remained relatively stable.

Tree Improvement Branch continues to be responsible for tree breeding, research, policy, decision support, seed orchards, and the processing, registering, testing, and storing of tree seed. The Forest Genetics Council of BC (FGC) also continues to provide advice to the Provincial Chief Forester respecting policy and funding priorities. Contributions of the BC tree improvement program to BC's forest sector and economy was recognized by the MLA Committee on Timber Supply in the summer/fall of 2013. As such, funding for research and incremental activities has also remained relatively stable. Staff retention and recruitment however are the most acute challenges with our current workforce.

David Reid, Manager, Seed Production, retired in January 2013 followed shortly by his administrative assistant Debbie Poldrugovac. Stephen Joyce was hired as the new manager and is based at the Kalamalka Forestry Centre. Long-term seed orchard supervisors Chris Walsh (Kalamalka Seed Orchard, Vernon) and Keith Cox (Skimikin Seed Orchard, Salmon Arm) also retired. Hilary Graham was hired as the new supervisor at Skimikin Seed Orchard and Penny May was hired as the new supervisor at the Bailey Road Seed Orchard near Vernon.

Regarding FGC, Kerry McGourlick, Industry Co-chair, retired from Western Forest Products Inc. and was replaced on FGC by Larry Gardner, Chief Forester, West Fraser. Tim Lee, Interior Technical Advisory Committee (ITAC) chair, FGC Councillor and Vernon Seed Orchard Co. Manager, passed away suddenly in July 2013 and his position on FGC and chair of ITAC was assumed by Stephen Joyce. A number of retirements are expected over the next 1–3 years. The face (and faces) of the tree improvement community in BC will therefore change considerably, but the historical legacies of Dr. Orr-Ewing and others, and our collaborative model between government, industry, and academia will continue to yield benefits for generations to come.

POLICY AND DECISION SUPPORT Lee Charleson, Leslie McAuley and Susan Zedel

The *Forest and Range Act* (FRPA) is the governing legislation that outlines the authority and regulatory requirements for forest and range practices, including silviculture obligations, forest regeneration, and

seed use. The Chief Forester's Standards for Seed Use sets out legally enforceable standards to maintain the identity, adaptability, diversity, and productivity of the province's forest tree genetic resources through the registration, collection, storage and testing, selection, and transfer of seed intended for use on Crown land. Over the past two years, opportunities for more flexible and/or innovative seed use practices have been sought through requests for alternatives to the seed use Standards. Seed use alternatives considered by the Chief Forester have generally fallen under seed transfer; however, recently there has been an increasing trend towards stand and landscape level alternative standards for seed selection. Chief Forester policy and decision support for seed use alternatives continues to be managed through the Chief Forester's Standards Technical Advisory Committee.

In response to the observed and anticipated impacts of climate change, a new policy development cycle has been initiated to transition BC from a geographically-based seed selection, transfer and deployment system to one that is climate-based. The Climate-Based Seed Transfer (CBST) project is a multi-year project comprised of four phases: Phase 1 – Science Foundation, Phase 2 – Policy Development, Phase 3 – Implementation, and Phase 4 – Monitoring and Revision. Currently, we are in year two of Phase 1, with efforts focussed primarily on the testing and validation of the proposed scientific methodology and approach. Seed transfer resource allocation and policy/business area oversight, external client and stakeholder engagement and consultation, priority setting, including support of research related to genecology, and the broader CBST project, continues to be provided through the provincial FGC, Seed Transfer Technical Advisory Committee (STTAC) with Lee Charleson as chair of this TAC.

This year FRPA is also nearing its 10 year anniversary and, together with a broader Ministry-wide Integrated Decision Making policy initiative, a number of changes are anticipated that will aim to bring all natural resource policy, practices, and decision-making under a 'one land, one manager' resource management framework.

Over the past two years headquarters policy, decision support, and seed specialist staff has also provided services outside the branch in the following business areas: Resource Management Objectives, Climate Change (Forest Stewardship), and BC Timber Sales. Services and work has included: 1) participation in the integrated decision-making and resource management objectives initiative, 2) verification of genetic gain assumptions identified in timber supply data packages, analyses, and/or rationale reports, and in strategic silviculture plans (Type 4) for a number of timber supply and tree farm license management units, 3) support of provincial parent tree and seed registries, and annual seedling request orders, and 4) integration of seed use policy with related forest stewardship and resource management policies (spanning the silviculture, forest health, and ecological interface).

Ongoing work in information management (data, spatial/non-spatial query tools, and web-based computer systems) included SPAR (Seed Planning and Registry) and SeedMap support to clients are conducted throughout the year, with the busiest time between September and February. The number of seedlings requested for planting on Crown and private land was 247 million in 2012 and 238 million in 2013. Maintenance and enhancements to SPAR and SeedMap are ongoing. Development of spatial data for each seedlot's "area of use" is nearing completion.

During this two year period, the GIS Analyst position was vacated, creating a large gap in the Branch's ability to provide GIS-based services to support initiatives such as the CBST project. Staff replacement is not contemplated at this time due to resourcing limitations and staffing priorities throughout the division and Ministry. GIS work will be done by contract in the foreseeable future.

On the climate change front, strategic planning, policy analysis, decision support, and business area expertise and services were provided to support Ministry, divisional, and branch level initiatives, projects and climate change actions (forest stewardship, tree improvement). This work included coordination of the Ministry's Climate Change Steering Committee, business area representation on the Ministry's Climate Change Adaptation team (current focus on Forest Stewardship); project design and content development for the ministry's climate change (adaptation) training module one 'Resilient Forests and Ecosystems' project leadership. It also includes policy, decision support, and information management, participation in the Climate Based Seed Transfer Phase 1 Science Foundation Working Group; and strategic planning, policy analysis and assessment of climate-related projects for the development of forest regeneration and stocking standards and guidance.

Extension and Forest Genetics 2013 (Diane Douglas)

Extension continues to be a cooperative program. Tree Improvement Branch (TIB) coordinates and develops extension activities. Diane Douglas is chair of the FGC Extension Technical Advisory Committee (ETAC).

A Resource Stewardship Division (RSD) learning series was facilitated: 1) to share what's going on in the division with the primary audience being RSD and 2) to get people together that normally wouldn't interact. There were 8 presentations with topics including "Deer browse resistance of western redcedar" by John Russell and "Impact of Mountain Pine Beetle on BC's Forests" by Albert Nussbaum.

Forest Genetics 2013 was held in Whistler, BC July 22 – 26, 2013. This conference was a joint meeting of the Canadian Forest Genetics Association (CFGA), Western Forest Genetics Association (WFGA), IUFRO Population, Ecological and Conservation Genetics (Working Group 2.04.01) and IUFRO Breeding and Genetic Resources of Pacific Northwest Conifers (Working Group 2.02.05). Lee Charleson, Tree Improvement Branch and Sally Aitken, UBC were co-chairs with 168 people attending from 22 countries. It was a highly successful conference.

There was considerable input from TIB: 1) John Russell was in invited speaker, 2) Alvin Yanchuk and Michael Stoehr, Scientific Committee, 3) Greg O'Neill and Michael Stoehr, Field Trip (July 26–27, southern Vancouver Island), 4) Diane Douglas and Lee Charleson, Registration, Logistics, and Finances, and 5) Dave Kolotelo, Tree Seed Working Group workshop facilitator, July 22 at the Tree Seed Centre and CFGA Student Sponsorship.

SEED PRODUCTION Stephen Joyce

The TIB operates five seed production sites (Prince George Tree Improvement Station (PGTIS), Skimikin, Kalamalka, Bailey Road, and Saanich Seed Orchards) and has managed seed orchards since 1963. TIB produces from 40 to 50% of BC's orchard seed from 40 seed orchards. These consist of nine species within several seed planning zones and elevations and contain 50,000 ramets.

The two Douglas-fir (*Pseudotsuga menziesii*) seed orchards at Bowser Seed Orchard have been retired and coastal Douglas-fir seed orchard production is now at Saanich Seed Orchard and industry sites. A red alder (*Alnus rubra*) orchard was grafted and readied for establishment at Saanich Seed Orchard in 2013. Existing orchards were maintained and enhanced through roguing of some orchards, and planting of additional ramets in young orchards still being established.

Seed production over the past two years was 165 kg in 2011 and 373 kg in 2012. This is down from the record crop of 1612 kg in 2010 and this lower production was expected after such a large bumper crop. To date, TIB orchards have produced over 13,500 kg of seed since 1974.

Table 1. Seed production in British Columbia for 2011 and 2012				
	No. orchards	Hectolitres of	Kilograms of	Millions of potential
Year	producing	cones	seed	seedlings
2012	25	590	373	36
2011	16	346	165	18

 Table 1. Seed production in British Columbia for 2011 and 2012

Seed Orchard Pest Management (Jim Corrigan)

The Interior Seed and Cone Pest Management Biologist provides services to the Tree Improvement Program including: 1) diagnoses of pest-based problems that affect propagule production coming from the province's network of seed orchards, 2) pest management advice to seed orchard personnel, 3) pest status reports to seed orchards and to the larger community-of-practice, 4) technical assistance and financial support through grants awarded by the FGC Pest Management Technical Advisory Committee

(PMTAC) for pest management research and operational trials, 5) representation at professional and operational meetings, and 6) maintenance and curation of collections of specimens relevant to seed orchard pest management.

Highlights from the past several years include holding spring field days and fall pest management workshops for the Interior seed orchard community. Through the PMTAC, support has been provided for the following projects and activities:

- 1) Trials to further efforts to obtain Minor-use pesticide registrations for new products for use in conifer seed orchards,
- 2) An attract-and-kill trial targeting *Synanthedon sequoia*, the Sequoia pitch moth,
- 3) Screening pesticides for control of the long-haired June beetle (*Polyphylla crinata*),
- 4) Operational-scale trials to evaluate the efficacy of Dimethoate, applied at several rates, to control *Dioryctria abietivorella*, the fir coneworm,
- 5) Detecting and quantifying direct and indirect feeding damage caused by the western conifer seed bug (*Leptoglossus occidentalis*) on lodgepole pine (*Pinus contorta*) seeds,
- 6) Operational Support for MFLNRO Cone & Seed Pest Research Laboratory (Dr. Ward Strong), and
- 7) Operational Support for MFLNRO Cone & Seed Pest Biologist (Jim Corrigan).

TREE SEED CENTRE David Kolotelo

The Tree Seed Working Group (TSWG) continues to provide extension on seed science and technology through its biannual Newsbulletin and its biennial workshop. Since the last Canadian Forest Genetics Association conference in 2011 at Thunder Bay, Ontario, we have produced News Bulletin editions 54, 55, and 56 with number 57 in preparation. Edition 57 will focus on abstracts from our recent TSWG workshop at the BC provincial Tree Seed Centre that attracted 48 people for presentations on the topic of 'Reproductive Biology'. Most talks focused on lodgepole pine seed set issues as this is our largest seed production issue. A dedicated webpage has been set up to house the abstracts and the pdf versions of the PowerPoint presentations.

From October 17, 2011 to May 20, 2012, Dave Kolotelo was Acting Manager of the BC Tree Seed Centre (TSC). From the TSC perspective our highest priority areas have been filling some of our succession issues due to some long-term employee retirements (Dawn Stubley, Dave Cripps, and Chuck Woodward). Operationally our biggest challenge is improving the efficiency of our kilning regime and in the fall of 2012 we had an Engineering co-op student perform some kiln evaluations and provide recommendations for kiln improvements. This kiln work will continue with a controlled assessment of optimal temperature and humidity setpoints to maximize extraction efficiency.

Dave Kolotelo continues to chair the FGC Genetic Conservation Technical Advisory Committee (GCTAC) which produced a new strategic plan during this time interval.

Dave Kolotelo attended the Whitebark Pine Ecosystem Foundation of Canada meeting in 2012 in Kimberley, BC and made a presentation on "Whitebark Pine: Seeds, X-Ray Viability Estimates and Propagation Options". He also served on the International Seed Testing Association (ISTA) Forest Tree and Shrub Seed Committee, FGC Applied Biotechnology Steering Committee, and both the Coastal and Interior Technical Advisory Committees. Dave was also on the Forest Genetics 2013 conference organizing committee responsible for the student sponsorship program and organizing the TSWG workshop.

TREE BREEDING

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

The BC tree breeding program, while still relatively well funded, continues to be under strain due to the lack of succession planning and hiring. In spite of the continued reduction in our technical capacity, we are nonetheless continuing to try and integrate some of the more recent tools and methodologies developing in the breeding and genetic improvement sciences. As will be seen in some of the program updates below, our main areas of new research direction are: 1) improving our phenotyping methods in

pest and disease resistance breeding, 2) continuing to develop and implement climate based seed transfer guidelines, 3) developing molecular genetic markers and marker-assisted breeding approaches that may be useful for early selection, and 4) using new mixed linear models that incorporate spatial environmental variation, genetic competition effects, and genetic groupings for genotype x environment interactions.

Not surprising to many people working in forest genetics over the last decade, is the rapid advance in the development of genotyping platforms and therefore new genomic tools that are expected to provide a major advance in tree breeding. Several genomics projects, funded by Genome BC and Genome Canada (e.g., the 'SMartForests' project on the genomics of spruce, the 'AdapTree' project looking at adaptive traits in pine and spruce, and 'PopCan' (genomics of poplar)) are well underway and working with populations of species in our current breeding programs. As such we are working closely with University of British Columbia (UBC) investigators and other agencies involved in these projects to look at approaches for applying the new tools and results in our genetic management programs. Also, new funding opportunities for more direct application of some of these genomics tools are becoming available in Canada. This will present us with interesting challenges, as well as opportunities, to change components of how we may want to do genetic selection in our breeding programs, as well as building more technical capacity in the field.

Coastal Douglas-fir (Michael Stoehr, Lisa Hayton and Keith Bird)

The current phase of testing and selection in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) is accomplished by the use of complimentary tests. A set of four General Combining Ability (GCA) tests of parents structured in sublines and planted in incomplete blocks (Alpha-design) is used for parental breeding value determination and selections are then made in 5x5 full-sib family block tests (planted on two sites). This year, selections were made in Series 2 (out of 4) in 7 sublines. Trees were measured at age 11 from seed by taking heights and diameters in the 71 polymix families (in GCA tests) and 81 full-sib families (in family blocks). Wood density was also determined on half of the trees on 3 GCA tests using the Resistograph. The primary selection criterion is growth. Secondary selection criteria include wood density, where selected candidates cannot have wood density values less than 5% below the population mean, based on mid-parent values and stem form (absence of ramicorns). Breeding values of selected in 2015 and in 2018 for Series 4. For the next generation of improvement, the breeding strategy is based on 4 parent diallels per subline with additional crossings across Series and possible open-pollinated testing followed by molecular paternal analysis. It is anticipated that 128 parents will form the breeding population (4 parents x 32 sublines) with an expected Ne of around 80 to 90.

Western Redcedar (John Russell)

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

Volume growth and cedar leaf blight resistance (CLB) Breeding for first-generation polycross testing, involving approximately 1000 parents for the Maritime Low Seed Planning Unit (SPU), is complete. Seven annual series of tests have been established totalling 46 sites covering six SPUs. Parental breeding values for volume at rotation and cedar leaf blight resistance are currently being estimated from all seven series based on 10-year data (1000 parents). Rogued and new seed orchards are currently producing seed with a genetic worth up to 20% for volume at rotation with partial resistance to CLB. Advanced generation breeding is currently ongoing using assortative mating with partial diallels.

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

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

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

Yellow Cypress (John Russell)

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

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

Western Hemlock and True Firs (Charlie Cartwright)

The forest genetics program for western hemlock (*Tsuga heterophylla*) is in the process of securing past investments and moving to inactive status. Although the species is the most common in the province's coastal timber inventory, few are planted, rendering a breeding program unnecessary. Final projects include analysis of polycross trials of top parents from first-generation testing of the Hemlock Tree Improvement Co-operative (HEMTIC), archiving of best HEMTIC second-generation forward selections, and analysis of clonal trials of more recently selected BC parent trees. Final analysis of 28 genecology installations and 9 realized gain trials are also underway.

The focus of true fir programs is on deriving seed transfer guidelines from genecology trials and identification of superior provenances for wild stand seed collection. Age 30 year data have been collected from grand fir (*Abies grandis*) provenance testing and similar data will be collected this year for noble fir (*A. procera*). Pacific silver fir (*A. amabilis*) seed transfer trials by contrast are being measured for age 10 results, while sub-alpine fir (*A. lasiocarpa*) test series are still in the establishment phase.

Broadleaves (Chang-Yi Xie, Lisa Hayton and Keith Bird)

Red Alder

Preparation for range-wide provenance-progeny testing is underway. Twenty four natural populations ranging from northern BC to California were sampled (Fig. 1) and seeds from 10 well-spaced mature trees were collected in each population. About 20,000 seedlings are growing in the greenhouse (Fig. 2) and will be ready for planting in the spring of 2014.



Figure 1. Locations of the sampled 24 natural populations of red alder.



Figure 2. Red alder seedlings of the 24 populations growing in the greenhouse at Cowichan Lake Research Station.

Six of the 10 sites have been selected (Table 2). Four more sites will be selected from the Chilliwack and Squamish districts.

Site Name	District	Latitude	Longitude	Elevation (m)	BEC Zone
Mt. Brenton	South Island	48 52 43	123 54 55	600	CWHxm1
Harry Rd.	Campbell River	50 05 35	125 23 29	50	CWHxm1
Vernon Lake	North Island-Central Coast	49 58 12	126 15 15	300	CWHmm1
Beaver Cove	North Island-Central Coast	50 31 20	126 51 34	200	CWHvm1
Lois Lake	Sunshine Coast	49 47 58	124 18 04	180	CWHdm1
Rainy River	Sunshine Coast	49 33 57	123 30 14	200	CWHvm1

Table 2. Six of the 10 sites that have been selected for red alder progeny tests

Big-leaf Maple

Trees of *Acer macrophyllum* are doing well on all three sites (Fig. 3). Brushing will be done and the second assessment will be completed by the end of 2013.



Figure 3. Four-year-old bigleaf maple trial at the Power River, BC site.

Black Cottonwood

A total of 108 superior clones of *Populus nigra* were selected based on 4-year performance at the Harrison site and cuttings were collected from UBC where no infection by *Septoria musiva* has been noticed. Cuttings are being rooted at CLRS and will be planted at Saanich Seed Orchard.

Interior Spruce (Barry Jaquish)

Interior spruce (*Picea glauca* x *engelmannii*) is the oldest tree improvement program in the BC Interior. Genetic testing and parent tree selection began in the late-1960s and expanded rapidly through the 1970s and 80s. Phase one of the program focussed on three ecologically and geographically unique regions: Prince George, Bulkley Valley, and East Kootenay. Phase two began in the mid-1970s and centred on the remaining geographic regions where Interior spruce was commercially and ecologically important. The program has progressed to the point where much of the current planting stock (50 – 90 million seedlings per year) comes from improved first-generation seed orchards. Full-sib second-generation progeny tests are in place for the Prince George, East Kootenay, and Smithers SPUs. In the Prince George Series 1 program, 65 second-generation forward selections have been grafted and established in clone banks and breeding orchards. In the East Kootenay SPU, 75 second-generation forward selections were made in 2009 based on 10-year measurements. Grafting was completed in early spring 2010 and a new breeding orchard was planted at Kalamalka in spring 2012. Breeding for second-generation selection is now focussing on the Nelson low and mid-elevation SPUs.

In 2011, measurements were made on the 40-year-old Aleza progeny test, the first interior spruce test established in BC. Family mean tree volume ranged from 0.08368 m³ (family PG110) to 0.41844 m³ (family PG29). Multiple correlation coefficients between breeding values estimated by BLP for height at age 15 and 40-year BLUPs for height, diameter, and volume were significant (R = .97, .91 and .40, respectively). Six-year measurements were also conducted on the four Prince George Series II full-sib progeny tests. Additional test plantation maintenance and measurement included: 1) four sites in the six-year-old Prince George realized gain tests, 2) five sites in the 15-year-old interior spruce somatic embling field trials, and 3) the 35-year-old comparative study of spruce families from three early BC seed planning zones and an Ottawa Valley population. Finally, seedlings from all of the parents in seed orchard 211 (Vernon Seed Orchard Company) were raised for planting in raised beds at Kalamalka for further screening for resistance to leader weevils.

Western Larch (Barry Jaquish)

In 2011, approximately six million western larch (*Larix occidentalis*) seedlings were planted in BC, 88 % of which originated from seed orchards. Presently, the second-generation crossing program in the East Kootenay and Nelson SPUs is now about 80 % complete and should be completed within three years.

In this reporting period, three series of western larch genetic tests were maintained and measured: windpollinated progeny tests (ten sites), the 20-year-old range-wide provenance test, and the 6-year-old Nelson SPU realized gain genetic tests. The realized gain genetic test includes four western larch seedlots of different genetic origin (composite control, two seed orchard seedlots, and elite) planted at three spacings (1.5 x 1.5m, 2.5 x 2.5m, 3.5 x 3.5m) on three contrasting sites (Bear Creek, Taurus Creek, and Burton Creek). The experiment was established in a replicated complete-block design with two replicate blocks on each site. Sampling units consisted of 144 trees planted in 12 x 12 tree square plots. After six growing seasons, site had the largest effect on tree growth. Mean tree height at Burton, the site with the greatest summer and annual precipitation, was 348 cm, while Taurus Creek, a cold and dry high elevation site, was 171 cm. Overall, the mean 6-year height of the elite and seed orchard seedlots were 25 and 13% greater than the control seedlot, respectively. Survival was about 83% across all sites and treatments. Tree spacing had little effect of tree height at this age.

Interior Douglas-fir (Barry Jaquish)

The BC Interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) tree breeding program began in 1982 with the objective of producing improved and genetically diverse seed for planting stock on productive forest lands in south-central BC. Within this wide-ranging and ecologically diverse land base, six SPUs were delineated for tree improvement. The first cycle of breeding in each SPU was based on: 1) phenotypic selection in wild stands, 2) establishment of grafted breeding orchards and clone banks, 3) progeny testing using open-pollinated (OP) seed collected from wild stand trees, 4) delayed clonal seed orchards established using backward selection based on early progeny test results, and 5) controlled mating to produce pedigree material for second-generation selection. The breeding goal is to improve

traits related to tree size (height, diameter, and volume) while maintaining wood relative density near old growth values. Moreover, the recent discovery of resistance to *Armillaria* root disease in Interior Douglas-fir suggests that resistance to *Armillaria* could become an important trait of interest. The first-generation progeny testing program includes 1466 OP families and 31 test sites across the six SPUs.

Seed orchards were established for each SPU in the north Okanagan in the early 1990s and are starting to come into production. In 2011, approximately 3 million of the 9.4 million Interior Douglas-fir seedlings planted in BC came from seed orchards. In 2010, selected parent trees from southern SPUs were identified to establish new 1.5 generation seed orchards for the Thompson Okanagan high and low elevation SPUs. The Thompson Okanagan region was excluded in the program's early stages because of low site productivity; however, recent increases in planting numbers combined with severe seed shortages for the area necessitates the establishment of orchards for these lands.

The second-generation crossing program focuses on the Nelson SPU and includes selected parents from the old West Kootenay, Shuswap Adams, and Mica regions. Moreover, since inter-varietal (coastal x interior Douglas-fir) hybrids have shown to be hardy and fast growing in the Nelson low elevation zone, the Nelson second-generation breeding population has been augmented with 16 high breeding value parents from the BC coastal breeding program and 16 forward selections from superior Submaritime provenances in the Trinity Valley range-wide Interior Douglas-fir provenance test.

Supportive research projects undertaken in this reporting period include: 1) measurement of the 20-yearold Douglas-fir coast/interior transition zone adaptation trial, 2) 25-year measurement and analyses of the Douglas-fir seedling/steckling trial, 3) measurement and analyses of the 35-year-old range-wide provenance test at the Trinity Valley tree breeding site, and 4) the initiation of a co-operative program with the Canadian Forest Service to expand the screening of Interior Douglas-fir parents for resistance to *Armillaria* root disease.

Lodgepole Pine (Nick Ukrainetz)

The lodgepole pine (*Pinus contorta* var. *latifolia*) breeding program has progressed from OP (firstgeneration) progeny testing to full seed production in several seed planning zones. Five zones were identified as high priority for advanced generation breeding. Information from OP testing was used to rank parents for growth and wood density. Top parents were selected for second-generation (F1) breeding and were crossed to produce a growth and a wood density breed. The second-generation progeny tests are now approaching 10 years from planting and will be used to select parents for next generation orchards and the breeding population. The highest priority trait continues to be volume at rotation, however, three pathogens have been identified for resistance breeding: western gall rust (*Endocronartium harknessii*), comandra blister rust (*Cronartium comandrae*), and dothistroma needle blight (*Dothistroma pini*). We are actively monitoring first- and second-generation progeny tests for signs of infection and pursuing opportunities for artificial inoculation trials. We are also continuing to survey attack by mountain pine beetle (*Dendroctonous ponderosae*) when progeny tests are attacked. There are two series of realized gain trials that will be monitored to verify breeding values and gain estimates for parent trees and seed orchards.

Interior Western White Pine (Nick Ukrainetz)

The western white pine (*Pinus monticola*) breeding program is divided into 2 breeding populations servicing coastal and interior regions of the province. The primary focus of the breeding program is blister rust (*Cronartium ribicola*) resistance and secondary priorities include maintaining adaptation to local biotic and abiotic factors, and enhancing growth. The program began with controlled inoculation trials of seedlings grown from seed collected from wild trees that had no active blister rust infection. Seedlings were monitored for infection for several years then selected for seed production in seed orchards. The coastal program began F1 crossing and deployed 4 rounds of progeny tests which are now serving as a source of material for seed orchards. The interior program acquired 50 trees from the Idaho breeding program which tested well and have formed a strong genetic base. Controlled crosses were created among the Idaho parents and the progeny screened in controlled inoculation trials. The focus of the interior program now is on F1 breeding among resistant parents and the establishment of progeny field trials to assess field level resistance.

Interior Hardwoods (Nick Ukrainetz)

Testing and selection of hybrid poplar in northern environments is ongoing. A clonal test at Red Rock was used to characterize several controlled crosses among individuals of *Populus trichocarpa*, *P. deltoides*, *P. maximowiczii*, and *P. nigra*. Several clones were identified with superior growth characteristics and tolerance to northern climates and these have been selected to create a stool bed which will be available for operational use and further research.

An extensive genecology and open-pollinated progeny testing program has been established for paper birch (*Betula papyrifera*). There was significant genetic variation for volume and several forward selections have been made and grafted into two seed orchards for operational seed production.

Ponderosa Pine (Nicholas Ukrainetz)

There is one active seed orchard for ponderosa pine (*Pinus ponderosa*) which was created based on information and selections from a provenance test which included material from the species' range in BC and northwestern United States. The provenance testing program will serve as a source of material for future progeny tests. We are now attempting to stimulate flowering in one provenance test for seed collection. Collections from the provenance test will be supplemented with seed from parent tree selections for future progeny testing.

TREE IMPROVEMENT ENTOMOLOGY RESEARCH PROGRAM Ward Strong

The entomology research program has two main areas of focus: research to improve understanding and management of seed orchard pests, and techniques to select for pest resistance in tree breeding programs. Recent projects include:

- 1) Continued investigation of *Leptoglossus occidentalis* as the cause of late-season seed loss in lodgepole pine,
- 2) Biological control of *Leptoglossus occidentalis* using a native egg parasitoid,
- 3) Pesticide trials leading to registration of new insecticides for use in seed orchards,
- 4) Attracticide technology for managing Sequoia pitch moth (Synanthedon sequoiae) in lodgepole pine,
- 5) White pine weevil (*Pissodes strobi*) resistance breeding in Interior spruce using genomics-based marker-assisted selection,
- 6) Influence of physical resistance mechanisms in Interior spruce on feeding and oviposition of white pine weevils,
- 7) Screening for spruce budworm (*Choristoneura occidentalis*) resistance in Interior Douglas-fir based on host selection by adult moths, and
- 8) Chemical basis of resistance to larval spruce budworm in Interior Douglas-fir.

FOREST GENETICS, SEED TRANSFER AND PHYSIOLOGY

Climate Change (Greg O'Neill)

The Assisted Migration Adaptation Trial (AMAT) (Fig. 4) is a long-term multi-species field trial intended to provide a better understanding of tree species' climate adaptation. The trial involves 48 seed sources (mostly orchard seedlots) from 15 species native to western North America planted at 48 test sites in BC, Yukon, and neighbouring US states. The final 12 test sites were established in spring 2012. Growth and health will be assessed every 5 years, beginning in fall 2013. Relationships of seedlot growth and health with plantation climate will be developed enabling identification of the seed sources most likely to be best adapted to current and future climates. The information will be used to revise BC's species and seed source selection guidelines, and help to ensure maximum health and productivity of BC's planted forests well into the future.

A large interior spruce climate change/genecology field trial established by Barry Jaquish and Greg O'Neill is beginning to provide valuable information that will guide seedlot selection for interior spruce.

The trial consists of 128 seedlots (99 wildstand and 29 orchard) from BC, Alberta, and neighbouring states and territories tested at 17 locations in BC, Alberta, and Yukon. Growth at six years of age was assessed in 2009. The trial will be re-assessed in 2014 and data used to refine seedlot selection of spruce in BC.

A group of geneticists, policy, and information management staff is developing a new seedlot selection system for BC. The project seeks to create a user-friendly system that is climate-based, incorporates assisted migration, and utilizes the latest provenance data to ensure that future plantations will be optimally adapted for the duration of the plantation.

Adaptive Physiology (Sylvia L'Hirondelle)

The Adaptive Physiology research program helps forest geneticists and forest nurseries produce stressresistant trees with high growth potential by testing genetic variation in frost hardiness, photosynthetic potential, and phenology, as well as heat, drought, and other stress tolerances. Recent and current work includes: 1) development of physiology tools to estimate population responses to a variety of climates, to assist in seed transfer under potential climate change conditions, 2) studying the relationship between patterns of genetic variation in adaptive traits and site ecology in populations of conifers such as *Picea* glauca x engelmannii and Abies lasiocarpa, 3) improving the speed and accuracy of tests to predict overwinter storability of seedlings, field performance potential, and balanced seedling growth of major conifer species used in reforestation, and 4) collaborating with a forest pathologist who is developing screening methods for resistance to important foliar pathogens of conifers in BC.



Figure 4. Assisted Migration Adaptation Trial test site and seed source locations.

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PROCEEDINGS

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OF THE

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PART 2

Symposium

Whistler, British Columbia July 22–25, 2013

Editors S. Aitken, L. Charleson, R. Mellway and J.D. Simpson

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TRENTE-TROISIÈME CONGRÈS

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L'ASSOCIATION CANADIENNE DE GÉNÉTIQUE FORESTIÈRE

2^e PARTIE

Colloque

Whistler, Colombie-Britannique 22–25 juillet 2013

Rédactrices/Rédacteur S. Aitken, L. Charleson, R. Mellway et J.D. Simpson

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INVITED SYMPOSIA PAPER ABSTRACTS

ECOLOGICAL GENOMICS OF BUD BURST IN TEMPERATE TREES

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Monitoring of the timing of bud burst (TBB) during the past decades in temperate trees has shown that TBB has shifted substantially due to global warming. Climatic predictions suggest that this trend will continue in the future. These observable variations in situ demonstrate the important plastic phenological response of trees to climate change, but raise the issue of the consequences they will have on the fitness of the trees. The various potential adaptive responses were explored by considering the different evolutionary drivers contributing to TBB variation (divergent selection, assortative mating and gene flow) and by using theoretical and experimental approaches. Computer simulations suggest that the phenotypic shifts will enlarge the genetic differentiation along the same direction than the phenotypic cline due to the combined effect of assortative mating and gene flow. Such consequences may reduce fitness for species exhibiting counter-gradient variation, and increase fitness for species that follow co-gradient variation. These predictions were compared to observed variations at the phenotypic and molecular level in populations sampled along altitudinal/latitudinal gradients reproducing temperature gradients. Contrasting slopes of genetic clines are indeed observed between species (oaks vs beech) exhibiting co- and counter- gradient variation along the altitudinal/latitudinal gradients. Clines at the phenotypic level translated in a limited number of cases to clinal variation of allelic frequencies at candidate gene loci.

FORECASTING FUTURE RANGES: CURRENT LIMITATIONS AND NEXT STEPS

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One of the most ubiquitous and well-documented types of biological responses to climate change is the shifting of species' geographic ranges to higher latitudes and altitudes. Because species are shifting at individualistic rates, changes in geographic ranges are rippling up to create important yet poorly understood changes in community structure and ecosystem functioning. Thus, a pressing challenge for ecologists, biogeographers, and conservation biologists is to more accurately predict the extent and rate of future range changes by different species. Tools that are currently available for forecasting future ranges often make simplistic assumptions about current range-limiting factors and fail to incorporate evolutionary processes. Case studies of the perennial wildflower *Mimulus cardinalis* were synthesized across its elevation and latitude ranges in western North America. Using field studies, lab experiments, and demographic models, these studies aim to determine the relative importance of different range-limiting factors and assess how local adaptation and eco-evolutionary dynamics influence the process of range shifts.

GENOMIC SELECTION IN *EUCALYPTUS*: CURRENT ADVANCES AND THE WAY FORWARD

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The prospects of accelerating breeding for complex traits in forest trees by Marker Assisted Selection (MAS), has been one of the driving rationales of large forest tree genome projects. We were assured that we would be able to look at the tree's alleles at quantitative trait loci (QTLs) or even directly at genes and determine its breeding or genotypic value. This included the implicit assumption that by the genetic dissection approach we would have already established the effects of all important alleles during the life of the tree, in every population and environment. However, despite important advances in QTL mapping and association genetics (AG) in forest trees, it seems that ascertaining these effects is proving much more elusive than originally thought. QTL numbers and QTL effects are under and overestimated respectively, while candidate-gene based AG captures negligible fractions of trait heritability to be useful to breeding. Notwithstanding the limitations of early genomic technologies, an overoptimistic outlook of the architecture of complex traits and an underestimation of the phenotypic plasticity of forest trees most likely explain why MAS has not made it yet in the 'real tree breeding world'.

The inability to ascertain the effects of QTLs has now caused a paradigm shift in molecular breeding. We are now moving from trying to discover genes or genomic regions and their individual effects, back to dealing with the aggregate of the genome, just like quantitative genetics, although with a much higher resolution driven by high density genotyping. We can now predict the Genomic Estimated Breeding Value (GEBV) of an individual using Genomic Selection (GS) (Meuwissen et al. 2001). Differently from MAS, where a small subset of marker-trait associations are declared using stringent statistical thresholds and then used for selection, GS estimates all marker effects simultaneously, retaining all of them as predictors of performance. The rise of GS was based on technical breakthroughs and declining costs of high-throughput genotyping, coupled to improved statistical methods to deal with large datasets. GS works on the principle that linkage disequilibrium (LD), provided by dense whole-genome genotyping, is sufficient to track all relevant QTL effects for the target trait(s) which are expected to be in LD with the queried markers. The extent of marker–QTL LD, dependent on the effective population size and marker density, has therefore the largest impact on the prospects of GS (Grattapaglia and Resende 2011). This impact will intensify as generations of selection advance because higher marker densities enable GS accuracy to persist over time due to a slower decay of LD (Long et al. 2011).

Experimental studies in two *Eucalyptus* breeding populations (Resende et al. 2012a) and in loblolly pine (Resende et al 2012b) started GS in forest trees. Experimental accuracies averaging 0.5 to 0.8 were estimated for traits such as volume growth, wood specific gravity, lignin content and composition, pulp yield, and fungal rust resistance, converging well with predictions from deterministic (Grattapaglia and Resende 2011) simulations and indicated potential gains of 50-200% in efficiency of selection. We are now at the brink of operational implementation of GS in eucalypt intensive forestry in Brazil. To this end we developed a fully public 60,000 SNP Illumina InfiniumTM genotyping chip for Eucalyptus species (EucHIP60K.br), based on whole genome resequencing and SNP discovery in 240 unrelated trees of 12 eucalyptus species. This powerful chip has now been successfully validated across all major species of the genus by genotyping almost 17,000 samples from 10 different breeding populations, yielding high quality polymorphic data for >50,000 SNPs. Designed with one SNP every 10kbp on average, the chip supplied an inter-marker spacing of polymorphic markers (MAF>0.01) between 15.4kbp to 32.3kbp. Substantial increases in prediction accuracies of GS models were obtained with SNP data generated with the EucHIP60k.br.

The prospects of GS in tree breeding are very promising (Grattapaglia 2013). GS can increase the rates of genetic gain per unit time through increased accuracy of estimated breeding (for parent selection) or genotypic (clone selection) values, reduction of generation time and optimization of genome-directed mate allocation. How to incorporate GS into a Eucalyptus improvement program will vary on a case by case basis, following a cost/benefit analysis coupled to the adoption of innovative breeding tactics. Several issues are currently the focus of GS research, including: 1) modeling of non-additive effects, 2) accuracy of predictions, inbreeding, and reduction of selection response as generations of GS advance, 3) GS modeling across different populations and environments, and 4) logistic issues such as special nursery infrastructure, sample collection, and tracking. Being that it a publicly accessible genotyping tool, the EucHIP60k.br will allow increasingly larger datasets to be assembled, opening unprecedented opportunities of large scale meta-analyses of genotypic and corresponding phenotypic data. Not only will this empower genomic selection, but it should also enhance genome-wide association studies, gene discovery, and population genomics experiments in many species of *Eucalyptus*.

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SITKA SPRUCE BREEDING IN BRITAIN: DAWN OF A NEW ERA

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Although Sitka spruce (*Picea sitchensis*) is native to the Pacific North West it is well suited to the moist warm climate of northern and western Britain. There are now 700,000 ha of Sitka spruce planted in Britain producing around 6 million cubic metres of timber annually over a roughly 40-year rotation. The most suitable origin of seed for most of Britain is from Queen Charlotte Islands, BC although seed sourced from Washington or even Oregon State, USA survives and grows well on milder sites in the extreme south and west of Britain.

The breeding program commenced in the early 1960s along traditional lines selecting superior phenotypes in plantations, half-sibling progeny testing, and re-selection of the best parents based on an index of growth rate, stem straightness, and an indirect measure of wood density. Tested clonal seed orchards are now mature and productive.

The first full-sibling progeny trials combining some of the early re-selected parents are now over 20 years old. The best progenies are re-created through controlled pollination and the resulting seed is sold to nurseries who multiply this scarce resource by striking roots on cuttings taken from stock hedges raised from the seed. Rooted cuttings are deployed as full-sibling family blocks.

Currently all the Sitka spruce planted is based on improved material derived either from seed orchards (approximately 75%) or from vegetatively propagated material (25%). Clonal forestry is not currently being practised due to perceived lack of demand and institutional resistance; technological problems are largely overcome but not yet proven to be economically viable.

Policy makers tend to hold the view that the achievement of further gains through breeding is not justified due to the cost that this would entail as well as the time it would take. This is particularly the case when research funding is generally being reduced and being diverted from Sitka spruce towards other species. There is however, an appreciation that we need to protect the Sitka resource we have created in terms of resilience to climate change and out-breaks of disease. At the same time there is a demand to make faster and cheaper progress in certain areas of breeding particularly wood quality and its component parts.

A Sitka spruce Marker Aided Selection project started in 2004 with the planting of a large scale clonal trial replicated on 3 contrasting site types. At each site there are 4,500 clones made up of 1,500 clones in each of 3 full-sibling families. There are 4 replicates of the same 4,500 clones on all 3 sites. Initially, work concentrated on looking for microsatellite markers and later moved onto SNPs. In 2007 we became an external partner of 'Genome BC'. In 2008 we became partners in 'NovelTree' a 4-year EU funded project which enabled us to collaborate with Roslin Institute. Within this project we used the RAD approach to genotype large numbers of SNP markers and used these to predict the performance of trees for 6-year height and 5-year bud-burst. These results provide the first DNA-markers that have been linked to a phenotypic trait of Sitka spruce growing in Britain, albeit for a large number of individuals within one full-sibling family. This demonstrates an important step forward for conifer tree breeding in Britain.

DNA-marker work continues under a new 4-year EU contract called 'ProCoGen' during which time Forest Research and Roslin Institute aim to find markers for a wood-quality traits such as density or micro-fibril angle in all three full-sib families in our trial.

BREEDING FOR MULTIPLE PEST RESISTANCE IN WESTERN REDCEDAR (Thuja plicata) UNDER A CHANGING CLIMATE

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Climate can be defined as prevailing conditions and/or sets of attitudes in human affairs, which include changes in long term weather patterns, economic and cultural globalization, land management practices, and environmental restrictions. These climatic conditions affect biotic threats to forest ecosystems and populations and influence management strategies to ameliorate these threats. Populations resistant to multiple pests, current and future, are imperative to ensure ecosystem health under a changing climate. Plants have an innate immune system that includes both basal and induced defences. These innate responses have been shown to have broad-spectrum resistance to multiple pests.

Western redcedar (*Thuja plicata*), a member of the Cupressaceae, is an important commercial species in British Columbia. Its naturally durable heartwood is used for outdoor building material, where rot resistant wood is essential. Current western redcedar pests include foliar blights, ungulates, and heartwood rot pathogens. Selection for broad-spectrum resistance against these pests requires increasingly detailed phenotyping in conjunction with identifying the genes underlying resistance loci. Understanding resistance mechanisms will assist in developing breeding strategies to ensure durable resistance to multiple pests. Favourable genetic and biological properties of western redcedar that will aid in population improvement include early precious flowering and short generations, close inbreeding with minimal trait depression, and ease of vegetative propagation and clonal deployment.

MODELLING AND EXPLORING GENOTYPE BY ENVIRONMENT INTERACTION USING FACTOR ANALYSIS, REDUCED TREE MODELS AND HIERARCHICAL CLUSTERING IN MULTI-ENVIRONMENT TREE BREEDING TRIALS

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Accurate prediction of the genetic value of individuals is crucial for all breeding programs, but none more-so than tree breeding programs which typically have extremely long generation intervals of up to 35 years. Prediction and selection for many traits is based on phenotypic data acquired from so-called multi-environment trials (METs). METs usually include (genetic) trials grown over many years and locations, the latter chosen to represent the environments for which deployment will occur.

One of the main reasons for use of a MET is to determine whether individuals (that is, genotypes) will perform similarly across the range of environments relevant to the objectives of the breeding program. The varying performance of genotypes in different environments is known as genotype by environment (GxE) interaction. It is well known that GxE interaction can be partitioned into heterogeneity of scale and cross-over interaction (Muir et al. 1992), and it is the latter that can ultimately affect rates of genetic gain unless selection and deployment strategies are developed to capture the associated specific adaptation.

In general, GxE interaction appears to be relatively important for tree growth rates (see for example Johnson and Burdon 1990; Carson 1991; Matheson and Wu 2005) but there is little evidence to suggest that this is being adequately described and exploited in tree breeding programs. Progress towards a solution has been hampered by practical and methodological issues. A key issue is that a comprehensive and genetically connected MET data-set is required to reliably estimate genetic correlations between environments (Apiolaza 2012). However, it is not uncommon in many historical tree breeding MET data-sets for there to be few parents in common between environments. There are various reasons for this, including limited resources, propagation issues or simply poor planning.

Due to the typically poor connectivity in tree breeding MET data-sets, historically only simplistic statistical models, such as compound symmetric models, or piecemeal approaches based on pair-wise bivariate analyses have been used for modelling GxE interaction.

A one-stage approach to the analysis of tree breeding MET data was presented that incorporates both genetic effects (that is, additive and where appropriate non-additive effects) and non-genetic effects

associated with aspects of the experimental designs (e.g., blocking, sets, and plots). GxE interaction is modelled using a factor analytic model (Smith et al. 2001) which simultaneously provides an estimate of the genetic correlation matrix for all environments. The genetic correlation between two environments is inversely proportional to the amount of cross-over interaction for these environments. We exploit this by using a model-based clustering of environments based on the estimated genetic correlations in order to produce meaningful "mega-environments". By definition all pair-wise genetic correlations within a mega-environment are similar so that "generalised" main effects may be obtained separately for each. The approach is illustrated using a large MET data-set from the Radiata Pine Breeding Company program, which spans 37 years with over 320,000 observational units.

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GENETIC CHANGES ACCOMPANYING POST GLACIAL COLONIZATION IN OUTCROSSING PERENNIALS: LESSONS FROM *Pinus* AND *Arabidopsis*

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All northern plants have undergone considerable evolution during colonization, partly due to demographic effects such as bottlenecks, partly due to selection to new conditions. The consequences of this process were compared between *Pinus sylvestris* (very large populations in Eurasia) and *Arabidopsis lyrata* (a short-lived perennial with a scattered distribution in Europe and in North America). In both cases, local populations show evidence of local adaptation, and there is high phenotypic divergence for example in photoperiodic reactions and timing of life history events. The underlying genetics were compared in the two cases.

BACK TO THE FUTURE: EVOLUTIONARY GENOMICS OF HYBRIDIZATION

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One of the longest running debates in evolutionary biology concerns the role of hybridization in evolution. During the previous century, many botanists argued that new gene combinations generated by hybridization contributed importantly to adaptive evolution and speciation. In contrast, others accorded little evolutionary significance to hybridization, viewing it as a primarily local phenomenon with only transient effects, a kind of "evolutionary noise". These views became more nuanced in recent decades as theoretical and empirical evidence showed that hybridization could have a broad range of evolutionary consequences, including the persistence of hybrid zones, the reinforcement or breakdown of reproductive barriers, the origin and transfer of adaptations, and the birth of new hybrid lineages. However, the frequency and importance of these different outcomes remains controversial.

The presentation covered what we have learned about the role of hybridization in evolution from genome-scale analyses, focusing on studies of sunflower (Helianthus) species. The presentation 1) reported on patterns of genomic divergence between populations or species that vary with respect to levels of gene flow, and discussed the causes of so-called "islands of speciation", 2) described a likely genomic signature of "speciation by reinforcement" that might be useful for establishing the frequency of this evolutionary mechanism, 3) presented the results of a long term selection experiment that validates a textbook example of adaptive introgression and identifies the traits and candidate genes targeted by selection, and 4) showed that hybridization may be incidental to adaptive evolution in newly arisen hybrid species. The presentation concluded by evaluating the overall importance of these different consequences of hybridization in the plant and animal kingdom and discussed how their evolutionary role may depend, in part, on the genetic architecture of adaptation.

ADAPTATION AND CLIMATE CHANGE

ESTIMATED REDUCTION OF SUITABLE CLIMATE HABITAT FOR *Pinus* pseudostrobus AND EXPERIMENTAL ALTITUDINAL UPWARD ASSISTED MIGRATION FOR ACCOMMODATING CLIMATE CHANGE

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A bioclimate model predicting the presence of *Pinus pseudostrobus*, the economically and ecologically most important conifer species in the Trans-Mexican Volcanic Belt and Guatemala highlands, was developed using the Random Forests classification tree, for contemporary (average 1961–1990) and future climates (decades centered in the years 2030, 2060 and 2090) using three General Circulation Models (Canadian, Hadley, and Geophysical Fluid Dynamics) and two emission scenarios (A2, elevated emissions and B1 or B2, low emissions). Results suggested, that for Mexico and Guatemala, the area occupied by the *Pinus psedostrobus* climatic niche should diminish 51.3% by 2030, 68.3% by 2060, and 89.6% by 2090. Areas remaining suitable in the future were always projected for higher and higher altitudes. Because altitudinal clines are well documented for this species, the results indicate the need to realign contemporary populations to the climate for which they are adapted, which will occur at different sites than today. We tested this hypothesis at the forest of the Native Indian Community of Nuevo San Juan Parangaricutiro, Michoacán, western Mexico, by experimentally shifting populations of *P. psedostrobus* and *P. devoniana* (= *P. michoacana*) upwards in altitude. The latter species is naturally distributed at altitudes below the former and thus would be the intuitive candidate to replace the former.

At two years, we found very high survival (> 98%) and a gradual decrease of growth as the altitudinal shift increased. When *P. pseudostrobus* populations were moved +300 m (the shift needed to accommodate the climate change projected for year 2030), relative growth in seedling height decreased 15% (5% per each 100 m of elevation shift) in comparison to local populations. *P. devoniana* grew as much as the local population when moved +300 m, but decreased 30% when moved +600 m (again, 5% per each 100 m of elevation shift). This suggested that assisted migration might be a viable strategy to realign genotypes to projected climates for year 2030, although achieving less growth than natural populations under contemporary climate in the short term.

LANDSCAPE GENOMICS OF ADAPTIVE GENETIC VARIATION IN LONG-LIVED POPULATIONS OF A CALIFORNIA OAK, Quercus lobata

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Quercus lobata (valley oak) is an endemic widespread oak in California that occurs in grasslands, woodlands, and riparian forests, but is now in serious jeopardy due to habitat loss and threat of global warming. These oak populations have occupied unglaciated regions for at least 120,000 years without a significant shift in the climate niche, although there were some local contractions and expansions. This stability provides ample opportunity for local adaptation in response to climate, though temporal fluctuations may reduce those effects. Using population samples from throughout the species range, we examined three types of evidence for local adaptation. First, the association of gradients in allele frequencies of SNPs at selected candidate genes with climate variables and identified several genes associated with budburst and cold tolerance that are significantly correlated with climate gradients. Second, the results of an environmental association analysis based on whole bud/leaf transcriptome data sampled from widely distributed localities were reported. When control for the background genetic structure was made, outlier SNPs associated with environmental gradients in growing season precipitation were found that suggested additional candidate genes. Third, the genome-wide association study of SNPs associated with climate gradients, based on SNPs discovered through reduced-representation genome sequencing approach was presented. Overall, these three approaches produced evidence that local adaptation is shaping the distribution of climate-associated adaptive genetic variation in natural populations.

ASSISTED MIGRATION OF TREE POPULATIONS TO ADDRESS CLIMATE CHANGE IN WESTERN CANADA

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Commercial forestry programs commonly use locally collected seed for reforestation under the assumption that tree populations are optimally adapted to the local environment. This assumption, however, may no longer be valid for many forest ecosystems in North America given the warming trends and changes in precipitation patterns observed over the last 25 years. We used a bioclimate envelope modeling approach to project suitable species habitat and estimated the adaptational lag for populations of 15 major commercial forestry species in western North America under observed climate trends (1970s to 2000s) and multi-model projections for the 2020s, 2050s, and 2080s.

We found that on average, populations already lag behind their optimal climate niche by approximately 130 km in latitude or 60 m in elevation (Gray and Hamann 2013 Climatic Change 117:289–303). Our models further showed an average lag of approximately 310 km in latitude or 140 m in elevation by the 2020s, with the most pronounced geographic lags identified in the Rocky Mountains and the boreal forest. For application, we were able to derive a general formula where 100 km northward shift is equivalent to an approximately 44 m upward shift in elevation, which in principle could be applied to guide assisted migration of planting stock in reforestation programs together with the projected species distribution maps provided.

Additionally, we generated detailed seed transfer matrices based on an ecosystem classification system, specifying seed source and planting target locations in Alberta to help guide reforestation decisions in the province (Gray and Hamann 2012 PLoS One 6(8):e22977). We found that genotypes of species that are adapted to drier climatic conditions will be the preferred planting stock over much of the boreal forest that is commercially managed. Interestingly, no alternative species that are currently not present in Alberta can be recommended with any confidence. Finally, we observed large uncertainties in projections of suitable habitat that make reforestation planning beyond the 2050s difficult for most species.

FST OUTLIER TESTS IN GENOME SCANS FOR LOCAL ADAPTATION: WHEN DO THEY TELL THE TRUTH AND WHAT ARE WE MISSING?

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Next-generation technology has made it possible to obtain large amounts of genomic data. But how do we find locally-adapted genes from this mountain of data? One method is to look for outliers in the distribution of FST (FST outlier tests, FDIST (Beaumont and Nichols 1996) and BAYESCAN (Foll and Gaggiotti 2008)). Using a large-scale landscape genetics simulator, we compared these two programs for three demographic histories that are common to tree populations: isolation-by-distance, expansion from one refuge, and expansion from two refugia with secondary contact. The latter two demographies were non-equilibrium scenarios. We found a large number of false positive FST outliers with these demographic histories, especially with the refugia scenarios. We showed that the default parameters in Bayescan produce more false positives than FDIST, but that the number of false positives can be decreased with the prior-odds parameter without affecting power. We also proposed that the FDIST method can be improved by simulating the actual number of populations in the data, rather than by the number of samples collected.

PERCEPTIONS OF ADAPTATION IN FOREST MANAGEMENT

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Forest management is presently undergoing major changes to adapt to a changing climate. The objective of this research was to examine the variation in perceived acceptability of potential forest management interventions that can mitigate the risks of climate change among stakeholders in British Columbia (BC) and Alberta (AB). A mixed method research design was developed that included surveys, focus groups, and Q-sort analysis. Online surveys were administered to the general public and leaders of forest-dependent communities in BC and AB to determine their levels of acceptability of various intervention strategies for forest adaptation (ranging from a "do nothing" approach, a status quo option, assisted migration, and genetically engineering optimal growth in future climates), as well as to assess their knowledge and perception of risk of climate change and reforestation technologies. Online surveys were also administered to registered professional foresters and biologists in BC, assessing their levels of knowledge and their perceptions of efficacy of assisted migration strategies. In addition to the surveys, four case-study communities were chosen for an in-depth analysis of stakeholder perceptions. Three qualitative focus groups were conducted in each community and an exit Q-sort was administered to measure perceived acceptability to a set of nine forest adaptation management scenarios. With analysis underway, preliminary results indicated that professional foresters perceive the status quo management option as the most acceptable, indicating in Q-sorts that the natural regeneration scenario is perceived better than scenarios featuring assisted migration in some cases. Business owners were observed to perceive the status quo scenario as most acceptable, in some cases remarking on the importance of adapting human behaviour to the changing environment. Environmentalists' responses varied widely by region with some regarding assisted migration as the most acceptable and some preferring the status quo. Ultimately, it appeared that a hesitance to accept human capability to project climatic and ecosystem changes on the landscape informs the perceived acceptability of all management scenarios.

EARLY SURVIVAL AND CLIMATIC DAMAGES OF WHITE, ENGELMANN AND INTERIOR SPRUCE POPULATIONS IN ALBERTA

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As part of the adaptation to climate change, British Columbia (BC) in collaboration with Alberta initiated a new series of provenance trials for white (*Picea glauca*), Engelmann (*P. engelamnnii*), and interior spruces (*P. glauca x engelmannii*) with populations sampled from across western USA and Canada. This series is replicated on eighteen sites (15 in BC, 2 in Alberta, and 1 in Yukon Territory). The series builds on the knowledge gained from previous provenance trials of white and interior spruce in Alberta and BC in the past 30 years. The new trials were designed to measure the climatic effects of transferring tree populations from as far south as latitude 36° in Arizona to as far north as latitude 65° in Yukon Territory. The trials located in Alberta at Whitecourt Mountain ($54^{\circ}N$, 817 m) and Hay River ($59^{\circ}N$, 370 m) are of great interest in that they provide an environment that is colder and more continental than trials in BC that are more maritime and located in the south.

When tree mortality is assessed in older field trials, it is impossible to identify its causes, which impedes our ability to link tree mortality to adaptation to climate and the potential effects of climate change on forestry. Limitations encountered when using data from older provenance and progeny trials have led us to modify trial assessment procedures so that the causes of tree mortality can be identified during the first five years of field establishment. For BC-Alberta spruce provenance trials we have found that much of the tree mortality occurs during the first few years of field establishment. After four years in the field at Hay River, survival was 30 - 67% for provenances from warmer environments in the USA and southern BC; 82 - 94% for provenances from cooler environments in northern BC, Alberta, and Northwest Territories; and 72 - 79% for provenances from Montana and Idaho. The correlation between survival and negative degree days (NDD) was 0.53 (P < 0.0001) showing that trees from regions with cooler winters had better survival at Hay River than provenances from regions with warmer winters.

At Hay River, assessment at age 3 years showed that on average, less than 7% (range 1 - 10%) of trees from provenances in northern BC, Alberta, and Northwest Territories suffered dieback. Provenances from the USA and southern BC had as high as 31% dieback. The correlation between percentage dieback incidences and NDD was -0.72 and -0.77 during the second and third field season, respectively. The corresponding correlations at the Whitecourt Mountain site were -0.55 and -0.53. This shows that provenances from cooler environments suffered less dieback than provenances from warmer environments. The correlation for dieback incidences between Hay River and Whitecourt were 0.59 and 0.63 during the second and third field season, respectively.

Generally, preliminary assessment of these two trials suggests that transferring populations for long climatic distances could potentially harm forest health and productivity through repeated dieback at least during the stand establishment stage.

BROAD- AND FINE-SCALE GENETIC STRUCTURE OF A SPRUCE (Picea sitchensis x P. glauca) HYBRID ZONE SPANNING A CLIMATIC GRADIENT

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Historic colonization and contemporary evolutionary processes contribute to broad- and fine-scale patterns of genetic variation and differentiation among populations. However, teasing apart the respective influences of these processes remains a challenge. This is particularly true of natural hybrid zones, where standing genetic variation may result from evolutionary processes both preceding and following contact, influencing the evolutionary trajectory of hybrid populations. Where adaptation to novel environments may be facilitated by interspecific hybridization, teasing apart these processes will have practical implications for forest management in changing environments.

In this study, we evaluated the genetic architecture of the economically and ecologically important Picea sitchensis (Sitka spruce) x P. glauca (white spruce) hybrid zone along the Nass and Skeena River valleys in northwestern British Columbia using chloroplast, mitochondrial, and microsatellite loci alongside a complementary study using single nucleotide polymorphisms (SNPs). Sitka spruce mitotype 'capture' throughout the introgression zone pointed toward asymmetric gene flow, congruent with patterns observed for microsatellites and SNPs, indicating extensive long-term introgression and widespread recombination with more Sitka spruce than white spruce ancestry in hybrid populations. Significant and complementary clinal variation for marker-based hybrid indices based on both neutral microsatellites and candidate gene SNPs suggested ancestry is strongly influenced by climate and geography. These results indicated extrinsic selection played a strong role in the distribution and structure of this hybrid zone. Indeed, highly parallel results for replicate transects along climatic gradients provide support for the importance of extrinsic selection at a broad scale, fitting expectations of the environmentally-determined bounded hybrid superiority model of hybrid zone maintenance. Finally, broad-scale patterns of variation, combined with fine-scale analysis of candidate SNP-specific patterns of introgression revealed a suite of candidate loci that may be targets of extrinsic or intrinsic selection, particularly along precipitation gradients. This broad- and fine-scale analysis of genetic structure indicated the importance of historic and contemporary gene flow, environmental selection, and their interaction in shaping genetic variation within this hybrid zone. These results have important implications for selection of seed sources for current reforestation within this ecologically transitional area, and appropriate scale and direction of seed transfer relating current genotype-climate associations to future climate predictions for this region.

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CHASING DROUGHT TOLERANCE IN Populus

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The cultivation of poplars (*Populus* spp.) is favored for forestry and reclamation purposes throughout the northern hemisphere where they represent a commercially important resource. More recently, hybrid poplars are planted and grown to provide feedstock for pulp and paper production and a carbon-neutral alternative to fossil fuels (Balatincz and Kretschmann 2001; Yuan et al. 2008). Poplars may also help Canada to meet its international commitments to reduce greenhouse gases by carbon sequestration. Poplars, however, are generally regarded as drought sensitive, which is a problem especially in the light of increasing summer droughts in the prairies. Several studies including one from the Intergovernmental Panel on Climate Change also predict increased drought frequencies on the Canadian prairies (IPCC, 2001).

Here, we sought to address four main objectives related to drought tolerance in poplar: 1) to evaluate drought responses in hybrid poplar clones commonly planted in the Canadian prairies and subsequently generate a drought-tolerance ranking, 2) to use the contrasting responses of the least and the most tolerant clones to identify gene expression responses that correlate with drought tolerance, 3) to rapidly screen the role of identified poplar genes in drought tolerance by overexpressing them in transgenic *Arabidopsis* plants and assess the plants for altered drought responses, and 4) to alter the expression of poplar genes identified in objective 3 in transgenic poplar in a manner that may improve drought tolerance.

We first compared nine hybrid poplar clones commonly grown on the Canadian prairies with respect to height growth, leaf formation, water potential, and relative water content during controlled drought in a greenhouse setting. We found significant differences in responses between clones for all four criteria. In particular, one clone stood out as being tolerant to drought. Likewise, one clone was particularly sensitive to drought. The ranking generated from this analysis can inform the choice of clones for deployment at drought-prone sites.

Secondly, a set of genes that are induced by drought was identified from the literature (Bogeat-Triboulot et al. 2007; Plomion et al. 2006; Street et al. 2006; Wilkins et al. 2009) and based on their sequence similarity, they may be involved in different aspects of drought responses such as stomatal movement, osmotic adjustment, ABA signaling, and cellular water conservation. We thereafter compared their expression in the least and the most drought tolerant poplar clones during drought stress. This comparison yielded a subset of genes whose expression differed significantly between the two clones, including three genes with high similarity to genes in *Arabidopsis* implicated in ABA signaling and drought tolerance.

To further test the function of these three differentially expressed poplar genes, we initially overexpressed them in transgenic *Arabidopsis*. We measured seed germination, root growth, fresh weight accumulation, and water loss in wild type and transgenic *Arabidopsis* plants. Based on these parameters, transgenic *Arabidopsis* plants overexpressing a poplar ortholog exhibited a drought hypersensitive and ABA-insensitive phenotype. In addition, we also observed a molecular phenotype in the form of reduced expression of genes with known function in drought tolerance providing evidence that the tested poplar gene is a negative regulator of drought tolerance also on the molecular level. Similarly, transgenic *Arabidopsis* overexpressing a second poplar gene showed a reduced water loss during drought stress indicating that this gene is a positive regulator of drought tolerance.

In light of these promising results, we have introduced the same DNA constructs into poplar plants. We presented our first analysis of drought responses of these plants as well as a perspective on how our results can be used for drought tolerance breeding in poplar based on both transgenic technology and marker-assisted selection based on natural variation.

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GEOGRAPHIC VARIATION IN PHENOLOGY BEHAVIOUR AND RESPONSE TO DROUGHT OF CORK OAK POPULATIONS IS CRUCIAL TO COPE WITH CLIMATE CHANGE

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Cork oak is widely distributed in the Western Mediterranean region, spanning a range of different environmental conditions, and frequently dominating open woodlands of high conservation and socio-economic value. Cork oak woodlands spread-out for over 715,000 ha in Portugal, covering 21% of the national forested area and 30% of the world's cork producing area. Cork oak is well adapted to the seasonal drought of the Mediterranean climate, following several decades of warming and frequent drought years. However, since the 1970's, maximum and minimum temperatures have risen in Portugal about 0.5°C each decade, corresponding to twice the average world temperature increment. In addition, since longer, more frequent, and more intense drought periods are expected, stress caused by the expansion of arid and semi-arid climate throughout the country will affect the species distribution. Consequently, not only established stands may be prone to tree mortality, but also the current reforestation effort may be jeopardized by low survival rates attributed to the use of unsuitable genetic material.

It is expected that, through genetic adaptation and/or phenotypic plasticity, cork oak populations may have developed significant differences in fitness and the traits related to it. In this context, provenance trials are the best resource of material to assess the variability between and within populations from seed sources sampled over a wide range of locations (stands) covering the geographical distribution of the species. This will enable the assessment of the levels and patterns of genetic variation for growth and traits determining adaptation to a specific environment, hence providing crucial information to select appropriate seed sources for planting, as well as to develop sustainable breeding and gene conservation programs. In 1998, multi-site provenance trials were established at three locations in Portugal, as a part of the "European Network for the Evaluation of Genetic Resources of Cork Oak for Appropriate Use in Breeding and Gene Conservation Strategies". Results from these field trials at 7 years from planting pointed out that seed origin must be considered in reforestation programs, since survival, growth, and adaptive traits (phenology, water use efficiency - wue) showed significant differences among populations. Responses to drought (a major limitation to cork oak regeneration) of four contrasting populations, selected according to their field performance for growth, phenology, and wue, were further studied under controlled-environment conditions where drought stress was induced. In addition, morphological (total height, root/shoot biomass), physiological (wue, water potential, relative water content, and gas exchange), and biochemical (maximum quantum yield of PSII) traits were assessed in 6-month-old seedlings produced from acorns collected in the stands representing the four contrasting provenances. Results from the controlled experiment provided an indication that drought adaptation was mainly related to early stomatal closure and root investment, with these traits showing significant differences between the studied populations. We have also observed significant differences in growth rhythm, as well as dissimilar temporal drought responses in the populations under study. In conclusion, both field trials and controlled environment studies showed consistent results and indicated an important influence of geographic origin on growth performance and wue.
SPECIES SPECIFIC VERSUS PARALLEL EVOLUTION OF LOCAL ADAPTATION IN TWO WHITE PINE SPECIES: Pinus monticola AND Pinus strobus

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Under rapid global warming, it is critical for us to better understand the capacity of forest trees to adapt to a changing climate, especially for species that are more at risk such as five-needle pines. Western white pine (Pinus monticola) and eastern white pine (Pinus strobus) have fragmented ranges and have suffered declines due to harvesting, fire suppression, and white pine blister rust (Chronartium ribicola). We identified and compared patterns of genetic diversity and adaptation to climate in these two species by using a set of 267 orthologous genes, including candidate genes for growth, bud phenology, and resistance to biotic and abiotic stresses. From those genes, assays for ~200 single nucleotide polymorphism (SNP) markers for each species were developed. Each set of SNPs was genotyped on range-wide samples of 362 P. monticola individuals (61 populations) and 840 P. strobus individuals (133 populations). Genotyping resulted in 158 and 153 successful SNPs for P. monticola and P. strobus, respectively. Depending on the species lineage and statistical models chosen, Fst outliers analyses revealed that from 4 to 25% of SNPs were candidates for natural selection, with the number of outliers being higher in P. strobus than in P. monticola. Similarly, ~25% of P. monticola SNPs and ~30% of P. strobus SNPs were significantly associated with one or more climate variables. Furthermore, out of the 79 genes containing SNPs in both species, $\sim 25\%$ showed signature of selection simultaneously in both species. These results indicated that a considerable amount of SNPs, genes, and gene families are under strong selection in both P. monticola and P. strobus. However, a larger number of SNPs and genes that have contributed to local adaptation are specific to each species. This indicates that parallel and species specific evolution can have occurred to shape adaptation to climate in these two conifer species, which diverged ~20 million years ago.

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Predicting effects of climate change on productivity of boreal and temperate forests requires a quantitative description of how cold adaptation mediates tree growth, yet this comes with the challenge of disentangling genetic differentiation from effects of ontogeny and climate. In this study, we calibrated a model of thermal and hydraulic limitations against 4.9 million periodic forest inventory measurements of relative growth rate (RGR), distributed across 34 North American species/subspecies sample groups, to develop a general framework for representing cold tolerance. Values of leaf-specific hydraulic conductance (K₁), derived from optimal fit against each sample, were correlated with the long-term average annual number of days with freezing (DWF) and the product of climate moisture index (CMI) and DWF ($R^2 = 0.90$, p < 0.001). We interpreted this as the selection of increased immunity against freezing-induced embolism at the expense of reduced hydraulic efficiency. Increased drought sensitivity in cold climates is partially balanced by increased cold hardiness, represented by setting reference growth at 10 °C in an exponential function of temperature (G_{10}) as an inverse function of DWF and setting direct sensitivity to freezing (\ddot{a}_F) as a function of K_L . The framework was equally applicable within the distributions of two tested species (Douglas-fir (*Pseudotsuga menziesii*) and white spruce (*Picea*) glauca)). Hence from a modeling perspective, the overall cold tolerance of trees predetermines how heat and water budgets limit immediate RGR by defining states of hydraulic efficiency and cold hardiness, while low hydraulic efficiency in cold-adapted populations controls the biomass sink by lowering the stem height and leaf area ratio that narrowly avoid drought-induced hydraulic failure over the long term. Estimating these traits using a climatological approach, by species or population, provides a foundation for representing maladaptation in spatially-explicit model predictions of tree growth under future climate change scenarios. The additional potential to map a cold tolerance index (CTI) based on geographically-weighted regression of satellite observations and ongoing efforts to test levels of hydraulic compensation and plasticity in G_{10} by comparing the model with tree-ring measurements were discussed.

GLACIAL REFUGIA AND MODERN GENETIC DIVERSITY OF WESTERN NORTH AMERICAN TREE SPECIES

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Interglacial warm periods, such as the current Holocene, are the exception to the Quaternary norm. The evolution of North American tree species, subspecies, and genetic varieties has therefore primarily taken place on the landscape with extensive continental ice and restricted temperate climate environments. Here, we reconstructed historical biogeographies and glacial refugia of western North American trees to evaluate the merit of biogeographic hypotheses regarding the existence of glacial refugia in Beringia and along the Pacific coast, the evolution of subspecies in widespread conifers, and the origin of Pacific Northwest inland rainforests. We used a species distribution modelling approach, validated against 3,571 fossil pollen and packrat (Neotoma sp.) midden records from 835 study sites. Genetic diversity was measured as allelic richness and expected heterozygosity, compiled from published studies for 473 populations of 22 tree species. We found that species with strong genetic differentiation into subspecies and varieties had widespread and large glacial refugia. In contrast, species with restricted refugia show no differentiation and little genetic diversity, despite being common over a wide range of environments today. In a regression tree analysis, 66% of allelic richness could be explained by the total size of glacial refugia. Expected heterozygosity was best explained by the number of glacial refugia as expected from genetic theory (27% variance explained). Habitat reconstructions further provide strong support for the existence of glacial refugia in Beringia and Haida Gwaii for many species, and suggest only a recent emergence of coastal Pacific Northwest tree species in inland rainforest.

MATCHING CURRENT FOREST GENETIC RESOURCES TO FUTURE CLIMATES

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One of the major concerns about the impact of climate change on ecosystems and forest trees is the mismatch between the climate that a forest ecosystem or a tree species is adapted to and the climate that the ecosystem will experience in the future. If such a mismatch occurs, the health and productivity of the ecosystem are likely to be compromised. Therefore, matching current forest trees with future climates is critical to ensure our future forests are adaptable to future climates. Niche-based bioclimate envelope models have been widely used to project future geographic distributions of ecosystem climate niches, which can be used as the basis for climate matching. However, challenges arising from model accuracy and the uncertainty of future climates make it difficult to apply the model projections with confidence in developing adaptive strategies in natural resource management.

We addressed these challenges through: 1) using high-resolution and high-quality climate data generated by ClimateWNA, 2) optimization of Random Forest modeling approach (a machine-learning classification approach), and 3) consensus projections based on multiple climate change scenarios. We found that our bioclimatic envelope model accurately predicted the distribution of current ecosystems in British Columbia (BC). Based on our predictions, over 20% of the BC landscape has already shifted to climate envelopes of different ecosystems since the 1970s. Projected shifts in BC ecosystem climates are substantial under climate change, which may seriously compromise the health of the ecosystems and impose challenges to land managers and policymakers. However, projected expansions of climate niches for several productive ecosystems may provide an opportunity to increase forest productivity and carbon sequestration capacity in BC if appropriate species and populations are matched for future climates. The consensus projections have been used in species selection and seed transfer and other projects. A web-based platform has also been developed to allow users to visualize and to have access to high-resolution climate data and the projections. To address the among-population variation within a species in the climate matching, we have developed population genecology functions, response functions, and a universal response function for lodgepole pine (Pinus contorta). A climate-based seed transfer system is being developed using both consensus projections and the universal response function.

PHOTOPERIODIC COMPETENCY FOR BUD DORMANCY INDUCTION IN Populus balsamifera

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Bud dormancy is an important overwintering mechanism for woody perennials and is induced in most species during late summer by a decrease in day length and/or low temperature. Adaptively, however, it would be important for newly flushed growth to avoid responding prematurely to a similar day-length that occurs in early spring. To investigate this phenomenon, two growth chamber experiments were conducted on four genotypes of balsam poplar (Populus balsamifera) originating from two latitudes. Rooted cuttings were moved into inductive, short-photoperiod conditions at weekly intervals after flushing at either 15°C or 20°C. Plant heights were measured every other day. Plants were harvested weekly and RNA was extracted for RT-qPCR on genes known to be involved in dormancy induction, and also for transcriptome sequencing followed by RT-qPCR to identify additional genes marking photoperiodic competency which were validated by qRT-PCR. Analysis of height growth cessation data showed that before a certain age, no matter how soon plants were transferred to short day conditions, they continued to grow until they became competent to respond to photoperiod. The different genotypes became competent at different times (18–40 days since flush), indicating possible genetic variation in this trait. Once competency was attained, it took plants 7-20 days to cease height growth under short photoperiod depending on genotype, experiment, and time since competency acquisition. Leaf number data revealed that competency acquisition and the transition from preformed leaf emergence to neoformed leaf production are two independent processes. Temperature did not appear to influence the development of competency, though it slightly increased the rate of height growth cessation. RT-qPCR results indicated three promising gene markers for competency: Potri.017G051100 (G6), Potri.001G222000 (G7), and CONSTANS 2 (CO2). Increased expression for G6 and G7 was observed post-competency relative to pre-competency, and changes in expression varied between leaves and stem tissue. In contrast, CO2 peaked in abundance at age 32d from bud flush, coinciding with competency acquisition. This research offers new insight into molecular mechanisms that may underlie the acquisition of photoperiodic competency, which, in a warming climate, may cause phenological mismatch in deciduous boreal tree species. By advancing dates of bud burst, earlier springs may result in plants becoming competent too early, causing premature height growth cessation and loss of productivity.

GEOGRAPHIC PATTERNS OF ADAPTIVE VARIATION IN INTERIOR SPRUCE AND LODGEPOLE PINE IN WESTERN CANADA

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In western Canada, both interior spruce (*Picea glauca x P. engelmannii*) and lodgepole pine (*Pinus contorta*) are important commercial forest tree species. However, a changing climate may lead to mal-adaptation and thereby decrease forest productivity and threaten forest health. Assessing the adaptive characteristics of populations within these species is important for the development of forest management strategies to match reforestation stocks with predicted future climates. This research, conducted as part of the AdapTree project, investigates phenotypic variation of lodgepole pine and interior spruce at the landscape level. Seeds were collected in British Columbia and Alberta from over 250 locations, germinated, and grown in environmental chambers with four different temperature and moisture regimes. Phenotypic traits (i.e., height, diameter, budbreak, budset, and cold hardiness) were measured to investigate variation among sampled populations. Central objectives in the analysis were to: 1) assess phenotype-climate correlations, 2) examine geographic patterns of phenotypic variation, and 3) determine groups of populations having similar phenotypes as the basis for seed zone delineations. Phenotypic traits will also be used in future genome-wide association studies to determine the genomic basis of local adaptation to climate.

Individual climate variables could explain between 5 and 25% of the variance in measured growth and adaptive traits. Variables such as mean warmest month temperature and growing degree-days best explained variance in growth, whereas variables related to winter temperatures correlated best with cold hardiness. Geographic distribution of phenotypic variation in interior spruce indicated a separation of cold hardy populations east of the Rocky Mountains from susceptible populations in the west. The most rapid growth was observed for seedlings originating from the interior valleys of British Columbia. High elevation interior spruce populations were distinguished by late budbreak and early budset. Lodgepole pine showed similar patterns with the exception of a few frost hardy and simultaneously well growing populations from Alberta. Provenances of *Pinus contorta* var. *contorta* from the BC coast stood out from interior var. *latifolia* with late budbreak and high susceptibility to frost. Multivariate partitioning of genetic variation using a regression tree approach suggested 11 seed zones for interior spruce and 9 seed zones for lodgepole pine that account for 18% and 14% of the total observed phenotypic variation, respectively.

IDENTIFYING PUTATIVE ADAPTIVE VARIATION AMONG AND WITHIN HYBRIDIZING LODGEPOLE PINE AND JACK PINE

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The most recent outbreak of the mountain pine beetle (Dendroctonus ponderosae) is unprecedented. Millions of hectares of pine forests in western Canada and the United States have been decimated, causing economic loss and affecting ecosystem health. The main host affected by this outbreak has been lodgepole pine (Pinus contorta var. latifolia), which shares a co-evolutionary history with mountain pine beetle. The outbreak range for mountain pine beetle underwent major range expansion into northern Alberta in 2006. In 2011, we used molecular markers to identify successfully attacked lodgepole x jack pine hybrids, and jack pine (Pinus banksiana), a new host species. With the range expansion into a new environment and host we are faced with many unknowns regarding spread-risk potential and tree response. For example, how will genetic differences between lodgepole pine, jack pine, and their hybrids affect mountain pine beetle population dynamics, including reproduction? Will the beetle become endemic in this expanded range? Will expansion continue east and north, beyond the borders of Alberta? How will changing climate affect these dynamics? With heightened uncertainty regarding spread-risk potential, our aim is to identify host genetic factors that may contribute to mountain pine beetle spread risk. To do this, we developed genomic resources using two-year-old lodgepole and jack pine seedlings exposed to biotic and abiotic stresses to increase the expression of potentially informative genes. From these we generated cDNA libraries from root, needle, and stem tissues that were sequenced on a 454 next-generation platform for each species. The resulting transcriptome assemblies were mined for SNPs. Following extensive filtering, 1,536 SNPs were selected for population typing using Illumina's Golden Gate assay. A total of 549 individuals were typed, comprising lodgepole pine, lodgepole x jack pine hybrids, and jack pine selected from both within and beyond the region currently affected by mountain pine beetle. From this analysis, 472 SNP loci were identified that were both variable and exhibited good amplification. Using these data, we have identified SNP loci that are putatively under selection by conducting three complementary approaches. First, we used outlier detection (BayeSCAN, Arlequin) to identify SNP loci that exhibit greater or lesser genetic differentiation than expectations based on all loci. Second, we used environmental correlation analysis (MatSAM and bayENV) to examine the relationship of alleles with a number of environmental variables. Finally, we used multinomial regression (INTROGRESS) to identify SNP loci that exhibited either more or less frequent degrees of introgression across the lodgepole x jack pine hybrid zone than expected. From consensus of these methods, we identified potential candidate loci across all samples (12), and within lodgepole pine (10) and jack pine (7). Comparison across the different methods highlights the potential for false positives and emphasizes the application of multiple methods as a best practice to ensure relevant results. Candidate loci identified by these methods are associated with genes that encode products putatively involved in important processes such as water transport, transcriptional regulation, metabolism, and stress response. Based on these findings we will carry out additional genotyping to better characterize the spatial distribution of these alleles which will allow us to test for associations with mountain pine beetle spread patterns. Our application of genomic resources to identify spread-risk factors in a non-model, complex system is a novel approach that could be extended to other forest-insect interactions.

NATURAL ADAPTATION IN *Populus trichocarpa* AND IMPLICATIONS FOR CLIMATE CHANGE

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Black cottonwood poplar (*Populus trichocarpa*) is a widespread, deciduous tree occurring west of the Rocky Mountains from Alaska to northern California and spanning a wide range in local growing season and climate. Populus trichocarpa shows strong relationships in many traits relating to its natural distribution along this latitudinal cline. We investigated inherent trait variation within P. trichocarpa using over 2,000 trees grown in a common garden at Vancouver (representing replicate plantings of 461 collected genotypes within 136 provenance localities). We focused on seasonal (phenological) events, biomass accumulation, growth rates, canopy traits, isotopes, and gas exchange-based traits. We also estimated broad-sense trait heritability and overall population patterns with ~29K single nucleotide polymorphisms (SNP) in 3,518 candidate genes using Illumina sequencing. Our findings showed that common garden trees demonstrated substantial variation in traits, many of which were repeatable year to year, and most traits correlated with latitude, length of day (photoperiod), and annual temperature but not with precipitation or moisture indices. Trait heritability estimates were highest in phenology traits and moderate to low in biomass and other ecophysiology traits. In general, traits with higher heritability also had higher correlation to biogeographic variables with few exceptions. Overall genetic population structure was clinal and related directly to the latitudinal cline from our accession collection. Many SNPs contributing to this structure are described genes, including clock genes and other photoperiodic related genes. The strong relationship between most traits in P. trichocarpa with latitude, length of day, and temperature suggests that selection is driven by a complex environment. This indicates that natural migration and/or inherent biological response to climate change in *P. trichocarpa* may be diminished by a strong photoperiodic requirement.

BLACK SPRUCE SEED SOURCE TRANSFERS FOR FUTURE ONTARIO CLIMATES

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Focal point seed zones are species-specific GIS algorithms developed to provide best-adapted, site-specific choices for seed transfer. They are based on regressions of growth/survival observed in common garden trials across a range of test climates. Thus, biological variation (e.g., height or survival) at the population level is expressed in terms of local climate patterns. Previous work with jack pine (Pinus banksiana) had demonstrated that temperature variables were most important for its future seed zone delineation. For black spruce (Picea mariana), however, precipitation variables were equally important to temperature variables for future seed source delineation. Based on these observations, three black spruce seed source portfolio trials 'preadapted to climate change' were established in northern and central Ontario based on four future climate models: CGCM, CSIRO, MIROC, and NCAR. Predicted best planting sites were: 1) quite variable depending upon which model they were based and 2) were increasingly distant from present day distributions over first and second 30-year periods.

EARLY RESULTS FROM A DOUGLAS-FIR RECIPROCAL TRANSPLANT STUDY

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Genecological studies of Douglas-fir (Pseudotsuga menziesii) have shown genetic variation in adaptive traits that track geographic gradients in temperature and aridity of seed sources, suggesting natural selection for phenology, cold hardiness, growth, and biomass partitioning. Although genecological studies are useful for delineating seed zones and population movement guidelines, they necessarily make assumptions about local adaptation and the degree to which population differences are important for productivity and adaptation. We set out to test hypotheses of adaptation of coastal Douglas-fir (Pseudotsuga menziesii var. menziesii) using a reciprocal transplant design in which 120 families from 60 populations from 12 diverse regions from northern California to western Washington were planted at test sites in 9 of those regions in western Washington and Oregon. Results from previous genecology studies were used to ensure sampling of a diversity of populations, the presumed climates in which they evolved, and the climates in which they are now being tested. A diversity of populations and climates is critical to modelling population responses to climate in order to understand the effects of climate change on adaptation and productivity of native populations, and to explore options for assisted migration. Preliminary results were presented for survival and height at age 6. Overall survival was high at 97%, although trees from the California Coast had poorer survival, particularly at the most northern and higher elevation sites. Analyses of variance for height indicated statistically significant differences among test sites, regions, and populations within regions, as well as significant test site x region and population interactions. As expected, greater differences were found among test sites than among populations within test sites, with warmer sites having the greatest growth. Response functions at individual sites indicated local adaptation with better growth from populations that came from climates most similar to the test sites. Other adaptive traits being evaluated include budburst, cambial phenology, drought hardiness, and cold hardiness.

GEOGRAPHIC VARIATION AND EVIDENCE FOR LOCAL GROWTH SUPERIORITY FOR COASTAL DOUGLAS-FIR: ROTATION-AGE GROWTH PERFORMANCE IN A SERIES OF DOUGLAS-FIR PROVENANCE TRIALS

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INTRODUCTION

Long-term provenance tests are commonly used to understand the geographic variation of the species, to select the best provenances for a specific region, and to explore provenance by environment interaction. Since two cycles of breeding have already occurred for Douglas-fir (*Pseudotsuga menziesii*) and genetically improved materials are widely used in the Pacific Northwest, selecting the best provenance is no longer the main purpose. Instead, quantifying provenance by environment interaction has become an important issue, as it will provide valuable information on long-term adaptation and breeding zone delineation.

Despite the fact that Douglas-fir is one of the world's most valuable timber species, it's provenance by environment interaction is not well understood. The only multiple-provenance, multiple-site, long-term provenance test in the Pacific Northwest was planted in 1959 in Oregon (OR), Washington, British Columbia (BC), and northern California (Ching 1965). Several publications based on this study were available at various ages, with inconsistent results (Ching and Bever 1960, Ching 1965, Rowe and Ching 1973, Ching and Hinz 1978, White and Ching 1985, and Krakowski and Stoehr 2009). Provenance by environment interaction appeared to be evident only after age 30 or later (Krakowski and Stoehr 2009). While White and Ching (1985) observed that the local provenances have greatly increased their growth rankings from age 9 to 25 at several locations, Krakowski and Stoehr (2009) concluded that provenances from relatively distant origins often grew as well or better than the local ones at age 45. No study focussed on quantifying the level of local growth superiority except for simple comparisons of local and non-local sources at each location.

The main objectives of this study were: 1) to investigate provenance x environment interaction and quantify the level of local growth superiority and 2) to verify if geographically or climatically closely located provenances have similar volume growth.

MATERIALS AND METHODS

Sixteen provenances were planted at each seed collection site using a complete block design with 4 blocks/ site. Individual provenances were planted in 7 x 7-tree square plots surrounded by two rows of buffer trees of the same source at 1.8 m spacing. Six of the 16 sites, located in OR and BC, were measured at age 46-52 years (from seed) and used in this study. Sites were thinned and 24-25 undamaged, well formed, and well-spaced trees were measured in each plot.Total height (ht) and diameter at breast height (dbh) at the final measurement (age 46-52) were collected. Stem volume (vol) was calculated using Bruce and DeMars (1974) volume equations. Total volume per plot (vol_sum) was calculated as the total vol in a plot for all living trees.

A set of 216 climate variables (including annual, seasonal, and monthly variables) was generated by ClimateWNA (v4.70).

Analysis of variance (ANOVA) pooled across sites was conducted for each trait using SAS Proc GLM. The linear model included the following random effects: site, block within site, provenance, provenance x site, provenance x block (for ht, dbh, and vol only), and random error. In addition,

single-degree-of-freedom contrasts were conducted for testing the relative performance between local and non-local provenances. Least-squares means (LSMs) were estimated for each provenance across sites as well as within site. Rank correlations were calculated to depict the pattern of provenance x site interaction.

Local superiority is defined as the difference in volume between the local provenance and each non-local provenance. At each site, only the provenance originating from that site was considered as local. Mantel tests were conducted to examine the existence of geographical (or climatic) spatial autocorrelation. The procedure was done using R package ade4 and based on 9,999 random permutations. Euclidean distances were based on weighted geographic coordinates (i.e., latitude, longitude, and elevation) or climate variables. Regression analyses were conducted to examine the relationship between local growth superiority and Euclidean distance between provenance's original location and the test site.

RESULTS AND DISCUSSION

While there were significant differences between provenances, provenance ranking also changed from site to site. The best provenances varied widely in growth ranking while the worst provenances performed relatively consistently across sites. The rank correlations between sites were generally low (average r = 0.31 for vol and 0.16 for vol_sum).

While local provenances were not always the best performers in growth, positive correlations were found between local growth superiority in vol_sum and geographic distance (between provenance's origin and the test site) at each site (r = 0.15-0.64) as well as across sites (r = 0.47). This revealed a general pattern that the closer the provenance is to the test site geographically, the better rotation-age volume growth the provenance has at this site. This pattern was statistically significant considering all sites, and at two (i.e., Haney and Nimpkish) of the six sites. At low-elevation sites, low-elevation provenances performed significantly better than high-elevation provenances. By contrast, high- and low-elevation provenances performed similarly at high-elevation sites. Across all sites, there was no difference between southern and northern provenances. For vol_sum, southern provenances generally performed better than northern provenances at the OR sites, while northern provenances performed better than southern provenances at the BC sites.

Across all test sites, results indicated that there was a significant spatial autocorrelation pattern (r = 0.3; P < 0.05) showing an overall trend of having similar rotation-age volume growth for geographically closely located provenances. Similar results were obtained when Euclidean distance was calculated using the climate variables instead of geographic variables (i.e., latitude, longitude, and elevation).

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GENETIC STRATEGIES FOR ADAPTIVE RESOURCE MANAGEMENT OF US SOUTHERN NATIONAL FORESTS IN A CHANGING WORLD

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National strategic goals have been established to guide adaptive resource management of the US's national forests in changing times. Those goals are: Restore, Protect, and Respond. Within the national genetics program the "National Genetics Strategic Plan", "Genetic Options for Adapting National Forests to Climate Change", and the "National Guideline on Conservation Genetics" have been written to provide genetic guidance to resource management activities.

Genetic principles have always supported and guided reforestation efforts on the fourteen national forests in the Southern Region (R8). These national forests are the most diverse forested ecosystems in the US, existing in very fragmented landscapes. Numerous challenges (e.g., increasing populaces, predicted climate change, and forest health issues) have been impacting and changing the way these national forests are managed. These challenges precipitated a new paradigm in R8's Genetic Resource Management Program (GRMP), whose role is to contribute and support healthy, productive, and sustainable tree populations on the national forests. As part of the adaptive process, R8's GRMP has evolved from traditional tree improvement to activities that include restoration, conservation, and partnerships.

Providing seed for operational reforestation and restoration has long been the priority goal of the GRMP. The guiding principle, thus far, has been through the use of local seed sources. However the current practice of relying on seed sources best suited to past and current climate will need to shift to allow consideration of the sources of seed that will be best suited to future climates. In many cases, research or management attention is lacking, as is basic genetic information that is needed to guide decisions. To address this, new seed zone modeling is being developed. A second priority goal for R8's GRMP is tree conservation. Over 140 tree species inhabit the southern landscapes. Using the ForGRAS model (Potter and Crane 2010) approximately twelve of those tree species are considered threatened. More aggressive gene conservation activities are needed for species and populations most vulnerable to the current and future challenges. A third priority goal is partnerships. The GRMP coordinates with partners in the development and implementation of projects, most notably in tree conservation.

SUB-OPTIMAL POPULATIONS: POSTGLACIAL REFUGIA-INDUCED MELTING-POT OF Populus tremuloides

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Species under suboptimal conditions presented strong evolutionary significance and are expected to be more vulnerable to climate change. The primary causes of sub-optimal populations are post-glacial refugia-induced population colonization and migration, adaptational-lag, margin-central population structure, and density-dependent selection. To test these hypotheses of sub-optimality, we investigated historical projections of species habitat with bioclimatic envelope modelling and intra-specific quantitative genetic variances of Populus tremuloides based on a series of reciprocal common garden experiments in western Canada and Minnesota State. We found two discernible refugia on the north and south margins of the *P. tremuloides* historical habitat range. An increasing trend appeared in the additive genetic variances of adaptive traits such as bud break in Alberta populations that suggests a possible admixture and outcrossing of north and south populations. The "melting-pot" genetic structure is promising among the sub-optimal Alberta populations. The most likely reason for current sub-optimal conditions was complex postglacial meta-population dynamics, but other potential explanations are margin-central population structure, or density-dependent selection, where two divergent populations of lineages colonized and mixed from Alaska and south of the Canada-US border although a clear boundary between different mechanisms rarely exists. Our findings can guide management strategies to ameliorate aspen productivity.

PATTERNS OF ADMIXTURE AND LOCAL ADAPTATION IN THE INTERIOR SPRUCE HYBRID ZONE: IMPLICATIONS FOR FOREST MANAGEMENT UNDER CLIMATE CHANGE

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The patterns of admixture and the nature of selection responsible for the maintenance of the economically and ecologically important *Picea glauca* x *P. engelmannii* hybrid zone in western North America were investigated.

Genome-wide estimates of admixture, based on a panel of 311 candidate gene single nucleotide polymorphisms (SNP) corresponding to 290 genes, were used to assess current levels of admixture and introgression and to identify loci involved in reproductive barriers and adaptive differences between species. These data were combined with long-term quantitative data (growth, survival, bud phenology, and cold hardiness) and climatic data, to test assumptions of hybrid zone maintenance and to model future scenarios under climate change.

Our results suggest that the *P. glauca* x *P. engelmannii* hybrid zone is maintained by local adaptation to growing season length and snowpack. Hybrids appeared to be fitter than pure species in intermediate environments, which fits expectations of the bounded hybrid superiority model of hybrid zone maintenance. Adaptive introgression from parental species has likely contributed to increased hybrid fitness in intermediate habitats.

A total of 12 outlier loci, corresponding to genes responsible for carbohydrate metabolism, signal transduction, transcription factors, and others were found. Levels of admixture are very high and introgression is asymmetric towards *P. engelmannii*, supporting recent studies using neutral markers.

While *P. engelmannii* ancestry is higher than *P. glauca* ancestry in hybrid populations, on average, selective breeding in managed hybrid populations is shifting genomic composition towards *P. glauca*, potentially pre-adapting managed populations to warmer climates.

GUIDING DOUGLAS-FIR SEED SELECTION IN EUROPE UNDER CHANGING CLIMATES: BIOCLIMATIC ENVELOPE MODEL PREDICTIONS VERSUS GROWTH OBSERVED IN PROVENANCE TRIALS

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Douglas-fir (*Pseudotsuga menziesii*) is an important tree species in its native range in North America, and in Europe, where it is was introduced more than 180 years ago due to its high productivity and wood quality. Many of these early plantations are experimental, with known origins of seed sources, and today they also serve as a unique experimental test bed to investigate how trees may react to climatic change. Here, we tested if a model developed to guide assisted migration of Douglas-fir in North America under climate change can accurately predict the success of provenance transfers to Europe.

We first evaluated the performance of more than 700 Douglas-fir provenances, originating from coastal British Columbia to the interior mountains of New Mexico, that were tested in 120 European sites, from boreal conditions in Finland to dry and hot climates of Spain and Turkey. Provenance performance was compared at each test site and regionally ranked. Second, a regression tree-based bioclimatic envelope model for Douglas-fir populations was developed using North American data, then applied to current and future climates in Europe to predict where populations may find a suitable climate match.

Model predictions of optimal climate matches for western and central Europe conformed well with observed provenance trial growth, but diverged under eastern Europe's continental climates. There was an expected north-south trend, with northern and southern coastal sources performing best at their corresponding western European latitudes. In Eastern Europe, however, interior Douglas-fir sources rarely outperformed coastal sources, even though they were predicted as best climate matches by the model.

Climate trends observed over the last three decades may warrant changes in seed selection, and the bioclimate envelope predictions developed here appear accurate enough to guide this selection in Europe under contemporary and changing climates. Where model predictions and observed growth data diverge, test plantations may not have experienced rare climate extreme events to which the best performers may in fact be maladapted. In such cases, a safe compromise would be to use slightly less productive provenances with a better match among source and planting environments.

GENETIC DIFFERENCES IN DOUGLAS-FIR PROVENANCES CONTROL WATER USE STRATEGIES AND STEM HYDROLOGY

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Douglas-fir (*Pseudotsuga menziesii*) is of outstanding economic importance in forestry in its natural distribution area in the Pacific Northwest as well as in western and central Europe. Since in those areas sites are almost expected to shift to significantly dryer conditions, selecting the most adapted planting material might be of increasing importance. In the present study we aimed to elucidate the basic physiological mechanisms leading to different growth performance and drought resistance between Douglas-fir provenances. Understanding those mechanisms of adaptation to drought is important for identifying populations better adapted to future climate and for improving forest management planning.

We used 50-year-old Douglas-fir trees in a common garden experiment in south-western Germany. Five provenances originated from British Columbia, Washington, and Oregon, representing a natural north-south transect, were selected for this study. We surveyed three individuals per provenance by electronic point dendrometers during the 2011 growing season to detect variation in stem hydrology. Gas exchange of these trees was measured in May and July 2011 under dry and moist conditions to assess stomatal conductance. An additional 5 individuals per provenance were felled for retrospective growth analysis. This was repeated on three sites of the same common garden experiment with different climatic conditions.

In some provenances the diurnal swelling and shrinking processes of the stems were correlated to variations in air humidity. This relation decreased gradually from the selected provenances of winter-cold to those of winter-warm habitats. No correlations were found with soil water content. However, stomatal conductance showed the same gradient from high values in the northernmost provenance to low values in the southernmost. Therefore, we assumed that diurnal swelling and shrinking processes are influenced by genetically fixed differences in stomatal regulation which are an adaptive trait to optimize photosynthesis and minimize water loss by transpiration under dry conditions. In contrast to previous work, our results indicated that minimum wood density is higher for populations originating from warmer habitats, even though the differences were not significant. This indicates a higher mechanical resistance of the hydraulic system to drought stress, and could carry important implications for forest regeneration strategies.

DISTRIBUTION AND EVOLUTION OF Pistacia IN ARID LAND

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The genus *Pistacia* L. (Anacardiaceae) consists of 11 species. Seven species are distributed from the Mediterranean basin to Central Asia (*P. atlantica*, *P. khinjuk*, *P. lentiscus*, *P. palaestina*, *P. terebinthus*, and *P. vera*), two species in Eastern Asia (*P. chinensis* and *P. weinmannifolia*), and two species from the Southwestern United States to Central America (*P. mexicana* and *P. texana*). *Pistacia aethiopica* was defined as a new species in 1980 by J. O. Kokwaro, however, its status has not been evaluated.

Pistacia is characterized by its dioecious reproductive system and homeochlamydic perianth (or naked flowers). The synapomorphies of a single apotropous ovule per locule place *Pistacia* within the Anacardiaceae.

The global distribution of species of the genus, in relation to arid and semiarid zones and long-term climate change, is the focus of our research. It was conducted by using available GIS data sets (climate, landscape), remote sensing variables (reflectance, the first derivative of the reflectance, RS vegetation indices), and high spatial resolution satellite imagery for single-tree crown extraction and mapping. We hypothesized that long-term global climate episodes differentially affect the different species, limiting the distribution of those that are more vulnerable to drought and heat and vice versa in relation to the tolerant species. A better understanding of the species distribution related to drought, temperature change tolerance, and nutritional requirements was studied.

The availability of an extensive germplasm collection of *Pistacia* from various geographical regions around the globe has made it possible to evaluate their genetic relationships and the evolution of the genus. Preliminary GIS data interpolation showed global distribution of the genus to be limited by climate and elevation. Additionally, biochemical content in the plants appeared to be an appropriate variable for species classification in separate clusters. High correlations were found between the specific portions of the first derivative of spectral reflectance and biochemical contents in the plants. Cluster analysis was done and dendrograms produced. They showed not only distinctive classes of the different species but also similar patterns to phylogenetic analyses.

Dehydration in higher plants, either as normal developmental processes such as what occurs during embryogenesis or induced by environmental stress, is accompanied by changes in gene expression.

We have identified and characterized a dehydrin protein in *Pistacia*, which appears to be associated with cold tolerance and drought. Dehydrins are abundant in all photosynthetic organisms from cyanobacteria to higher plants. Much dehydrin research has been directed at herbaceous species, but reports concerning dehydrins in woody plants are less numerous. Dehydrin accumulation in *P. vera* was found to be associated with cold tolerance, dormancy, and/or seasonal changes.

The adaptation of *Pistacia* trees to harsh desert conditions and their longevity make them ideal candidates for reforestation in arid zones. A better understanding of the genetic relationships among the species would be an advantage for breeding programs in this genus.

BREEDING PACIFIC NORTHWEST CONIFERS

COOPERATIVE ADVANCED-GENERATION BREEDING AND TESTING OF WESTERN HEMLOCK IN OREGON AND WASHINGTON

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FIRST-GENERATION TESTING

The IFA-PNW "Progressive Tree Improvement System" was launched in 1966 due to the failure of grafted Douglas-fir clonal orchards at that time (Silen and Wheat 1979). The emphasis was on forming local cooperatives to share costs and on progeny testing large numbers of trees using wind-pollinated seed on multiple sites in small testing zones.

Western hemlock (*Tsuga heterophylla*) is a prolific and dominant species in the coastal forests of the US Pacific Northwest and often outcompetes coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) in the highest-rainfall areas close to the coast. It is the second most important timber conifer in the US Pacific Northwest. Interest in this species increased markedly in the 1990s after growing incidence of Swiss needle cast (*Rhabdocline pseudotsugae*) disease on Douglas-fir on the Oregon Coast and the white pine weevil (*Pissodes strobi*) on Sitka spruce (*Picea sitchensis*) from Oregon to Alaska.

The first-generation testing phase for western hemlock ran from 1972 until 1995, during which over 1,300 first-generation parents were tested in five breeding units. Height was measured at five and 10 years from seed, while diameter was measured at age 10 and sometimes at age 14 or 15. Unlike Douglas-fir, branching traits (e.g., forking and ramicorns) and stem sinuosity were not scored. Age-5 results from one of these programs are presented in Foster and Lester (1983).

SECOND-CYCLE TESTING

Design and Implementation

A single second-cycle breeding and testing program (HEMTIC) was developed in 1992 (Fig. 1) covering an area from the middle of the Oregon coast (44°30'N) to northern Vancouver Island (51°00'N). Genetic materials were selected from six first-generation programs based on age 10 height growth. HEMTIC differed from the Douglas-fir second-cycle cooperatives in several ways: 1) in addition to Oregon and Washington, there was active involvement and leadership from the province of British Columbia, 2) no forward selections from open-pollinated tests were used due to lower heritabilities compared to Douglas-fir, and 3) resources were divided into a main and an elite population. The main population had 150 parents (chosen from over 1,500 parents) crossed in six-parent disconnected diallels forming 342 crosses. The elite population of 30 parents had 166 crosses (Jayawickrama 2003) and was tested in both single-tree plots and full-sib family blocks. The number of crosses per parent was much higher, 5 in the local diallels and up to 18 in total. Test sites were not fenced. Neither intensive mechanical site preparation nor frequent herbicide application were used, due to western hemlock's sensitivity to these treatments.



Figure 1. Location of second-cycle western hemlock sites in Oregon and Washington. Over 130,000 test trees were planted and tested on 19 sites between 1997 and 2001.

Data collection, analysis and selections

Age 5 heights and incidence of basal forking were measured and analyzed from 86,375 progeny trees and checklots by the end of 2004, and age-10 data were analyzed by early 2010. Heritabilities for these traits are shown in Table 1 and are generally considerably lower than for coastal Douglas-fir at the same ages. As a result of the lower heritabilities predicted gains were also lower than for coastal Douglas-fir, but there was still solid evidence for an overall gain for the second-cycle population over unimproved controls (4.1% gain for HT10, 11.3% gain for VOL10).

Third-Cycle Breeding and Testing Cooperators in Oregon and Washington have supported moving forward to a third-cycle program. The strategy is similar to the second-cycle strategy. Differences between the 3rd- and 2nd-cycle strategies include: 1) the "elite" population will be much smaller, 2) a maximum of four crosses per parent, 3) fewer progeny planted per cross per site, 4) use of a standard group of Oregon and Washington woodsrun controls on every site, 5) test sites will be fenced, 6) a slightly smaller number of selections (about 120), 7) the 3rd-cycle breeding population is comprised mostly of untested 2nd-cycle forward selections from full-sib tests (the largest component), with perhaps 30 high-gain 1st-generation parents unused in second-cycle HEMTIC, 8) some germplasm from higher elevations in the Coast Range and

Heritabilities	Trait								
	Age 5		Age 10						
	HT	Basal forking	HT	HT510	DBH	VOL	Stem forking	Ramicorn branch	Stem break
Range of within-site	.0417	.0315	.0726	.0425	.0317	.0519	.0102	.0208	.0204
Across-site narrow- sense	0.058	0.062	0.077	0.067	0.048	0.057	0.011	0.023	0.016
Across-site family mean	0.655	0.639	0.743	0.758	0.665	0.695	0.249	0.461	0.191

Table 1. Heritabilities of second-cycle western hemlock (HEMTIC) sites

Ages calculated from seed. HT= height, HT510 = height increment from age 5 to 10, DBH= diameter at breast height, VOL= $DBH^2 * HT$, Stem forking = incidences of stem forking, Ramicorn branching = incidences of ramicorn branching.

Cascades will be included, 9) a small cloned breeding population may be included, and 10) additional traits such as wood stiffness may be used as selection criteria. A total of 152 forward selection candidates were identified for the third-cycle breeding population and grafted in 2006, 2010, and 2013. This will be reduced to 90–100 selections based on predicted gains, response to stimulation, and to restrict the number of related entries. Stimulation and pollen collection began in 2012.

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SIXTY YEARS OF GENETIC IMPROVEMENT FOR COASTAL DOUGLAS-FIR IN OREGON AND WASHINGTON

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FIRST-GENERATION TESTING OF DOUGLAS-FIR

Forest tree improvement got underway in the US Pacific Northwest in 1954 with several organizations starting tree improvement programs by selecting trees and grafting them into clonal orchards. Graft incompatibility soon became evident and dampened enthusiasm for grafted clonal orchards (Silen and Copes 1972). The IFA-PNW "Progressive Tree Improvement System" was launched in 1966 (Silen 1966, Silen and Wheat 1979), emphasizing the formation of local cooperatives to share costs and progeny testing large numbers of trees using wind-pollinated seed in small testing zones (believing that it was not appropriate to move Douglas-fir (*Pseudotsuga menziesii*) seed far from the source). Improved seed was to be delivered from seed orchards established using full-sib crosses made on the parent trees.

First-generation test establishment continued until 1993, during which over 25,500 first-generation Douglas-fir parents were tested in 115 breeding units and ranked based on 2.4 million surviving progeny trees. Most trials were established using either "reps-in-sets" or "sets-in-reps" design. The typical measurement schedule was 5, 10, and 15 years from seed. Height was usually measured each time and diameter at age-15 and sometimes age-10 as well. The incidence of ramicorn branches, forks, and stem sinuosity was assessed in some testing program, once the inheritance of these traits was established (e.g., Temel and Adams 2000).

SECOND-CYCLE COOPERATIVE BREEDING AND TESTING

The second-cycle breeding and testing strategy was developed around 1997 based on global analyses of first-generation data, computer simulation, and inferences from other breeding programs (Johnson 1997, 1998a, 1998b; Johnson et al. 1997). It was concluded that full-sib breeding had several advantages over open-pollinated breeding, that there would be little marginal gain per test beyond six successful progeny tests (in terms of ranking families), that two or three crosses would give a reasonable estimate of a parent's GCA, and that final selection around age 12 years would be efficient. Eighty-six first-generation testing programs were merged, mostly in the north-south direction, into 11 larger testing zones operated by eight second-cycle cooperatives.

Breeding populations for second-generation cooperatives ranged from 112 to 429 crosses. Within a breeding population, breeding groups were constructed with each breeding group originating from a single first-generation program. This resulted in sublines to manage inbreeding and multiple populations to maintain locally adapted gene complexes. About 1 in 10 first-generation parents or families were selected to move forward into the second cycle. Most selections were made on age-15 height; information on DBH, stem form and wood specific gravity were also considered. The breeding population for each testing zone included parents or progeny from the "local" breeding groups within the testing zone and only the highest-ranked selections from breeding groups originating further away from the zone. Each selection was generally used in two crosses, with some elite selections used up to four times. Some elite crosses were made across first-generation zones.

A total of 2,640 pair-crosses were planted in 115 tests between 1999 and 2012 (see Fig. 1) and about 455,000 test trees planted of which about 364,000 were measurable trees. Five to eight tests were established per test series using an "alpha" design (a few in "sets-in-reps design") with 20 trees per cross per site in single-tree plots. Test sites contained 50–283 full-sib crosses. Only six of the 115 sites were

established over 900 meters elevation. Multiple phases within a testing program were linked by at least 10 common crosses and additional linker parents. Unimproved checklots were included in all tests planted after 2001. No polymix tests were established.



Figure 1. Distribution of cooperative second-cycle Douglas-fir test sites in Oregon and Washington.

All sites were established with one-year old containerized seedlings and fenced to prevent browse. The goal was to control herbaceous competition for three years and more harmful competitors (such as Scotchbroom (*Cytisus scoparius*) and aggressive hardwoods) until crown closure. Tests are being measured twice, at seven and 10–12 years. Recent research has established little benefit to waiting beyond age-10 for final selections for growth rate (Ye and Jayawickrama 2012). Height, diameter, forking, ramicorns, sinuosity, and budburst rating are scored by age-7, fall cold hardiness has been scored in four programs and acoustic velocity is currently being scored in one program. Genetic gains are predicted using BLUP after adjusting for spatial trends (Ye and Jayawickrama 2008).

THIRD-CYCLE COOPERATIVE BREEDING AND TESTING

The strategy is largely the same as in the second cycle. Further consolidation of breeding zones will take place, the merits of adding polymix tests is being debated, and test sites will be kept small to maximize heritability. Over 960 forward selections from second-cycle tests have been grafted in seven breeding orchards. Elite first-generation selections, omitted from second-cycle programs or only involved in suboptimal pairings, are also being included. Selections are organized in sublines of 10–15 trees with all

relatedness confined to within those sublines. Over 270 crosses have already been attempted. Test establishment may start as early as 2016.

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THE LODGEPOLE PINE BREEDING PROGRAM IN BRITISH COLUMBIA: BREEDING FOR MULTIPLE TRAITS

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Lodgepole pine (*Pinus contorta* var. *latifolia*) is a very important commercial tree species in British Columbia (BC) east of the Coastal Mountains. In 2012, 107 MM seedlings were planted which represented 53% of the total provincial planting program. Population and quantitative genetics research has been conducted for over 45 years and has focused on growth as a target trait for breeding activities and an indicator of adaptive differences among populations. Currently there are five seed zones that have progressed to the second-generation of progeny testing. The second-generation tests are composed of F1 crosses derived from parents which were selected for growth and wood density based on data from open-pollinated progeny tests. These tests are approaching 10-years of field testing and are being assessed for growth, infection by pathogens, and attack by insects. While stem volume continues to be the primary trait of interest for breeding activities, we are investigating the feasibility of integrating other traits into the breeding program.

Three pathogens have been identified as high priority for breeding activities based on consultation with foresters and pathologists: comandra blister rust (Cronartium comandrae), dothistroma needle blight (Dothistroma pini), and western gall rust (Endocronartium harknessii). We are actively monitoring progeny tests for the occurrence of these pathogens and continue to survey mature progeny tests that have been attacked by mountain pine beetle (Dendroctonous ponderosae). Resistance and tolerance to western gall rust and dothistroma needle blight are the most promising with heritability estimates of similar magnitude to other traits such as growth and wood density. Resistance and tolerance to comandra blister rust infection has very low heritability. Symptoms caused by comandra blister rust are similar to those of white pine blister rust (Cronartium ribicola) on western white pine (Pinus monticola) and the life-cycles of both rusts are very similar with multiple spore stages and alternating hosts. There is no significant genetic correlation between growth traits and resistance to these three pathogens. We are currently investigating the possibility of screening progeny for resistance using field tests established in high hazard areas and controlled inoculation experiments. We are also conducting controlled crosses among parent trees selected for tolerance and resistance. The heritability of resistance and tolerance to mountain pine beetle and its associated fungi is similar in magnitude to other traits of interest but the relationship with growth is more complex. There was strong selection by beetles for large trees in southern progeny tests but less in northern progeny tests. The infrequent nature of epidemic outbreaks of mountain pine beetle makes it a low priority for selection and breeding activities.

A very significant challenge for the lodgepole pine breeding program in BC is poor seed production from established seed orchards in the north Okanagan. Seed production in the north Okanagan has historically been much lower than from seed orchards located in other regions of the province with lower seed-per-cone yields and higher numbers of cones-per-ramet. Seed loss occurs at the end of July and beginning of August of the second year of cone development but the mechanism is not yet clear. Ongoing research is now focusing on the short window in which seed loss occurs to help determine the possible causes and actions necessary to help boost seed production in established north Okanagan orchards.

The integration of innovative techniques to help set priorities and improve efficiencies in the breeding program is key to the advancement of the lodgepole pine genetics program in BC. We are using climate envelope modelling to help prioritize future breeding activities by ranking breeding populations for

importance based on changes in the size of seed zones. A similar approach is being used to model the relationship between pathogen infection and climate to help predict the impact of diseases at the landscape level and prioritize disease screening research. We are adapting controlled inoculation procedures from other conifer-pathogen systems to improve efficiency in screening for resistance and tolerance and help to identify mechanisms of resistance. We are also investigating the use of genomic selection to improve efficiencies in the breeding program by reducing testing time and costs for phenotyping complex traits. The future of the lodgepole pine breeding program will continue to build on the legacy left to us by the hard work and dedication of the many researchers and technicians that preceded us.

THE PAST, THE PRESENT AND FUTURE OF THE COASTAL DOUGLAS-FIR **TREE IMPROVEMENT PROGRAM IN BRITISH COLUMBIA**

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Coastal Douglas-fir (Pseudotsuga menziesii var. menziesii) was the first conifer subjected to tree improvement efforts in British Columbia. Intensive phenotypic selections were made by Alan Orr-Ewing starting in 1957. It was expected that these intense selections would translate into immediate gains in seed orchards and as a consequence, progeny testing was not considered an integral part of the program. By 1966, a total of 455 selected trees had been grafted and were growing in breeding arboreta and seed orchards. In 1972/73, it was decided to produce a pedigreed population for the testing and selection of superior genotypes as well as for the estimation of genetic parameters and the establishment of a long-term breeding population. This gave rise to EP708, which formed the basis for all future improvement of coastal Douglas-fir. EP708 was a 6-tree partial, disconnected diallel breeding design extending over 8 years (11 sites/year), crossing a total 372 parents in 62 diallels. By the early 1990s, a total of 991 trees were forward selected from many of the 88 test sites planted. Breeding for the next generation was structured into sublines to manage future inbreeding. A total of 32 sublines were established with up to 12 parents per subline. Crossings were done in four series. Series 1 was planted in 1999 and Series 4 was planted in 2010. Series 1 to 3 were complimentary tests (GCA testing of parents and full-sib family blocks), while Series 4 is a full-sib test using single tree plots on four sites. GCA tests were planted on four sites/Series, while the full-sib blocks were established as 25-tree plots per full-sib family on two sites for each Series. Forward selections (age 12 years from seed) were based on mid-parent BVs obtained from the GCA test for Series 1 and based on combined BLUP analysis for Series 2. Wood density was either estimated using the resistograph (Series 1) or increment cores (Series 2) taken from half of the GCA test seedlings. Breeding values of selected trees average around 25% gain, expressed as volume gain at age 60. Trees with BVs for wood density below -5% were not selected. Breeding for the next generation will be done using 4-parent diallels for each of the 32 sublines. It is anticipated that the Ne for this breeding population will be around 70 to 80.

GENETIC AND ENVIRONMENTAL CONTROL OF DOUGLAS-FIR STEM FORM IN THE PACIFIC NORTHWEST

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The value of wood products is determined by tree volume and stem quality. Stem form defects, such as forks and ramicorn branches, reduce stem quality and, therefore, tree value. Foresters in the Pacific Northwest have observed that the frequency of stem form defects seems to be associated with rapid growth and proximity to the coast. In addition, past research studies have indicated a positive genetic correlation between growth and stem defects. Nonetheless, the relative roles of genotype and environment on the frequency of stem form defects are still unclear. The objectives of this study were to: 1) identify environmental characteristics that explain variation in the frequency of forks and ramicorn branches, 2) examine whether rapid plantation growth is associated with an increase in stem defects, 3) determine how much variation in stem defects can be explained by differences in growth, 4) examine whether there is a relationship between stem form and proximity to the coast, 5) estimate genetic and environmental correlations between stem defects and growth traits, and 6) estimate heritabilities and genetic gains for stem defects and growth traits.

To achieve these objectives, data from 22 first-generation operational breeding programs within the Northwest Tree Improvement Cooperative (NWTIC) were analyzed. We examined 40 environmental characteristics (climate, soils, and topography) and did not find any evidence that they explain variation in stem form defects. We found that the frequency of stem form defects increased with increased growth and increased proximity to the coast. We also found that forks and ramicorn branches were heritable and were generally positively genetically correlated with growth. However, genetic correlations varied among programs. Direct backward selection on stem form traits could result in a decrease in defect frequency between 3 and 28%. Selection solely on growth traits (i.e., indirect backward selection) had a small potential to increase the frequency of stem form defects (1-4%). Because of the variability in genetic correlations between growth traits and stem form defects, it is also possible to simultaneously select for growth and fewer defects.

We now have a better understanding of the genetic and environmental control of Douglas-fir (*Pseudotsuga menziesii*) stem form. There is ample evidence to suggest that stem form should be included as a selection criterion in operational breeding programs, particularly in breeding programs that plan to deploy material close to the coast or on "high" sites. The genetic correlations between stem form and growth traits should be examined in later generations, as the potential to increase the frequency of stem form defects may change, particularly in programs that had large positive genetic correlations between growth and the frequency of stem defects.

RESULTS FROM DOUGLAS-FIR PROVENANCE TESTS: THE BASE FOR A BREEDING PROGRAM IN GERMANY

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In the first part of the presentation selected results from two provenance trails were presented. Seed from 54 provenances of Douglas-fir (*Pseudotsuga menziesii*) from eight USA states and two provenances from Canada, collected within the natural range of the species, was sown in 1962 for the first trial. In spring 1965, a field trial was established in the forest district of Nordhorn, north-western Germany, to test the suitability of Douglas-fir provenances on former agricultural land. In the field trial, several traits were measured or assessed regarding growth performance and susceptibility to biotic agents. Regarding these characters the provenances exhibited great variation. Regarding growth, considerable changes in ranking were still observed between age 11 and age 33. At age 33, provenances from coastal Oregon and Washington had the largest diameters. These provenances were as well resistant to Rhabdocline needle cast disease (*Rhabdocline weirii*). Provenances from the southern interior were partly heavily attacked by the fungus. Some of them failed totally by age 33. To assess the quality of provenances, especially for exotic tree species, older aged provenance trials are essential.

The second trial was established with provenances from Washington and British Columbia and progenies from stands in Germany. Most of the offspring from the German stands had better height growth than those from North America. Survival of the German progenies was average.

Based on the results of the previous trial and further tests, a breeding program was projected for the next 15 years. This was presented in the second part of the presentation. The program consists of 4 components: 1) analysis of existing provenance trials, 2) evaluation of gene flow, 3) establishment of progeny trials with off-spring from seed orchards, and 4) establishment of breeding populations and tests on drought stress and frost tolerance under controlled conditions. The estimated cost of the program is 7 million \notin (9.2 million US\$). Grants for about 2/3 of the cost are necessary. To initiate the program a proposal was submitted for a 3.5 year period (2 million \notin).

DOUGLAS-FIR: A HISTORY OF BREEDING IN NEW ZEALAND AND SOME CHALLENGES FOR THE FUTURE WITH CLIMATE CHANGE

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Douglas-fir (*Pseudotsuga menziesii*) has been grown in New Zealand since 1859 and is now the second most important planted exotic forest tree. With around 109,000 ha planted, the species represents 6% of New Zealand's planted forest estate. Douglas-fir is grown mainly in the South Island of New Zealand, in colder, wetter environments.

Douglas-fir breeding was initiated between 1957 and 1959 when provenance trials were established across a large number of sites in both the North and South Islands of New Zealand. Origin of the material was from Washington and Oregon (1957), and from coastal California and Oregon (1959). Following an analysis that demonstrated the superior growth rate of Oregon- and California-origin material, further collections were made from these areas. Additional breeding trials ranging in location the central North Island to the south of the South Island were established in 1996.

There are three environmental challenges to increasing the profitability from Douglas-fir in New Zealand. First, resource quality varies with temperature and water availability. Cooler temperatures lower the density and stiffness of the wood. Second, Swiss needle cast (SNC), caused by the pathogen *Phaeocryptopus gaeumannii*, results in a 32% reduction in tree growth (New Zealand-wide average). This productivity loss is greatest in the warmer, wetter regions of the North Island. Third, New Zealand temperatures are projected to increase with climate change. Temperatures are predicted to rise, particularly on the east coasts of both the North and South Islands, by approximately 2°C.

The challenge to tree breeders is how to deal with the loss of productivity in warmer regions due to SNC and with the lower wood stiffness in the cooler areas, all in a changing climate. In particular, we are interested whether the gain achievable in wood stiffness from breeding will be sufficient to compensate from lower wood stiffness in cooler sites now and into the future. Breeding for wood stiffness is important to achieve wood quality on some sites. Wood stiffness has not been measured routinely in the breeding program. A sub-set of 60 families at three sites has been assessed in the 1996 trials. Wood stiffness was found to be highly heritable (narrow-sense heritability of 0.49, age 11–13 years). Importantly, little change in rankings was found across three sites. This result needed confirmation. We also needed to know if wood stiffness would still be highly heritable across all the 238 open-pollinated families tested so that selections would definitely deliver heritable variation.

New wood stiffness assessments are currently underway. We will use the new data to confirm the high heritability across a wider genetic sample. We will also examine the effect that sub-sampling of families has on genetic parameters and genetic gain for wood stiffness in NZ Douglas-fir. We will estimate genetic gain and determine if the gain achievable will be sufficient to compensate for lower wood stiffness on cooler sites.

Understanding the relationship between gains achievable in wood stiffness (even with climate change) and needle loss due to SNC will be vital for the future profitability of Douglas-fir production. Currently, SNC damage is restricted to the North Island and the northern areas of the South Island. Modelling under different climate scenarios indicates that SNC will move down further into the South Island, into the core growing region for this species. We will examine the genetic relationship between these growth, wood quality, and disease resistance traits and determine trade-offs necessary (if any) to provide robust trees for planting. Changes to breeding and deployment plans were discussed.

BREEDING OF RADIATA PINE IN AUSTRALIA

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The objective of the national tree improvement program for *Pinus radiata* in Australia is to breed, select, and deploy genetic material with improved biological characteristics for traits of commercial importance. Breeding began in the 1950s with plus tree selection and the establishment of seed orchards by various companies and state and federal agencies. The Southern Tree Breeding Association (STBA) breeding cooperative, established in 1983 in South Australia, continues to expand, consolidating genetic resources on a national basis, providing cost efficiencies, and enhancing the rate of genetic gain for this major plantation species.

Economic objectives for plantation growing for structural timber markets are fundamental to the breeding and deployment programs. Changes in the environment due to changing climate and markets are evaluated within this framework.

There is effectively a single breeding population with a single national objective. We are still deciding on how best to operationally manage the population for regional performance, different products, and risk traits associated with some pests and diseases. New software tools (SELECT and MATE) have helped breeders manage relatedness and limit the change in group co-ancestry to ensure inbreeding does not build up too quickly in the advanced generation population. Some effort is also directed towards conserving native provenance material for risk management purposes as well as infusion of genes from tested selections to broaden the genetic base.

The breeding program moved to a rolling front strategy in 2000, where breeding, testing, and selection activities are done on an annual basis. This has reduced costs and generation interval by avoiding resource and biological bottlenecks, and maintained the skills of field technicians and breeders (particularly in reproductive biology). It has enhanced the rate of genetic gain per unit time, as well as delivering new information and genetic material (tangibles) to cooperative members on a regular basis. Infusion of 30–50 new selections each year into a dedicated multi-age breeding arboretum allows about 250 crosses per year; many more than was previously achieved per generation.

Progeny tests and deployment trials are established on an annual basis, and across years to sample all of the plantation estate. Families are also being exchanged with the Radiate Pine Breeding Company in New Zealand for collaborative testing in a series of trials across Australasia. Families with parents in common provide genetic linkage among trials across sites and years. Treatments are stratified into groups of families with similar breeding values for growth to reduce competition among single-tree plots and provide large plots to monitor long term stand growth. Traits measured at various ages include growth, tree form, wood properties (density and stiffness using acoustic tools), and damage due to pests (pine aphid) and diseases (dothistroma, pine pitch canker, phytophthora, and spring needle cast).

All data and information are stored in the national DATAPLAN database. The web based system has been operating since 2001 and currently stores ten million measurements on 650,000 *Pinus radiata* genotypes in over 300 trials, with a total of 60 million measurements on four million genotypes for 11 species. We are currently incorporating new trial data (more than 80 trials) for radiata pine from a previously independent state based breeding program in Western Australia, following the Forest Products Commission joining the national cooperative in 2013. This will further broaden the genetic base and increase selection intensity for all members.

The TREEPLAN genetic evaluation system has been used routinely since 2001 for breeding value prediction using multi-generation multivariate (-site, -trait, and -age) BLUP. BLUP allows us to account for previous selection and monitor trends in genetic improvement over time by having objective comparisons across time and space. Variance components are estimated within trials (and across subsets of trials) using ASREML and other tools. TREEPLAN genetic values (EBVs, EGVs, and SCAs) are currently predicted for 32 selection criteria (measured traits) using data from more than 160 trials in the multi-variate analysis. Genetic values are generated for the clearfall harvest age breeding objective traits of growth (MAI on a regional basis, m3/ha/yr), stem straightness (SWEEP, mm/m), BRANCH size (cm), and timber STIFFNESS (GPa). Economic indices using appropriate economic weights (based on various production systems and end use processing) are produced for each genotype, family, aggregate, and genetic group.

Standard errors and accuracies are now routinely reported as measures of reliability for all types of genetic values for all pedigree entities (individuals, families, aggregates, and genetic groups), and for all trait types (selection criteria, objective traits, and profit indices). Genetic values for all the population are updated regularly (at least annually) as new data are collected. Realised gain trials are used to audit the predictions and ensure scaling factors are appropriate, particularly for MAI. Implementation of changes in economic models and other assumptions derived from research projects is straightforward within this framework.

The STBA does not itself produce seed and plants for deployment. This is done by members and a licensee. STBA provides selections for use in propagation systems, as well as objective information to assist seed producers, nurserymen, and forest growers to make informed decisions about what material is best for their situation. This is not a simple task, as the breeding program generates complex and vast amounts of information for consideration in deployment.

The SEEDPLAN decision support system has been developed within the DATAPLAN and TREEPLAN framework for industry use after pilot testing. Its purpose is to improve the use of improved seed and clones in deployment under different situations. Tools assist with selecting genotypes to best balance gain and diversity (SELECT), placement of ramets in seed orchards (DESIGN), estimating the composition of seed harvested (COMPOSE), and customising the economic indices for the different growing environments and production systems (INDEX). A tool for allocating seed and plant lots to stand types in an optimal manner to maximise enterprise profitability (MATCH) has been prototyped, but is still under development.

Other studies have focused on juvenile wood and timber stiffness. The potential impact of climate change is given some consideration, but mainly in terms of characterising the genetic resource for a greater range of environments. A better understanding of GxE patterns and associated environmental factors is helping classify site types across Australasia for breeding and deployment. Current research suggests rainfall, temperature, and altitude are largely responsible for observed patterns of genotype by environment interactions. It is anticipated some refinement of site type classifications for screening and deployment of genetic material will improve overall gain.

Molecular markers have been used in the past for fingerprinting to check pedigree error rates in the breeding and deployment populations. Genomic selection is not being used operationally in the tree improvement program for radiata pine at this time. Research projects have generated some marker data, but it has been too little to warrant incorporation in routine evaluation. It is anticipated next generation genetic values will increasingly rely on additional information from molecular genetic studies.
Breeding values incorporating non-neutral marker information can currently be done in TREEPLAN by using a synthetic pseudo-continuous trait. Multi-locus marker-trait responses are combined into a prediction of genetic merit for a trait based on genotypes which have phenotypic and/or marker information. This approach has been used successfully in *Eucalyptus nitens*. We anticipate advances in genotyping technology will make it feasible to test individuals for hundreds of thousands of single nucleotide polymorphisms simultaneously at an affordable price. A genomic relationship matrix based on neutral markers can be merged with the average co-ancestry matrix currently used. This should increase the accuracy of breeding value prediction, by better accounting for the Mendelian sampling term.

Alleles of large effect can be incorporated directly into the model, utilising all phenotypic data to better identify their effects. The opportunity for radiata pine is largely with harvest age traits that are only expressed later in the life of the tree, or are expressed rarely, such as with pests and diseases, or are very expensive or impractical to measure. It will be necessary to continue collecting phenotypic data to ensure precision of estimates.

In summary, the radiate pine program has undergone substantial change in the past decade with the development and adoption of economically defined breeding objectives; screening for new pest (essigella pine aphid) and disease traits (pine pitch canker), increased measurement of stiffness; development of DATAPLAN software and data consolidation in national database(s); development of TREEPLAN software for comprehensive industry-wide genetic evaluation with regionalised genetic values; a rolling front operational program; development of selection, breeding, and deployment (SEEDPLAN) tools; gene conservation coordinated nationally; improved security of the national genetic resource (breeding population); and a focused research portfolio with rapid adoption of results and findings.

GAINS IN WEEVIL RESISTANCE FOR SITKA SPRUCE: COMPARISONS BETWEEN FIRST-GENERATION AND F1 TRIALS AND IMPLICATIONS FOR SITKA SPRUCE IN THE PACIFIC NORTHWEST

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White pine weevil (Pissodes strobi) is one of the most devastating pests of young spruce (Picea spp.) and pines (Pinus spp.) in North America. The weevil is a native insect that occurs across Canada and the northern United States. In eastern North America it is a major pest of Eastern white pine (Pinus strobus) and introduced Norway spruce (Picea abies), but in the west it mainly attacks spruce species. Sitka spruce (*Picea sitchensis*) is particularly susceptible. Finding and incorporating resistance to this pest has been the main focus of a breeding and research program for Sitka spruce, carried out for over two decades by the BC provincial and the Canadian federal forest services and universities. First-generation screening of Sitka spruce parents occurred in open-pollinated or clonal trials. Thirty of these firstgeneration trial sites were monitored annually for up to 10 years for weevil attack. Over one-third of these had been artificially infested by augmenting the trial trees with weevils. This was quite effective in getting early screening results and parents were chosen from these trial screenings to produce an F1 population. At this stage results are being monitored from both augmented and non-augmented F1 trials. A marked reduction in weevil damage is noted in the F1 compared to the first-generation trials, even in the F1 trials that were augmented. Work is ongoing at discovering the mechanisms behind this resistance and current knowledge and concerns were discussed. This improvement for weevil resistance, together with silvicultural changes means that Sitka spruce can once again be considered as a viable plantation species in British Columbia and the Pacific Northwest. This program is arguably one of the most successful pest resistance breeding programs for plantation forest species in North America.

BREEDING AND SEED PRODUCTION OF CONIFERS FROM THE PACIFIC NORTHWEST IN SWEDEN

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Three conifers from the Pacific Northwest are currently of serious interest for use and genetic improvement in Sweden.

LODGEPOLE PINE

Lodgepole pine (LP) (*Pinus contorta*) is the most frequently used introduced tree species in Swedish forestry. It covers some 600,000 hectares and is the third largest species to be planted with 16 million seedlings sold in 2011, which is about 7% of the total planting stock. Prolific early flowering and ease of clonal propagation make LP a good species for tree improvement, with shorter generations and higher rates of genetic improvement than for Norway spruce (*Picea abies*) or Scots pine (*Pinus sylvestris*). In addition, lack of background pollen enhances realised gain in seed orchards. After a peak of planting LP in the late 1980s, the annual planting decreased – a trend that has only recently reversed. Today's interest in greater forest production has led to increased planting of LP. This has also initiated the production of new seed orchards, which are expected to give a total gain in growth rate of about 25% compared to unimproved trees.

The first plus tree selection in natural forests in Canada for grafting trees into seed orchards in Sweden was initiated in the early 1970s. In the late 1970s, a cooperative of forest companies initiated and financed a seed orchard program to make Sweden self-sufficient in improved LP seed. This also initiated the establishment of a long-term breeding program, which included new plus tree selection in natural forests in Canada, progeny testing, and establishment of seedling seed orchards. There are a total of 3,232 plus trees available as candidates for the LP breeding population. These include plus trees imported as grafts, selected progenies from plus trees, and selections in commercial Swedish plantations. This material covers much of the native distribution range of LP north of latitude 50°N in Canada.

A new breeding plan for LP has evolved and been put into practice. Based on research findings, operational experience and analysis of different breeding strategies, the new strategy will be by forward selection within clonally replicated full-sib families after field testing. This option best utilises resources already invested in the breeding, and provides options for long-term breeding and selection into seed orchards. A new generation of trees for forward selection is formed without delay. Breeding gains for LP under alternative long-term strategies have been quantified. Long-term gain for a program initiated with 50 trees would give 5% gain per generation in the breeding population for individual forward selection and 11% gain for clone testing, corresponding to an annual gain of 0.26 and 0.65% per year, respectively.

Currently, the plan is to structure the LP breeding populations as 11 advanced-generation sub-populations which cover the climate variation in northern Sweden. Each closed sub-population has at least 50 unrelated trees, resulting in a meta population of some 550 trees. In general terms, the breeding of LP focuses on trees that are efficient in using limited resources and grow stem wood more rapidly. A selection index is constructed from the measured traits, their heritabilities, and economic weights of the breeding objectives.

The interest for Douglas-fir (*Pseudotsuga menziesii*) and Sitka spruce (*Picea sitchensis*) is increasing but from a low level. The main reason for these species is a desire to spread risks to more species than Norway spruce due to the expected impact of global warming, especially in southern Sweden.

SITKA SPRUCE

For Sitka spruce, 65 new plus trees were selected in the 1990's and tested together with materials from Danish, Norwegian, and British breeding programs. Progenies from the new Swedish selections turned out comparatively well indicating a land race effect in those selections. Experiment means showed 25–50% superiority over Norway spruce checklots. British improved materials also look promising for south Sweden. The Norwegian material did not perform well due to the Alaskan origin being too far north. Two seed orchards will be established with backwards selected tested plus tree clones and some forward selections among progeny. Long term strategies for breeding and seed production are being developed.

DOUGLAS-FIR

Intermittent breeding activities have been carried out from time to time. In 1995, 65 plus trees were selected in good performing stands in south Sweden. Progeny tests were established and forward selections were carried out after analysis.

A series of three combined provenance and progeny trials in south and central Sweden were analyzed 18–19 years after planting. Results showed a general pattern of significant differences for growth traits between provenance groups of inland and coastal origin and even larger differences between provenances within groups. The most useful variation, however, was detected between open-pollinated half sib families. No serious adverse correlation estimates were found between growth and stem quality traits. Growth traits and susceptibility to Rhabdocline needle cast (*Rhabdocline weirii*) seemed to be favourably correlated. Strong additive genetic correlation estimates between the two southern trials suggested that the same regeneration material can be used all over the southern part of Sweden.

Grafted seed orchards will be used as a first step for capturing the genetic gain by forward selection of plus trees within the best open-pollinated families in good provenances. Seedling seed orchards, established after seed collection from selected individuals, is an alternative low cost method for mass propagation and would also serve as clone archives for the breeding population in a low budget breeding program.

WESTERN HEMLOCK FOREST GENETICS PROGRAM FOR BRITISH COLUMBIA

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Western hemlock (*Tsuga heterophylla*) is the most common tree on the British Columbia (BC) coast. It is roughly 30% of the annual volume harvested and estimated to be about 60% of the timber inventory for the Maritime region. As a consequence of its potential, tree improvement activities commenced with parent tree selections starting in the late 1950s. The first progeny trials were established in the early 1970s and a provincial breeding program was launched in the second half of that decade under Mike Meagher. With the recession of the early 1980s and consequential budget restrictions, activities were restricted to maintenance of existing sites and provenance seed collections were placed in the hands of the Douglas-fir (*Pseudotsuga menziesii*) breeder, Jack Woods. The hemlock program revived with the economy, as forest industry put weight behind their investment in the species requesting, in the words of Western Forest Product's John Barker, that "if they were to have seed orchards they should be tested". John King was engaged as the breeder, and seeing that few parent trees were tested to that point, set out with others to develop the Hemlock Tree Improvement Co-operative (HEMTIC) in the 1990s. Its course is well described by Jayawickrama and Ye in their description of endeavors in the US Pacific Northwest.

For hemlock in BC, unstable economics of the new millennium combined with the fecundity of the species led to reliance on natural regeneration. Planting numbers have fallen from a high of about 9 million a year in the early 1990s to as little as 10% of that recently. As a consequence, it was decided by the guiding committee for tree improvement for the province, the Forest Genetics Council of BC, that the program should be put on hold. This has meant once again activities are limited to securing long term research installations and finishing planned measurements of field trials.

The current program has several facets. Gene conservation status has been assessed and as expected for a prolific, invasive, shade tolerant species, risk of loss of genetic variation is relatively low. For genecology research there is a broad network of almost 40 field installations covering most of the environments in which hemlock occurs in BC. Measurements to age 10 years are fairly complete for the trials and reporting on features of the genetic architecture and adaptation is planned for the near future. Preliminary analysis suggests a latitudinal cline of decreasing growth capacity from South to North. With elevation, at least for coastal seed sources, there appears to be a step cline with a fairly abrupt differentiation of low and high altitude genotypes. Longitudinal trends within the coast, transition, and interior regions are not detectable, but movement of seed from one of these regions to another does not have positive outcomes.

For investigation of growth and yield of improved genotypes versus wild type plants, 3 series (9 test sites) were established. These tests confirm gain estimates from single tree progeny trial plots by area based 0.1 ha plots. As well, 6 of the trials have varied planting densities in order to predict effects from competition. Results to date confirm expectations for gain. In addition, comparison of wild stand progeny to controlled-cross orchard mean lots suggests inbreeding depression results in a loss of about 5% in early height growth.

Breeding to identify genotypes superior in desired traits ceased almost a decade ago, but progeny tests established then are just now yielding reliable data. Polycross (Px) trials of high elevation parents age 12 years are due for measurement in 2013, with new breeding values subsequently. Similarly, measurements of polycrosses of backwards selections from the HEMTIC program have been carried out recently, with final rankings to be determined in 2013. Forward selections from HEMTIC second-generation testing

have been ranked and the best 120 genotypes grafted for inclusion in clonebanks, but planned Px trials to confirm the breeding values will not to be carried out. Also, tests of best BC parent trees not ranked in time to be included in the HEMTIC second-generation will have a final measurement in 2013. Unlike the earlier second-generation trials, the parents were cloned to allow for more effective within-family selection.

Prospects for the future of the hemlock forest genetics program are likely limited. Despite two year delays for natural regeneration versus planting, inbreeding depression, patchiness, clear evidence that local seed is surpassed by more southerly sources, and increasing genetic gains in seed orchards, the method is inexpensive and often fairly successful. Perhaps declining rotation lengths, or rising genetic worth of seed, either from our orchards or those of the Pacific Northwest, will lead to a resurgence of this program once again.

AGE-7 RESULTS FROM A DOUGLAS-FIR REALIZED GENETIC GAIN/STAND MANAGEMENT TRIAL

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INTRODUCTION

Realized genetic gain is vitally important to those who fund tree improvement and use its results. Single-tree-plot progeny tests do not serve the purpose well following crown closure because of high competition among trees with different sizes (Magnussen 1989, Ye et al. 2010). Evaluating how genetically improved materials respond to different silvicultural treatments (e.g., stand density, weed control, etc.) can only be done in large-block field trials. Verifying realized genetic gain is expensive and takes a long time. In the mid-1990s, two realized gain trails of Douglas-fir (*Pseudotsuga menziesii*) were established in the north Oregon Cascades and British Columbia (St. Clair et al. 2004, Stoehr et al. 2010, Ye et al. 2010). Realized gains were evident for growth rate in these studies.

The Grays Harbor realized genetic gain / Type IV trial was the first large block-plot genetic gain testing effort in the public domain for Douglas-fir in the Washington coastal area and was established by the Stand Management Cooperative, the NW Tree Improvement Cooperative, and the USDA Forest Service PNW Research Station.

TRIAL OVERVIEW

Three genetic levels (G) of seedlots were tested from the Grays Harbor breeding zone: 1) Unimproved (a random sample of 50 wild trees distributed throughout the zone). 2) Intermediate (pair crosses among 20 parent trees with an intermediate level of genetic gain). This seedlot is only tested in the genetic gain trial portion (i.e., 3 m spacing with standard vegetation control). 3) Elite (pair crosses among 20 highly ranked parent trees). All parents in the Elite and Intermediate populations originated from the Grays Harbor vicinity and were selected based on their growth performance in the progeny tests.

The trial has six sites, three each established in 2005 and 2006 using one-year old containerized seedlings. Each site contains 22 square plots (100 trees / plot). There are three planting densities (S): 1) low - 4.5 x 4.5 m, 500 stems per hectare (SPH); 2) intermediate - $3 \times 3 m$, 1100 SPH; and 3) high - 2.1 x 2.1 m, 2,222 SPH. Two types of vegetation control (V) were applied. Nineteen of the 22 plots are common to all sites and received complete weed control to maintain at least 80% bare ground until crown closure. These 19 plots at each site form a response surface design with greatest replication of all gain levels at 3 m spacing. The remaining three plots received standard weed control (i.e., one complete weed kill during site preparation). These plots form a randomized incomplete block design. Thus, a total of 132

plots, comprising 14,800 measure trees were established within fenced plantations. Each gain level was represented by 10 trees from each of 10 families. A schematic of one site is shown below in Fig. 1.



Figure 1. Plot layout of one of the Grays Harbor Genetic Gain/Type IV trials. G1, G2, G3 – unimproved, intermediate, and elite seedlot, respectively; V1, V2 – standard and complete weed control, respectively; S1, S2, S3 – 4.5 x 4.5 m, 3 x 3 m, 2.1 x 2.1 m, respectively.

Data were collected, from all sites six seasons after planting, for height (ht7), diameter at breast height (dbh7), average crown width (acw7), crown base height (cbh7), number of incidents of forking (fk7), number of incidents of ramicorn branching (rc7), and stem sinuosity score (su7). The last thee traits (fk7, rc7, su7) were subjected to square root transformation before mixed model analysis.

RESULTS

By age-7 there were 12,289 surviving trees. Survival (surv7) varied from 72 to 90% within sites. Analyzed across sites, the genetically improved seedlots had significantly greater height and diameter than the unimproved seedlot (P < 0.001), with larger crown base height (P = 0.01) and fewer incidences of ramicorn branching (P = 0.001). While differences between gain levels for forking were non-significant, least-square means for forking were marginally lower in the intermediate and elite lots. Realized genetic gains were slightly higher for diameter (12.5% - intermediate, 12.2% - elite) than for height (10.0% - intermediate, 10.7% - elite), and were 27.6% (intermediate) and 29.5% (elite) for tree volume.

No significant difference was found between elite and intermediate seedlots for growth rate. We expect a difference to appear after the onset of strong inter-tree competition. The lack of significance in seedlot x spacing and spacing x survival may imply that inter-tree competition at age 7 is still weak.

Trees planted at the narrow spacings (2.1 m and 3 m) were on average taller (~5%) and had greater diameter (~4%). Spacing had not, however, significantly impacted tree survival (P = 0.25) yet. Differences between the two levels of vegetation control were insignificant except for dbh7 (P = 0.05), cbh7 (P = 0.02), and surv7 (P = 0.05), with the complete control having slightly better diameter growth, smaller crown base height, and higher survival. We speculate that there would be larger gains from complete weed control in areas subject to more severe summer droughts.

Realized gains varied greatly among full-sib families within each improved seedlot for all traits and within-seedlot variation was much larger than among-seedlot variation. The highest realized gains for age-7 height, diameter, and volume for a full-sib cross were 23.6%, 27.3%, and 73.8%, respectively, based on about 480 surviving trees for that cross.

The overall predicted gains were quite comparable to the realized gains. The average realized gains (elite + intermediate) were 10.4% for ht7, 12.3% for dbh7, and 28.3% for vol7. The corresponding average predicted gains from the progeny trials were 11.7% for ht10, 11.8% for dbh10, and 30.8% for vol10.

CONCLUSIONS

Results indicated that genetic gains are readily obtainable from Douglas-fir tree improvement programs and, averaged over the crosses, are close to those predicted from progeny trials.

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POPULATION GENETICS AND GENOMICS

THE TRIA PROJECT: USING GENETICS AND GENOMICS TO ASSESS THE VULNERABILITY OF THE PINE HOST LANDSCAPE IN THE FACE OF MOUNTAIN PINE BEETLE RANGE EXPANSION

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The current epidemic of the mountain pine beetle (Dendroctonus ponderosae) (MPB) is unprecedented in recorded history, impacting more than 28 million hectares of pine forests in western North America. Lodgepole pine (Pinus contorta var. latifolia), with a range overlapping that of MPB, has been the main species of pine affected by the present outbreak. From its historic range in the interior of British Columbia, MPB has spread across the Rocky Mountains into more northerly, easterly, and higher elevation forests than previously documented. Long distance dispersal events marked the arrival of MPB into northern Alberta in 2006, where lodgepole pine naturally hybridizes with jack pine (Pinus banksiana), a boreal species whose range spans eastward to the Atlantic coast. We used species-distinguishing markers to redefine this hybrid zone and demonstrate that MPB has undergone host range expansion to pure jack pine. Lodgepole pine shares a co-evolutionary history with MPB, and is thought to have acquired constitutive and induced defences that render greater protection against MPB than evolutionarily naive hosts such as jack pine. We are using biochemical, physiological, and functional genomics to test the hypothesis that molecular aspects of host suitability differ between lodgepole and jack pine. There is evidence that host suitability is influenced by environmental factors, so we are also testing the hypothesis that water limitation affects host suitability features of both species. The development of lesions in both seedlings and mature trees following inoculation with the MPB fungal associate, Grosmannia clavigera, was slower in jack pine than lodgepole pine; lesion development in both species was delayed by water deficit. Terpenoid oleoresins are an important component of the pine defense arsenal that the tree invokes against MPB and other pests and pathogens. The monoterpene profiles of lodgepole and jack pine differ, leading to the possibility that these dissimilarities contribute to differences in host quality between these two species. Towards identifying how these differences are manifested at the genetic, molecular, and biochemical level, transcriptomic resources have been mined to identify and characterize genes whose products are implicated in biosynthesis of these monoterpenes. Transcriptome-scale analyses by microarray reveal that thousands of genes are invoked in the response of pines to G. clavigera infection, that there are substantial differences in responses of lodgepole and jack pine, and that water limitation alters this transcriptional programme. In tandem with this research, we are using population genomics to characterize the lodgepole pine, jack pine, and lodgepole – jack pine zone of introgression as a means to develop an "index of naiveté" that can be used to inform risk analysis frameworks. Information arising from this integrated research program also has the potential to inform tree improvement and reforestation strategies in the wake of this destructive forest insect pest.

COMPARATIVE GENOMICS OF SPRUCE AND PINE

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Using two types of genomics resources, full-length cDNAs (ESTs) and BAC libraries, we studied several aspects of evolution along the white spruce - loblolly pine (Picea glauca - Pinus taeda) lineage. Previous studies have documented conservation of chromosome number and linkage groups in several species of this family. Our analyses of ESTs indicate a quite dramatic slowdown of nucleotide substitution rates, perhaps up to ten fold relative to angiosperms, in line with reduced nucleotide diversity within each species. Conifers also have higher dN/dS ratios ("slow but not low"). A simple model is presented that illustrates that natural selection is more effective at a single locus than when two loci are selected at once. providing one possible explanation of higher dN/dS in species with lower diversity. We sequenced 96 random BACs in each species (ca. 10 mb in each species) and also an apparent homologous region containing the lignin biosynthesis gene, CCoAMT. Identification of transposons with long terminal repeats shows that transposition has been ongoing at comparable rates in both species since their divergence (over 120 MYA), but in both species, there seems to be a peak at the start of the Cenozoic era (65 MYA), corresponding to a period of climate change (Paleocene-Eocene Thermal Maximum). CCoAMT coding regions were almost 100% identical, but flanking regions showed low homology. Comparisons of random BACs with draft genome sequence assemblies in both species illustrate that BACs containing complex large repeat structures are left out of the spruce and pine genome assemblies, while other BACs aligned quite well with the draft genome sequences.

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GENETIC DISSECTION OF EXPRESSION VARIATION IN WHITE SPRUCE (Picea glauca)

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In forest biology, two diciplines stand out as benefitting from high-throughput genomics analysis. One of them is ecological and evolutionary genomics, the goal of which is to find genes linked to evolutionary processes in ecological contexts (Feder and Mitchell-Olds, Nature Reviews Genetics 4:649-655). The other is genetical genomics, which aims at dissecting the genetic architectures underlying variation in complex molecular phenotypes such as global gene or protein expression (Jansen and Nap, Trends in Genetics 17:288–391). Bridging these two disciplines would give scientists greater resolution in studying the genetic basis of ecologically important evolutionary questions. This however requires that the studied species is amenable to the assessment of genetic effects on phenotypes. Although genomics analysis can now be applied to almost any organism, the list of species that are genetically tractable has remained relatively restricted. This problem is even more pronounced regarding species with dominant roles in their respective ecosystems. In conifer trees, for example, standard genetic experiments that address the phenotypic effects of genetic variants have remained largely unfeasible. Such studies would however be essential to our understanding of the eco-evolutionary dynamics that drive genetic variation.

We have explored an approach to track genetic effects on gene expression that is based on the analysis of global gene expression levels in the haploid, meiotic seed tissue of gymnosperms, the megagametophyte. Microarray analysis of megagametophyte sets from two white spruce (Picea glauca) trees allowed the identification of gene expression differences between maternal alleles that segregated in Mendelian ratios, and which were therefore most likely caused by single genetic variants (Verta et al., Molecular Ecology 22:2369-2379). A relatively high frequency of analyzed genes exhibited simple Mendelian segregation in expression levels in our white spruce dataset, compared to public datasets of plant, insect, and mammal model species. While being consistent with high levels of heterozygosity in white spruce, the higher frequency of Mendelian over polygenic effects on gene expression points towards a lower level of connectedness in gene networks in the case of white spruce. On the other hand, as well as being over represented in genes involved in stress response, expression variation showed a bias towards duplicated genes. Partitioning of expression variation between duplicated genes was consistent with a transition from relaxed selection on young copies to unequal selection pressures on older paralogs. Furthermore, the evolutionary implications of expression variation are underlined by our observation that expression variation in most genes was affected by independent genetic variants. Because the effects of single genetic variants seem to be limited to only a few expression traits, natural selection could act on the expression variants independently. Our results therefore support the idea that adaptation in some traits may be achieved through alternative molecular solutions in different subpopulations of wide-spread conifers.

We are currently conducting a RNA-seq experiment using megagametophytes and the associated, self-fertilized embryos in order to gain insight into the genetic loci that are associated with heritable expression variation, and to examine some of the above predictions in more detail. Both genotypes and gene expression levels can be estimated in a single RNA-seq experiment, enabling the identification of genetic variants that co-segregate with gene expression levels. Our approach takes full advantage of the sequence data because the haploid nature of the megagametophyte tissue allows unequivocal identification of haplotypes. In addition, gymnosperm embryos are produced by fertilized archegonia which are derived from cells of the megagametophyte, and thus the maternal genome of the embryo is

identical to that of the megagametophyte. Megagametophyte haplotype information can therefore be used as *a priori* information in calling genotypes in the associated embryos. Analysis of self-fertilized embryos will allow us to characterize the phenotypic effects of genetic variants in diploid tissue. In addition, comparisons between homo- and heterozygote embryos allow the determination of dominance effects between allelic expression levels. This aspect is important when predicting the evolutionary dynamics of genetic variants affecting gene expression, because the balance between drift and natural selection as drivers of allele frequencies and phenotypic evolution depends on dominance relationships between alleles (Lemos et al., PNAS 105:14471– 14476).

In total, we have generated over 1.3 billion 100 bp paired-end Illumina reads, distributed among 66 sample pairs. To date, we have aligned the reads to the white spruce gene catalogue, called segregating variants, defined linkage groups, and performed association tests of gene expression against local SNP variants in the megagametophyte samples. We identified over 10,800 genes that contained segregating SNP variants between the maternal alleles. Allele-specific (i.e., local) effects on transcript accumulation seem to be common, affecting nearly one-third of the genes, with the p-value threshold of 0.001. We expect to be in a position to give accurate estimates on the frequencies of local and distant genetic effects on gene expression, their effect sizes, and heritabilities in the two studied tissues, as well as their dominance relationships, within the next two months.

Taken together, our analyses indicate that RNA-seq coupled with megagametophyte sampling provides a very powerful and cost-efficient method to identify segregating genetic variants in coding regions in conjunction with defining gene expression levels. In addition to serving as a proof-of-concept for linking genetic variants to molecular phenotypes in spruce, our analyses will build knowledge on the network effects and dynamics of genetic variation with phenotypic impacts.

LANDSCAPE GENETIC CONSEQUENCES OF BIRD, GRAVITY AND WIND DISPERSED VALLEY OAK PROPAGULES

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We have invested heavily in studying propagule dispersal over the last decade, on the premise that it determines both the amount and pattern of genetic variation across the landscape. We have learned that pollen generally moves farther than seed and dispersal asymmetries should have implications for male and female gametic affinity patterns across that landscape. We have examined natural recruiting patches in Q. lobata: 1) canopy shadow patches (CS) of maternal plants (gravity and small rodent-vectored) and 2) open patches (OP) (bird-vectored). These vector differences impact the patterns of female gametic affinity. Using microsat assay techniques, we have genotyped both the seed coats and embryos of multiple seeds in both types of patches and have determined male and female gametophytic genotypes. We reported seed and pollen dispersal outcomes elsewhere. The point is that the open patches involve recruits dispersed further from their maternal sources. We anticipate more rapid decay of megagametophtic relationship with increasing distance for canopy patch than for avian-dispersed open patch recruits. What to expect from the male gametes is unclear. We computed autocorrelograms for both male and female gametes for both types of patches. Female gametes show striking patterns of IBD. For (CS), the progression was (r = 0.45) at close quarters \rightarrow (r < 0.04) at 500 m. For (OP), the same progression was (r = 0.27) \rightarrow (r = 0.07). Male gametes, by constrast, show virtually no difference between patch types and no real decay with distance: for (CS), we found $(r = 0.03) \rightarrow (r < 0.01)$; for (OP), we found $(r = 0.03) \rightarrow (r < 0.02)$. For each patch type, we also computed within patch (á), among patch (\hat{a}), and total (\tilde{a}) allelic diversities. For (CS) and female gametes, the partition is ($\hat{a} = 1.54$, $\hat{a} = 1.53$, $\tilde{a} = 1.53$ 2.36). For (OP), the same partition is (a = 2.18, a = 1.31, a = 2.86). Greater seed dispersal increases a, decreases b, and increases g). For male gametes, the corresponding partition is ($\dot{a} = 3.29$, $\hat{a} = 1.08$, $\tilde{a} =$ 3.56) for (CS) and ($\dot{a} = 3.27$, $\dot{a} = 1.10$, $\ddot{a} = 3.56$) for (OP), no meaningful difference, but larger \dot{a} , smaller â, and larger ã than for female gametes. In overview and to a first approximation, seed flow determines IBD; pollen flow represents the homogenizing glue that holds the species together.

POPULATION GENOMICS IN *Populus trichocarpa* (BLACK COTTONWOOD) REVEALS A CANDIDATE GENE FOR LOCAL ADAPTATION

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Black cottonwood (Populus trichocarpa Torr. & A. Gray) is an ecologically and economically important forest tree distributed throughout western US and Canada. Along its range, poplar exhibits variation in several adaptive traits including growth, phenology, and disease susceptibility. To understand the genetic basis of this adaptation, the genomes of P. trichocarpa accessions were scanned from across its range for signatures of selection. Despite low levels of population genetic structure at most genetic markers, COMT1 (encoding caffeic acid 3-O-methyltransferase), an enzyme involved in phenylpropanoid metabolism, showed strong levels of population differentiation. Here we analysed 302 COMT1 DNA sequences based on whole genome resequencing in individuals from 29 locations in the north, center, interior, and south of British Columbia, spanning over 14° in latitude, to ascertain whether increased population differentiation could be driven by adaptation. From 17 COMT1 SNPs (Single Nucleotide Polymorphisms), three were found to be nonsynonymous (nsSNP) and two of these were located in the O-methyltransferase domain (amino acids 158 and 287, respectively). The geographic distribution of haplotypes revealed that alleles with glutamine at position 287 (Q287) are restricted to northern and interior populations, while alleles with proline at this position (P287) occur at all locations. These results were supported by further population genetic analyses that showed relatively low levels of COMT1 allele exchange between southern and northern populations (Fst = 0.193) and between southern and interior populations (Fst =0.151). The Fst between northern and interior populations was not different from zero. Although statistical tests for selection revealed that COMT1 overall is under purifying selection, certain amino acids may have adaptive significance. While COMT1 E287 is restricted to northern and central populations of P. trichocarpa, it is common across the range of P. balsamifera, a closely related boreal species. COMT1 P287, on the other hand, is exclusively found in COMTs from Arabidopsis, tobacco, Medicago, and other Populus species, as well as in the P. trichocarpa COMT2, a gene involved in developmental lignification. We hypothesize that Q287 causes changes in COMT1 enzyme activity, and affects the P. trichocarpa phenotype, resulting in a competitive disadvantage of this allele at lower latitudes, where both temperature and precipitation are higher. To test if the introgression of COMT1 from P. balsamifera to P. trichocarpa is being selected against at southern locations, we will perform further selection analyses and phenotypic studies of individuals with different COMT1 genotypes. We are also functionally characterizing COMT1 by transforming an Arabidopsis comt mutant with different poplar COMT1 and COMT2 alleles.

GENOMIC SIGNATURES OF LATE PLEISTOCENE RANGE DYNAMICS AND DIVERGENT SELECTION REVEALED BY RE-SEQUENCING OF THE BLACK COTTONWOOD EXOME

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Temperate and boreal tree species have undergone repeated range expansion and contraction coincident with glacial cycles during the late quaternary. For northern hemisphere species, refugial populations typically existed south of the maximum extent of glaciation, though there has long been interest in the degree to which additional refugia were present in northern ice-free areas, for example, on coastal islands or interior boreal deserts where low precipitation limited ice formation. High phenotypic differentiation among contemporary populations implies rapid local adaptation along these migration routes, but these adaptations may have been hindered by re-colonization bottlenecks and central-peripheral structure. Populus trichocarpa (black cottonwood or 'poplar') is a temperate species with a wide contemporary latitudinal range that extends from northern California, USA north through coastal southeast Alaska, USA. We recently reported exome re-sequencing of a range-wide sample of poplar genotypes. Here we use these data to elucidate how neutral and adaptive forces have shaped genomic variation within and among populations of this species. The most parsimonious solution to population structure in our range-wide sample of 47 genotypes resulted in three groups arrayed latitudinally (which we refer to as north, central, and south). Mean nucleotide diversity was highest in the northern population and lowest in central population, while linkage disequilbrium was highest in center and lowest in the north. These patterns were consistent across the genome. Estimation of mutation-scaled effective population sizes (Ne) revealed that the north had the largest Ne, followed by the center and south populations. Taken together, these results are consistent with a glacial refugium in the vicinity of the northern population, which is supported by the paleobotanical record indicating the presence of cottonwoods in Beringia during the last glacial maxima. Genome scans of population differentiation reveal a very narrow distribution of FST for intergenic regions, with much broader distributions for different classes of sites (synonymous, replacement, promoter, UTR) within genes. Promoter regions harbored significantly more outliers than other classes of sites within genes, suggesting the role regulatory variation in adaptation to climate.

TOWARDS UNDERSTANDING THE GENOMIC BASIS OF CHEMICAL DEFENSE TRAITS IN EUROPEAN *Populus* SPECIES

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Trees are long-lived organisms that cannot move to escape environmental challenges such as herbivores, pathogens, or climate change. Their survival and fitness thus depend critically on adaptive responses to biotic and abiotic stressors. Tree adaptation to changing environments is likely to involve genetic variation supplied by migration within species and introgression from related species, although these hypotheses have rarely been tested in a rigorous manner.

We studied patterns of expression of key secondary metabolites involved in biotic and abiotic responses (salicinoids, flavonoids, and chlorogenic acids) in three large natural hybrid zones and a common garden composed of open pollinated families of two ecologically divergent *Populus* species, *P. alba* and *P. tremula*. The same plant material is also being genotyped with mapped microsatellites, Restriction site Associated DNA (RAD) sequencing and related methods, and population samples of each species have been subjected to whole genome resequencing.

Uni- and multivariate analysis of metabolomic profiles in recombinant hybrids reveals the potential and limits of wide recombination to generate novel combinations of functionally important chemical traits. By combining admixture mapping of chemical traits in natural hybrid zones with common garden measurements, we provide first insights into the genomic architecture of chemical defense traits that differ between these ecologically divergent, hybridizing species.

The implications of our findings for the genetics and chemical ecology of phenotypic species differences with potential community and ecosystem effects were discussed. The benefits of combining genomics, metabolomics, and ecology in fundamental and applied studies of forest tree biology were highlighted, using *Populus* as an example.

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COMPARATIVE PHYLOGEOGRAPHY AND GENETIC STRUCTURE OF THE EASTERN NORTH AMERICAN BIRCHES: Betula alleghaniensis, B. papyrifera and B. lenta

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We examined the phylogeography and genetic structure of birches (*Betula* spp.) in eastern North America, with a specific focus on the widespread, ecologically, and economically important species, *B. papyrifera* Marsh. (4x=56, 5x=70, 6x=84), *B. alleghaniensis* Britt. (6x=84), and *B. lenta* L. (2x=28). Chloroplast DNA (cpDNA) and nuclear microsatellite (nSSR) markers were used for inferring patterns of population structure in relation to historic glacial and postglacial population dynamics as well as contemporary gene flow. Six chloroplast microsatellites and the psbA-trnH intergenic spacer region were analysed for *B. papyrifera*, *B. alleghaniensis*, and *B. lenta* sampled from 65, 80, and 12 populations, respectively. A subset of these populations was used for examining the population genetic structure at eight polymorphic nSSR markers. Genetic diversity within and among species was compared using rarefaction analysis and haplotype richness maps. Haplotype networks, spatial analysis of molecular variance (SAMOVA), and comparisons of NST and GST values were used to evaluate phylogeographic structure at nSSR markers.

Betula papyrifera and B. alleghaniensis demonstrated relatively high allelic richness at cpDNA and nSSR markers, while B. lenta was fixed for a single cpDNA haplotype and demonstrated low nSSR diversity. The highest cpDNA and nSSR diversity for B. alleghaniensis was detected in the western Great Lakes region and B. papyrifera also had high cpDNA diversity in that region. Populations of B. papyrifera and B. alleghaniensis were strongly differentiated at cpDNA markers (GST=0.526 and 0.601, respectively), and the SAMOVA revealed two main phylogeographic groups for these species corresponding to populations of eastern and western refugial origin. Betula papyrifera and B. alleghaniensis showed extensive regional haplotype sharing indicating that introgression within glacial refugia or during postglacial colonization may have contributed to the shared phylogeographic pattern. In contrast, no phylogeographic structure was observed in B. lenta at cpDNA markers.

No clear pattern of population genetic substructure was found for any of the species at nSSR makers. Populations were weakly differentiated (FST=0.008-0.015, RST=0.011-0.032) and differed significantly only over large geographic scales. The relatively high genetic diversity of *B. papyrifera* and *B. alleghaniensis* in the western Great Lakes region might be explained in terms of haplotype mixing in this region which is a postulated zone of biogeographic contact between eastern and western glacial lineages. The high level of cpDNA-based population differentiation in *B. papyrifera* and *B. alleghaniensis* could be attributable to long-term isolation within glacial refugia leading to differentiation through genetic drift. In contrast, the low level of nSSR-based population differentiation observed in birches could be due to high rates of contemporary gene flow via pollen. High levels of genetic diversity observed in *B. alleghaniensis* and *B. papyrifera* could be attributed to relatively large effective population sizes in glacial and contemporary landscapes facilitated by their relatively broad ecological amplitude or their polyploid origins, which may contribute to high heterozygosity at nSSR loci.

The low genetic diversity detected in *B. lenta* could be due to its relatively narrow geographic distribution and lower cold tolerance, which may have led to population bottlenecks during the last glacial maximum. The low genetic diversity at both chloroplast and nuclear genomes suggests that *B. lenta* may possess low adaptive potential to the changing climate and should potentially be given a high priority in conservation and management programs. In contrast, the relatively high genetic diversity of *B. papyrifera* and *B. alleghaniensis* suggests that they may have maintained relatively large effective population sizes during past glacial/interglacial periods and may possess genetic resilience to climate change.

GEOGRAPHIC CLINES IN NUCLEAR MICROSATELLITE DIVERSITY AND STRUCTURE AND CONE CHARACTERISTICS VARIATION OF *Pinus densiflora* NATURAL POPULATIONS IN JAPAN

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Pinus densiflora (Japanese red pine) is both an economically and ecologically important conifer whose widespread natural distribution is mainly in the semi-mountainous area which constitutes one of the major Japanese forest landscapes. Since this species is suffering from serious reduction in population sizes in Japan due to the introduction of pine wood nematode, the design of programs to conserve or manage its regional genetic clustering have been evaluated for populations of many other major tree species in Japan (e.g., Takahashi et al. 2005 for *Cryptomeria japonica*; Hiraoka and Tomaru 2009 for *Fagus crenata*), almost no information has been obtained on its current population genetic structure based on molecular markers as well as variation in its adaptive traits. In the present study, the geographic pattern of genetic diversity and structure of 62 *P. densiflora* natural populations (a total of 1,883 trees) across their distribution in Japan was examined using eight nuclear microsatellite markers. The geographic variation in cone characteristics was also investigated for 28 out of the 62 genotyped populations (a total of 628 trees).

The allelic richness was significantly lower in both northern and eastern marginal populations. Analysis of recent bottlenecks detected a significant heterozygosity excess more frequently in the northern and eastern populations. The overall value of the standardized measure of population differentiation ($G'_{ST} = 0.122$) was similar both to other widespread Japanese tree species and to continental *Pinus* species. STRUCTURE software analysis revealed a gradual cline in the genetic structure, with three main clusters corresponding to the western, central, and northeastern populations; the northeastern cluster showed the highest F value. We also found that cone size (length, width) as well as the seed-ovule ratio per cone was significantly larger in both northern and eastern populations.

The microsatellite results indicated strong genetic drift through rapid population expansion of P. *densiflora* in northeastern Japan, associated with recent changes in distribution suggested by anthropological and paleoecological studies. The geographic cline in cone characteristics might also be related to the climatic or environmental conditions of populations such as temperature. Results obtained on these neutral genetic and morphological variations provides fundamental information for designing conservation units and breeding zones as well as an evaluation of adaptive genetic variation for future studies.

USING ANCIENT DNA FROM LAKE SEDIMENTS TO EXAMINE FOREST RESPONSES TO HOLOCENE ENVIRONMENTAL CHANGES

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Paleoecological reconstructions based on fossil pollen records have given us much insight on Holocene shifts in forest community composition in North America. Such long-term records are necessary to make better prediction of forest responses to future climatic change. However, pollen analysis also has well-understood limitations including long-distance dispersal and similarities in pollen morphotypes in some species. Ancient DNA (aDNA) is a potentially useful complementary source of information to pollen records in understanding historical vegetation shifts. Apart from providing links between Quaternary paleoecology and fields such as evolutionary biology and population genetics, aDNA might be able to provide higher taxonomic resolution than fossil morphology. This potential requires overcoming three obstacles: 1) Ancient DNA must be preserved and recoverable in lake sediments, 2) molecular variation in these aDNA assemblages must contain information of high taxonomic resolution (e.g., identification of species or subspecific lineages), and 3) the bioinformatics problems of assemblages of damaged aDNA must be resolvable. We addressed these issues in one of the most common contexts in Holocene paleoecology: inference about changing forest composition from lake sediments. Working in Upper Michigan lakes with excellent fossil pollen and macrofossil records, we extracted chloroplast DNA (cpDNA) from macrofossils and bulk sediments as old as 6,000 yr BP (Before Present). We used the novel sequence capture method and high throughput sequencing to extract and sequence large numbers (on the order of 107) of DNA fragments per sample. We are currently addressing bioinformatics challenges of these assemblages of fragmented and damaged DNA using a new bioinformatics pipeline and assembly algorithms. Preliminary results indicate we can identify cpDNA at a level equivalent to that provided by fossil morphology, with higher confidence at the genus level. We can also identify interspecific variation - species that are not resolved via macrofossil morphology (e.g., species of Betula) as well as intraspecific variation unavailable via fossil morphology (e.g., distinct lineages of Fagus grandifolia). These results, when combined with paleoecological data (e.g., pollen and macrofossil analysis), provide a better and more comprehensive signal for reconstruction of past shifts in forest community in response to environmental changes.

USING RNAseq TO CHARACTERIZE GENE EXPRESSION IN LODGEPOLE PINE AND INTERIOR SPRUCE

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Understanding the genomic basis of how forest trees adapt to climate will be important to making management decisions to prepare for climate change. The AdapTree project aims to characterize the genomic basis of local adaptation to climate in Pinus contorta (lodgepole pine) and Picea glauca, P. engelmanii, and their hybrids (interior spruce), which are key species of economic importance in British Columbia and Alberta. Plasticity is one important component of how species cope with variable environments, and studying gene expression provides one approach to characterize which genes are most involved in responding to climate. Here, we explore how gene expression varies among 48 accessions of lodgepole pine and 41 accessions of interior spruce, each from a single population. Each individual in the experiment was grown under one of seven treatments in growth chambers, representing a range of moisture, temperature, and light regimes. We then used RNAseq methods to sequence RNA extracted from root, stem, and leaf tissue, yielding approximately 3.8 Gb of data per library. To assemble a reference transcriptome for each species, we combined one library from each of the seven treatments and ran the combined data on Trinity. We used RSEM and EdgeR to estimate expression levels for each gene in our transcriptome, and used WGCNA to group these genes into clusters based on similarity of expression profiles. We identified 8,894 and 11,618 differentially expressed genes in spruce and pine, respectively, at an FDR = 0.01. Our cluster analysis identified 13 groups, which correspond to genes that have similar patterns of expression across the different treatments. These characterizations of gene expression will provide a basis to test hypotheses about the importance of plasticity in adaptation. Subsequent work in the AdapTree project aims to characterize which genes are involved in local adaptation to climate. Using these data, it will be possible to test whether the most plastic genes also tend to be the most or least likely to be involved in local adaptation.

FINDING THE GENES FOR LOCAL ADAPTATION: TRIMMING TO ACCOUNT FOR POPULATION STRUCTURE

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The genes responsible for local adaptation are likely to have more genetic differentiation among populations than do neutral genes. In principle, a good clue that a gene is affected by evolution to local conditions is that it has a higher F_{st} than the rest of the genome. However, testing this statistically is challenging because some population samples are not evolutionarily independent of other samples. Typical F_{st} outlier approaches have high false positive rates. A new method of F_{st} outlier analysis–called LookWell– adjusts for non-independent sampling. Simulation demonstrated that LookWell gives fewer false positives and much more reliable inference.

QUANTITATIVE GENETICS AND TREE BREEDING

MATCHING GENOTYPES TO CURRENT AND FUTURE PRODUCTION ENVIRONMENTS TO MAXIMISE RADIATA PINE PRODUCTIVITY AND PROFITABILITY

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Radiata pine (*Pinus radiata*) is the most important commercial conifer species in Australia and New Zealand. Significant progress has been made in the improvement of growth, form, and wood quality traits through the understanding of their genetic control. However, the benefits of long-term investments in genetic tree improvement have not been fully realised, as improved genotypes should be matched to their specific growing environments and production systems to fully realize their genetic potential.

Current radiata pine breeding and deployment in Australia and New Zealand is based largely on state and regional boundaries or forest inventory zones rather than on rigorously mapped climatic and physiographic parameters. Current zones may not be delivering optimal genetic gains across the whole estate. To further improve radiata pine plantation productivity and to maximize realised genetic gain from breeding and deployment populations under current and future climates, it is necessary to: 1) delineate breeding and deployment zones based on site and climatic factors and 2) match genotypes with current and future production environments considering multiple objectives, such as maximizing growth, improving form, branching, resistance, and wood quality.

Optimal breeding and deployment zones must consider genotypic responses together with physiographic and climatic information. Genetic information on breeding stock was combined with soil and climatic data of more than 300 test sites to pinpoint environmental factors affecting the response of radiata pine to environmental variation, and to provide a better understanding of the factors contributing to the observed genotype x environment interaction (GxE). An analysis of GxE was performed using multivariate analytical techniques and mapped using geographic information system (GIS) tools.

We explored different methodologies to account for GxE. In practice, the Radiata Pine Breeding Company (RPBC) in New Zealand prefers clustering of sites based on a factor analytic model involving a GxE term. To explain the clustering of sites in New Zealand based on geo-climatic variables we used several analytical techniques including visualisation based on Random Forest Classification and Multi Dimensional Scaling, Multiple Regression on Distance Matrices, and Multiple Regression Trees.

The Southern Tree Breeding Association (STBA) in Australia prefers site classification based on modelling of genetic correlations. For trials in Australia, we created a database of genetic parameters as a

basis for the modelling of genetic correlations. Site classification involved grouping trials into site types based on certain goodness of fit criteria to reduce the so called residual GxE, or minimise GxE within site types. Genetic evaluations with the TREEPLAN system, incorporating the new site classification, will demonstrate the genetic gain resulting from the improved classification.

The environmental variables determining the GxE patterns or, so called, "drivers of GxE" were identified, and at the broadest, trans-continental scale, climatic variables such as temperature and rainfall were the most significant. However, at a local regional scale soils and topographical factors were of more significance. New site classification will be defined across Australia and New Zealand and genetic gains that can potentially be obtained by accounting for $G \times E$ evaluated. Our ultimate goal is to recommend best strategies to maximise genetic gain by matching genotypes with production environments and ensure immediate adoption of results into selection and deployment programs of the radiata pine industry.

Predictions of plantation yield at different plantation locations based on future climate forecasts typically do not consider potential for adaptation by using genetic selection. Climate change scenarios will be superimposed on the "response functions" or "norms of reaction" models developed in this study, and responses of different genotypes will be extrapolated beyond current climatic conditions. Trials at the extremes of the radiata pine climate envelope will be particularly valuable to assess the effects of future climate change on growth and form traits. Climate change impacts on deployment zones will then be evaluated using GIS tools that we are currently developing. This study will determine the extent to which we can use genetic selection to mitigate some of the predicted negative effects of climate change on timber supply from the radiata pine plantation estate in Australia and New Zealand.

INTERACTIONS OF FAMILY BY FUSIFORM RUST INOCULUM IN LOBLOLLY PINE PROGENY TESTS AND IMPLICATIONS FOR BREEDING IN THE SOUTHERN US

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Fusiform rust disease in loblolly pine (*Pinus taeda*) is caused by the fungus (*Cronartium quercuum* f. sp. *fusiforme*), an endemic pathogen in the southern US. The disease causes multi-million dollar annual losses in southern pine plantations and can be partially controlled via deployment of resistant genotypes of loblolly pine. Significant improvements in rust resistance have been made in loblolly pine breeding programs. For example, in the Atlantic and Gulf Coastal Plains, the predicted performance of the top 30% of open-pollinated families ranges from 5 to 24% rust disease at sites where non-improved loblolly pine would have 50% disease.

The stability and utility of rust resistance across diverse sites for given families have been questioned by breeders and pathologists for decades. Major gene resistance for rust resistance has been well documented and significant interactions between host and pathogen genotypes have been found in greenhouse assessments using bulked inocula as well as single acciospore inocula. In field trials established across a wide range of sites, we have found mixed results for interactions of family and site for resistance to fusiform rust. One study showed that resistant families showed variable degrees of rust infection across wide-ranging sites and other trials have similarly shown that some families do interact with site. The majority of our field trials, however, have shown little to no interaction of family by site for resistance to the disease. We have recently compared the rust incidence of families that were tested in narrow geographic trials to those same families tested across wide-ranging trials. The performance of families in the local trials appears to be generally predictive of performance across wide test environments, again suggesting stability of resistance across sites.

The lack of predictable interactions in field sites implies that the mix of fungal pathotypes at each field site is complex and that multiple different pathotypes are present at most sites. Any individual pathotype may be at a higher or lower frequency at any particular site, and occasionally a family that is generally resistant will be infected at a high frequency. A breeding strategy that selects pine families resistant to rust disease across diverse environments has worked and will be implemented in the NC State University Cooperative Tree Improvement Program's fourth-cycle breeding strategy.

PEDIGREE ANALYSIS AND FOURTH-CYCLE BREEDING STRATEGY IN THE NORTH CAROLINA STATE UNIVERSITY COOPERATIVE TREE IMPROVEMENT PROGRAM

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The build-up of inbreeding is expected to occur over time in any recurrently-selected breeding population. Breeding strategies aim to control coancestry and inbreeding while increasing genetic gain. As more cycles of selection are completed, the pedigree structure of a population becomes more complex. Designing crosses to optimize genetic gain and keeping the inbreeding at a desired level is challenging but necessary. We evaluated a number of software packages for their usefulness for analyzing pedigrees in the NC State University Cooperative Tree Improvement Program. Pedigree analyses suggest that there is minimal inbreeding in our loblolly pine (*Pinus taeda*) breeding populations after three cycles of selection. Out of 258 individuals tested in third cycle polymix trials with one or more known parents, only one individual (a self with F=0.5) has an inbreeding value greater than zero.

We used a Differential Evolution algorithm developed for animal breeding (Kinghorn 2011) to design the mating for the Cooperative's fourth cycle. Simulations were carried out, testing various combinations of three different 'balancing' strategies between gain and coancestry, two different candidate mating pools, and two different maximum levels of parental use. Crosses between full- and half-sibs were discouraged by weighting against solutions that contain any recommended crosses with F>0.124. As expected, scenarios resulting in lower population diversity also achieved higher mean index values (mean breeding value). The mean F and the predicted progeny index of the fourth-cycle population changed depending on the level of constraint placed on coancestry. For all the scenarios tested, mean progeny F for the recommended crosses ranged from 0 to 0.0018 and is not a problem. This is expected given the large number of parents in the breeding population and an initial mean F=0. Using the Differential Evolution algorithm to design crosses in sublines over several generations resulted in rapid increase in mean F and rapid leveling out of the expected mean index. In conclusion, pedigree analysis and the Differential Evolution for the Cooperative.

HOW TO ADD MORE SEED CONES ON LODGEPOLE PINE

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Low seed cone number can limit seed production. By studying hormone profiles of parts of lodgepole pine (*Pinus contorta*) long-shoot buds, we were able to devise a method to enhance seed cone production. In a long-shoot bud, developing seed cones and male cones are separated. High performance liquid chromatography-electrospray ionization tandem mass spectrometry in multiple-reaction monitoring mode was used to investigate phytohormone profiles in the proximal and distal portions of long-shoot buds. Higher concentrations of trans-zeatin riboside (t-ZR) and dihydrozeatin (dhZR) were found in the distal parts of long-shoot buds, whereas concentrations of isopentenyl adenosine (iPA), indole-3-acetic acid, abscisic acid (ABA), ABA glucose ester (ABA-GE), and phaseic acid (PA) were higher in the proximal parts. In long-shoot buds of genotypes with a history of high seed cone yield, concentrations of t-ZR and the ratio of zeatin-type to isopentenyl-type cytokinins (CKs) were higher in the entire buds. In low seed cone yielding genotypes, concentrations of c-ZR, iPA, ABA-GE and PA were higher throughout the long-shoot bud. ABA was higher in the distal portion. Exogenous gibberellins (GAs) applied by stem-injection or branch paste reduced endogenous concentrations of ABA and/or some of its metabolites, such as ABA-GE. Treatments that combined GAs with CKs, such as thidiazuron or 6-benzylaminopurine, altered endogenous CK concentrations more than treatment by CK or GA alone. Application of bud paste with a mixture of GA₄₊₇ and CK resulted in many female cones. Many of these were found in locations where previously only male cones are found. These ectopic PGR-induced seed cones developed normally.

INHERITANCE OF PARTIAL RESISTANCE OF WHITE PINE BLISTER RUST IN SUGAR PINE

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Sugar pine (*Pinus lambertiana*), one of the white pine species that is most susceptible to the blister rust fungus (*Cronartium ribicola*), has a major gene (Cr1) at low frequency in populations that confers immunity to the disease. Sugar pines bearing at least one copy of Cr1, however, are vulnerable to specific virulence from a complementary gene (vcr1) in the pathogen. In the years since the appearance of the virulence gene, a form of resistance, partial (PR; aka, slow-rusting), was discovered, which is a suite of traits that reduces susceptibility to blister rust in pines challenged by either the avirulent (Avcr1) or virulent (vcr1) forms of the pathogen.

Sixteen sugar pine parents with known, suspected, or low PR were control-pollinated in a mixed factorial-diallel design, resulting in 75 families. In 2006, a plantation was established at Happy Camp in northwestern California to assess the degree and inheritance of PR. Progenies consisted of open-pollinated seedlings from survivors of previous epidemic waves on the same site (all presumed to carry some degree of PR), along with the control-pollinated families. Since 2006, all progenies have been naturally exposed to blister rust at the test- ite, in which alternate host *Ribes* spp. have been interplanted among the rows. Progenies were evaluated in 2010-2012 after successive wave years of C. ribicola caused overall infection to reach 82%. In 2010, numbers of separate infections were counted on each seedling and characterized as normal, susceptible, or reactive PR responses; in subsequent years, only the presence of reaction type was noted. Mean infections per family ranged from 0.39 to 12 per tree, with numbers of infections in families with low PR increasing exponentially, suggesting epistatic effects. In 2012, families varied from 36 to 100 in the percentage of progeny with normal, susceptible infections; mortality is now ~60%. Families with the least infection tended to come from F1 PR parent survivors from prior epidemics. As expected, the presence of the Cr1 gene in a parental genotype had no effect on PR responses of the progeny. We analyzed the data in the manner of Cockerham and Weir (1977) using SAS' PROC GLIMMIX. In addition to estimating general and specific combining ability effects, the C&W model also estimates extranuclear paternal and maternal effects and general and specific reciprocal effects. However, because of imbalance in our data, reciprocal effects could not be estimated. Additive genetic variation was ~80% of the total genetic variation and narrow-sense heritability was ~0.35 in the 2011 and 2012 evaluations; non-nuclear maternal effects were 8% of genetic variances. We regressed bi-parental expected values against family means. In the 2011 data, this relationship was linear. However, in the 2012 data, the relationship was quadratic, showing underdominance and with families from the best parents deviating positively from their expected values. In spite of this, only three families deviated outside the 90% confidence interval for individual values. Thus selection can be largely based on general combining abilities. These results present opportunities for combining PR with single gene resistance to develop long term, durable resistance.
PREDICTION OF WOOD QUALITY BASED ON AGE-AGE CORRELATION FOR WOOD DENSITY COMPONENTS

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Wood density is considered one of the most important characteristics of timber quality, therefore, the possibility of its formation through population selection can be fundamental for wood quality improvement. Trees need a long time to achieve a proper age to be considered appropriate for use. Therefore it is difficult to quickly assess the efficiency of the process of population selection. The observed high correlation between the properties of juvenile wood and mature wood allows for the acceleration and improvement of efficiency of selective actions. Studies, run by the State Forests in Poland, aimed at selecting the main forest tree species, integrated genetic aspects of wood structure. For conducting studies on heritability and genetic correlation of components of wood density of European larch (Larix decidua) we chose progeny of plus trees from a seed orchard. Such a choice was dictated by the requirements of analysing trees with a confirmed origin. The studies included eight families of European larch aged 25, however, in the age-age correlation analyses, the annual rings corresponding to agees 8-19 were also taken into account. This allowed for the observation of the relations between juvenile and mature wood and for the assessment of the possibility to predict the properties of mature wood based on the values observed in juvenile wood. This is particularly important in the case of European larch growing in plantation conditions characterized by a rapid growth rate. Components of wood density for each annual ring were determined using a X-ray densitometer. Analyses were preceded by the preparation of wood samples including the extraction of resins. Genetic correlation coefficients were estimated on the basis of the MANOVA option of SAS PROC GLM. For most wood density components, including the overall density of the annual ring, high correlation coefficients were observed (>0.8). These results were confirmed in previous studies conducted on other tree species (Fujimoto et al. 2006; Hannrup et al. 1998; Hannrup et al. 2001; Hyllen 1999; Kumar et al. 2002). The highest values of the age-age genetic correlation were observed between the age of 8 and 19, however, the high correlation coefficients were observed also for the later age. These results indicate the possibility of making efficient decisions on the selection of individuals after the first 10 years of growth to improve wood properties.

FAMILY REFORESTATION USING PORTFOLIO THEORY TO IMPROVE YIELD STABILITY AND REDUCE RISK IN BLACK SPRUCE

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Family forestry, here defined as the deployment of family mixtures into plantations, is becoming an attractive option for black spruce (*Picea mariana*) in New Brunswick. While there are many elite families of black spruce available, there is a knowledge gap regarding how to determine a mixture of families that optimally balances the objectives of increased yield and reduced risk. This study, based on real field test data, investigated the application of a model based on modern portfolio theory to optimally balance yield and risk when selecting a portfolio (mixture) of black spruce families to deploy in reforestation. The risk was expressed as the variance of the family portfolio, an effective indicator of yield stability. This approach was applied for the first time in forestry practice, and it is compared to the currently used method, truncation-deployment, i.e., the equal deployment of seed of selected families. Results showed that the portfolio maximizing yield at a given stability, or conversely minimizing instability at a given yield. By comparison, the new portfolio theory approach was never inferior in maximizing yield to the truncation-deployment approach when yield stability was the concern. While this study used portfolio theory to determine family portfolios for family forestry, the results may be applicable to other deployment strategies where stability is a concern, such as clonal forestry.

USING ACOUSTIC VELOCITY FOR GENETIC IMPROVEMENT OF MECHANICAL WOOD QUALITY IN BOREAL CONIFERS: AN EXAMPLE OF EARLY SCREENING IN EASTERN WHITE SPRUCE

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There is growing interest in using acoustic sensors for selection in tree breeding in order to ensure high wood quality of future plantations. We assessed acoustic velocity as a selection trait for the improvement of mechanical wood properties in two 15- and 30-year-old white spruce (*Picea glauca*) genetic trials in eastern Canada.

Previous studies, mostly conducted in fast-growing pine species such as radiata pine (*Pinus radiata*), loblolly pine (*P. taeda*), and slash pine (*P. elliottii*), identified strong correlations between acoustic velocity and mechanical wood traits. This led to the general conclusion that sound velocity can be used for cost-effective indirect selection of mechanical wood traits such as microfibril angle (MFA) and stiffness (modulus of elasticity, MOE). But at the start of this current study, it was not known how a combination of slow growth, small diameter stems, and a high incidence of live branches along the whole length of the stem would influence the accuracy of the velocity measurements and, consequently, the suitability of acoustic tools for use in breeding programs for slow-growing boreal conifers.

For the young trees, genetic analyses included 1,534 trees from 59 half-sib families from a genetic trial planted on two sites. Tree height and breast height diameter (DBH) were recorded for each tree. Acoustic velocity was measured in each stem and increment cores served to determine wood density, ring width, and MFA. Individual heritability of acoustic velocity was moderate (h²=0.38) and of the same magnitude as heritability of wood density (h²=0.32). However, genetic control of cellulose MFA was rather low (h²=0.22) and similar in magnitude to genetic control of early wood width and DBH (h²=0.22 and 0.23, respectively). As a result, considerable genetic gain was estimated for acoustic velocity and a measure combining velocity and wood density. The relationship between acoustic velocity improve MFA, which is one of the most important determinants of wood mechanical properties. Phenotypic and genotypic correlations between velocity and growth traits were low, which is in contrast to the moderate negative relationship between growth and other wood traits such as density (DBH and density $r_{\rm g}$ =-0.41, $r_{\rm p}$ =-0.40). Although low, the positive relationship between acoustic velocity and tree height ($r_{\rm g}$ =0.17) presents an interesting opportunity for the mutual improvement of both tree growth and wood quality.

On the phenotypic level, MFA was more strongly correlated to acoustic velocity in mature trees than in young trees ($R^2=0.54$ and $R^2=0.24$, fixed velocity effect only). The difference was probably related to the presence of reaction wood in the juvenile stem. The evolution of the link between both traits with tree age suggests that age-age correlations should be more closely monitored in future studies. Multivariate regression models showed that velocity was the most important trait for prediction of MFA or wood stiffness, which is in agreement with previous studies. The addition of other easily obtainable traits such as DBH, height-to-diameter ratio as well as wood density to velocity could slightly improve models of MFA at the young and the mature age. We concluded that juvenile acoustic velocity is an appropriate trait to indirectly select for wood quality in a tree breeding context.

NOVEL REMOTE SENSING PHENOTYPING PLATFORM AND GENOMIC SELECTION WILL BOOST THE DELIVERY OF GENETIC GAIN OF RADIATA PINE IN NEW ZEALAND

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Radiata pine (*Pinus radiata*) breeding has at least 60 years of history in New Zealand. The national program is based on phenotypic data collected through on-site assessment of genetic improvement trials. Two new initiatives will ensure a step-change in the speed of delivery of improved genotypes to the forest industry: the utilisation of remote-sensing to phenotype individual trees and the integration of genomic selection techniques.

Developments in the types and resolution of remote sensing technologies are creating opportunities for researchers. Satellite images can capture spectral information that can detect areas of poor forest health. LiDAR (Light Detection And Ranging) captures spatial information and is being rapidly adopted for developing spatial surfaces of forest height. These technologies have obvious applications in forest inventory systems and have the potential to be applied to phenotype radiata pine both in replicated field trials and in the forest itself. We plan to evaluate the application of data collected using remote sensing technologies to phenotype radiata pine in large-plot genetic trials and large-scale forests. In-forest data will be interrogated to determine the effectiveness of LiDAR and RapidEye relative to field-based measurements. The ability to identify outstanding individuals or groups of individuals (e.g., healthy trees in areas under stress) and apply mass selection in the forest estate will also be investigated.

Genomic selection and the utility of single nucleotide polymorphisms (SNPs) have tremendous potential for increasing the speed of delivery of genetic gain to the forest. In New Zealand, we believe that we will be able to halve the time (from 25-30 to 10-15 years) it takes to establish genetically improved trees in the forest by reducing the breeding cycle and facilitating the use of forward selection in the breeding program. Clonal breeding populations will be trained for key traits, and non-key traits and SNP assays developed for selection on seedlings, with the eventual likelihood of skipping the testing phase altogether. Early selection will finally be possible and traits that are expensive to measure can have assays developed at a fraction of the cost of conventional phenotyping (e.g., wood quality traits). Increased selection intensities are also possible, assuming sufficient diversity and SNP assays will be cost-effective.

These two programs will come together with the identification of outstanding phenotypes through remote sensing. In order to take advantage of these phenotypes, we intend to identify their parents using a SNP assay developed from the initial stages of a genomic selection program. Once good parents are known, they will inform further breeding and deployment. Final SNP assays for breeding traits will also be applied once available, again, to inform the breeding and deployment of radiata pine genotypes.

The SNP resource created for the development of genomic selection is being undertaken as a partnership between Scion and the Radiata Pine Breeding Company. SNPs will be extracted from DNA sequence data currently being generated to build the first genome sequence of radiata pine, in collaboration with the University of California, Davis and likely to include the University of Concepción, Chile.

These new directions will see an acceleration of delivery of genetic gain for radiata pine in New Zealand and an operational-level integration of molecular and quantitative genetics in the breeding cycle.

APPLICATIONS OF GENOMIC TOOLS

DISSECTING THE GENETIC COMPONENT OF INSECT RESISTANCE IN SPRUCE

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Here we aim to identify both DNA markers and patterns of gene expression that correspond to interior spruce trees (*Picea glauca* x engelmannii) that are resistant to insect pests, specifically from attacks by the white pine weevil (Pissodes strobi) and the western spruce budworm (Choristoneura occidentalis). We are using an association genetics approach to map insect-resistant phenotypes to DNA markers within the spruce genome, which has now been sequenced within the wider SMarTForests Project (http://www.smartforests.ca). The discovery population is located near Salmon Arm, BC and represents the F1 generation of the Prince George breeding population. These trees were derived from control crosses made between the top 144 parents that were selected based on growth. From this F1 population we have sampled one tree per cross (n=251), plus seven genotypes from control seedlots. To disentangle the multifaceted mechanisms of insect resistance in spruce, we performed phenotyping of monoterpenes and sesqiterpenes (using gas chromatography-mass spectrometry), diterpenes and phenolics (using high-performance liquid chromatography), histology (using tissue sectioning, staining, and light microscopy), as well as growth measurements and natural field attack by P. strobi. By sequencing the messenger RNA (using Illumina RNA-seq) of each individual in the population, differences in gene expression have been identified between trees with contrasting phenotypes, and these differentially expressed transcripts are good candidate genes underlying resistance phenotypes. Applying a "genotyping by sequencing" analysis to the same RNA-seq data, 331k Single Nucleotide Polymorphisms (SNPs) were identified, located within 23k genes. Association testing was performed in TASSEL 4.1.8 using all SNPs and target traits, both the General Linear Model (GLM) and the Mixed Linear Model (MLM) which corrected for kinship and population structure. Associations between the SNPs in several terpene synthase genes and the monoterpenes myrcene, 3-carene, as well as the sesquiterpene B-caryophyllene were presented. We also demonstrated how panels of SNP markers can be used to calculate Genomic Estimated Breeding Values (GEBV) that predict the target traits from the DNA sequence, opposed to traditional Breeding Values (BV) calculated from observed phenotypes.

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A GENOMICS APPROACH TOWARDS SELECTION FOR BIOTIC RESISTANCE IN WESTERN REDCEDAR (*Thuja plicata*)

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Thuja plicata silviculture is hampered by two quite different biotic stressors, deer browsing of planted seedlings render reforestation efforts inefficient and expensive, while fungal heartwood rot reduces yield at the time of harvesting. Resistance to both stressors has been linked to production of monoterpenoid compounds. High levels of thujone deter browsing of foliage, whereas high levels of tropolones, in particular thujaplicins, correlate with rot resistance in living trees. In addition, lignans contribute heavily to rot resistance of wood in service, which in turn is one of the main reasons behind the high price and popularity of lumber from this species. While selection for high foliar content of thujone is well under way, generational selection for individuals with high lignan and especially thujaplicin content in heartwood is not yet feasible due to the long time (10–20 years) it takes before the levels of these compounds can be scored and compared reliably. Thus, there is a need for early prediction of lignan and thujaplicin content, as well as simultaneous scoring with other traits such as thujone content and growth.

Recent advances in DNA sequencing technology now provide avenues for large-scale gene discovery as well as genotyping. In addition, Genome-Wide Association Studies and more recently, Genomic Selection technology has shown that breeding values for allele and allele combinations can be assessed in species with large and unknown genomes such as *T. plicata* and be applied directly in the selection of superior genotypes.

Towards this goal we are identifying large sets of genes that are expressed at sites and times of thujone, thujaplicin, and lignan biosynthesis. We have used a natural phenotypic variant that lacks foliar resin glands to identify, by Illumina tag profiling, greater than 600 genes whose expression is associated with foliar resin glands producing and storing thujones and other terpenoid compounds (Foster et al. 2013). In situ RNA hybridization showed that a putative monoterpene synthase-encoding gene is expressed in the epithelium of foliar resin glands. Furthermore, in vitro enzyme assays showed that the corresponding protein converts geranyl diphosphate into sabinene, a known precursor of thujone. The 600 genes also include candidate genes for the conversion of sabinene into thujone, as well as potential orthologs of proteins involved in the induction of biotic defences. We have also identified ray cells in the sapwood-heartwood transition zone that secrete hydrophobic, possibly resin and thujaplicin-containing, droplets into adjacent xylary parenchyma cells. The red color of these secretions also indicates that it contains plicatic acid. High concentration of this lignan correlates with decay resistance in lumber (Morris and Stirling, 2012). We have used Illumina RNA sequencing to identify greater than 7000 mRNA transcripts that are expressed in this transition zone. This large set contains transcripts with predicted open reading frames for putative terpene synthases and other enzymes that may be involved in thujaplicin biosynthesis, as well as dirigent proteins and other enzymes that may be involved in lignan biosynthesis. We are currently comparing this population of transcripts to those that we found were expressed in sapwood fractions that lack thujaplicin and lignans, to identify a subset of candidate genes.

Together, the foliar gland and heartwood-sapwood transition zone-specific populations of transcripts will provide the core of candidate genes for genotype-phenotype association studies. We have generated 36 transcriptome libraries from foliage of trees with a range of thujone levels that will be used for SNP and indel discovery, and are making similar plans for genes expressed in the sapwood-heartwood transition zone.

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SEQUENCE CAPTURE IN LODGEPOLE PINE AND INTERIOR SPRUCE

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Local adaptation is common in widespread conifer species and current reforestation policy reflects this through local seed sourcing and breeding programs. However, as climate changes, local tree populations may become maladapted to their environments. Our goal is to identify the genes responsible for climatic adaptation in western Canada's two most economically important conifers, lodgepole pine (Pinus contorta) and interior spruce (Picea glauca, P. engelmannnii, and their hybrids). As the genomes of these species are very large (>20Gb), we are using sequence capture methods to target our sequencing efforts to regions of interest. To identify these regions, we developed a *de novo* transcriptome for each species and conducted an RNAseq expression study. We retained a single isoform of each gene that was expressed in our RNAseq study, or had gene ontology terms potentially related to climate. We also included candidate adaptive loci identified in previous studies and loci currently being mapped in white spruce (Picea glauca) (Arborea project). To avoid reduced hybridization efficiency resulting from probes spanning intron/exon boundaries, we aligned the transcripts to the draft white spruce genome (SMarTForests Project) and the draft loblolly pine (Pinus taeda) genome (PineRefSeq Project) and identified these boundaries. We removed repetitive sequences as well as mitochondrial and chloroplast genes to avoid capturing sequences that have many copies within a single cell. We were able to identify 70,834 exons from 28,437 genes in pine (6,732 sequences with unidentified exon boundaries) and 75,799 exons from 35,957 genes in spruce (10,531 sequences with unidentified exon boundaries) for resequencing. We also identified non-coding, non-repetitive sequences from the draft genome of white spruce and from low-coverage whole genome shotgun sequence of lodgepole pine. These putatively neutral target regions will allow us to control for demographic history during our search for loci under selection. Our preliminary test of the NimbleGen sequence capture protocol was run on 12 interior spruce and 12 lodgepole pine. More than 90% of the target sequences had reads aligning to them and 40-60% of the reads were on target, demonstrating that the sequence capture protocol is enriching our libraries for the regions of interest. From these data we were able to identify ~600,000 SNPs in each species. These SNPs will be used for analyzing allele-environment and allele-phenotype associations. We will select \sim 25k of these SNPs and genotype a further \sim 5000 individuals per species. The objective of this project is to improve seed-transfer policy in response to climate change by comparing the adaptive genetic portfolio of seedlots from seed orchards and breeding programs to the climatic distribution and landscape genomics of natural populations.

IDENTIFICATION OF GENES INVOLVED IN STROBILI DEVELOPMENTAL REGULATION USING AN ABERRANT BISPORANGIATE SYSTEM OF *Pinus tabuliformis* Carr.

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Reproductive control is an important part of the genetic improvement and seed production process of conifer species that has accumulated a great deal of practical experience. However, there is still a considerable lack of molecular biology research on the development and regulation of conifer male and female cones. Conifer cones are typically of restricted growth and either exclusively pollen-bearing (male) or exclusively ovule-bearing (female). Male flowers are borne in clusters at the base of the shoot bud while the female flowers are borne as one conelet at the bud apex. However, several reports indicate that bisporangiate cones (also called bisexual cones or hermaphroditic strobili) occur in gymnosperms which possess both male and female structures, but the cause of this anomaly is not well understood. Chinese pine (Pinus tabuliformis Carr.) is a widespread indigenous species and an economically and ecologically important hard pine in northern China. Recurrent bisporangiate cones were detected on six trees in a seed orchard during a three year period. Based on the Roche 454 GS-FLX Titanium pyrosequencing platform, the first P. tabuliformis transcriptome from a normalised cDNA library of multiple tissues (xylem, phloem, vascular cambium, needles, cones, and strobili) was sequenced in a full 454 run, producing 911,302 sequencing reads. The high quality overlapping expressed sequence tags (ESTs) were assembled into 46,584 putative transcripts, and based on these information, microarrays were designed. Through the microarray analysis of large scale gene expression profiles between bisporangiate and normal cones, we found that the transcriptome of bisexual cones had undergone great remodeling compared with the normal male cones as well as the cones in adjacent clusters. Interestingly, the correlation analysis of gene expression between different cones showed that the transcriptome of male structures below the bisexual cones are more like female cones. However, male and female functions are not altered in bisporangiate structures. Based on a very strict cut-off criterion as P < 0.001 in the t tests and fold change (FC) ≥ 5 , 1059 differentially expressed genes were obtained. When a 50 fold change (FC) was used to filter our gene list, 157 genes were obtained; 30 of them were upregulated while the other 127 genes were downregulated. This result indicated that the feminization of male cones is primarily by suppressing the expression of specific genes. Three up-regulated genes and three down-regulated genes with unknown function were chosen from differentially expressed genes to analyze their expression pattern in male and female cones at the different developmental stages. The results showed that all these genes have a similar expression in female and male strobili at early developmental stages and have a relatively stable expression in female cones at different developmental stages, but were drastically changed in male cones at late developmental stages. These results indicate that the disorder of gene expression regulation at early developmental stages in male cones results in feminization and the ultimate formation of bisexual cones. These genes may play a crucial role in sex determination and developmental process of the male and female cones. This study gained insight on the transcriptome remodeling of aberrant bisporangiate cones, and more than one hundred candidate genes were identified that associated with sex determination and development of strobili. These data will facilitate future molecular biology studies on the development of male and female cones of *P. tabulaeformis* and other related species.

RESISTANCE OF SITKA SPRUCE AGAINST WHITE PINE WEEVIL: A GENOMIC AND HISTOLOGICAL APPROACH TO DECIPHER STONE CELL DEVELOPMENT

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The white pine weevil (WPW; *Pissodes strobi*) is a major pest of Sitka spruce (*Picea sitchensis*) and interior spruce (*P. glauca* x *engelmannii*) in British Columbia and also destroys Norway spruce (*P. abies*) plantations in eastern Canada. Breeding naturally resistant trees has proven to be the most effective control for WPW. High densities of cortical sclereids (stone cells) were found to have the strongest correlation with resistance to WPW among the traits examined in resistant Sitka spruce populations. Stone cells are highly lignified cells, which occur in clusters and are hypothesized to serve as a constitutive physical defense against WPW attack. However, the precise mechanism by which they affect WPW is not currently known, and the genes and mechanisms that control sclereid development are also unknown. Identification of genes, metabolites, and molecular processes involved in the formation of stone cells will lead to a better understanding of natural resistance mechanisms against WPW, and can support breeding for resistance. Building on the genomics resources developed in the Treenomix (http://www.treenomix.ca) and SMarTForests Project (http://www.smartforests.ca), we are using transcriptome and metabolite profiling of developing stone cells isolated via laser-microdissection (LMD), as well as fluorescence and electron microscopy to decipher the mechanisms governing stone cell development in WPW resistant spruce genotypes.

SELECTION OF GROWTH AND WOOD DENSITY IN RADIATA PINE USING A PANEL OF SINGLE NUCLEIC POLYMORPHISMS ASSOCIATED WITH CANDIDATE GENES

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Molecular technique advancement has made it possible to incorporate DNA variation into the design of breeding programs to increase the accuracy of selection. Genomic selection has been proposed as an approach to accelerate plant genetic improvement. It is especially valuable to reduce the generation interval in tree breeding. The accuracy of genomic selection heavily depends on the number of single nucleotide polymorphisms (SNPs) used in the development of prediction equations. But with the increase in the number of SNPs included in a genomic selection panel, genotyping cost can be a limiting factor. This may limit the application of genomic selection on a large scale. A SNP panel with a small number of SNPs with acceptable accuracy may be a solution. The objective of this analysis is to examine the possibility of developing an inexpensive SNP panel for marker-assisted selection of growth and wood density in radiata pine (*Pinus radiata*).

Sixty-seven SNPs from 47 genes were selected from a SNP discovery resequencing program. Two breeding populations of the Radiata Pine Breeding Company, one control-pollinated progeny trial, a clonal trial, and their parents were successfully genotyped with these SNPs. Fifty-one SNPs remained after removing SNPs with minor allele frequency less than 0.05. The progeny trial had 1,849 individuals from 44 parents and was planted at three sites. The clonal trial had 520 individual genotypes from 63 parents planted at two sites with ten clones per family and six ramets per clone planted in six replicates on each site.

Breeding values of diameter-at-breast-height (DBH) and wood density (WD) were estimated separately within each trial series using an individual tree linear mixed model, implemented with ASREML (Gilmour et al. 2009). Effects for site and replicate were fitted as fixed effects and sets-within-replicate effects, additive genetic effects, and residual effects were fitted as random effects in the statistical model for both the progeny trial and the clonal trial. Clonal effects were also fitted for the clonal trial. The heritabilities of DBH and WD were 0.15 and 0.52 in the progeny trial and 0.25 and 0.22 in the clonal trial, respectively.

In this analysis the clonal population was used as the calibration population and the progeny population as the validation population. The estimated breeding values (EBVs) were deregressed using reliability and the amount of information available from relatives (Garrick et al. 2009). The prediction equations of the 51 SNPs were developed with Bayesian ridge regression (Pérez et al. 2010) using the deregressed EBVs of the calibration population. Marker-assisted EBVs (MEBVs) were calculated as the summation of the products of the prediction equations and their genotypic values. The accuracy of MEBVs, which was equal to the correlation between deregressed EBVs and marker-assisted EBVs, ranged from 0.60 to 0.66 in the calibration population and ranged from 0.36 to 0.46 in the validation population. The percentage of genetic variation explained by the panel in the validation population ranged from 13% to 21% for DBH and WD. One application of this SNP panel is to conduct pre-selection in the nursery. Seedlings can be screened at large scale in the nursery to select the best individuals for planting in the field.

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GENOMICS-ASSISTED SELECTION FOR GROWTH AND WOOD TRAITS IN WHITE SPRUCE COULD BE A REALITY IN THE NEAR FUTURE

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Spruces are the most important forest resource for the Canadian forest industry and their natural ranges extend across Canada. With sizeable gains having been obtained for growth, spruce breeders are now putting more emphasis on wood quality. Given the time delays and high costs for evaluating these traits, genomics-assisted selection is expected to contribute positively to increasing gains per unit of time. To test the potential of genomic prediction for wood and growth traits in white spruce (*Picea glauca*), a discovery population of about 1,700 white spruce trees has been assembled from a 17-year-old progeny test replicated on two sites and comprising 59 full-sib families belonging to two unrelated sublines of a breeding population. Increment cores were collected and wood density and microfibril angle (MFA) were assessed for each tree. Tree height and DBH were also analyzed. A genotyping assay with one SNP per gene locus yielded genotypes for 6,900 genes segregating in the population.

First, SNP-by-SNP association tests were carried out using a mixed-linear model approach and indicated that between 500 and 700 SNPs were significantly associated ($P \le 0.05$) with single traits related to wood quality or growth before correction for false-discovery rate. Next, genomic selection models for growth and wood traits were built using either all available SNPs or only those that were significant at $P \le 0.05$ after association tests. The predictive value of the models was estimated by cross-validation with within and between sublines sampling and assuming same site or different sites. The accuracy of genomic prediction for within-subline selection varied from 0.67 to 0.84 depending of the trait when considering all SNPs, and from 0.61 to 0.78 when using only the subset of significant SNPs. When predicting genomic values across sublines, the accuracy of prediction of genomic values was much lower, except when using only the significant SNPs (for instance 0.58 and 0.49 for wood density and MFA, respectively). These results suggest that significant gene SNPs cover a major part of the quantitative trait loci involved and that shared ancestry might be present between sublines. When cross-validation was made across test sites, the accuracy estimates were also in the same range, reflecting the small genotype-by-environment interactions usually seen with eastern white spruce. Gains per unit of time were also estimated, and their amplitude suggests that economically useful gains could be obtained if selection delays are reduced. For instance, when assuming a breeding cycle of 30 years for the traditional approach versus 10 years when using genomic selection, everything else being equal, the gains per unit of time for a selection intensity of 5% obtained from genomic selection where 1.7 to 2.6 times higher that those from traditional selection. These positive results indicate that genomics-assisted selection could likely be successfully implemented for white spruce.

MINING SPRUCE GENOMES FOR DEFENCE GENES AND FUNCTIONS

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Draft genome sequence assemblies of two spruce species, white spruce (Picea glauca) (Birol et al. 2013. Bioinformatics 29:1492–1497) and Norway spruce (P. abies) (Nystedt et al. 2013. Nature 497:579–584), have recently been reported. Birol et al. reported innovative strategies for building sequence assemblies of a very large (~ 20 gigabase) conifer genome sequences and provided insights into the tree's gene space. Biological insights from the Norway spruce genome, for example insights into the nature of large introns, repeat elements, interpretation of processes that contributed to the very large genome size, and comparison with low coverage genome sequences of other gymnosperm plants are reported by Nystedt et al. Both studies used the Sitka spruce (P. sitchensis) full length cDNA reference data set (Ralph et al. 2008 BMC Genomics 9:484) to assess the quality and completeness of gene predictions, with conservative estimates suggesting the number of functional protein coding spruce genes to be in the order of 30,000 to 60,000. A summary of the status and challenges of the spruce genome assemblies and initial genome annotations was presented. Other presentations by members of the SMarTForests Project (e.g., see presentations by Drs. Beaulieu, Bousquet, Sexton, and Whitehill) addressed aspects of the integration of the genome sequence with genetic mapping as well as application of spruce genomics tools in spruce tree breeding. This presentation added to the former presentations information from the identification of genes and gene functions involved in spruce resistance against spruce budworm (Choristoneura occidentalis) or white pine weevil (Pissodes strobi). This included the characterization of diterpene oleoresin defenses as well as a newly discovered glucosyl hydrolase gene function that is important for spruce budworm resistance.

ACKNOWLEDGEMENT

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POPCAN: LINKING TRAIT VARIATION WITH WHOLE GENOME VARIATION IN *Populus* TO FACILITATE ACCELERATED TREE IMPROVEMENT

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Black cottonwood (Populus trichocarpa) and balsam poplar (P. balsamifera) are two closely related Populus species with large north-south and east-west ranges in western and northern North America. P. trichocarpa and P. balsamifera, along with their hybrids, are well suited for high yielding biomass plantations. We are investigating the reservoirs of natural genotypic and phenotypic variation in these species using range-wide collections assembled by the BC Ministry of Forests, Lands and Natural Resource Operations and Agriculture and Agri-food Canada's Agroforestry Development Centre. The primary aim is to identify allelic variants that could be used for accelerated domestication of poplars including their potential use as bioenergy feedstock. We carried out extensive population-wide transcriptome and whole genome re-sequencing of P. trichocarpa individuals to identify SNPs that were used to develop a 34K SNP genotyping array covering 3,500 candidate genes, and SNP genotypes were obtained for 450 trees grown in common gardens. Next, in order to reveal the full extent of allelic variation across the genome, we re-sequenced the genomes of approximately 300 accessions of each species from across their ranges. In parallel, the same genotypes were subjected to extensive phenotyping for a suite of wood chemistry and ultrastructure traits and for multiple traits related to growth, photosynthesis, phenology, foliar chemistry, and pathogen susceptibility. These large phenotype and genotype datasets have allowed us to assess population structure, identify candidate genes related to adaptation, and to use association genetics to identify sets of SNP markers with potential utility in marker-assisted breeding and genomic selection for population improvement.

PROTECTING CANADA'S FORESTS: THE TAIGA PROJECT

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Forest health is under continuous threat from attacks by exotic invasive pathogens, apart from a combination of climate change and anthropogenic mismanagement. A pro-active strategy of disease prevention can help avoid new outbreaks and ensure continued forest health. This approach for forest disease prevention must involve early detection and identification of the causal organism, followed by mitigation measures and pathogen surveillance on local, regional, national, and global scales. Additionally, identifying the infection source is essential for discovering the dissemination pathways and preventing further introductions of potential emerging diseases. Canada's regulatory system requires that all imported plant material and wood products be inspected. Plant health monitoring and pathogen detection is widely based on visual inspections. A significant limitation is a particularly high rate of false negatives, as asymptomatic plants with latent infections cannot be detected. Our Genome Canada funded TAIGA (Tree Aggressor Identification using Genomics Approaches) project proposes to address many of these challenges in pathogen detection and identification by harnessing the power of genomics. A new generation of genomics-enhanced pathogen tools will be developed and deployed to detect and monitor different classes of pathogens. In addition to a robust and specific detection of known pathogens, our method will allow for the discovery of lesser known and latent fungal pathogens. An inbuilt redundancy mechanism in the assays will enable a higher confidence in the test results. With a minimal turn-around time for establishing a pathogen profile, the TAIGA project will serve a valuable role in keeping Canadian forests healthy.

GENETIC CONSERVATION

GENETIC IMPACTS OF FOREST MANAGEMENT PRACTICES IN SOUTHEAST ASIAN FORESTS

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The major threat to genetic viability of the Southeast Asian forest species is commercial logging and fragmentation to a lesser extent. Species' vulnerability to the threat of genetic viability posed by commercial logging is highly correlated with its abundance in a particular forest management unit. Tree density for the species can be a useful indicator in reflecting the risk of genetic viability rather than the overall disturbance level based on a reduction in basal area of all trees. Mating and gene flow patterns tend to be similar in species with similar ecological characteristics. Therefore, information on the mating system, gene flow, and inbreeding depression relating to major species could be relevant to closely related species, and used to determine the minimum number of mother trees to be retained for successful breeding. Knowledge of the biological attributes of species including the main pollinators, flowering phenology, and synchrony of individual species can be used to develop field guides for harvesting operations to ensure genetic quality of residuals left behind in the post-harvest stands. There is a general lack of understanding on structure of genetic variation and on the evolution of adaptability of the affected populations. Though genetic diversity of a number of tropical tree species has been determined, the critical levels of the genetic parameters that render a population more susceptible to extinction are known for a few species only. DNA-based systems for the analysis of gene expression for the understanding of gene expression in different tissues and in response to biotic and abiotic stresses have not been deployed in genetic viability studies of tropical forest species. Genetic mapping of quantitative trait loci will enable rapid assessment of ecosystem integrity for sustainable management of ecosystems.

GENETIC IMPACTS OF FOREST MANAGEMENT PRACTICES IN NORTH AMERICAN BOREAL AND TEMPERATE FORESTS

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Genetic diversity is the basis of all biodiversity because it provides the raw material for survival, adaptation, and evolution of all organisms, especially under changed environment, climate, and disease conditions. Conservation of genetic diversity in forest trees is especially critical for the stability and functioning of forest ecosystems because forest trees are normally the keystone species of many ecosystems, and many faunal and floral associations depend on their existence. Therefore, genetic diversity of forest trees could be viewed as the foundation of ecological and economic forest sustainability and ecosystem stability. Forest management practices based on partial and clearcuts followed by natural or artificial regeneration systems can impact genetic diversity, population structure, mating system, gene flow, and effective population size. Thus, adaptation, fitness, survival, and productivity of forest trees may be adversely affected. Therefore, knowledge of the genetic impacts of forest management practices is crucial for the conservation and management of forest genetic resources.

North America has about 17% of the world's forest resources as boreal and temperate forests. The boreal forest region is the largest of all forest regions in North America extending from Alaska to Newfoundland. Boreal forests are usually managed by clearcut harvesting followed by natural and/or artificial regeneration. Forest trees in other North American temperate forest regions are managed by both clearcut and partial-cut harvesting followed by natural and/or artificial regeneration systems. Several forms of partial-cut systems, such as high-grading, shelterwood, seed tree, patch cut, and group selection are implemented. Of these, shelterwood and seed tree methods have been commonly used. The harvesting and tree retention intensities in partial-cut systems vary from species to species and region to region.

Synthesis of the results and conclusions from studies conducted on genetic impacts of forest management practices on boreal and temperate forest trees of North America were discussed. The extent of genetic impacts depends upon the population characteristics and reproductive biology of the species and the harvesting and reforestation practices used. For example, early successional, widely distributed conifer species with serotinous or semiserotinous cones and a good seed bank on the ground, such as black spruce (*Picea mariana*) and lodepole pine (*Pinus contorta*), will likely have very little or no genetic effects of forest harvesting and reforestation practices. On the other hand, late successional or species that do not regenerate well after clearcuts or do not have a good bank of viable seeds on the ground, such as white spruce (Picea glauca) and eastern white pine (Pinus strobus), can have significant genetic impacts of forest harvesting and reforestation practices. Therefore, the genetic impacts of forest management practices cannot be generalized to all boreal and temperate conifer species. This is evident from the mixed patterns observed so far. Silvicultural practices should take the population and reproductive biology and silvics of a species into account to ensure that the genetic diversity and structure and evolutionary processes are maintained in forest populations. The predominantly outcrossing mating system, severe inbreeding depression, and long distance pollen dispersal in North American conifers can help buffer the negative genetic impacts of harvesting, such as bottleneck, genetic drift, and inbreeding. More than one molecular marker type should be used for assessing genetic impacts of forest management practices as different markers may provide different results. Allelic diversity measures are more suitable than expected heterozygosity (He) in assessing the genetic impacts of forest management practices because He is not very sensitive to bottlenecks and perturbations in populations.

HIGH GENETIC DIVERSITY OF A DEMOGRAPHICALLY VULNERABLE RAINFOREST TREE IN AN URBANIZED LANDSCAPE

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Over the last 150 years, Singapore's primary forest has been reduced to less than 0.2% of its previous area, resulting in extinctions of native flora and fauna and leading to an expectation of genetic erosion in primary forest species. We surveyed greater than 95% of the remaining primary forest in Singapore and used eight highly polymorphic microsatellite loci to assess genetic diversity indices of 179 adults (>30 cm dbh), 193 saplings (>1 yr), and 1,822 seedlings (<1 yr) of the canopy tree Koompassia malaccensis (Fabaceae). We tested the hypotheses that 1) the K. malaccensis population underwent a genetic bottleneck and 2) recruits may exhibit genetic erosion and inbreeding. Contrary to expectations, we detected neither a population bottleneck nor a reduction in effective population size and high genetic diversity in all three age classes. Genetic diversity indices among age classes were not significantly different; we detected overall high heterozygosity (mean He > 0.850), high allelic richness (mean R = 19.7), a large proportion of rare alleles (30.1%), and a low Inbreeding Co-efficient (mean FIS = 0.068). However, long-lived species such as trees might need several generations for detrimental genetic effects of deforestation and/or fragmentation to accumulate. The spatial genetic structure (SGS) showed higher relatedness of saplings and seedlings compared to the adult cohort, as well as increasing SGS intensity in younger cohorts. In addition, demographic factors for this population (i.e., <200 adult K. malaccensis trees) are a cause for concern, as rare alleles could easily be lost due to stochastic factors. The high outcrossing rate (calculated from seedlings) (tm = 0.924) may be an underlying factor for maintenance of genetic diversity and supports the hypothesis that effective pollination may increase resilience to acute habitat loss.

PONDEROSA PINE EVOLUTIONARY HISTORY AND GENETIC VARIATION: RESULTS FROM RANGE-WIDE MITOCHONDRIAL AND NUCLEAR MARKER STUDIES

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Ponderosa pine (Pinus ponderosa) is the most broadly distributed pine species of the Western Hemisphere, where it has considerable ecological and economic importance. It exhibits complicated patterns of morphological and genetic variation, suggesting it may be in the early stages of differentiation into multiple species. These patterns have created confusion about evolutionary relationships within the ponderosa pine complex, while the near absence of paleoecological data for the species during the Pleistocene obscures important phylogeographic processes that have influenced the evolutionary history of the complex. To clarify ponderosa pine evolutionary history and phylogeography, we amplified the highly polymorphic mitochondrial DNA second intron minisatellite region in 3,100 trees representing 104 populations across the range of the species. We estimated population-level haplotype diversity and determined diversity partitioning among varieties, races, and populations. After sequencing all length variants and aligning sequences of minisatellite repeat motifs, we evaluated evolutionary relationships among haplotypes. The results suggest a complex phylogeographic history not revealed by other genetic and morphological data, or by the sparse paleoecological record. The geographical structuring of the 10 haplotypes corresponded with division between Pacific and Rocky Mountain varieties. Pacific haplotypes clustered with high bootstrap support, and appear to have descended from Rocky Mountain haplotypes. These results appear consistent with long-term divergence between the Pacific and Rocky Mountain varieties, along with more recent divergences not well-associated with race. Pleistocene refugia may have existed in areas of high haplotype diversity (the southern Sierra Nevada, northwestern California, and southern Nevada), as well as in the Great Basin, the Southwestern United States/northern Mexico, and the High Plains. Separately, we used six nuclear microsatellite markers and 19 allozymes to assess patterns of genetic variation across the range of ponderosa pine. Preliminary results show low levels of inbreeding within populations and moderate differentiation among populations. When individual trees are separated based on mitochondrial DNA haplotypes, those in the core of the species range have somewhat higher nuclear marker genetic variation. The results of both the mtDNA and nuclear marker studies should assist in management decision-making and conservation planning relating to ponderosa pine subordinate taxa and populations in the face of multiple threats, including those associated with climate change. For

example, each haplotype may represent an evolutionarily distinct unit that may respond differently to climate change because of differences in adaptation to climatic conditions. Measures such as seed archiving, silvicultural treatments, and prescribed fires should take into consideration the presence of rare haplotypes or nuclear alleles and of high haplotype or nuclear diversity.

ADAPTATION AND CLIMATE CHANGE

THE ROLE OF SYMBIOTIC ASSOCIATION BETWEEN MYCORRHIZAL FUNGI (AMF) AND WILLOWS ON COPPER UPTAKE AND STORAGE: A HYDRAULIC PERSPECTIVE

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Concern is emerging worldwide related to the management and restoration of natural and industrial sites affected by the accumulation of complex mixtures of trace-metals, and toxic inorganic and organic compounds through mining, agricultural, and industrial activities. Soil and water remediation using plants and their associated microbes has become a lower cost, versatile, and non-invasive alternative for moderated decontamination of those sites, in which shrubs of the genus Salix (willows) are widely used. In the case of plants growing under trace-metal polluted soils, different plant mechanisms have been described for overcoming stress when metals are uptaken and accumulated in plant tissues, including the production of intra-cellular metal-binding compounds and their storage into the vacuole or apoplast. In willows growing in copper (Cu) polluted soils, accumulation of Cu has been observed in various tissues, with roots accumulating the most. In order for plants to maintain their cellular homeostasis while accumulating trace-metals, changes in their water relations must occur, which should have an impact on their Aquaporin (AQP) activity. In this study, we investigated the effect of symbiotic associations between mycorrhizal fungi (AMF) on the uptake and storage of Cu and on hydraulic traits and AQP expression patterns in roots of Salix purpurea (Fish creek) saplings. Rooted saplings that were either inoculated or not with Rhizophagus irregularis were grown under three Cu treatments: no-Cu (Control), low (66.7 ppm of free Cu), and moderate concentrations of Cu (156.78 ppm of free Cu). We found that root hydraulic conductance (Lp) of small roots was gradually reduced in plants exposed to Cu when compared to controls in non-innoculated plants. In contrast, plants inoculated with the fungus showed a steady Lp within the different levels of Cu, suggesting that the symbiotic relationship might be beneficial for maintaining root homeostasis during trace-metal stress. Lp was reduced when exposed to an AQP inhibitor in all cases (up to 92%), showing a physiological role of AQPs in root hydraulics. A phylogenetic analysis of functional Salix AQPs was conducted, comparing them with their closest related heterologous genes from *Populus trichocarpa* for further classification within the subfamilies of the Major Intrinsic Proteins. In addition, AQPs belonging to two subfamilies, Plasma Membrane Intrinsic Proteins (PIPs) and Tonoplast Intrinsic Proteins (TIPs), were transcriptionally profiled in roots to identify AQPs candidates involved in homeostasis balance in Salix during copper accumulation.

GENOMIC ARCHITECTURE OF APICAL BUD FORMATION IN Picea glauca (Moench) Voss: AN eQTL MAPPING APPROACH

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Apical bud formation is an important adaptive trait that permits conifer trees to synchronize their phenology to seasonal changes and may determine the fitness of individuals under variable climatic conditions. Recently, 4,500 genes that were differentially expressed during bud formation were identified by comparing gene expression profiles of unrelated individuals of white spruce (*Picea glauca*) (Kayal et al. 2011). Among these genes, 108 were differentially expressed exclusively in developing buds and were expressed at higher levels in buds than in other tissues (Kayal et al. 2011). Several of these genes fell within robust QTL clusters obtained for bud burst and bud set across pedigrees, years, and environments (Pelgas et al. 2011).

We undertook an eOTL (expression quantitative loci) mapping approach that integrates expression profiling experiments and our most recent genetic maps to gain a deeper understanding on how genomic architecture influences bud phenology and related regulatory pathways. First, we isolated total RNA from terminal spruce shoots for two biological replicates at two developmental stages: 1) first sign of bud formation and 2) midpoint of bud development. Samples were collected from 145 full-sib progeny that were a subset of cross C94-1-2516 (Pavy et al. 2012; Pelgas et al. 2011). Transcript levels were quantified using the spruce large-scale oligonucleotide microarray which contained 25,045 oligonucleotide probes representing 23,853 unique genes (Raherison et al. 2012). An analysis of variance of the normalized expression data identified 10,985 gene-probes with significantly different expression levels between the two developmental stages (P \leq 0.05). In parallel, we genotyped 1,800 full-sib progeny for 16,720 single nucleotide polymorphisms (SNPs, Arborea PgLM3 array) with an Illumina Infinium bead array and assembled these data into two parental genetic maps (Pelgas et al. in preparation). We are currently using these genetic maps to screen the expression profiling data of a subset of the mapped gene-probes from the spruce oligonucleotide microarray (8,839 of the 25,045 gene-probes), to identify eQTL using a composite interval mapping approach (Michaelson et al. 2009). Ultimately, we hope to use this integrated QTL mapping approach to identify genes and/or network of genes directly (cis-regulatory genes) or indirectly (trans-regulatory genes) involved in bud set.

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UTILIZING THE INTRA-SPECIFIC VARIATION IN GROWTH TO DEVELOP MANAGEMENT GUIDELINES FOR DOUGLAS-FIR UNDER CLIMATE CHANGE: A PROPOSED STUDY IN AUSTRIA AND BAVARIA

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Climate change poses serious challenges to sustainable management of forests throughout the world. Planting of alternative and non-native tree species or different provenances better adapted or having a higher potential for adaptation to future climate conditions has been discussed as an important silvicultural measure to adapt forests to climate change. Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is considered as one of the most promising species in Austria and elsewhere because it exhibits superior productivity, high wood quality, and low risk for storm damage. Particularly, it grows well under warm and dry conditions where currently native conifers such as *Picea abies* are considered vulnerable to an array of insect and disease organisms. Douglas-fir is known to have a wide natural range and grows under varied environmental conditions. Therefore a detailed study on provenance-specific growth performance in response to climate is crucial. In the present study, we will utilize 63 Douglas-fir provenance trials throughout Austria and more than 20 trials from Bavaria, Germany to understand intraspecific variation in growth in response to climate.

The aims of the project are: 1) to understand the interaction of intra specific genetic variation and climate on growth of Douglas-fir, 2) to identify the most important climatic factors that pose a risk for growth of Douglas-fir in the study area, and 3) to integrate Douglas-fir provenances into the dynamic forest ecosystem model PICUS in order to assess forest management strategies for different sites and provenances.

The methods include: 1) multivariate analyses to determine climate-related growth and stress factors that constrain the growth of Douglas-fir in the study area, 2) developing provenance-specific transfer and

response functions and ultimately combining these two functions into a Universal response function, and 3) parameterization and evaluation of the forest ecosystem model PICUS (e.g., Lexer and Hönninger 2001; Seidl et al. 2005) with several Douglas-fir provenances in order to simulate and assess forest management strategies for different sites and provenances.

Preliminary analysis from 33 provenance trials in summer-dry eastern regions of Austria reveals strong correlations of provenance-specific growth of diameter at breast height to climate of the site where introduced. The expected project outputs (e.g., suitability maps, guidelines, etc.) will help to define management guidelines and provenance recommendations for Douglas-fir as an alternative conifer species in Austria.

GENETIC RESOURCE MANAGEMENT AND CLIMATE CHANGE: GROWING HEALTHY FORESTS FOR THE FUTURE

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Providing seed for operational reforestation and restoration has long been the principal focus of the U.S. Forest Service's Genetic Resource Management Program. Although this work will continue into the future, climate change predictions will require changes in the ways these needs are met.

The guiding principle for managing the genetic resources of National Forests has been through the use of local seed sources in reforestation and restoration. The advent of a rapidly changing climate, however, means that a new paradigm will be required to maintain healthy and productive vegetation in National Forests and to preserve at-risk species and populations. At a minimum, the current practice of relying on seed sources that were best suited to the past climate will need to shift to allow consideration of the source, or sources, of seed that will be best suited to predict future climates. In many cases, species and seed sources that may be optimal under climate change scenarios have not received adequate research or management attention and thus lack basic genetic information as well as sufficient representation in forest seed banks. More aggressive gene conservation programs, especially *ex situ* seed collection, will also be needed for species and populations most vulnerable to climate change impacts.

At present, there is no generally applicable national guidance for incorporating climate change impacts into the management of National Forest genetic resources. In the spring of 2010, Forest Service and university geneticists convened to share background information and develop a consensus for revising National Forests System genetic resource management guidelines. The goals of the meeting were to: 1) provide information on climate change scenarios and potential effects on vegetation and forest genetic resources, 2) facilitate the interaction and exchange between climate scientists and geneticists to develop strategies for responding to climate change in Forest Service genetic resource management programs, 3) identify genetic options for responding to climate change and its effects on vegetation and genetic resources, with an emphasis on the next 5-10 years, and 4) identify gaps related to information, resources, research, and tools needed to manage effectively within a changing climate. A whitepaper on key genetic issues, guidelines, and program gaps/needs in light of climate change has been written and published.

INFERRING PARENTAL GENETIC EFFECTS AND GENETIC CONTROL OF TREMBLING ASPEN (*Populus tremuloides* Michx.) FOR GROWTH AND ADAPTIVE TRAITS IN A HYBRID POPULATION UNDER A WARMING CLIMATE

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Although parental genetic effects and their temporal variation in plants have been recognized for a long time, their ecological and evolutionary roles remain unclear for forest tree species in terms of early growth, and natural and artificial selection responses under rapidly changing climates. Recent studies underscore an accelerating mortality of *Populus tremuloides* natural stands that are widely-distributed in North America with high potentials in biomass and biofuel production as well as various ecological values such as carbon sequestration and animal shelter.

Here, we evaluated the maternal and paternal additive genetic effects of *Populus tremuloides* in growth and adaptive traits based on progeny trials with factorial mating designs, within which ten common garden trials were established with 58 female and 64 male half-sib families as well as 100 full-sib families. Ratios of additive genetic variances for both parental sources were compared within sites and the age effects of these ratios were also investigated. General combining ability, specific combining ability, narrow-sense heritability, and genetic correlations of these traits were estimated for single site and combined age groups of trees.

We found a discernible decreasing trend of inferred maternal genetic effects on tree height and fall senescence by ages while the ratios of paternal effects were maintained or rose. The maternal genetic effect of spring bud break had an opposite increasing trend during developmental stages. The varying weights of the maternal effect were revealed by the proportions of parental variances in multiple cohorts. Within the same cohort, site effect was significant for tree height and adaptive traits. The dominance genetic effect decreased when trees grew older. Also high additive genetic correlation existed between height growth and fall adaptive trait.

The diminishing trend of inferred maternal genetic effect during juvenile tree growth had important values for predicting tree performance under natural and artificial selection. For such a hybrid population, decreasing maternal genetic effect combing with pollen flow from the southern population would potentially increase the risk of frost damage because of delayed senescence timing and extended growing season.

PREDICTING THE RISK OF CEDAR LEAF BLIGHT (Didymascella thujina) IN BRITISH COLUMBIA UNDER FUTURE CLIMATE CHANGE

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Cedar leaf blight (*Didymascella thujina*) is considered to be the most important disease of western redcedar (*Thuja plicata*) in British Columbia. The disease is most prevalent in warm-moist coastal low-elevation environments causing mortality among seedlings and significant loss of incremental growth and branch death among mature trees. In this study we used a principle component regression model to spatially project the disease risk under observed climate (2003 to 2008) and multiple future climate scenarios for the 2020s, 2050s, and 2080s. We found that while the majority of future climate scenarios predicted coastal environments will continue to favour occurrence of the disease, intensity is predicted to decrease towards the 2080s. Projected reductions of available summer climate moisture (cumulative precipitation – potential evapotranspiration), corresponding to the time of ascospore discharge and germination, contribute significantly to this finding. Disease intensity is, however, projected to increase under moderate temperature and precipitation increases for the 2020s. We therefore recommend current reforestation efforts deploy disease resistant western redcedar seedlots in high risk environments common to hypermaritime coastal regions such as Haida Gwaii and northern Maritime, to avoid significant mortality and growth reduction.
COMMON GARDEN EXPERIMENTS IN CONTRASTING CLIMATES REVEAL ADAPTIVE VARIATION IN GROWTH, PHENOLOGY, AND PHYSIOLOGY OF Alnus rubra

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To explore adaptive variation and genotype x environment interactions in red alder (*Alnus rubra*), we examined the pattern and range of variation in growth, phenological, and physiological traits among 59 families of red alder. We related this variation to the climates of family origin to identify the climatic variables associated with adaptive variation. Red alder families from six regions in coastal BC were grown in common garden experiments at two sites with contrasting climates. We found significant variation among regions in growth, canopy cover, cold hardiness, and nitrogen nutrition of red alder families. Differences in continentality and available moisture of the climate of origin explained most of the among-family variation in phenology and cold hardiness, whereas temperature and growing season length were associated with among-family differences in cold hardiness and growth. Red alder families from the north coast had earlier bud burst at the southern test site, and less autumn canopy cover and higher nitrogen fixation rates at both test sites. A trade-off between growth and cold hardiness was clearly evident. Families vary in the degree of phenotypic plasticity however, most families are relatively tightly adapted to their climate of origin and may perform sub-optimally in contrasting climates.

RISK AND BENEFIT ANALYSIS OF INTRA-SPECIFIC ASSISTED MIGRATION OF WHITE SPRUCE (*Picea glauca* [Moench] Voss) IN ONTARIO

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The 410-series of white spruce (Picea glauca) range-wide provenance tests, cooperatively established by the Canadian Forest Service and the Ontario Ministry of Natural Resources in the 1970s - 1980s, provided a good source of information for assessing risk and benefit associated with intra-specific assisted migration (ISAM) with the species. The 410-series of experiments included 245 range-wide provenances of white spruce at 16 Ontario field sites, representing the species' growing environments in Ontario. Trial measurement data at ages 19 - 28 years (in 2001) were analyzed for adaptation (indicated by tree survival) and growth potential (height, DBH, and volume). Results indicated that while most white spruce provenances survived reasonably well across the 16 Ontario sites, those performing better were usually from milder environments (i.e., lower latitude), especially those from south-central Ontario and southern Quebec. Southern provenances were more responsive to improving thermo-conditions of planting sites and were significantly superior to northern provenances at warmer sites. Southern provenances performed better or equivalent to local provenances at northern Ontario sites under the climate of the past 30 - 40 years. In light of climate change, ISAM seemed to be a good strategy to enhance genetic diversity and climatic adaptation of white spruce in northern Ontario, which may bring about genetic gain in growth equivalent to that achievable from Ontario's first-generation tree improvement programs with boreal conifer species. Future work is to re-measure the 410-series trials and determine the safe distances of ISAM by incorporating data from both long-term provenance trials and short-term farm field tests with the assistance of climate-based software like Focal Point Seed Zones.

TOWARD MORE RESILIENT FORESTS AND ECOSYSTEMS: BC MFLNRO FOREST STEWARDSHIP ACTION PLAN FOR CLIMATE CHANGE ADAPTATION

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Adapting BC's forest practices to a changing climate is both an immediate imperative and long term proposition that must begin now. In February 2012, the BC Ministry of Forests, Lands and Natural Resource Operations released a Forest Stewardship Action Plan for Climate Change Adaptation. This Action Plan signals an important new policy direction for climate change adaptation within the sphere of forest management in British Columbia. It identifies goals, objectives, and initial actions the Ministry will take between now and 2017 to adapt BC's forest management framework to a changing climate. This is the first step in a broader adaptation strategy for the natural resource sector that will grow and evolve as we move forward.

The Forest Stewardship Action Plan aims to reduce risks, capture opportunities, and improve the ability of BC forests to withstand, recover from, and adapt to the full amplitude of climate change and variability. The plan outlines actions underway and planned to adjust BC's existing forest management framework to foster resilient forests and improve the sustainability of BC forests, communities, and industries.

The Ministry's Climate Change Adaptation Team sought input from a broad array of partners, including e.g., industry, government, and community forest managers. The most promising opportunities were identified through four regional workshops in Creston, Kamloops, Nanaimo, and Prince George.

Actions were identified under three goals: 1) Foster Resilient Forests, 2) Maintain Future Options and Benefits, and 3) Build Adaptive Capacity. Actions to foster resilient forests will also enhance other values such as water quality and wildlife. For more information, visit Adaptation Action Plan (http://www.for.gov.bc.ca/het/climate/index.htm) and the new BC MFLNRO Adaptation Knowledge and Tools website (https://www.for.gov.bc.ca/het/climate/knowledge/index.htm).

Executive Sponsors: Dave Peterson (BC MFLNRO A/Deputy Minister, Chief Forester) and Tom Ethier (BC MFLNRO ADM, Resource Stewardship Division).

Climate Change Steering Committee: Tom Ethier (chair), Rory Annett, Brian Barber (former), and Normand Bilodeau (CAS rep); Alec Dale and Glen Davidson (TOPFND Rep); Paul Knowles, Lynn Kriwoken, Allan Lidstone, Madeline Maley, Leslie McAuley, Diane Nicholls, Albert Nussbaum (former), and Jim Sutherland (former) (IROD Rep); Andrew Wilson.

Climate Change Adaptation Team: Kathy Hopkins (chair); Frank Barber and Rob Bowden (former); Tim Ebata, Christine Fletcher, and Rein Kahlke (former); Leslie McAuley, Katharine McCallion, Deb MacKillop, Don Morgan, James Sandland, Dave Spittlehouse, Kristine Weese, and Ralph Winter.

TREE RING ANALYSIS INDICATES GENETIC COMPONENT IN DROUGHT RESPONSE OF DOUGLAS-FIR

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Douglas-fir (*Pseudotsuga menziesii*) is among the most important economical and ecological conifer species in western North America. These functions may, however, be jeopardized by recent and projected warming. The expected increase in the frequency and severity of drought events may exceed the adaptive capacity of local populations. To mitigate the negative effects of an expected increase in drought, the transfer of tolerant seed sources has been proposed. We used a dendro-ecological approach to identify drought tolerant genotypes using samples from a provenance trial in British Columbia.

The study site, located approximately 60 km east of Vancouver, was subjected to a severe drought event in the summer of 1985. We selected two provenances from British Columbia, one provenance from Washington, and two provenances from Oregon according to latitudinal, altitudinal, and summer drought gradients. A total of 304 increment cores from 76 trees were sampled from a completely randomized block design with four replications. As a measure of drought tolerance, we used growth depression and subsequent post-drought recovery as well as correlation coefficients of annual growth to summer temperature and precipitation.

The drought event of 1985 caused reductions in increment ranging between 40 and 60%, as compared to a two year pre-drought reference period. Counterintuitively, we found that a cold, high-elevation provenance from Washington was least affected by this drought event. The second least affected provenance was a source from southern Oregon, which also showed the fastest post-drought recovery. A provenance from the wet area of northern Vancouver Island and a provenance from northern Oregon were the most highly affected, as indicated by reduced increments for several years. These results were mirrored by the climate-growth correlations. The southern Oregon and the high elevation source from Washington exhibited lower correlations with summer precipitation and temperature. In contrast, the provenances from British Columbia and the source from northern Oregon showed moderate correlations with these two variables.

We could not fully verify that provenances from the southern coastal range of Douglas-fir are more drought tolerant than provenances from the north, as we have found a northern Oregon provenance strongly affected by drought and a high elevation seed source from Washington with superior drought tolerance. Both drought-tolerant provenances were among the below-average performers in the studied trial, with the southern Oregon source being the least productive. The provenance from Washington might, however, represent a practical compromise between risk avoidance and productivity.

A PROPOSAL TO EXPLORE RESPONSES OF DOUGLAS-FIR POPULATIONS TO CURRENT AND FUTURE CLIMATES USING DATA FROM PROVENANCE TESTS ESTABLISHED WORLDWIDE

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Douglas-fir (Pseudotsuga menziesii) is a valuable tree species that is grown in temperate regions around the world. A large number of provenance tests have been established worldwide with the primary objective of identifying those provenances that do well in a particular region. With increased interest in climate change, however, researchers are revisiting questions of appropriate provenances and are beginning to take a fresh look at provenance test data to explore the relationship between population performance and climate. With this in mind, we began gathering Douglas-fir provenance test data from around the world with the goal of developing response functions to predict the performance of populations given information about the climate of seed sources and planting locations. Response functions will be used to predict productivity of Douglas-fir stands in current and future climates, and to evaluate management options for maintaining or enhancing productivity by choosing populations appropriate to future climates. Beginning in 2010 we began by putting together a list of people and organizations that might currently be responsible for Douglas-fir provenance tests, particularly those established in the 1970s as part of the collections undertaken and organized by IUFRO beginning in 1967, and used the list to solicit data. To date we have data from provenance tests established in the United States, Canada, Belgium, France, Germany, Netherlands, and Spain. Much of the data collected so far have been incorporated into the Center for Forest Provenance Data, a centralized, web-based data management and information system designed to archive and make available data from provenance tests with the hope of encouraging collaboration among researchers for evaluating the data in new ways.

IS THERE A RELATIONSHIP BETWEEN CLIMATE AND SOIL FACTORS AND GENETIC STRUCTURE IN DIFFERENT TREE SPECIES ON THE SIERRA MADRE OCCIDENTAL, MEXICO?

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Climate and soil factors are likely to impose strong directional selection in many plant populations, which must therefore adapt if they are to survive. Within populations, microgeographic genetic differentiation of individuals with respect to climate and soil suggests that populations may adapt to certain types of climate and soil through changes in gene structure. Such adaptation may also apply to tree species in the structurally rich Sierra Madre Occidental, Mexico. In this study, several populations of Picea chihuahuana Martínez, Pinus durangensis M., Pinus cooperi Blanco, Pinus engelmannii Carr., and Pinus ayacahuite Ehrenb. ex Schltdl. in Durango State, Mexico were examined with regard to adaptive genetic differentiation, which was presumably caused by different altitude, temperature, aridity, and concentrations of essential elements in the topsoil. Needle samples were analyzed using modified AFLP technology. Outlier loci were detected using the genotype differentiation *ä* among populations and its components, D_i , and permutation tests considering false discovery rates. The covariation C was used to measure how the relative frequency of the recessive genotypes was related to altitude, temperature, aridity, and soil element concentrations. On the basis of the results obtained, we concluded that an evolutionary response to climate and soil factors is detectable in the studied populations, and also that interactions between soil factors and genetic structure are of potential interest for future research related to forest trees.

EVOLUTIONARY HISTORY AND SPECIATION OF *Pinus armandii* AND ITS CHINESE RELATIVES: INFLUENCES OF THE UPLIFT OF QINGHAI-TIBETAN PLATEAU AND QUATERNARY CLIMATE CHANGES ON THE HIGH PLANT SPECIES DIVERSITY OF CHINA

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The Tertiary geological event and Quaternary climate change are two major drivers of speciation and evolution. In this study, we used Pinus armandii and ten other Chinese white pines (subsection Strobus) as a system to explore the relative influences of uplift of the Qinghai-Tibetan Plateau (QTP) and Quaternary climate change on the exceptionally high plant diversity in the south of China. We conducted a chloroplast and mitochondrial DNA survey of these species with range-wide population sampling of P. armandii, an ecologically and economically important species with the broadest geographical distribution in Central and Southwest China. Four geographically and genetically distinct subdivisions were detected in P. armandii, i.e., Qin-Daba Mountains (QDM), Hengduan Mountains (HDM), Yungui Plateau (YGP), and Taiwan. In contrast to the strongly structured cpDNA of P. armandii, the mtDNA genetic structure was surprisingly much lower at the range-wide scale. Coupled with the distribution patterns of the chlorotypes and mitotypes, we inferred two eastward migratory routes during the glacial periods, one along the Qing Ling-Daba Mountains in the north and the other along the Nanling Mountains in the south, probably extending as far as East China and Taiwan. Repeated range shifts during the Quaternary would promote the speciation of the white pines in Eastern (P. dabeshanensis) and Southern China (P. fenzeliana, P. kwantungensis, P. wangii), and Taiwan (P. armandii var. masteriana, P. morrisonicola). The recent divergence time was consistent with their genetic similarity and close phylogenetic relationship to P. armandii. Chloroplast introgression would occur among the Taiwanese white pines (P. pumila and P. sibirica) as well as the HDM populations of P. armandii and P. bhutanica. Molecular dating estimates showed that Asian white pines originated in the early Miocene (~20.04 Ma), but the initial diversification took place during the late Miocene (7.41 Ma), suggesting the important role of the rapid uplift of the QTP in the allopatric speciation of Asian white pines. Within P. armandii, the divergence of the north and south lineage was estimated to the Pliocene. Divergence time partly explained the contrasts in the strong intraspecific variation between the two lineages of P. armandii and low interspecific differentiation among P. armandii and Southern and Eastern China white pines. Our study suggested that the uplift of the QTP and Pleistocene climate change played a complex role at different spatial and temporal scales in the speciation and diversification of Asian white pines.

EXOTIC PLANTATIONS OF FOREST TREES IN BRITISH COLUMBIA: A VALUABLE SOURCE OF INFORMATION FOR ASSISTED MIGRATION

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Assisted migration of tree species and genotypes has been suggested as a method for climate change adaptation in forestry. To realize and implement this kind of strategy on larger scales, information from migration trials is needed for all stages of tree growth, from seed germination and seedlings to mature trees. Regarding mature trees, provenance trials including exotic species and genotypes were established throughout Canada during the last century, in various efforts searching for improved productivity, pest resistance, etc. There is a potential to 'repurpose' these kinds of plantation experiments for studying climate adaptation and potential implications for assisted migration. We studied a series of 'XP' exotic plantations established by the Canadian Forest Service (CFS) in British Columbia starting in the 1930s, in many cases using stock originating from provenance experiments started in the late 19th Century that were eventually incorporated into CFS experiment stations. We assessed migration distances versus tree survival for 146 provenances (56 endemic and exotic species) that were migrated within North America to 55 plantation sites. Genera included Abies, Acer, Betula, Chamaeocyparis, Fraxinus, Juglans, Larix, Liriodendron, Picea, Pinus, Populus, Prunus, Pseudotsuga, Robinia, Quercus, Salix, Sequoia, Thuja, and Ulmus. For the overall dataset, linear regression analysis only showed weak trends. There was a nearly significant reduction in survival vs. increasing latitudinal migration distance (P=0.056), but the trend only explained a minor part of the variance ($R^2 = 0.0251$) and there were no significant effects versus total (P = 0.1112) or longitudinal (P = 0.1884) migration distance. More northerly migration tracks (mean migration latitude) generally had increased survival (P = 0.0486), but again the trend only explained a very small amount of the variation ($R^2 = 0.0267$). For particular genera, Quercus spp. (Q. macrocarpa and Q. rubra) collectively showed decreasing survival with increasing total (P = 0.0041, R² = 0.83) and longitudinal (P = 0.0009, $R^2 = 0.90$) migration distance. Robinia pseudoacacia also showed decreased survival with total migration distance (P = 0.0009, $R^2 = 0.90$), while Pinus spp. (P. banksiana, P. contorta, P. echinata, P. engelmannii, P. flexilis, P. jeffrevi, P. monticola, P. ponderosa, P. pungens, P. radiata, P. resinosa, P. strobus, P. sylvestris, and P. taeda x rigida) collectively showed a nearly significant, slight (P = 0.0592, R² = 0.08) reduction in survival with increasing longitudinal migration distance. Regarding the distribution of tree survival results, there was a spike in mortality at 7-8 years after planting, nearly double that of other age categories. When the distribution of mean migration latitudes was compared in trees with less than 9 years of survival versus those with greater survival, no clear patterns emerged; trees with poor survival were often represented at shorter migration distances, while trees with good survival were often represented at the greatest migration distances. These results indicate that trees may generally have a highly variable tolerance to assisted migration that only partly depends on migration distances. More extensive studies incorporating other long-term field data and differences in climates at sources and destinations are needed before we can determine how these trends apply to assisted migration of particular species and genotypes.

CASE STUDY: IMPACT OF CLIMATE CHANGE ON INDUSTRIALLY RELEVANT WOOD TRAITS DETERMINED WITH SilviScanTM

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Global climate is expected to change further with intensifying weather extremes due to an increase in greenhouse gas concentration. Climate change affects not only tree growth and survival (Andalo et al. 1999, Rehfeldt et al. 1999, Reich and Oleksyn 2008), but also wood quality (Ceulemans et al. 2002). It is well-known that wood quality traits are strongly influenced by growing conditions including climate conditions. There is a great deal of information about the response of tree growth and survival to climate change; however the impact of climate change on wood quality traits is not well studied. Given the importance of wood quality and resulting product performance to the economic values of our forests, it is imperative to understand how wood quality responds to climate change. Advanced technologies such as SilviScan are now available for research into impacts of climate change on industrially relevant wood traits.

SilviScan rapidly and cost-effectively determines multiple wood quality traits on the same increment core or wood disc sample. It offers high-resolution pith-to-bark measurements of fibre dimensions, wood density, ring width, and microfibril angle, from which modulus of elasticity, cell wall thickness, fibre coarseness, and other industrially relevant wood traits can be determined. Compared to conventional densitometry, SilviScan technology integrates growth-ring orientation information and automated sample-stage rotation. This provides precise and distinct densitometric and diffraction measurements from early-wood and late-wood fibres within a sample, resulting in sharp definition of growth-ring boundaries.

In this case study, transfer models were developed to establish the relationship of wood quality traits with transfer distances for 17 annual climate variables. The wood quality traits were measured with SilviScan on wood discs from 3 planting sites and 21 provenances in Quebec. Quadratic regression analyses showed that variation in wood quality traits between provenances is large, reflecting the significant genetic control of wood traits. The study also revealed a significant impact of climate change on wood quality traits, even with the restricted range of climate values and small number of provenances available in the study. For example, total annual precipitation alone accounted for 18% of the variation in fibre coarseness, one of the key fibre attributes impacting pulp and paper product performance.

In summary, SilviScan efficiently measures wood quality traits that are of crucial importance to the forest sector and is a valuable tool for providing measurements for various aspects of forest genetics, genomics, and climate change related research.

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BREEDING PACIFIC NORTHWEST CONIFERS

WESTERN HEMLOCK FOREST GENETICS PROGRAM FOR BRITISH COLUMBIA

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Western hemlock (*Tsuga heterophylla*) is the most common species on the British Columbia (BC) coast. It represents approximately 30% of the annual volume harvested and estimated to be about 60% of the timber inventory for the Maritime Region. As a consequence of its potential, tree improvement activities commenced early with parent tree selections starting in the late 1950's. The first progeny trials were established in the early 1970s and a provincial breeding program was launched in the second half of that decade under Mike Meagher. With the recession of the early 1980s and consequential budget restrictions, activities were restricted to maintenance of existing sites and provenance seed collections were placed in the hands of the Douglas-fir (*Pseudotsuga menziesii*) breeder, Jack Woods. The hemlock program revived with the economy, as forest industry put weight behind their investment in the species requesting, in the words of Western Forest Product's John Barker, "that if they were to have seed orchards they should be tested". John King was engaged as breeder, and because few parent trees were tested to that point, set out with others, to develop the Hemlock Tree Improvement Co-operative (HEMTIC) in the 1990s. Its course is well described by Jayawickrama and Ye in their description of endeavors in the US Pacific Northwest.

For hemlock in BC, unstable economics of the new millennium combined with the fecundity of the species led to reliance on natural regeneration. Planting numbers have fallen from a high of about nine million a year in the early 1990s to as little as 10% of that recently. As a consequence, it was decided by the guiding committee for tree improvement for the province, the Forest Genetics Council of BC, that the program should be put on hold. This has meant once again activities are limited to securing long term research installations and finishing planned measurements of field trials.

The current program has several facets. Gene conservation status has been assessed and as expected for a prolific, invasive, shade tolerant species, risk of loss of genetic variation is relatively low. For genecology research there is a broad network of almost 40 field installations covering most of the environments in which hemlock occurs in BC. Measurements to age 10 years are fairly complete for the trials and reporting on features of the genetic architecture and adaptation is planned for the near future. Preliminary analysis suggests a latitudinal cline of decreasing growth capacity from South to North. With elevation, at least for coastal seed sources, there appears to be a step cline with a fairly abrupt differentiation of low and high altitude genotypes. Longitudinal trends within the coast, transition, and interior regions are not detectable, but movement of seed from one of these regions to another does not have positive outcomes.

For investigation of growth and yield of improved genotypes versus wild type plants, three series (nine test sites) were established. These tests confirm gain estimates from single tree progeny trial plots by area based 0.1 ha plots. As well, six of the trials have varied planting densities in order to predict effects from competition. Results to date confirm expectations for gain. In addition, comparison of wild stand progeny to controlled-cross orchard mean lots suggests inbreeding depression results in a loss of about 5% in early height growth.

Breeding to identify genotypes superior in desired traits ceased almost a decade ago, but progeny tests established then are just now yielding reliable data. Poly-cross (Px) trials of high elevation parents age 12 years are due for measurement in 2013, with new breeding values subsequently. Similarly, measurements

of polycrosses of backwards selections from the HEMTIC program have been carried out recently, with final rankings due to be tabled in 2013. Forward selections from HEMTIC second-generation testing have been ranked and the best 120 genotypes grafted for inclusion in clonebanks, but planned Px trials to confirm the breeding values will not be carried out. Also in 2013, tests of best BC parent trees not ranked in time to be included in the HEMTIC second-generation will have a final measurement. Unlike the earlier second-generation trials, the parents were cloned to allow for more effective within family selection.

Prospects for the future of the hemlock forest genetics program are likely limited. Despite two year delays for natural regeneration versus planting, inbreeding depression, patchiness, clear evidence that local seed is surpassed by more southerly sources, and increasing genetic gains in seed orchards, the method is inexpensive and often fairly successful. Perhaps declining rotation lengths or rising genetic worth of seed, either from our orchards or those of the Pacific Northwest, will lead to resurgence of this program once again.

OPERATIONAL DEPLOYMENT TRIALS OF FOLIAR TERPENE ENHANCED WESTERN REDCEDAR (*Thuja plicata*) TO DETER UNGULATE BROWSE DAMAGE

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Western redcedar (Thuja plicata) is an ecologically and economically important tree species in coastal British Columbia. Post-harvest establishment of western redcedar seedlings in areas that are densely populated with mule deer (Odocoileus hemionus) is an operational challenge because the seedlings are preferentially browsed by ungulates. Browse damage is most severe early in the season of seedling establishment. Existing strategies to mitigate seedling growth losses in newly established forests include barrier installations, chemical repellant applications, planting with large stock, enhanced fertilization regimes, late season plantings, and obstacle plantings. These applications can be costly and marginally effective. Previous research trials show that ungulate browse preference is negatively correlated to increased foliar monoterpene concentrations, particularly the monoterpenoid á-thujone. Testing of western redcedar planting stock with enhanced monoterpenes commenced in six operational reforestation trials established by Western Forest Products on Vancouver Island in spring 2013. The objective of this study was to assess ungulate browse of western redcedar A-Class seedlots having low to average terpene concentrations compared to trial seedlings and rooted cuttings that were produced from pedigreed seed and selected for mid and high foliar terpene concentrations. Each of the six replicates consisted of two minimum 5 ha areas that have similar aspect, slope, mature and immature adjacent stands, accessibility, and predicted mule deer browse pressure. Seed orchard seedling stock was randomly mixed with mid-level seedlings and high-level terpene clones in each respective area. Terpene enhanced stock was marked, and 50 m² plots were staked and numbered. At least two months after establishment, seedlings and cuttings at each plot will be assessed for extent of ungulate browse. We hypothesize that mid-level and high-level terpene stocks will be browsed less than A-Class orchard stock. Further operational studies are needed to determine the long term effects of enhanced terpene stocks on browse resistance. Browse measurements will be taken annually to monitor the operational deployment trials.

PROGENY TESTING, ORCHARD SEED PRODUCTION AND DEPLOYMENT OF NOBLE FIR IN OREGON AND WASHINGTON

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INTRODUCTION

Noble fir (*Abies procera*) is a conifer species native to western Oregon and Washington, and northern California. While definitely one of the less important timber species compared to the dominant coastal Douglas-fir (*Pseudotsuga menziesii*), it has some interesting features that have led to tree improvement work in the Pacific Northwest (PNW): 1) it is an important component of higher elevation stands and, with its short stout branches, is adapted to handling frequent wet snows, 2) forest practices rules require successful reforestation of harvested land and natural seed crops are unreliable, 3) lumber from this species forms part of the "white wood" component of PNW timber production, 4) foliage is harvested in an economically important bough production industry, and 5) Noble fir Christmas trees are highly valued (Landgren et al. 2003) and generate about US \$80 million in annual sales from these two states. Yearly, planting in Oregon since 2004 averages 3.2 million seedlings (NASS 2011).

In addition to lower log values compared with coastal Douglas-fir, stem cracking is an unfortunate phenomenon which affects lumber recovery and reduces deployment of this species for timber production. It is widely believed, though not conclusively proven, that severe or unseasonal cold is the primary factor. Low moisture in late summer has also been suggested as a cause.

FIRST-GENERATION TESTING

Lipow et al. (2002) reported that 1,460 parents were put into tests between 1981 and 1987. We present one example. The Bureau of Land Management (BLM) selected 195 first-generation parents in two Oregon testing programs, breeding units 50 (Cascades, 11 test sites) and 51 (Coast Range, four test sites).

It was unusual for first-generation programs in PNW conifer species to have extensive cross-testing between the coast range and the Cascades. Test sites were established in 1981 and 1987, between 897 and 1,270 m elevation. Total height (HT, cm) was assessed at ages 5, 10, and 15 years. Diameter at breast height (DBH, mm), number of forks, and number of ramicorns were also assessed on each tree at the latest measurement. A total of 19,736 trees (14,724 from BU50 and 5,012 tfrom BU51) was used for analysis. Individual-tree heritabilities for HT10, HT15, DBH15 and VOL15 were 0.28 ± 0.03 , 0.34 ± 0.04 , 0.26 ± 0.03 , and 0.26 ± 0.03 , respectively. Type B genetic correlations among sites were relatively high (0.74~0.82), indicating good stability of families despite sites being located both in the Coast Range and the Cascades. Parents originating from the northwest and higher elevation had slightly higher predicted gain, but this trend was weak (r < 0.3). Little stem cracking was seen in the progeny tests, but grafted parents of those progeny showed cracking in a seed orchard.

Christmas tree growers have made their own seed collections and tests (Brown 1988, Landgren and Bays 2011). Progeny tests established for the Christmas tree program have also included a number of families from the BLM's program, with focuses on BU51. These tests are usually small and established on grower farms, operate on a Christmas tree rotation, and important selection criteria include rapid growth and bud development, foliage traits, and resistance to current season needle necrosis. Trees are evaluated for insect problems. Christmas tree growers have strong preferences for seed from certain provenances (Brown 1988, Landgren and Bays 2011). Currently there are approximately 250 parents in test programs (Landgren unpublished data).

SEED PRODUCTION AND DEPLOYMENT

Seed orchards have been established by the US Forest Service (USFS), BLM, Weyerhaeuser Company, the PNW Christmas Tree Growers Association, and Cascade Timber Consulting (CTC). The USFS established the largest extent of orchards (65.8 ha) (Lipow et al. 2002), but these are no longer managed because of greatly reduced regeneration harvests on the National Forests. The BLM manages two mature Noble fir orchard blocks (established in 1973 and 1978) at their Horning seed orchard complex.

Noble Fir grafts easily and does not need specialized rootstock, although initial growth of the grafted ramets is slow. After 15–20 years sizable orchard crops are produced about every three or four years. Response to stimulation is inconsistent (Landgren et al. 2008). Mature orchards can produce a lot of seed: the BLM orchards produced 309 kg in 2012 from 5.2 ha (873 kg between 2000 and 2008 even without collecting all the cones). Trees eventually get too large for cost-effective cone collection, leading to re-establishment of new orchards.

Noble fir seed are large (18,700-20,900 per kg) and germination at the outset is moderately high (85%). However, the seed is easily damaged during cone processing and germination begins to drop significantly after 5–6 years. This means that orchards have to be maintained over long periods since long-term seed storage is not viable. Recent developments in seed processing and stratification, and upgrading seed lots with low germination potential may allow nurseries to make better use of low germination seedlots.

Most forest growers in Oregon and Washington with high-elevation land plant some Noble fir. For example on units 760 m elevation and above Oregon Department of Forestry plants 20–30% Noble fir, using 1-0 container stock, while CTC plants about 30% above 910 m. Expected rotations are in the order of 50–60 years.

FUTURE WORK

There are no plans to move forward to advanced-generation breeding and testing. The main unresolved questions are the cause and prevention (if possible) of stem cracking and a reliable seed stimulation procedure.

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BREEDING WESTERN WHITE PINE AND PONDEROSA PINE IN BRITISH COLUMBIA: DISEASE RESISTANCE AND ANTICIPATED RANGE EXPANSION

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The main objective for the western white pine (Pinus monticola) breeding program in British Columbia (BC) has been resistance to white pine blister rust (Cronartium ribicola). Results from provenance testing revealed little population level variation within the species, however, enough variation was observed between coastal (west of the Coastal Mountains) and interior (east of the Coastal Mountains) populations so that two seed zones and breeding populations were established. The program began with the selection of disease-free parent trees from stands that had been exposed to blister rust. Seed was collected from parent trees and seedlings were inoculated with blister rust at the Cowichan Lake Research Station. Surviving seedlings were monitored for several years then selected and grafted into seed orchards for seed production. The approach taken in the two breeding programs began to diverge. The interior program supplemented local BC selections with 50 trees selected from the Idaho, USA breeding program. The Idaho trees tested very well in BC and formed a strong foundation for the seed production program. Crossing was conducted among the 50 Idaho clones and progeny were screened for disease resistance in inoculation experiments. Surviving seedlings were monitored for several years before being selected for the seed orchard and breeding population. The coastal program began controlled crossing among local parent trees and established several field trials to screen for disease resistance. These field trials are now a source of resistant material for seed orchards. A final series of F1 field trials will be established across both seed zones with material from both breeding populations. The coastal program will continue to rely on the F1 field trials for information and material for seed orchards and the interior program will begin an F1 breeding and field testing program. There are currently one coastal and three interior seed orchards that supply nearly 100% of the demand for white pine seed.

Ponderosa pine (*Pinus ponderosa*) will become a more ecologically important species as the climate changes. Many areas of the province are expected to become hotter and drier and will become climatically more appropriate for ponderosa pine. There is no formal breeding program for ponderosa pine, however, provenance testing has led to the selection of progeny from elite provenances and the establishment of a provenance based seed orchard. We are attempting to stimulate flower production in a mature provenance test in order to collect seed that will form the basis of a formal progeny testing program. This material will be supplemented by wild-stand collections and parent tree selections. A formal progeny testing program will allow us to better understand genotype x environment interactions and family variation, and calculate accurate breeding values for our orchard and breeding programs.

GENETIC CONSERVATION

CONSERVATION GENETICS OF BAKER CYPRESS (Cupressus bakerii)

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Cupressus bakerii (Baker cypress or Modoc cypress) is found in appoximately twelve isolated stands in northern California and southwestern Oregon. It has serotinous cones and reproduction is facilitated by fire. Many of the existing stands are declining because fire suppression has allowed other conifer species to encroach into these stands. This species was also rated highly vulnerable to the potential effects of climate change in a vulnerability assessment for southwestern Oregon. To-date no information has been available on the level of genetic diversity or population structure. We used 12 polymorphic isozyme loci to assess genetic diversity and population structure of eight geographically separate populations. Genetic diversity (H_o) was generally high (>0.15) for most populations (7 of 8) but most populations also exhibited a deficiency of heterozygotes (F>0.09) (6 of 8). Over all sites, approximately 17% of the genetic variation was among sites ($F_{st} = 0.171$), although this parameter is likely inflated by the northernmost site (Flounce Rock), which also had the lowest diversity. Implications of these results for manangement of the species were discussed.

FRAGMENTATION OF A POPULATION AND ITS EFFECT ON EFFECTIVE POPULATION SIZE FOR GENETIC CONSERVATION OF *Taxus cuspidata* IN MT. SOBAEK, SOUTH KOREA

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Japanese yew (Taxus cuspidata var. cuspidate) is an endangered species on subalpine mountains and a keystone species at the protected area of forest genetic resources at Mt. Sobaek, South Korea. The yews in this region had grown vigorously until the 1960s, but were reduced in number during the 1970s to early 1980s due to illegal logging, stealing for ornamental trees, and damage from excess numbers of climbers. The yew forests were designated as a protected area of forest genetic resources in the 1990s. However, the yew forests have a problem due to lack of seedlings or saplings and poorly growing adults. The region can be divided into three management units according to their legal status: the core zone (BR), the buffer zone (YW1), and two remnant sites (YW2 and KM). They also were geographically fragmented, especially for the remnant sites. The total number of individual yews in the 1980s was reported as 1,999 on 15 ha at BR, 1,633 on 119 ha at YW1, 90 on 20 ha at YW2, and 218 on 110 ha at KM, respectively, by the local management office. We collected needles from 111, 59, 23, and 31 trees per site, respectively, according to their relative densities, for genetic analysis using a nuclear microsatellite marker system. A total of 57 alleles of 8 nSSR loci were acquired from 224 samples. The observed and the expected heterozygosity were 0.269 and 0.532 respectively, and the population was under Hardy-Weinberg disequilibrium. The average number of alleles was 5.3. Considering the four sites, the private alleles were found in KM with a high frequency of 0.5. In Bayesian clustering, four sites showed quite different genetic compositions so the proportions of individuals reallocated to the clusters were not similar between the sites. The existence of four sites could explain 9% of the total genetic variation in the population from AMOVA. The effective population size, estimated from individual genotypes based on linkage disequilibrium, was 32.6 with a range of 28.0 to 37.9. When the sites were considered separately, effective population size was 41.5 at BR, 60.2 at YW1, 28.4 at YW2, and 41.5 at KM. Genetic patch size from spatial autocorrelation was detected as 20 m at BR and 10 m at YW1. At the remnant forests, YW2 and KM, there was no spatial genetic patchiness. The differences in the spatial genetic patterns in Mt. Sobaek might be the result of environmental conditions. We have to consider not only the core or the buffer zone but also the remnant forests when developing a conservation strategy for the yews at Mt. Sobeak.

PRESERVATION STANDS AS A FORM OF PROTECTION OF FOREST GENE RESOURCES IN POLAND

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The protection of forest gene resources has gained significance in recent decades. All around the world, numerous programs to preserve the most valuable populations of native species have been initiated. Autochthonicity of a population becomes the determinant of its genetic value. At the same time, diverse methods of forest management and the stand composition prevent the formulation of a uniform definition of old stands. At present, overmature stands cover an area of 277,522 ha (as of January 1, 2008), which represents 4% of the total forest area in Poland. This proportion is likely to increase in the coming years. The major part (60%) is covered by pine stands. The share of seed stands in relation to the entire area of protected forest stands reaches 42%. The selected stands are not subject to logging when fulfilling their basic function, i.e., to provide a valuable source of seed. These stands are also, according to current knowledge, the best populations in terms of phenotype and probably also in terms of genes. However, doubts appear more and more often as to the native character of these stands.

When choosing preservation stands one of the main selection criteria is the age of the population which enables the determination of a native character of the population. For coniferous species the minimum stand age was determined to be 150 years. For deciduous species, for which seeds have not been traded in the past, the age was determined to be 200 years. The continuous presence of a species within the area results in the formation of species best adapted to local habitat and climate. Preservation stands, which are the remnants of natural populations and which are adapted to local conditions through centuries of selection, are most valuable for the protection of forest gene resources. There are, however, no studies on the recognition of the breeding value and the genetic structure of such populations.

Results of studies on the variability of traits in seeds and seedlings of selected preservation populations of Scots pine (*Pinus sylvestris*) presented in this work serve for the first and only attempt to determine the level of variability in those stands at the anatomical and morphological level. A crucial element of the presented study is the analysis of seed and seedling traits in relation to the age of the parental stands and the verification of a generally accepted approach about the decrease of seed life and of the quality of seedlings along with age.

A PRELIMINARY EVALUATION OF GENETIC VARIATION OF *Litsea cubeba* (Lours.) Pers. IN NORTHERN THAILAND

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Litsea cubeba (Ta krai ton) occurs widely within 700–1,600 m above sea level in the lower montane coniferous forest in the northern part of Thailand. It produces a fruit that is processed for its lemony essential oil. According to a survey, two varieties of *L. cubeba* were found in Chiangmai province namely *L. cubeba* var. *cubeba* and var. *formosana*. However, in Phitsanulok, *L. cubeba* var. *cubeba* was only found. For utilization, volatile oil can be developed as a mosquito (*Aedes aegipti*) repellent effective for 6.33 hours. Due to the high demand for agricultural land, the forest in northern Thailand has been degraded and deforested which has resulted in habitat loss and has reduced the gene pool of this species. Therefore, it is important to investigate the status of genetic resources of this species.

Preliminary results showed that three out of seventeen ISSR (Inter Simple Sequence Repeat) primers can be used to evaluate genetic diversity of these two varieties. Forty ISSR loci were identified and used to evaluate genetic diversity in nine natural populations of *L. cubeba* in northern Thailand. One hundred and five samples from nine populations from two provenances, Chiang Mai (8) and Phitsanulok (1), were evaluated. The results showed that Nei's gene diversity (H_e) was 0.24 and the Shannon Index (I) was 0.38 whereas G_{st} was 0.34. The UPGMA dendrogram based on Nei's genetic distance illustrated that the nine populations were divided into two groups. Based on these results, the need for futher study and the conservation management of this species was discussed.

ASSESSING RANGE-WIDE GENETIC VARIATION AND STRUCTURE IN SUBALPINE LARCH (Larix lyallii)

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Subalpine larch (*Larix lyallii*) populations are already restricted to a relatively small geographical and altitudinal range. Changing climate could further reduce available habitat, either by increasing levels of late-summer desiccation or by encouraging the upward migration of more competitive timberline species. Recent studies have also suggested that subalpine larch is poorly equipped to adapt to changes in its environment. In the northern part of its range, *L. lyallii* is genetically depauperate, most likely due to the combined effects of recent bottleneck events and reproductive isolation among spatially discontinuous populations. To examine genetic diversity in this species, we are collecting foliage samples from populations distributed throughout the species' natural range. Variation in the maternally inherited nuclear genome will be assessed. By evaluating levels and patterns of genetic diversity in *L. lyallii* we hope to identify genetically unique populations which may be of particular importance for planning future management and conservation efforts.

RELATIONSHIP BETWEEN TREE SPECIES DIVERSITY AND GENETIC STRUCTURE IN *Picea chihuahuana* **Martinez**

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Genetic structure is the distribution of the genetic variation determined by diverse factors such as mutation rate, genetic derivatives, natural selection, and gene flow. Genetic diversity and species diversity are two basic components of biodiversity. Populations evolve within a community context and the diversity of the species in the community can influence the genetic structure of a population via selection. However, until now, the selective effect of the diversity of species within a community on the genetic structure in the population or individual species has not been explored. The objective of the present study was therefore to identify any relationships between tree species and genetic structure in Picea chihuahuana Martinez. With this aim, 14 populations of P. chihuahuana located in the Sierra Madre Occidental (states of Chihuahua and Durango, Mexico) were analyzed. Within each population, data from a permanent field plot covering an area of one quarter ha (50 x 50 m) were used to measure the diversity of tree species. Needles of P. chihuahuana were collected (50 samples per population) in the plots for analysis by AFLP (Amplified Fragment Length Polymorphism). The tree species diversity was calculated by the well-known measure i_2 ($i_2 = 1/Oni^2$), which is frequently referred to as the "effective number" of variants. This measure is one of an infinite family of measures, i_a, where "a" is a real number ranging from zero to infinity. The results revealed significant relationships between tree species diversity and genetic structure in P. chihuahuana. On the basis of our findings, it appears that an interaction between genotype and diversity of tree species may be an important mechanism shaping the composition of plant communities. The present results may help us understand the interactions between ecological and evolutionary processes that determine community structure and dynamics.

GENETIC DIFFERENTIATION OF THE ENDEMIC MEXICAN SPECIES Picea chihuahuana Martínez

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A primary goal of empirical population genetic studies is the identification, quantification, and comparison of genetic differentiation among loci, individuals, populations, species, and studies. Genetic differentiation is the accumulation of differences in allelic or genotypic frequencies between populations and individuals due to evolutionary forces. The distribution of genetic variation among populations is known as the genetic structure and is determined by genetic factors such as rate of mutation, genetic drift, natural selection, gene flow, and mating systems, as well as by ecological factors including life history, geographic distribution, and the dispersal mechanisms of the species. The adaptation of species to different ecological conditions under strong selection pressures has led to changes in genetic structure, morphology, and physiology of populations. The analysis of genetic differentiation enables us to find out more about the influence of evolutionary factors in the genetic structure of the species. The most widely used means of genetic differentiation has been Wright's fixation index, F_{ST} , which was developed as part of a set of hierarchical parameters (F_{ST} , F_{IS} , and F_{IT}) to assess how genetic variation is hierarchically partitioned in natural populations. However, the frequently used fixation index, F_{ST} , and its derivatives produce errors as descriptors of genetic differentiation, principally when applied to highly variable genetic markers. Moreover, this index is not applicable at the genotypic level. Hence, the use of other parameters has been suggested for accurate measurement of genetic differentiation. Picea chihuahuana Martínez is endemic in Mexico and is considered endangered, according to the Mexican Official Norm (NOM-ECOL-059-2010). The species is therefore considered fundamental for determining the degree of genetic differentiation between populations. The principal aim of the present study was to determine the genetic differentiation of 14 populations of P. chihuahuana located in the State of Durango, Mexico. Needles were sampled from about 700 randomly chosen individuals of P. chihuahuana from these 14 populations, and DNA data were obtained through AFLP technology. A data matrix was constructed according to the presence or absence of bands. To measure the genetic differentiation, parameters such as genotypic differentiation ä and its components Dj were determined for each AFLP locus, thus enabling indirect, accurate measurement of the presence of direct forces that increase or decrease the differentiation among populations. A value of zero indicates that populations are genetically identical, and conversely, a value of one indicates that populations are genetically different. Significant genotypic differences between populations of P. chihuahuana were found that are probably caused by effects in small isolated populations and by selection.

GENETICS OF WOOD QUALITY

COEXPRESSION NETWORK ANALYSIS OF CANDIDATE GENES RELATED TO WOOD PHYSICAL TRAITS IN WHITE SPRUCE

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The molecular functions and biological connectivity of candidate genes related to wood formation have rarely been explored. The aim of this study was to conduct a network analysis of gene expression data and to identify sets of functionally related candidate genes based on correlated patterns of expression. The anticipated results should help highlight the nature and biological role of candidate genes, determine what are the main expression profiles represented among candidate genes, and verify if there is an over-representation of specific profiles. To identify coexpression modules, a gene correlation network for 2,660 candidate genes was generated using gene expression data from seven white spruce (*Picea glauca*) tissues obtained with microarray measuring the accumulation of transcripts of 23,853 genes (Raherison et al. in preparation). The data were submitted to the weighted gene coexpression network analysis (WGCNA) package (Langfelder and Horvath 2008). We carried out gene ontology (GO) enrichment analyses for all coexpression modules obtained to identify genes containing particular GO terms that are over-represented in these modules.

A total of nine modules of correlated genes were detected in the WGCNA correlation network analysis. The modules varied in size from 62 to 551 genes and had an average size of 280 genes. The largest modules were composed of genes whose expression was contrasted between needles and secondary vascular tissues (both xylem and phloem). The network analysis also showed that genes associated with wood traits had diverse expression profiles. For example, module 2 consisted of 511 genes, of which 367 genes had high secondary vascular tissue expression and 144 had low secondary vascular tissue expression. The functional enrichment showed that genes that were highly expressed in vascular tissues were enriched with gene products that target the Golgi apparatus (GO:0005794), plasma membrane (GO:0005886), and endoplasmic reticulum (GO:0005783). The distribution of genes significantly associated with wood traits in these modules might be the key to understanding the genetic architecture of these complex traits.

INBREEDING IN MID-ROTATION COASTAL DOUGLAS-FIR

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The effects of inbreeding on growth traits are fairly well understood in young conifers. However, in trees approaching mid-rotation, this information is not widely available. To evaluate inbreeding in coastal Douglas-fir (Pseudotsuga menziesii) trees, a 9-clone founder population was used to produce 20 outcross, 66 half-sib, 36 full-sib, 17 parent-offspring, and 9 selfed families. The trees were planted on two farm-field sites in coastal BC and were re-assessed at age 26 from seed for this study. Height, dbh, survival, and wood density were recorded and single-tree volume calculated. Previous results obtained from this test population showed negative linear effects with levels of inbreeding in seed production, nursery growth performance, and growth traits in the field assessed at age 10. At age 26, inbreeding depression was highest in survival, ranging from 20 to 80% for f=0.125 and f=0.5, respectively. In contrast, the most severe inbreeding depression among the three levels of inbreeding was only 4% for wood density at f=0.5 (selfing). Inbreeding depression in height, dbh, and volume increased linearly from f=0 to f=0.25 then leveled off. Founder genotypes had varied responses to inbreeding as parental breeding values across inbreeding levels were inconsistent (in magnitude, sign, and trait). Differences in levels of inbreeding depression were also found between full-sib matings and parent-offspring matings, despite having the same level of inbreeding (f=0.25). These differences also depended on the trait and field site. Heritability estimates varied among traits and inbreeding levels.

GENOMICS-ASSISTED SELECTION OF WHITE SPRUCE BENEFITS FROM SilviScanTM: ADVANCED PHENOTYPING TECHNOLOGY FOR MEASURING INDUSTRIALLY RELEVANT WOOD TRAITS

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With the rapid advancement in genotyping technologies, genotyping is becoming more affordable and will no longer be a limiting factor in forest genetics research. Consequently, to keep pace with these developments, there is an increasing need for precise, high throughput and cost effective phenotyping for wood and fibre traits in genomics research. Advanced phenotyping technologies such as SilviScan can facilitate the acquisition of wood traits accurately, efficiently, and cost effectively for large sample sets.

SilviScan rapidly and cost-effectively determines multiple wood traits on the same increment core or wood disc sample. It offers high-resolution pith-to-bark measurements of fibre diameter, wood density, microfibril angle, modulus of elasticity, cell wall thickness, specific surface area, ring width and area, fibre coarseness, and cell population. The data have applications in many fields, such as resource characterization, forest inventory enhancement, forest management decision-making, forest genetics research, tree improvement, and adaptation to climate change.

One example of the benefits of SilviScan in forest genetics research is illustrated in a pilot study to test for association between single nucleotide polymorphisms (SNPs) of candidate genes and wood traits in white spruce (*Picea glauca*) (Beaulieu et al. 2011). Genomic selection in boreal conifers appears particularly promising for traits that must be assessed at a more mature stage, such as wood quality. Preliminary results indicate that genomic selection could likely be applied to spruces using only several tens of gene SNPs found to be associated with wood traits. In this study, SilviScan played a key role in the measurement of industrially relevant wood traits that are significant to the performance of wood, pulp, and paper products.

In summary, SilviScan efficiently measures wood quality traits that are of crucial importance to the forest sector, and is a valuable tool for providing measurements for various aspects of forest genetics and genomics research.

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APPLICATIONS OF GENOMIC TOOLS

GENETIC PURITY CERTIFICATION THROUGH MOLECULAR MARKER TECHNIQUES IN Eucalyptus grandis

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The correct identification in the genus Eucalyptus is hindered due to morphological similarities between species. Furthermore, the high capacity of interspecific hybridization in this genus also hinders correct identification. Molecular techniques have been incorporated into forest improvement programs, as well as used in varietal identification and genetic purity certification. Eucalyptus grandis Hill ex Maiden seeds from Coff"s Harbour, New South Wales, Australia were sowed in Mogi-Guaçu (SP) and Salto (SP), creating a base-population. Some individuals of the base-population were selected and subjected to a progeny test. Based on this test new individual selection was performed and, by vegetative propagation, a clonal seed orchard of E. grandis was created. A new progeny test was performed, in which E. grandis individuals were planted next to individuals of E. urophylla (from Timor). The seeds that were created from open-pollination between E. grandis and E. urophylla were harvested from E. grandis. These seeds were sown in a nursery and individuals with characters typical of E. grandis were selected and transferred to an experimental field. New selection according to growth and stem form was performed and the selected individuals were cloned and planted in a clone bank located at Morrinhos farm (Itatinga/SP). However, morphological analysis of the bark suggested that some individuals were not E. grandis. Thus, it is necessary to conduct a complementary analysis using molecular techniques. The aim of this work was to confirm the identification of *E. grandis* that was carried out by phenotypic characters (bark) using molecular tools.

Sixteen selected trees were characterized by bark phenotype as *E. grandis* or *E. urograndis*. Genomic DNA extraction was based on the Ferreira and Grattapaglia protocol, with a reduction of CTAB concentration of 10 to 5%, and by using the volume twice. PCR reactions were performed with a specific SCAR (Sequence Characterized Amplified Regions) primer, called Elig, that amplifies in individuals without a lignotuber character (99% of *E.grandis*). The amplicons were analyzed on agarose gel 1.5% (w / v) in Tris-borate (TBE 1X, pH 8.0), stained with ethidium bromide 1% (v / v).

The SCAR Elig primer was developed to identify *E. grandis* individuals with the absence of lignotuber. More than 99% of the *E. grandis* population does not possess lignotuber. However, some individuals can possess this structure due to unknown interspecific hybridizations, defining the individual as not pure.

This work aimed to confirm the identification of some individuals from the open-pollinated progeny test, whereupon the female parent is *E. grandis*. Once we sought only *E. grandis* individuals to comprise the clone bank and some selected individuals displayed uncharacteristic features, we realized a test with the SCAR Elig primer was required to identify *E. grandis* without lignotuber.

The test resulted in four situations:

1) Typical bark and band presence: Confirms the identification as *E. grandis*.

2) Typical bark and band absence: The individual can be a hybrid with *E. urograndis*, with the characteristic bark inherited from the *E. grandis* parent. The absence of the molecular marker can be explained by remote and unknown interspecific crossing.

3) Untypical bark and band presence: The individual can be considered a *E. urograndis* hybrid with the phenotypic character (bark) inherited from the *E. urophylla* male parent and the genome possesses the molecular marker inherited from the *E. grandis* female parent.

4) Untypical bark and band absence: The individual can be a *E. urograndis* hybrid, with the *E. grandis* female parent possessing in its genome traces from *E. urophylla* that originated from remote and unknown interspecific crosses.

The capacity of interspecific hybridization with the genome similarities in this genus hinders the exact identification of pure species and hybrids. Even though molecular tools complement morphological analysis, it is necessary to conduct a more extensive analysis in order to have better reliability of the results.

VALIDATION OF SNP-TRAIT ASSOCIATIONS IDENTIFIED IN BLACK COTTONWOOD (Populus trichocarpa)

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Populus trichocarpa is a tree species with high economic and ecologic relevance. Hence, it is an important model species in forest tree research. Extensive genomic resources, e.g., the full genome sequence and tools for the identification of single nucleotide polymorphisms (SNP) are available. Furthermore, with the advent of SNP genotyping arrays and more recently, low-priced sequencing technologies, genome-wide SNP profiles from hundreds of individuals can be assayed. We and others are using this information in combination with extensive phenotype data from trees grown in common gardens for genome-wide association studies (GWAS) to study the correlation between genetic variants and quantitative trait differences based on linkage disequilibrium (LD). These studies identify a considerable number of SNPs that are significantly associated with various traits. However, our GWAS have revealed many SNP-trait associations in genes of unknown function or genes not previously known to be related to the associated trait. Also, many SNPs appear not to affect protein function or are located on non-coding regions. Major questions thus include: How can we corroborate the allelic effects of these SNPs? Which strategies can be applied to identify causal SNPs (OTN) that directly impact trait variation? In our work we are analyzing selected genes and SNPs identified in two *Populus* GWAS that focused on wood properties and adaptive traits with respect to these questions. Our strategies were discussed and initial results were presented for experimental validation of the SNP-trait associations including analysis of expression level variation and/or transgenic expression of the target alleles.

WESTERN WHITE PINE QUANTITATIVE RESISTANCE TO BLISTER RUST FUNGUS: UNDERSTANDING THE ROLE OF KEY PROTEINS TO FACILITATE TREE IMPROVEMENT

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Western white pine (*Pinus monticola*) is a commercially and environmentally important species in North America that has been threatened since the early 1900s by an introduced pathogen, white pine blister rust fungus (WPBR, Cronartium ribicola). Natural Resources Canada in collaboration with the BC Ministry of Forests, Lands and Natural Resource Operations and the USA government established a breeding program in the 1970s which has incorporated qualitative and quantitative resistance mechanisms. However, screening trials for quantitative resistance can take up to seven years. The objective of our research is to identify the functional gene candidates and genetic markers associated with resistance to C. ribicola in P. monticola to expedite this screening process. As quantitative resistance is expected to be multigenic, several defense-related gene candidates were previously identified by our group in P. monticola needles using a proteomic strategy including: antimicrobial proteins, pathogenesis-related protein 10, chitinases, and thaumatin-like proteins. Chitinases are enzymes hydrolyzing the glycosidic bonds in chitin, which is the major component of cell walls of fungi, subsequently resulting in cell lysis. However, the exact mechanism of thaumatin-like protein action is still unclear. To further investigate the function of these genes in resistance, recombinant proteins were generated for two gene families: class IV chitinase and thaumatin-like protein. Indeed, we overexpressed these genes in an E. coli expression system and used affinity chromatography to purify the recombinant proteins to assess their inhibitory activity against different fungal pathogens. We found that proteins of both gene families have antifungal activity. These data provide a better understanding of host-pathogen interactions and potential molecular markers for selection of white pine blister rust resistance.

IDENTIFICATION OF SNPs IN CANDIDATE GENES RELATED TO WATER STRESS, TRANSPORT AND ABSORPTION OF BORON IN Eucalyptus

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Forestry companies have established Eucalyptus plantations in northern and northeastern Brazil. However, limitations on water supply and boron (B) are affecting biomass production and reducing yield significantly. Tolerant plants develop defense mechanisms like hormone production of abscisic acid (ABA) and osmoprotector glycine betaine (GB) when exposed to drought conditions. Polyol sorbitol with Bor1 carrier are correlated with boron translocation and absorption. Identifying and studying genomic regions related to water stress tolerance, boron transport and translocation are important for forest breeding programs. We identified SNPs (single nucleotide polymorphisms) in four Eucalyptus candidate genes, 9-cis-epoxy-carotenoid dioxygenase (NCED) and choline monooxygenase (CMO) related to drought tolerance, and Sorbitol dehydrogenase and Bor1 transporter related to boron absorption and transport.

Contrasting plants of *E. grandis*, *E. urophylla*, and the hybrid *E. grandis* x *urophylla* were selected according to their physiology. Specific primers were designed from homology sequences from a Eucalyptus ESTs databank and amplification products submitted to sequencing which allowed for SNPs identification and plants genotyping.

In the four studied genes, 110 SNPs were found, of which 54 were in the BOR1 and Sorbitol dehydrogenase genes. At 3860 bp in the BOR1 gene, 35 SNPs were found, of which, 16 were located in exons with synonymous mutations in 12 (75%) and non-synonymous mutations in four (25%), and 19 SNPs were located in introns. Nineteen SNPs were found in the sorbitol dehydrogenase gene in the 2889 bp region. Seven are located in exons, with synonymous mutations in 4 (57%) and non-synonymous mutations in 3. Ten SNPs were located in íntrons and two at UTRs regions. In the NCED e CMO genes were found for 66 SNPs; seven SNPs in a 1230 bp region on the NCED gene, from which five (71.5%) were present in codified regions and generated synonymous mutations and two (28.5%) in UTRs regions. For the CMO gene, 49 SNPs were identified in the 3885 bp region, with 12 in the codified regions. For these codified regions, ten (83.3%) of the mutations were synonymous and two (16.7%) were non-synonymous with 37 in UTRs and intron regions. For the BOR1 gene, were found 16 haplotypes with 0.957 haplotypic diversity and 0.00308 nucleotide diversity Pi(ð), averaging one SNP for each 120 bp. For the Sorbitol dehydrogenase gene, 18 haplotypes were found with 0.967 haplotypic diversity and nucleotide diversity Pi (ð) of 0.00296, averaging one SNP for each 152 bp. The NCED gene presented seven haplotypes which generated 15 different genotypes. For the CMO gene, these polymorphic sites constituted 18 haplotypes producing 16 diverse haplotype combinations. Nevertheless, the CMO gene showed some unique genotypes for E. grandis and E. urophylla. As a result, the genotyping of individuals by the allele-specific extension technique demonstrated to be efficient; in addition the SNPs primers designed can decrease costs and permit the genotyping of these mutations in a large scale of contrasting populations to water deficit and in Eucalyptus population studies.

GENOME-WIDE ASSOCIATION MAPPING AND GENOMIC PREDICTION FOR WOOD PROPERTY AND QUANTITY OF MALE STROBILI IN Cryptomeria japonica

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Genome-wide association studies (GWAS) and Genomic selection (GS) can be powerful technology in conifer breeding because conifers have long generation intervals, protracted evaluation times, and high costs of breeding inputs. In the present study, we examined the potential of GWAS and GS in conifers using 367 unrelated plus trees of Cryptomeria japonica D. Don, which is the most widely planted and commercially important tree species in Japan, and tried to detect significant associations between wood property traits and quantity of male strobili on the one hand, and 5,055 single nucleotide polymorphisms (SNPs) assigned to 5,055 genes on the other. The mixed linear model, taking into account kinship relationships and subpopulation structure, and Ridge-regression model were used for GWAS and GS, respectively. In total, dozens of SNPs were found to have significant associations with variations in phenotype. These SNPs were not associated with the positions of known genes and QTLs that have been reported to date, thus they may identify novel QTLs. Eighty percent of these SNPs were found in sequences showing similarities with known genes, although further analysis is required to dissect the ways in which they affect wood property traits and abundance of male strobili. These presumptive QTL loci provide opportunities for improvement of C. japonica, based on a marker approach. Genome-wide predictions for GS were accurate at the medium level (0.54) in quantity of male strobili and at low levels (0.2) in wood property traits. The results suggest that GWAS and GS have potential for use in future C. japonica breeding programs.

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PATERNITY RECOVERY IN A MARITIME PINE POLYCROSS TRIAL

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Maritime pine (*Pinus pinaster* Ait.) is the main plantation species in France with nearly one million ha of cultivated forests in south western France. A breeding program has been conducted since the early 1960s using a recurrent selection scheme. The breeding strategy combines two successive experimental designs: polymix crossing to evaluate parental breeding values and full-sib crossing from which selections are made for the next generation of breeding. This strategy was efficient both to increase genetic gains (maritime pine varieties reach 30% expected gain in volume and in stem straightness) and to maintain genetic variability in the breeding populations (Bouffier et al. 2008).

Future improved varieties must be adapted to environmental evolution (climate change, development of some pests) and to wood industry diversification. In that changing context, forest tree breeding programs have to integrate more and more selection criteria and develop different varieties adapted to various environments and diversified uses. The most important aspect is to accelerate the selection cycles in order to frequently renew variety composition. Fast development of molecular genetic tools and their decline in cost can be of great help to cope with these challenges. Shortening the selection cycle could be achieved by polymix breeding with parental analysis of progeny as proposed by Lambeth et al. (2001) instead of the current breeding strategy of full-sib breeding and testing.

In that perspective, a maritime pine polymix progeny trial is currently under study. Trees have been highly phenotyped for various traits relative to growth (diameter, height), wood quality (stem straightness, spiral grain, wood density) and adaptation (water use efficiency). We are now genotyping this population with 60 SNPs (Sequenom technology) to recover the paternity identity. The poster presented first results of this study which aims to answer the following questions:

Is there differential reproductive success among pollen donors?

What is the gain for breeding value accuracy when analyses are carried out with the full pedigree instead of with only the mother identity?

Which new breeding strategies integrating pedigree recovery can be implemented?

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POPULATION GENETICS AND GENOMICS

GENOMICS RESOURCES FOR NORTH AMERICAN HARDWOODS

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The hardwood forests of eastern North America are complex biological systems, covering millions of acres, providing habitat for wildlife, long-term carbon sequestration, fiber, recreational uses, and other essential services. The sustainability of these forests is increasingly threatened by forest health issues such as exotic pests, diseases, invasive plants, climate change, and fragmentation. In this project, researchers at seven universities are collaborating to develop genomic resources and genetic tools for a broad taxonomic distribution of hardwood tree species, including northern red oak (*Quercus rubra*), black walnut (*Juglans nigra*), white oak (*Quercus alba*), yellow poplar (*Liriodendron tulipifera*), sweetgum (*Liquidambar styraciflua*), honey locust (*Gleditsia triacanthos*), sugar maple (*Acer saccharum*), blackgum (*Nyssa sylvatica*), and green ash (*Fraxinus pennsylvannica*). We are producing expressed gene sequence databases, DNA markers, genetic linkage maps, and reference populations with an emphasis on tools for assessing genetic variation in growth, adaptation, and responses to abiotic environmental stresses. These resources are being made available through the project website

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(www.hardwoodgenomics.org). Sequence data will be deposited in GenBank, and the genetic linkage maps and associated marker data will be available at the Dendrome database (http://dendrome.ucdavis.edu/). This project is supported by grant # TRPGR IOS-1025974 from the National Science Foundation's Plant Genome Research Program.

THE CHESTNUT GENOME PROJECT

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Hardwood trees in the northern hemisphere are under increasing threat from invasive pests and diseases. The devastation of American chestnut (Castanea dentata) populations in eastern North America by the chestnut blight fungus (Cryphonectria parasitica) is a well-chronicled environmental disaster. Unlike the American chestnut, the closely related Chinese chestnut (*Castanea mollissima*) evolved resistance to the sympatric chestnut blight fungus. Hence the Chinese chestnut is an important species for decoding the evolution of disease resistance, especially in hardwood species. Thus, we have undertaken a project to sequence the Chinese chestnut genome to provide a high quality reference genome for the discovery of blight resistance genes and for studying the evolution of disease resistance in forest trees. Using mostly next generation sequencing data, we produced over 61 billion bases of NG DNA sequence data providing 76 X coverage of the estimated 800 Mbp chestnut genome. The sequence data were assembled into 41,270 scaffolds, with an N50 scaffold length of 39,580 bp, covering 724.4 Mbp of the estimated 800 Mb chestnut genome. A total of 38,146 genes have been predicted and annotated in the genome scaffolds using Chinese chestnut and American chestnut RNA sequences. To ensure that all of the blight resistance genes are identified, we also sequenced BAC clones covering the three major blight resistance OTL from the Chinese chestnut physical map. Over 400 candidate disease resistance genes were identified in the OTL sequences, of which 22 have been selected as high priority candidate genes for blight resistance. This project was supported by The Forest Health Initiative (http://www.foresthealthinitiative.org).

GENETIC VARIATION IN CHLOROPLAST DNA OF Dalbergia cochinchinensis Pierre IN THAILAND

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The Siamese rosewood (*Dalbergia cochinchinensis* Pierre) is an economically important tree species throughout Laos, Cambodia, Vietnam, Myanmar, and Thailand. This species has been illegally logged since the last few decades which may affect the gene pool, genetic diversity, and genetic structure. In this study the cpDNA variation of *D. cochinchinensis* was investigated by using cpDNA sequencing to characterize the spatial genetic variation and pattern of haplotypes of investigated populations in Thailand. *D. cochinchinensis* was sampled from 9 populations in central and northeastern Thailand.

The 1,841 bp nucleotide sequences from three cpDNA regions (trnS-trnG: 469 bp, trnV-trnM: 664 bp, and trnC-petN1R:708 bp) were obtained from 98 sampled trees. A total of 11 haplotypes with 10 polymorphic sites, including 8 single nucleotide polymorphisms (SNPs), and 2 insertions/deletions were observed. The haplotype diversity (h) for each population ranged from 0 to 0.697 and the nucleotide diversity (ð) from 0 to 0.00056. The Na Dun population in Maha Sarakam Province (NM) had the highest haplotype diversity and nucleotide diversity. This may be because this population is the forest community which has been reforested by different sources of *D. cochinchinensis*. However, it was revealed that Chuen Chom population in Maha Sarakham Province (CM), Phu Laenkha population in Chaiyaphum Province (PC), Tapraya population in Buriram Province (TB), Nadun population in Maha Sarakham Province (NM), and Muakleknai population in Saraburi Province (MS) have different unique haplotypes. Therefore, the unique haplotype information of the various populations can be used as a tool to identify the origin of illegally logged wood in the future.

Therefore, more molecular markers, number of samples, and populations of *D.cochinchinensis* in Thailand and the Asian region should be collected and investigated for the indicative haplotype for specific populations, sub-regions, and countries. Then it can be used to identify the origin of genetic resources of illegally logged wood, since Thailand is facing this problem due to high demand by the illegal trade.

FINE-SCALE SPATIAL GENETIC STRUCTURE OF A Quercus gilva POPULATION IN SOUTH KOREA BY MICROSATELLITE MARKERS

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Quercus gilva is an evergreen and endangered oak species in Korea. This species is distributed in Japan and southern China, but grows naturally only in South Korea on the volcanic Jeju Island. We conducted a total inspection of growth performance and analyzed the genetic variation of a Q. gilva population using microsatellite makers. A total of 668 individuals were found in the surveyed region (38.5 km²) and the mean height and dbh of O. gilva trees were 9.5 m and 21.7 cm, respectively. Mean distance to the nearest neighbour was 41.7 m and the distribution was highly aggregated ($R = 0.15^{***}$). We randomly sampled 192 individuals for genetic analysis with eight nSSR markers. The number of observed alleles and the effective number of alleles were 10.1 and 4.1, respectively. There was little difference between the observed and the expected heterozygosity ($H_0 = 0.617$ and $H_e = 0.702$) so the fixation index was small (f=0.112) as compared to those of other oak species. This population was out of Hardy-Weinberg equilibrium. The sampled individuals were spatially aggregated ($R = 0.58^{***}$). From spatial autocorrelation analysis, the size of genetic patchiness was estimated as 50 m in diameter. The distribution pattern of genetic variation determined by the genetic bandwidth mapping method showed that individuals in the southern part of the population were genetically heterogeneous, while others in the northern part were homogeneous. This difference of spatial genetic composition might be explained by topographic characteristics and by land use history. The northern part is at the foot of a mountain and the southern part is flat land which was once used for farming. These two parts are also physically divided into two sites along a road. Acorn distribution by birds or rodents was surmised to be a major factor contributing to spatial genetic structure in the region rather than dispersal by gravity.

COMPARATIVE GENOMICS OF P. glauca AND P. abies

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Picea glauca and *P. abies* are economically and ecologically important tree species that dominate temperate and boreal forests in North America and Northern Europe. The recent sequencing of their complex and large genomes provides a unique opportunity to study the genome structure evolution in conifer species.

We assessed the levels of synteny and colinearity between *Picea glauca* and *P. abies*. Sequences from 38 fully sequenced bacterial artificial chromosomes (BACs) from *P. glauca* were compared to 450 *P. abies* fosmid-pools (representing aproximately 50% of the complete genome sequences) and diploid whole genome shotgun data (*P. abies* 1.0 assembly, 19.6 Gb).

Data analysis was done using customized perl and shell scripts. Selected fosmid-pools' contigs were correctly oriented and ordered using nucmer and displayed with mummerplot.

We found that the number of BACs with at least one match to *P. abies* varied from 28 (fosmid-pools) to 30 (whole-genome) from a total of 38. The percentage of coverage in the BAC scaffolds by one or several fosmid-pools varied from 0.57 to 47.48%. Significant synteny was found in seven BACs, in which > 20% of the BAC sequence was contained in one or several *P. abies* fosmid-pools' contigs. Five of these BACs contain important genes in wood formation or nitrogen metabolism in *P. glauca*.

FINE-SCALE TRANSCRIPTOME RECONFIGURATION IN RESPONSE TO A CLIMATE-INDUCED STRESS: TESTING THE NAÏVE HOST HYPOTHESIS

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The current outbreak of mountain pine beetle (MPB; *Dendroctonus ponderasae*) and its associated pathogenic fungi (i.e., *Grosmannia clavigera*) have had devastating impacts on the health of western North American pine forests. Approximately 28 million ha of lodgepole pine (*Pinus contorta* var. *latifolia*), the historic host for MPB, have been destroyed within western North America (1–3). In recent years, MPB has undergone marked range expansion into novel habitats northward, eastward, and into higher elevations attacking a new host species, jack pine (*P. banksiana*), an invasion facilitated by a hybrid zone between the two pine species (4). This expansion has, in part, been driven by warmer winters associated with global climate change that have occurred at different stages of the epidemic (5) allowing further spread into novel habitats of the boreal forest. Consequently, understanding the evolutionarily naïve host defense response relative to the evolutionarily co-evolved host may aid in evaluating risk of MPB spread in novel habitats (6).

Given the history of selection and adaptation of constitutive and induced defenses of lodgepole pine and MPB, we expect the defense response of lodgepole pine to differ from that of the novel host, jack pine. Furthermore, as stressed trees are more prone to successful MPB attack under endemic conditions, we expect differential partitioning of resources to defense and other aspects of host suitability in response to environmental stress. We used microarray transcriptome profiling to evaluate the transcriptional response of two-year old lodgepole pine and jack pine seedlings subjected to either well-watered or water deficit treatments in concert with inoculation of G. clavigera, as a proxy for MPB attack. RNA was extracted from phloem harvested over a time course of 1, 7, and 28 days post-inoculation (dpi). Loblolly pine (P. taeda) cDNA microarrays containing 26,496 elements (7) were hybridized with aRNA according to El Kayal et al. (8). The loblolly pine sequences represented on the microarrays were cross-annotated with the corresponding lodgepole pine and jack pine transcript sequences, in addition to Arabidopsis TAIR annotations. Statistically over-represented gene ontology terms categorized from differentially expressed pine gene putative orthologs, were integrated into Ontologizer (9). Differentially expressed genes were clustered using K-means in MeV (10) across all dpi and treatments. Identification of functional categories or 'bins' were developed using the Mercator Automated Sequence Annotation Pipeline (Lohse et al. unpublished) for loblolly pine sequences. These data were used to visualize functional categorization of differentially expressed genes across dpi and treatments for the two pine species within MapMan (11).

Microarray analyses revealed significant transcriptional differentiation across treatments, including a considerable influence of water limitation between species over time. Several thousand significantly differentially expressed genes were observed across treatment combinations, including defense-related processes such as terpenoid, phenylpropanoid, and phenolic compound biosynthesis, and cell wall modification. These categories were statistically over-represented within the differentially expressed gene set of jack pine, although similar patterns were not observed for lodgepole pine. Cluster analysis revealed similar gene expression patterns between species over time, with the greatest number of differentially expressed genes suggests species-level differences in the functional categories represented by those differentially expressed genes in functional bins associated with defence that are unique to jack pine. These findings identify fine-scale patterns of differentiation in the regulation of defence-related genes. In addition, these data suggest that water limitation modulates the defence response, suggesting abiotic and biotic factors may interact to influence the transition of MPB from its historic host, lodgepole pine to its naïve host, jack pine.

This study identified functionally important environment and species-specific transcriptional changes during defence. While timing of defence is conserved between species, fine scale differentiation among functional categories of differentially expressed genes point towards species-specific strategies associated with climate-induced stresses. These unique responses to both abiotic and biotic stresses within the native and novel host may influence evaluation of MPB spread into novel habitats and ultimately management of the new host species.

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PHYLOGEOGRAPHICAL STUDY OF *Quercus phillyraeoides* A. Grey IN THE WESTERN PART OF JAPAN REVEALED BY MICROSATELLITE MARKERS

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Quercus phillyraeoides is an evergreen shrubby oak species growing on the Pacific coast and the Seto Inland Sea of southwestern Japan. Most famously this oak species has been utilized for producing high-quality charcoal, Bincho-tan, and it is an important component of the dry hill vegetation of Japanese coastlines. In a previous study we showed some of the isolated populations on the Pacific coast (Kushimoto (Ks), Ashizuri (Az), Sata (St), and Ryugadake (Rd)) were refugial populations of the last glacial maximum (LGM) using cpDNA non-coding regions.

In this study 414 individual samples collected from the same 19 populations of the previous study were examined by using 11 microsatellite loci. Eight of the 19 populations were located in the Seto Inland Sea area and four were along the East China Sea (Fig. 1). A total of 104 alleles were found (average = 9.45 alleles per locus), and 12 of them were private alleles. Average observed heterozygosity was 0.560 ± 0.021 (SE) and average expected heterozygosity was 0.551 ± 0.017 (SE). The expected heterozyogsity for populations ranged from 0.422 ± 0.081 in Izena-jima (Iz) in Okinawa to 0.608 ± 0.056 in Nagahama (Ng) at the Seto Inland Sea. AMOVA by Arlequin software showed small, but highly significant F_{ST} (= 0.07656, P<0.0001). Pairwise F_{ST} showed the easternmost population in Iwai (Iw), the southernmost population in Sata (St) in Kyushu, and Iz were found to be the most isolated populations. Neighbour Joining tree was constructed using pairwise genetic distance. The tree showed Iz, the southern and the westernmost population, was paraphyletic to the other populations which suggested it is the oldest population. Whereas, Iw, the easternmost population, was at the tip of the furthest branch showing it to be the most recent population. These observations suggested that *Q. phillyraeoides* has migrated to the Japanese Archipelago from mainland China through the Ryukyu Islands to the east on the Pacific coast.

We examined population structure using STRUCTURE software. The most probable number of clusters (K) was four. These clusters appeared in a series (i.e., cluster 1 - cluster 2 - cluster 3 - cluster 4) from west in Iz (cluster 1) to the eastern population of Iw (cluster 4) on the Pacific coast, but the populations in the Seto Inland Sea were a mixture of clusters 2 and 3. This observation, together with the result of the phylogenic tree, suggested that migration and expansion of Q. phillyraeoides has occurred in a series producing new clusters one by one. Because at the LGM, when the sea level was 150 m lower than today, the Seto Inland Sea was dry land and the populations here were the newly migrated ones from the Pacific coast through the opening of Bungo and Kii straits, which are dividing Kyushu and Shikoku and Shikoku and Honshu, respectively. This scenario is also supported by cpDNA data in the previous study. Geological studies have shown that the Ryukyu Islands were once a land bridge at the end of the Pleistocene about 1.7 to 2.0 million years ago, connecting mainland China and the Japanese Archipelago and provided a migration route for animals and plants from the subtropical area of China. The land bridge was broken by the newly opened Tokara gap between the southern part of Kyushu and Okinawa Islands about 1.7 to 1.3 million years ago and was never closed. The Iz population has been isolated since then and the southwestern populations in Kyushu may have expanded further east during the later Quarternary period. Clustering may have occurred during the periodic glacial events.



Figure 1. Collection sites of *Quercus phillyraeodes* (dots). Location names are shown by tow-letter abbreviations.

GENETIC DIVERGENCE BETWEEN POPULATIONS ALONG THE JAPAN SEA AND PACIFIC OCEAN SIDE OF JAPAN IN Cryptomeria japonica USING NUCLEAR GENOMES

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Genetic diversity and population genetic structure in plant species are influenced by various factors, including their life history and ecological traits (e.g., mating system, modes of seed and pollen dispersal) and historical shifts in distribution caused by climatic change, especially those associated with ice ages. In particular, climatic fluctuations during the Quaternary appear to have caused profound changes in the distribution ranges of many plant species and thus strongly affected their genetic diversity and structure. In this study, genetic diversity and structure were examined using 20 nuclear microsatellite markers in 37 natural forests of *Cryptomeria japonica*, the most economically important forestry tree in Japan. *C. japonica* is an allogamous coniferous species with wind-mediated pollen and seed dispersal systems. Natural forests of the species are distributed under variable environmental conditions, from Aomori Prefecture (40° 42' N) to Yakushima Island (30° 15' N) in the Japanese Archipelago. Geographical variation between natural forests of *C. japonica* has been investigated, focusing on both morphological traits (e.g., needle length or curvature) and diterpene components and clonality. The results of these studies suggested that there are two main lines: Japan Sea lineage and Pacific Ocean lineage.

Bayesian clustering analyses provided clear evidence of genetic divergence among four genetic clusters: 1) the populations in the north region, 2) the populations on the Japan Sea side, 3) the populations on the Pacific Ocean side, and 4) the populations on Yakushima Island. Each linage was divided into two genetic clusters. This genetic structure was not inconsistent with previous reports of refugia from fossil pollen data. Based on the coalescent theory, the DIY ABC analysis suggested that the four genetic clusters diverged at the same time in the late-glacial period. We also analysed the relationships between genotypes from several SNPs loci and environmental variables to obtain information on the selective pressures acting on individual populations. These results indicated that local adaptation and/or physical barriers to migration and gene flow among refugial populations have promoted genetic divergence among these populations.

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ADAPTIVE DIVERGENCE OF CANDIDATE INSECT DEFENSE GENES IN **INTERIOR SPRUCE**

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Plant resistance to insect attack involves a complex mechanism, controlled by many genes. In this study, we selected 210 candidate genes for insect defence, and sequenced 18 individuals from each of three Interior spruce (Picea glauca x engelmannii) provenances: East Kootney (EK), Fort Nelson (FN), and Prince George (PG). These provenances span the range of Interior spruce in British Columbia. The first objective of our study was to find evidence for positive selection as given by dN/dS ratios (the ratio of non-synonymous codon changes to synonymous changes). We found dN/dS of most genes to be less than one, however it should be noted dN/dS is normally estimated for individual sites across a phylogeny; at least the ranking of dN/dS is informative about which genes are more subject to positive selection. These are presented. Interestingly, four genes had dN/dS values greater than one in EK, five in FN, and four in PG. Moreover, when the three provenances were merged, dN/dS of five genes were greater than unity. These genes are strong candidates for positive selection. A second objective of our study was to detect correlated changes between SNP loci across the three provenances; this is termed "selection covariance analysis". The correlation of SNP frequency changes between adjacent SNPs on the same gene were significant for five genes, showing that SNP changes in response to environmental pressures can be complex.

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POPULATION STRUCTURE AND MIGRATION HISTORY OF A POPLAR PATHOGEN, Mycospharella populorum

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Due to the need for wood and wood products, the hybrid poplar industry has expanded rapidly over the last 100 years. The fast growing nature of hybrid poplars has enabled rapid breeding of desirable characteristics and bulk up of preferred genotypes via clonal propagation. This has encouraged frequent and massive exchange of poplar material (via cuttings) between Canadian provinces and American states. Mycosphaerella populorum Thompson is a leafspot and canker causing pathogen of poplar. The disease affects both quality and quantity of wood produced in hybrid poplar production and is considered the most important disease of poplar where it occurs. Inevitably, the intensification of poplar culture has resulted in intensification of the disease. The latest discovery of this pathogen was in the lower Fraser Valley in British Columbia (BC) in 2006. In order to manage disease spread, prevent further incursions, and ensure the sustainability of breeding programs, we need to discern the pathways of pathogen movement. We sequenced the genomes of 88 M. *populorum* strains collected from plantations in five Canadian provinces and six American states. Genetic clustering revealed a mid-US center of diversity for M. populorum with colonization pathways eastward following the distribution of the native host Populus deltoides. The best-supported modeling scenario of the spread of the pathogen to BC is a recent introduction from an eastern source via massive or repeated introductions. This suggests that the movement of the pathogen is linked to the distribution of poplar cuttings.

GENOTYPING BY SEQUENCING AS AN ECONOMICAL METHOD OF SNP DISCOVERY IN Pinus contorta AND Picea glauca

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Genotyping-by-Sequencing is an economical way to reduce the complexity of a genome before sequencing to make the best use of expensive sequencing space. Here we present a modified protocol to produce GBS libraries that reduces hands-on sample preparation time and increases the total amount of library produced. We used the restriction enzyme Pst1 to cleave the genomic DNA sample and then we ligated double stranded adapters onto each end of the cleaved fragments. We then amplified the fragments that have the adapters correctly ligated using a high-fidelity polymerase and performed a final size selection for fragments 400-600 bp using magnetic beads. We have sequenced a preliminary plate of 96 lodgepole pine (*Pinus contorta*) samples using this technique. We aligned the GBS sequences to a draft of the draft lobolly pine (P. taeda) genome (PineRefSeq Project) using bwa. After filtering we identified 168,326 SNPs, but only 34,025 SNPs representing 9437 genes, were shared across 50 or more individuals. We plan to fine-tune our modified protocol for interior spruce (Picea glauca x engelmannii) and lodgepole pine and use the SNPs we discover to complement our sequence capture data to assess the genetic basis of adaptation to climate in these species. This will complement a large sequence capture data set that will capture SNPs across much of the exome. The GBS will allow us to expand our search to other areas of the genome, such as regulatory regions or other genes not included in our re-sequencing project. It will also allow us to identify more putatively neutral loci that will allow us to assess the effects of demographic history on the genome.

QUANTITATIVE GENETICS AND TREE BREEDING

GENETIC VARIATION IN SECOND-GENERATION Pinus elliottii var. elliottii

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Genetic parameters were estimated for growth traits in *Pinus elliottii* var. *elliottii* second-generation half-sib families. Twenty five families harvested from a first-generation seed orchard established in Colombo, PR, Brazil, were used in the study. The experiment was established in a randomized complete block design with thirty two replications of single plant plots. Height and DBH (diameter at breast height) were measured four years after planting. Deviance analysis and genetic parameter estimations were performed by using the best linear unbiased predictor (BLUP) and the residual maximum likelihood (REML) methods. A highly significant family variation in growth traits was detected. Narrow-sense individual heritabilities were estimated at 0.32, 0.14 and 0.37 for total height, DBH and volume, respectively. Average family heritability estimates ranged from 0.54 to 0.76 in dbh and volume, respectively. These results show promising perspectives to obtain substantial genetic gain, especially in wood volume growth, through either individual or family selection.

PHYTOPATHOLOGY OF A QUANTITATIVE TRAIT: IDENTIFICATION OF POTENTIAL RESISTANCE MECHANISMS TO Didymascella thujina IN Thuja plicata

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Thuja plicata (western redcedar, WRC) is one of the most valuable trees in British Columbia (BC) due to the durability, dimensional stability, and beauty of its wood. WRC is prone to infection by the foliar fungus *Didymascella thujina* (Cedar Leaf Blight, CLB), an airborne disease that is especially problematic in forest nurseries. Early studies with CLB in Europe noted that another species in the genus, *Thuja standishii*, was completely resistant to the blight and that hybrids of the two species were equally resistant to the blight. However, all WRC trees studied were susceptible but with varying amounts of the disease between individual trees. More recently, the BC Ministry of Forests, Lands and Natural Resource Operations research staff have shown quantitative variation among iWRC populations and families in CLB disease symptoms and that such potential quantitative resistance is significantly related to population or family origin. Trees originating in either drier, hotter or colder ecosystems tend to exhibit more disease symptoms than those from humid, cool environments.

Most studies on CLB have focused on chemical methods to control the disease, but little research exists on the underlying resistance or tolerance mechanisms. Our objective was to examine the histological characteristics of WRC that may be related to CLB resistance or tolerance. Two groups of WRC F_1 families, one that exhibited less disease symptoms and the other more, were assessed for stomatal density, epidermis thickness, leaf thicknesses, cuticle thickness, and lignin and flavonoid deposition by using compound-specific stains and a combination of light and fluorescence microscopy techniques. Preliminary analysis of the data depicted differences among families in cuticle thickness, lignin, and flavonoid deposition. Cuticles were thicker in families exhibiting more disease symptoms, which may be related to parental origin rather than a defence system against CLB. Differences in lignin and flavonoid deposition have also been seen, which may represent a complex defence system in resistance to CLB.

APPLICATION OF JOINT MODELLING OF COMPETITION EFFECTS AND ENVIRONMENTAL HETEROGENEITY IN DOUGLAS-FIR TRIALS USING AN INDIVIDUAL-TREE MIXED MODEL AND BAYESIAN INFERENCE

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Forest genetic evaluation involves the use of mixed linear models to calculate "best linear unbiased predictors" (BLUP) of tree breeding values (BV). As BLUP depends on the values of the (co)variance matrices for the assumed model, the specification of the dispersion parameters should take into account both the negative correlation caused by competition among individuals and the positive spatial correlation due to the environmental heterogeneity. Both phenomena, in any given experiment, are dynamic and coexist simultaneously. Therefore, modelling only one of these effects may lead to biases in the estimation of genetic parameters and the prediction of breeding values, and a joint model must be fitted to account for both sources of potential bias. As a first step in the analysis, we used several approaches to identify and quantify the competition effects (at the genetic and environmental level) or/and environmental heterogeneity. Then, a joint individual-tree mixed model with direct genetic effects, genetic and environmental competition effects, and a two dimensional B-spline smoothing surface to account for environmental heterogeneity (competition + spatial model), was applied to three Douglas-fir (Pseudotsuga menziesii) progeny tests. This model was compared to three reduced individual-tree mixed models: a standard model with direct genetic effects only, a competition model including direct genetic and genetic and environmental competition effects, and a spatial model with a smoothing surface and direct genetic effects only. Three growth traits: diameter at breast height (DBH), total height (TH), and volume (VOL) were assessed at two ages (12 and 35 years old). This data set was composed of 78 parents and 165 families arranged within 10 diallels and included in a random complete block design with 4 replicates of 4-tree row plots.

The traits DBH and VOL at age 35 years revealed strong competition effects at both genetic and environmental levels. There was also evidence of environmental heterogeneity for these traits. In general, the joint competition + spatial model gave a better fit (lower DIC value) than the simpler models on the three test sites. With strong competition genetic effects (i.e., correlation between direct and competition additive genetic effects higher than -0.3) the standard model yielded additive variance estimates that were smaller (from 39.5 to 50.6%) and residual variances that were higher (from 16.3 to 45.0%) than those estimated from the competition + spatial model. Ignoring the genetic and environmental competition effects leads to overestimating environmental heterogeneity; i.e., the spatial model yielded variance estimates of the random knots effects that were higher than those of the competition + spatial model. Ignoring the environmental heterogeneity leads to underestimating genetic and environmental competition estimates that were smaller (from 6.0 to 71.5%) and environmental competition variance that was higher (from 6.3 to 29.4%) than those of the competition + spatial model yielded direct and competition variance that the simultaneously adjusting for competition genetic effects and environmental heterogeneity has on the selection were discussed with respect to the Douglas-fir genetic improvement program.

GENOME-WIDE EFFECTS OF SELECTIVE BREEDING ON ADAPTATION OF REFORESTATION SEEDLOTS FOR FUTURE CLIMATES

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Climatic change is predicted to cause a lag in the productivity of western Canada's forests that will have negative economic, ecological, and social impacts. Such a lag in productivity is caused by shifting climates that are expected to disconnect locally adapted provenances from their established climatic optima. To assess the impacts of climate change on forests in British Columbia (BC) and Alberta, the AdapTree project is utilizing a combination of genomic and phenotypic approaches to quantify the genetic architecture of local adaptation to climate and to assess climate-based seed transfer approaches.

The component of AdapTree described here will investigate the effects of selection in tree breeding programs on adaptive diversity and climate-related phenotypic traits in the economically and ecologically important species lodgepole pine (Pinus contorta) and interior spruce (Picea glauca, P. engelmannii, and their natural hybrids). In western Canada the use of reforestation seedlots from advanced generation selective breeding programs is increasing rapidly and accounts for a majority of reforestation. The genetic effects of selective breeding on long-domesticated crop species are well documented, but the genomic consequences of selective breeding on adaptive forest diversity and divergence, as well as the suitability of reforestation seedlots to future climates remain unclear. We are evaluating how selective breeding affects the adaptive phenotypic and genomic architecture of reforestation seedlots. The primary questions that guide this research are: 1) how much do climatically-linked phenotypic traits differ between natural and seed orchard populations, 2) do correlated responses to phenotypic selection exist between height as the primary artificial selection trait and other phenotypic traits of importance to local adaptation, 3) what are the effects of selective tree breeding on adaptive genetic diversity in reforestation seedlots, and 4) how much molecular genomic divergence exists among natural and seed orchard populations from the same geographic areas, and does the amount of adaptive genomic divergence reflect phenotypic divergence?

A combination of analysis techniques that dissect the effects of selective breeding on adaptive phenotypic and genomic diversity and divergence in both species are being applied. Seedlots have been sampled across BC and Alberta to obtain representative natural (>250 seedlots per species) and selectively bred reforestation seedlots (~20 orchard lots per species). Seedling common gardens containing N > 2,500 individuals per species have been established at the University of British Columbia and at a field site in the central interior of BC. Phenotypic data are being collected on several climatically relevant phenological and growth traits. All trees will be genotyped for a suite of ~25,000 to 50,000 candidate adaptive SNPs being identified through exome capture and re-sequencing, and analyses including associations with provenance climate or phenotypes and outlier tests in other activities within AdapTree.

This research will allow evaluation of current provincial reforestation seedlot diversity standards in the context of adaptive rather than neutral genetic variation, and prediction of future climatic seedlot transfer ranges. This will contribute vital information to developing provincial policies on assisted genotype migration that aim to maintain forest productivity in BC and Alberta under a changing climate.

GENOTYPIC SELECTION IN Dipteryx alata Vog. IN THE MIDWEST REGION OF BRAZIL

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The success of forestry breeding programs is closely linked to the quality of seed trees selected to constitute their respective base population. The seed trees selected, according to the evaluation of progeny tests, gathers, among its features, the genetic merit transmitted to subsequent generations. The choice of species to be improved is also one of the important points to think about when conducting a breeding program because it has to possess qualities that make it attractive to the market. Thus, it was deemed advisable to study *Dipteryx alata*, also known as baru, which belongs to the family Fabaceae. It is an arboreal species of the cerrado and is characterized by possessing excellent qualities of products like wood and chestnuts. This work aimed to select progeny of D. alata, aiming at genetic improvement of the base population, consisting of three provenances. At eight years of age diameter at breast height (DBH) was measured. The experimental design was random blocks, with three populations, (16 progeny from GO, 42 progeny from MG, and 39 progeny from MS), five repetitions, six plants per plot, planted at 3.0 x 3.0 m. Estimates of variance components and genetic parameters were obtained by REML/BLUP. From these estimates 50% of the best individuals were selected; 232 plants from GO, 561 plants from MG, and 570 plants from MS. The average estimate for DBH of 463 individuals analyzed for GO was 7.42 cm, 7.80 cm of 1,121 individuals analyzed for MG, and 9.45 cm of 1,140 individuals analyzed for MS. Thus, selecting 50% the best genotypes will result in the DBH of the new populations becoming 9.63 cm for GO, 8.93 cm for MG, and 10.29 cm for MS. This results in a gain of 29.8% for the new generation for GO, 14.5% for MG, and 8.9% for MS. The selection, based on DBH, at eight years of age, in this progeny/provenances test, would result in considerable genetic gains and still keep the populations with sufficient genetic variation for new selections to be conducted in the future in order to produce wood or chestnuts of high nutritional value.

GENETIC VARIABILITY IN PROGENIES OF Dipteryx alata Vog. GROWING IN THREE STATES OF BRAZIL

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Dipteryx alata, known as baru, is an arboreal species of the cerrado characterized by having relatively fast growth, able to grow and develop in poor soils, and produces high quality wood primarily for use in the construction industry. In adulthood, the baru fruits annually and produces nuts with high commercial value. By having different qualities, this species has been exploited intensively resulting in it being endangered. Thus, this study aims to evaluate the genetic variability for diameter at breast height (DBH) and survival of progeny from three natural populations of D. alata, to provide information for conservation programs and breeding. In April 2004 we installed a progeny test with three natural populations of D. alata coming from the states of Minas Gerais (MG), Goiás (GO), and Mato Grosso do Sul (MS) in Farm of Teaching, Research and Extension of the Faculty of Engineering of Ilha Solteira/UNESP, located in Selvíria-MS. At eight years of age DBH was measured and survival assessed. The experimental design was random blocks with three populations, (16 progeny from GO, 42 progeny from MG, and 39 progeny from MS), five repetitions, six plants per plot, planted at 3.0 x 3.0 m. Estimates of genetic parameters were obtained by using the linear mixed model univariate additive software SELEGEN - REML/BLUP (restricted maximum likelihood / best linear unbiased prediction). The progenies of all populations showed good adaptation, with survival around 97%. The progenies coming from MS showed the greater DBH of 9.45 cm, corresponding to a mean annual increment (MAI) of 1.18 cm. Progeny from MG averaged 7.80 cm (MAI = 0.98 cm), and GO had 7.42 cm (MAI = 0.93 cm). The variation coefficient was good for all populations ranging from 13.23% (MS) to 17.77% (MG). The accuracy, which represents the ratio between the true genetic value and the estimated was high, ranging from 82.20% (MS) to 92.61% (GO).

Based on the likelihood ratio test (LTR) it was noted that the three populations showed variation for DBH. The same was not true for survival in which there wasn't a difference between the progenies coming from MG and GO, but there was variation among progenies coming from MS. In other words, survival was different for at least one of the progenies. For DBH, the coefficient of heritability of individual additive effects, coefficient of heritability of mean progeny, and heritabilities of additive effects within plots were 0.48, 0.70 and 0.49 for provenance MG, 0.72, 0.86 and 0.73 for provenance GO, and 0.35, 0.68 and 0.31 for provenance MS, respectively. Comparing the values of heritability, the coefficients of heritability on a progeny mean basis were higher than within progeny, indicating that selection based on plot rather than individual plants will result in greater gain. The coefficient of additive genetic variation for individuals was greater than the coefficient of genotypic variation among progenies in the three populations, with 24.34% and 12.17% for MG, 36.04% and 18.02% for GO, and 17.07% and 8.54% for MS, respectively. In the joint analysis there was a significant difference between the populations, with the population of Brasilândia (MS) contributing to positive genotypic values and Campina Verde (MG) and Itarumã (GO) producing negative values. Thus, a breeding program should emphasize the importance of provenance of the material to be collected. Thus, it was found that the populations of D. alata have a genetic basis and significant difference between them, which highlights the importance of preserving these as an active germplasm bank to serve as the base population for breeding programs.

INVESTIGATION OF INTRA-SPECIES VARIATION IN LODGEPOLE PINE (Pinus contorta) SECONDARY METABOLITE SYNTHESIS AS A DEFENSE AGAINST Dothistroma septosporum

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Over the past century the world's forests have faced increasing pressure from a variety of sources. From a forestry perspective, fungal diseases alone have caused considerable damage in terms of growth loss and costs of control measures. The damage caused by fungal pathogens has been especially severe in British Columbia, where native lodgepole pine (*Pinus contorta*) has suffered high mortality to foliar fungal pathogens such as *Dothistroma septosporum*. While chemical agents can be used to combat these pathogens in small plantations, their use over larger areas is considered impractical, and poses a potential threat to the native ecosystem. The preferred methods of control include enhancement of species and age class diversity, or selection for resistant species.

Resistance to fungal pathogens can be conferred through a variety of mechanisms. For lodgepole pine these have been found to include higher levels of defence chemicals. Greater levels of some secondary metabolites have been correlated with heightened resistance to foliar fungal infection. A high-throughput assay system was developed to determine the toxicity of these compounds to *D. septosporum* in media. Using this system, we have shown that a number of secondary metabolites associated with disease resistance also inhibit growth of *D. septosporum* in broth culture. Current work aims to correlate provenance metabolite profile differences with expression changes of producer synthases and the disease history of the original provenance locations.

An improved knowledge of the molecular basis for *Dothistroma* resistance will aid in the development of resistant varieties, and future correlation with disease history will improve prediction of outbreaks in a changing climate.
TREE SEED WORKING GROUP WORKSHOP

CONIFER REPRODUCTIVE BIOLOGY EVOLUTIONARY PATHWAYS

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The intent of this talk was to provide a baseline for conifer reproductive biology from its early beginnings to the current diversity found among north-temperate conifers. I consider it a work in progress. Time estimates of significant events are primarily based on fossil evidence and are therefore restricted to samples which have been 'fossilized' under appropriate conditions and have been found. Land plants appeared approximately 450 million years ago (MYA) and coincided with the appearance of mycorrhizal associations. These early land plants reproduced like ferns and were dependent on water for spore dissemination. Xylem evolution preceded seeds and this allowed plants to reduce potential for embolism under freezing or drought conditions, in addition to significant height increases and an increased competitive ability for sunlight.

The evolution of seeds is generally considered in terms of three large-scale evolutionary changes:

1) Heterospory – The differentiation of two separate spore types differing in size and sex. Considered to have occurred multiple times in plant evolution.

2) Megaspore Reduction / Retention in Sporophyte – The reduction to one female sprore (megaspore) from many and maintenance of the structure within the body of the parent plant (endospory).

3) Integument Evolution – The evolution of protective tissues to protect the innermost structures.

In conifers, a variety of pollen reception mechanisms evolved and these finalized the conversion to the water independence of spores for movement and fertilization. Seeds, in addition to mycorrhiza and xylem, are considered the major evolutionary factors allowing conifers to dominate large land areas, especially those with stressful or sub-optimal environments. Seeds can be thought of as "concentrated life" as the seed contains the future diploid tree, the initial food reserves, and the seed coat for protection. Seeds are the dispersal package and they also 'allowed' for the development of seed dormancy to synchronize germination with the appropriate environmental conditions to maximize survival and growth.

The two main groups of seed plants, Angiosperms (about 250,000 species) and Gymnosperms (600-700 species) diverged approximately 310 MYA. Inverted ovules, pointing towards the central axis, is a characteristic of most conifers and evolved approximately 265 MYA. The inverted ovules' orientation (up or downwards pointing) is related to the type of pollen present (saccate or non-saccate). Subsequent to ovule inversion there was also a division of the Pangeaen supercontinent about 200 MYA resulting in species isolation and evolution of some highly variable reproductive biology mechanisms among conifers. One third of all conifers are attributed to two genera: *Pinus* in the Northern hemisphere and *Podocarpus* in the Southern hemisphere.

Conifer cones are basically structures that house ovules, allow for pollen to enter, and then close to protect the embryo. In many species this is a woody structure, but *Taxus* and *Podacarpus* are examples of conifer genera with fleshy structures to house the seed. In general, pollen cones (microsporangia arranged spirally along a central axis) are relatively simple and each microsporangium is considered to be a modified leaf. These pollen cones do not show a great deal of diversification among genera. The seed cones (bract scale complexes arranged spirally along a central axis) are considered to be more complicated as each bract-scale complex is considered to be a modified shoot vs. a modified leaf as for pollen cones. In contract, seed cones have differentiated greatly in terms of gross morphology, ovule orientation, and type of ovular secretion mechanism.

BROADLEAF REPRODUCTIVE BIOLOGY

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Seed plants started to evolve 340 million years ago with Angiosperms arising about 130 million years ago, but it was about 100 million years ago that the number of species began to rapidly evolve. Angiosperms produce true flowers which can be either perfect or imperfect. Perfect flowers contain both male and female reproductive structures whereas imperfect flowers are either sex. Flowers occur in a variety of arrangements such as: spike, raceme, panicle, corymb, cyme, umbel, catkin, and head. Some genera or species are monoecious where both males and females occur on the same tree as opposed to dioecious species where males and females occur on separate trees. Pollen that is dispersed by wind (anemophilous) is small and is dispersed over great distances. Pollen from species that produce showy, fragrant flowers is generally dispersed by insects (entomophilous). This pollen is larger and its dispersal is generally limited. Broadleaved species produce fruit that contains the seed. The type of fruit produced is species-dependent. Examples include: achene - sycamore (Platanus occidentalis); double samara maple (Acer spp.); single samara – elm (Ulmus spp.), ash (Fraxinus spp.); acorn – oak (Quercus spp.); nut – hickory (Carya spp.), butternut (Juglans cinerea); legume - Kentucky coffeetree (Gymnocladus dioicus); capsule – poplar (Populus spp.); pome – apple (Malus spp.), mountain ash (Sorbus spp.); and drupe – cherry (Prunus spp.). Fruits are shed by most species in late summer and fall and are primarily dispersed by wind. Dispersal by animals is important for species such as mountain ash, cherries, oaks, and butternut. Seed of broadleaved species often exhibits dormancy due to seed coat, embryo, morphological or a combination. Seed coat dormancy can be alleviated by soaking seed in cold or hot water or treating the seed with acid to allow moisture to enter. Moist chilling or combinations of warm, moist incubation and moist chilling alleviate embryo and morphological dormancy.

REPRODUCTIVE BIOLOGY SYSTEM AND ITS IMPACT ON GENETIC ARCHITECTURE

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Since the days of Darwin, scientists have observed that life history traits are associated with patterns of population adaptation and diversity. Significant relationships between genotypic and adaptive diversity and genetic architecture have been demonstrated for numerous life history traits, the most consistent being: mating system, taxonomy, life form, successional status, range extent and contiguity, and dispersal mechanism of seed and pollen. In nature, many of these traits are correlated and few studies have evaluated the individual factors. Generally, longer-lived, late-successional, outcrossing, animal-dispersed perennials that are not endemic have higher expected and observed heterozygosity than annual, endemic or narrowly distributed, gravity- or wind-dispersed, pioneer species. The former group generally have less differentiated populations than the latter. However, there is wide variation among species. In addition to life history traits, many studies have quantified equally strong - sometimes even stronger external influences on plant genetic architecture. Dominant influences include: refugia and post-glacial range expansion, spatial and temporal disturbance patterns, soil and geology, hybridization, management interventions, sample breadth and size for genetic studies, and type of genetic marker used. Adding to the complexity, these factors can also have significant interactions with life history traits. While general patterns of total heterozygosity, population differentiation, and heterozygote deficiency are associated with life history characteristics, species-specific data based on a carefully designed study with clear objectives are needed to answer questions about any particular species.

CONIFER POLLINATION MECHANISMS REVISITED

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Pollination mechanisms in Gymnosperms can be divided into types, depending on the shape of pollen and ovule micropyle and whether pollination drops are present. Two general types exist: pollen capture mechanisms (PCM) and extra-ovular capture and germination (ECG). Of these, PCM has the greatest diversity, as these show six different sub-types. Phylogenetic analysis of modern and extinct gymnosperms reveals that pollination drops were fundamental to pollination capture mechanisms of the earliest seed plants. The most derived types of PCM are found within Pinaceae. ECG, also derived, is restricted to a few conifer families and genera. Pollination drops provide a nectar function in gnetophytes and possibly in cycads. This provides an independent origin to study convergent evolutionary patterns in plant-insect relations.

SEED PRODUCTION IN LODGEPOLE PINE

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Seed yields (filled seeds per cone) from north Okanagan (NO) lodgepole pine (*Pinus contorta* var. *latifolia*) orchards have not met expectations compared to seed yields routinely realized at Prince George (PG). Since the NO is substantially hotter and drier than PG, early efforts focused on better irrigation systems (broad cast sprayers) and crown cooling (misting). Data collected from Kalamalka Seed Orchard 307 over the period of 2000 to 2005 did not show any significant improvement in either cone numbers or seed yields. However, cones protected by insect bags had consistently more seed.

Since most NO orchards were experiencing poor seed production, in 2006 we began to collect standardized orchard statistics from eight orchards on four NO sites and two orchards at the PG Tree Improvement site. All 10 orchards produce seed for three PG and three southern interior seed planning units. We also compared production from two orchards of the same provenance base at PG and NO.

Over the period of 2006 to 2012, the trend for higher seed yields per cone but fewer cones at PG continued. Since the number of filled seed per cone (FSPC) was high and remained fairly consistent (20-25 FSPC) at PG, variation in the number of seed per tree principally resulted from variation in the number of cones per tree (100-300 cones per tree). Year-to-year variation occurred in all orchards but those NO orchards producing seed for the PG area (with one notable exception) consistently produced lower yields than those NO orchards producing seed for the southern interior. Orchard site also was important. Southern interior orchards in Armstrong, BC (Pacific Regeneration Technology Inc.) (PRT) equalled or exceeded that from PG and for the last two years have consistently out-produced all other NO orchards.

Over the seven years of observation, the loss of seed from un-bagged cones in all NO orchards ranged from about 2 to 11 FSPC. There was no seed loss from un-bagged cones at PG. Of the four NO orchard sites, Kalamalka and Tolko had the greatest losses (about 10) with the two PRT and Vernon Seed Orchard Company orchards each losing about 5 and 4 FSPC, respectively. The greatest loss of seed from un-bagged cones appeared to occur in August. Cones exposed for a two week period from April to the end of July were not significantly affected but cones exposed in August had significantly fewer seed per cone. Again, bagged and un-bagged cones from PG did not show any differences in seed yields.

There is no general agreement about the bagging affect. One side of the argument suggests the losses are too large to be caused by insects. However, bagged cones and insect management (spraying) all show better seed yields than untreated cones. Insect-protected cones can account for some of the losses but on average, we expect seed yields from lodgepole pine to be in the order of 20-25 FSPC. However, production from NO orchards ranges from 10-15 FSPC. If we account for 5-10 FSPC losses from unprotected cones, we still must account for the other 5-10 FSPC that may not be attributed to insect predation.

VARIATION IN REPRODUCTIVE CAPACITY OF LODGEPOLE PINE (Pinus contorta var. latifolia) IN BRITISH COLUMBIA

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Lodgepole pine (*Pinus contorta* var. *latifolia*) is the most wide-ranging pine in North America. Populations in British Columbia vary widely in phenotypic and genotypic characteristics and differences between populations can be linked to local climate or to geographic predictors. The effect of climate on variation in reproductive characteristics has never been examined, yet is vital to the production of seed necessary for reforestation. This study aims to determine the relationship between climate and variation in female cone and seed characteristics. The study makes use of the Illingworth provenance trial, sixty common garden plots that are distributed throughout British Columbia. Female cones from seven source populations were collected at 22 sites during the summer of 2012. Data processing to date includes measuring cone length, and determining the number of scales per cone. Initial results indicate wide variation between sites for both variables. Further data processing will include determining the number of seeds per cone. The final product of this study will be a response function relating climate variables to cone morphology and seed yield.

LODGEPOLE PINE CROP STATISTICS FROM SelectSeed Ltd. ORCHARDS

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SelectSeed Ltd. is wholly owned by the Forest Genetics Council of BC, a government – industry cooperative. It's mandate is the production of genetically selected tree seed within a financial objective of long-term sustainable profit and a modest return on capital employed. Nine of the 14 seed orchards operated by SelectSeed under contract are lodgepole pine (Pli) (*Pinus contorta* var. *latifolia*) (about 29 of 35 thousand ramets). As a result, Pli seed production and sales are key to the SelectSeed's long-term business prospects.

The original seed production and cash-flow expectations used for SelectSeed's business plan were based on the small amount of information available in about 2000. More recent information clearly shows issues with low seed production (filled seeds per cone) from Pli orchards located in the north Okanagan valley of south-central BC relative to orchards located near Prince George in central BC. Using seed production statistics from the nine SelectSeed orchards, comparisons are made between forecast and actual seed production statistics, with breakdowns for Pli derived from naturally occurring stands from seed planning units (SPU) located in southern BC vs. central BC, and between north Okanagan orchards vs. those located in slightly cooler environments just outside the Okanagan valley. A SPU is a geographic area where tree populations are genetically similar enough to allow seed transfer within the geographic and elevation boundaries of the SPU)

Overall seed production across the nine SelectSeed Pli orchards does not vary substantially from initial forecasts (Fig. 1). Orchards with selected trees from SPUs in central BC, however, have had poorer seed production than those from SPUs in southern BC. There is a confounding of SPU origin and orchard location that makes direct comparison difficult, but there is evidence that Pli populations from central BC SPUs, planted in orchard locations cooler than the Okanagan valley but not cool enough to be within naturally occurring lodgepole pine forests, are producing more seed than the same populations in north Okanagan orchard locations. Reduced seed production relative to potential is a significant financial concern to the company, even though actual production levels are close to initial forecasts.



Figure 1. Age-adjusted grams of lodgepole pine seed produced per orchard position for selected trees from northern and southern seed planning units (SPU).

QUESTIONS ABOUT THE ROLE OF SEED BUG HERBIVORY IN PRODUCTION COMING FROM INTERIOR LODGEPOLE PINE SEED ORCHARDS

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Herbivory by the western conifer seed bug (*Leptoglossus occidentalis*) has been assumed to be the principal cause of substantial annual shortfalls of seed coming from lodgepole pine orchards located in the interior of British Columbia. The difference between the number of filled seeds per cone (FSPC) produced in cones protected from seed bug attacks (by being enclosed inside nylon-mesh bags) and the numbers of viable seeds coming from cones left open in the environment has been used as an indicator of the amount of seed bug herbivory taking place in these orchards. This is the 'Seed Bug Model' for losses occurring in this cropping system. Operational production records from Kalamalka lodgepole pine Seed Orchard 307 for 2001 to 2010, taken with bagging trial results from this orchard for the same years, allow us to make numerical estimates of whole-orchard FSPC shortcomings occurring annually during the past decade. These results are discussed in the context of whether they are, or are not, consistent with a Seed Bug Model.

For over fifteen years, concerted efforts have been made to establish a relationship between the number of seed bugs observed in Orchard 307 and the FSPC losses attributed to seed bug herbivory. These efforts have not been successful as no seed bug density:crop damage relationship and no economic threshold have ever been established for this cropping system. Without an economic threshold, pesticide treatments for seed bugs have never been conducted in a manner consistent with the modern practice of Integrated Pest Management. Beyond this consideration, comparisons of FSPC values derived from annual crop production statistics with FSPC values for cones held in bags show that applications of the pesticide Sevin for seed bug control never have raised operational seed yields to the levels obtained inside the bags. In fact, seed deficits of one-third or more of the crop occurred in each of eight years in which pesticides were applied for seed bug control. These results were not consistent with expectations coming from the Seed Bug Model. If FSPC losses were being caused by insect herbivory, why did treatments by an insecticide fail to increase FSPC numbers close to those seen inside the bags?

Work done by Dr. Joe Webber and colleagues in 2010 showed that major FSPC losses occurred in cones that were not enclosed inside bags during the month of August. Being exposed to the environment through any two-week period from the beginning of May to the end of July did not significantly reduce FSPC values. Timing of harvest trials done in 2011 showed a similar temporal profile for FSPC declines. However, none of the cones used in the 2011 trial were held in bags during any point of the growing season. Versions of both experiments have been replicated across multiple years at several locations. Now, it is recognized that the majority of the FSPC losses observed in north Okanagan lodgepole pine orchards occur in August.

By using the FSPC deficits derived from bagging trials and multiplying this by the number of cones harvested in any given year (derived from crop production statistics), we can calculate the number of seeds 'lost' during a growing season. For 2010, this number was estimated to be about 5.4 million seeds. Bagging experiments indicate that these FSPC shortcomings occurred in August. Using this information, we can calculate the number of seed bugs needed to cause the FSPC deficits observed for Orchard 307 in 2010:

Over the course of 30 days, 5,374,000 seeds were lost, eaten by seed bugs with a maximum individual daily herbivory rate of 5 seeds per day. This loss would require a minimum of 35,826 seed bugs to be actively feeding, each eating their daily maximum number of seeds on every day of the month.

Orchard 307 was harvested through August 2010. Staff members spent a minimum of 175 person-hours per week in the orchard in August and they physically handled every cone that was picked. Although workers reported seeing seed bugs, we never received any reports of seed bugs being seen in anything approaching the numbers that would be required to cause these FSPC losses under the Seed Bug Model.

In summary, there never has been a relationship established between seed bug population sizes and the FSPC damage that they reputedly cause. Pesticide treatments have never raised FSPC levels in the operational harvests to those predicted (from bagging trials) to be possible if seed bugs were causing the FSPC losses. With the recently acquired knowledge of when the FSPC declines occur during the growing season, we can calculate how many seed bugs must be present in Orchard 307 to cause the losses attributed to them by the Seed Bug Model. Nothing even close to the number of seed bugs required to cause these deficits was observed in 2010, nor have they been observed in any other year during the past decade. It is for these reasons that I question the Seed Bug Model. I do not believe that the differences in FSPC observed between cones held inside bags and cones left exposed in the environment can be solely attributed to herbivory by seed bugs.

Currently, there are a number of investigations that are examining other aspects of this problem:

1) seed samples taken through the decline period are being examined microscopically to observe the physiological aspects of losses of filled seed,

2) the relationship between seed set, area of origin of the orchards, temperature and seed declines is being investigated in field trials,

3) in-cone temperatures and seed bug herbivory are being monitored on selected cones throughout the growing season,

4) harvesting schedules that incorporate the timing of FSPC declines by clone are being developed to maximize the yields coming from interior lodgepole pine seed orchards, and

5) a method to detect seed bug herbivory on individual lodgepole pine seeds is being developed.

It is hoped that this multi-faceted approach will help to address the unanswered questions that remain about seed losses occurring in interior lodgepole pine orchards.

LEPTO-CAMS AND THERMOCOUPLES: WHY DO INSECT BAGS PREVENT AUGUST PII SEED LOSS IN THE INTERIOR OF BC?

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Lodgepole pine (*Pinus contorta* var. *latifolia*) seed orchards in the Southern Interior of BC have suffered from low seedset for many years. The causes largely remain a mystery, despite years of research. In 2011 and 2012, it was discovered through bagging studies that much of the seed loss occured in August. The August seed loss is entirely preventable by enclosing the cones in mesh bags, while seed loss earlier in the season was not affected by mesh bags.

Only two theories exist as to why mesh bags prevent seed loss. One is that the bags alter the microclimate, protecting the cones from, for example, extreme heat events. The other is that the bags exclude *Leptoglossus occidentalis*, the western conifer seedbug, which might feed on seeds during August. Both theories were tested during the summer of 2013.

The microclimate theory was tested by choosing 4 cones on each of 10 lodgepole pine trees in commercial seed orchards in Vernon, BC. Cones were close together, with similar aspect and tree height. A one millimetre hole was drilled into the base of each cone, and a small K-type thermocouple was inserted and held in place with zip-ties. All four thermocouples on each tree were connected to a 4-channel datalogger that recorded the internal cone temperatures at 5 minute intervals. Two of the cones on each tree were enclosed in a mesh bag; the other two were left unbagged. Internal cone temperatures were compared between bagged and unbagged cones, examining maximum temperatures and times spent above various temperature thresholds.

The seedbug theory was tested by installing one time-lapse camera in each of the 10 trees used in the thermocouple experiment. Each camera was aimed at a cluster of 3-4 cones and set to take photos at 5 minute intervals. A similar cone cluster on a nearby branch, with the same aspect and tree height, was enclosed in a mesh bag. Photos were examined for the presence of seedbugs, which were categorized by activity: feeding, walking, mating, etc. At season's end, bagged and unbagged cones will be harvested and seeds extracted to determine the extent of filled seed loss.

As of July 3, 79,206 cone temperature measurements had been taken. Mean cone temperatures were always slightly higher in bagged than in unbagged cones. Bagged cones also had more hours above any given threshold temperature, such as 35°C. There is more variation in cone temperatures between trees and between cones within a treatment, than between treatments. No statistical analysis has been conducted yet, but it appears that cone temperatures are unlikely to be the cause of the August seed loss.

By July 3, 81,425 images had been collected and examined. Of these, 1,703 images showed seedbugs on or near the cones. Feeding was a dominant activity. Each cone cluster was fed upon from 0 to 19.7 hours. Seedbugs were never observed feeding on two cone clusters. These sightings confirm previous evidence that there is a clonal difference in seedbug host preference. It also confirms the likelihood that seedbugs are responsible for the August seed loss.

However, these data only record events up to July 3. Things might change during the month of August. Bags might shield cones from extreme heat events not experienced during May or June. Seedbugs may not feed upon cones during August. Though this evidence is suggestive, we must wait until our August data have been collected before drawing conclusions.

SEED SHORTFALL IN LODGEPOLE PINE

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Samples for histological study were collected from a seed orchard located at Kalamalka Research Station, Vernon, BC. Weekly sample collections were completed between mid-July and mid-September, 2012. A big increase in damaged seed was confirmed during August. The percentage of filled seed per cone (FSPC) diminished progressively over the course of the first two weeks, reaching levels of under 50% FSPC, where it stayed until mid-September. Under a dissecting microscope, seed death occurred in two steps: death of the megagametophyte was followed by that of the embryo. Dying tissues ranged in colour from yellowish-brown to dark brown. Both gametophyte and sporophyte, i.e., embryo, were soft in texture, unlike their healthy counterparts, which were not only firm, but of much lighter hue.

Histological analysis revealed tissue degeneration in the seed. Healthy tissue was composed of storage cells that had abundant reserves of protein bodies, starch grains, and lipid bodies. The cells were tightly appressed to one another. Tissue degeneration was categorized as three types: Type I began with the appearance of tiny intercellular spaces. These spaces increased gradually in size and then the tissue developed large holes. Fungal hyphae were frequently observed in cells as well as intercellular spaces. Type II degeneration had the appearance of cell liquidization. Cell walls were dissolved and cell contents were amorphously coagulated. Yellow particulate structures were frequently observed. Type III was progressive loss of cell contents until only cell walls remained. Protein body breakdown was followed by vacuolation and nuclear disintegration. Tissue integrity failed with cells showing signs of cytoplasmic collapse and cell wall rupture. Types I and II occurred randomly at multiple loci in a megagametophyte. Different stages of Type I degeneration were sometimes present in the same tissue. Types I and II were found in samples from all collection dates, whereas Type III was observed in all dying megagametophytes that had softened tissue.

Our histological study does not support the possibility of Leptoglossus occidentalis-related seed loss. However, our study did not exclude the possibility of other organisms that might contribute to seed loss. The presence of vellow particles found in many of the samples suggests the presence of an unidentified organism or an unknown aggregation phenomenon. A major reason for degeneration in seed was the presence of a fungus. Hyphae were widespread in holes that developed in the degenerating megagametophyte in many of the samples. In conclusion, seed shortfall in lodgepole pine shows a progressive degeneration of the seed and there are different types of tissue degeneration which can occur in the absence or presence of a fungus and/or some unidentified particles implying that there is more than one biotic or abiotic factor responsible for seed death. High temperatures in August may accelerate the process of seed degeneration.

METHODOLOGY TO ORGANIZE OPERATIONAL LODGEPOLE PINE CONE HARVESTS BY USING OBSERVATIONS OF SEED SET DECLINES

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Poor seed set in North Okanagan lodgepole pine (*Pinus contorta* var. *latifolia*) seed orchards has been an issue for a number of years. Results from numerous trials indicate that seed set levels are acceptable until early in August. After this point seed set declines rapidly. Each lodgepole pine clone experiences this sudden loss of seed at a different point in time. We relate this period to growing degree days.

In order to increase seed production, each clone must be harvested at the correct time. If the cones from a particular clone are harvested too early seed quality is poor. If the same clone is picked after its unique seed decline period has started seed production is poor.

We are working on developing a method to identify the best time to harvest each clone. We collect weekly cone samples from throughout the summer and x-ray the seed in order to identify the onset of sudden seed decline. Relating this information to degree days will allow us to schedule our cone harvest timing so that each clone is collected when seed yield and seed quality are at acceptable levels.

ATTENDEES OF THE 33rd CFGA/ACGF MEETING

Aguiar, Ananda Embrapa Florestas Brazil

Aitken, Sally University of British Columbia Canada

Aldana, Juan University of Victoria Canada

Alger, Genoa University of Victoria Canada

Almeida, Maria Technical University of Lisbon Portugal

Almeida-Rodriguez, Adriana University of Montreal Canada

Angert, Amy University of British Columbia Canada

Arneiro, Lidia Universidade Estadual Paulista Julio de Mesquito Filho Brazil

Arneiro, Joao Universidade Estadual Paulista Julio de Mesquito Filho Brazil

Arshad, Muhammad Simon Fraser University Canada

Baltunis, Brian Weyerhaeuser USA

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