

2007

PROCEEDINGS OF THE THIRTIETH MEETING
OF THE
CANADIAN TREE IMPROVEMENT ASSOCIATION

PART 1 Minutes and Members' Reports
PART 2 Symposium

Canada's Forests – Enhancing Productivity,
Protection & Conservation



Les forêts du Canada : mise en valeur
de la productivité, de la protection et
de la conservation

COMPTES RENDUS DU TRENTIÈME CONGRÈS
DE
L'ASSOCIATION CANADIENNE POUR
L'AMÉLIORATION DES ARBRES

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° PARTIE Procès-verbaux et rapports des membres
PARTIE Colloque

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

**Jean Beaulieu, Gaëtan Daoust, Nathalie Isabel, Marie Deslauriers
and Manuel Lamothe**

**Natural Resources Canada
Canadian Forest Service
Laurentian Forestry Centre
PO Box 10380, Stn. Sainte-Foy
Québec, QC
G1V 4C7**
E-mail: Jean.Beaulieu2@nrcan.gc.ca

Keywords: white spruce, black spruce, eastern white pine, Canada yew, Norway spruce, larches and poplars, *Picea glauca*, *Picea mariana*, *Picea abies*, *Pinus strobus*, *Taxus canadensis*, *Larix* spp., *Populus* spp., growth-related traits, wood quality, warp, shrinkage, lumber, open-pollinated families, climate change, pedigree, ESTPs, AFLPs, microsatellites, RAPDs, SNPs, mtDNA markers, genomic mapping, association mapping, QTLs, species-specific markers

This report summarizes the progress of the Canadian Forest Service, Laurentian Forestry Centre (CFS) research in forest genetics, genomics, molecular ecology, and biodiversity for the period 2004–2006. Our activities have mainly focussed on advanced genetics and breeding work in three spruces and eastern white pine (*Pinus strobus*). We have also continued to invest considerable time and effort in population genetics studies of forest tree species and in genomics in collaboration with J. Bousquet from Université Laval and as part of our participation in the activities of the Canada Research Chair in Forest and Environmental Genomics (Tier I) and via a strategic grant obtained from Natural Sciences and Engineering Research Council (NSERC) for the development of a phylogeographic atlas of Canadian conifer tree species. Research was also done in collaboration with our colleagues from Forintek Canada Corporation and Université Laval on genetic variation and control of wood characteristics. One of the projects was funded by the Fonds québécois de recherche sur la nature et les technologies. We are also co-investigators of the genomics research program ARBOREA (Mackay, Bousquet, et al.), a major initiative, funded by Genome Canada and Genome Quebec for developing molecular breeding in softwood trees. On another front, in collaboration with Université Laval (J. Bousquet and D. Khasa) and Forêt Québec (P. Périnet et al.), nuclear diagnostic markers for the recognition of *Populus* species and their hybrids have been developed.

Research accomplishments were made possible with the generous contributions of dedicated collaborators and staff who deserve our thanks. They are: René Pâquet, Daniel Plourde, Jean-Paul Bilodeau, Sébastien Clément, France Gagnon, Marie-Claude Gros-Louis, Patrick Meirmans, Karine Plante, Esther Pouliot, Patricia Sylvestre, Michèle Bernier-Cardou, Pamela Cheers, and many summer students.

WHITE SPRUCE

Quantitative Genetics

Over the last 2 years, we completed a study initiated in collaboration with Tony Zhang (Forintek Canada Corp.) and André Rainville (Ministère des Ressources naturelles et de la Faune (MRNF)) to estimate genetic parameters of white spruce (*Picea glauca*) wood traits in order to develop a breeding program for growth performance and quality end-products. A genecological test, including 40 open-pollinated families from the Great Lakes-St. Lawrence region and replicated on two sites, was thinned in 2001. Two 210 cm logs were taken from each of the 320 36-year-old trees sampled and transported to the Forintek Canada Corp. facilities in Sainte-Foy, Québec. Logs were processed into 2 x 4 studs for analysis of lumber mechanical properties. As expected, the mechanical properties of lumber from young plantation-grown trees were low (Beaulieu et al. 2006, in press). The low wood density, the occurrence of numerous large dimension knots, and a high proportion of juvenile wood are the main factors contributing to low lumber stiffness and strength properties. The narrow-sense heritability for lumber stiffness was low to moderate (0.279) whereas that of strength was hardly different from zero (0.016). The environmental growing conditions appear to highly influence young white spruce wood mechanical properties. Strong negative correlations between stem volume and lumber stiffness and strength at the family means (-0.43 and -0.34, respectively) suggest that selection for stem

volume would have an indirect negative effect on lumber quality. The white spruce reforestation program is one of the most important in Québec with close to 30 million seedlings planted yearly. This indigenous species is valued for its high yield (over 420 m³/ha were obtained at 38 years with a fast-growing progeny test established at Valcartier, for instance) and its general tolerance to insects and disease.

In order to estimate the potential impact of climate change on growth of white spruce populations, transfer models were developed to predict the performance of seed sources based on temperature and summer precipitation differentials between the geographical origin of seed sources and experimental site locations. To do so, data were collected in a genecological test, replicated on three sites, and involving 45 distinct geographical seed sources from the province of Québec, most of them represented by five open-pollinated families. We found that white spruce populations located within the sampled area were optimally adapted to their local environment for thermal conditions but not for moisture conditions; populations that originated from sites receiving more precipitation generally showed higher tree growth than the local sources (Andalo et al. 2005). We predicted that the adaptive lag related to precipitation would increase under global warming conditions. Simulations of growth under various climate change scenarios indicated that it would be tangibly diminished under more intense warming.

Thanks to a grant from the Fonds québécois de recherche sur la nature et les technologies awarded to one of us and J. Bousquet, a biophysical site index model was developed for white spruce plantations in Québec. This model makes it possible to predict the white spruce site index (average height of dominant trees at 25 years) for any location based on degree-days and summer precipitation. A GIS-tool was then developed to map both site index and plantation yield at any age for a given spacing. Moreover, using data of selected families from a series of progeny tests, we estimated a genetic-gain multiplier for height which can be used to multiply the predicted site index in order to estimate genetically improved plantation yield. Results will be presented in a scientific paper that is in preparation.

The seed source transfer model and the biophysical site index model described above, both based on temperature and precipitation, were combined to obtain realistic estimates of white spruce plantation yield under future global warming conditions. Both models as well as white spruce yield tables were incorporated into BioSIM, a climate simulator developed by J. Régnière from CFS, to estimate and map plantation yield up to 2070 (Beaulieu and Rainville 2005). Our simulation results predict that global warming should favour a slight increase in white spruce plantation yield in southern Québec. However, one cannot expect to obtain similar yields from a seed source rapidly exposed to warmer conditions compared with a seed source that is presently growing under climatic conditions to which it has become adapted. The simulator is used to revise seed source transfer rules.

A comparison of early height growth between seedlings and rooted cuttings was carried out using annual measurements collected over 5 years in a farm-field test replicated on three sites. The experiment included 148 full-sib families and 15 open-pollinated families used as controls. Average total heights of seedlings and rooted cuttings were 186 cm and 171 cm, respectively, after the 5-year period. The difference in height between both types of plants at the end of the testing period was about of the same magnitude as that at out-planting. The average growth rate of seedlings was slightly larger than that of rooted cuttings and the yearly growth rate increased over time. It did so at a somewhat higher rate for the seedlings. Variance components associated with families suggested that important gains in early growth might be achieved by bulking up superior families through cuttings. Producing rooted cuttings is more expensive than producing seedlings. This is why this vegetative propagation method can be efficient only when there is a need to bulk-up scarce seed supplies. However, as family ranking based on rooted cuttings is similar to that of seedlings, it could be used to obtain better estimates of breeding values.

Genomics

During the period under review, two grants were obtained from the Canadian Biotechnology Strategy to initiate one project on QTL mapping and an association study of growth-related traits and another one on association mapping of wood traits, both in white spruce. These grants were also used as co-funding for two of the research activities of the ARBOREA Project, a major initiative, funded by Genome Canada and Genome Québec for developing molecular breeding in softwood trees and co-led by J. Mackay and J. Bousquet from Université Laval. So far, primer pairs to amplify DNA sequences of candidate genes for wood traits have been developed for over 200 genes. Discovery of SNPs in gene sequences is underway. Our goal is to design primers to amplify DNA sequences of over 500 candidate genes for both growth-related traits and wood traits. We will identify favourable alleles responsible for a significant proportion of variation in traits related to growth (budset), wood formation, and quality. For growth-related traits, two mapping populations of more than 300 individuals each were developed. For wood traits, a discovery population of 495 trees was assembled as well as a validation population of 200 trees. Increment cores were collected from these trees and phenotypic

values for a variety of wood traits and properties are being assessed at Paprican's facilities in Vancouver. Association studies will be carried out over the next 2 to 3 years thanks to the grant from Genome Canada and Genome Québec.

The development of pedigree populations continued. Single-pair matings were carried out in order to have access to a large number of unrelated families for the genomics studies. A population of 1,000 trees, one from each of 1,000 unrelated families, was assembled to carry out an association study between candidate genes and growth and phenology traits. Seeds were sown in February 2005 and seedlings were submitted to accelerated growth in a heated greenhouse during winter 2006. Cuttings will be collected from each of the seedlings and rooted during summer 2006 with the collaboration of Michel Rioux from the Saint-Modeste cutting centre.

BLACK SPRUCE

Quantitative Genetics

There is still some controversy about the determination of transition from juvenile wood to mature wood in conifers. Several methods have been proposed including visual examination of pith-to-bark profiles and mathematical approaches such as Gomperts function, segmented regression techniques or polynomial regression models. A study was carried out to characterize the average trend in growth and wood density as a function of age and to obtain consistent estimates of transition age from juvenile to mature wood in black spruce (*Picea mariana*) using about 1000 trees from a 50-year-old plantation (Koubaa et al. 2005). Radial patterns of wood density, ring width, latewood density, and latewood proportion obtained by X-ray densitometry of increment cores were modeled with polynomial regressions. It was found for all traits measured that the radial profiles were typical of those previously reported for *Picea* species. Significant differences among the various classes of diameter observed in the plantation were also disclosed for all the traits. The juvenile wood production period varied with growth rate and transition age for a given diameter class also varied, depending on the trait. Hence, it appears that in black spruce, transition age needs to be defined based on physiological processes.

Structural Genomics

In collaboration with J. Bousquet (Université Laval), individual and composite linkage maps of the black-red spruce complex species have been published (Pelgas et al. 2005). Over the past two years, PhD student B. Pelgas and MSc student S. Beauseigle, supervised by J. Bousquet (Université Laval) and co-supervised by one of us, have focused on further mapping the genome of white spruce (one composite map) and establishing a composite map for black spruce. In collaboration with colleagues from the Université de Nancy (S. Jeandroz and V. Achere), macro-synteny and macro-collinearity among divergent spruce species, including black spruce, white spruce, and Norway spruce (*Picea abies*), were estimated (Pelgas et al. submitted). This joint project with J. Bousquet was funded by a NSERC-genomic grant and the Canadian Biotechnology Strategy.

JACK PINE

Phylogeography

A highly variable minisatellite locus was discovered in the mitochondrial DNA of jack pine (*Pinus banksiana*) and the pattern of intra- and interpopulation variation was studied using this marker by Julie Godbout, a PhD student supervised by J. Bousquet and one of us (Godbout et al. 2005). By examining the modern population structure of mitochondrial DNA diversity along its natural range, three relatively homogeneous groups of populations could be delineated and they are presumably representative of genetically distinct glacial populations. The first one covers the region extending from Lake Huron to the Yukon. The second one is located in the southeast and mainly gathers populations located in eastern Ontario and south of the St. Lawrence River in Québec. The third group is located in the Maritimes. The last distinct group was observed in central Québec, which harbours a higher level of genetic diversity within populations and a lower degree of population differentiation than other groups. This region is likely a zone of suture between migration fronts of the three ancestral populations colonizing the three other zones. Based on these results and on the available fossil record, it was possible to put forward hypotheses regarding the number and the location of glacial populations and refugia for jack pine during the Last Glacial Maximum. Because of startling differences between western and southeastern populations, it is likely that there was a glacial population located west of the Appalachian Mountains in the United States, which was genetically distinct from another one located

east of the mountain range. Because of the genetic distinctiveness of Maritimes populations, a third glacial population was presumably located on the unglaciated northeastern coastal area in Canada. This last hypothesis is also supported by botanists and entomologists. Recommendations about seed source movement were also made. Hence, it was recommended to avoid any transfer between the Maritimes and the regions west of the province of Québec, and vice versa. Moreover, any *in situ* conservation decisions should take into account the phylogeographical structure reported in that study.

ENVIRONMENTAL GENOMICS

Estimation of effective gene flow levels towards native species represents the first step in the assessment of ecological risks linked to the introduction of GMO trees in Canada. In order to evaluate the risks of such gene flow, several authors have recently suggested assessing spontaneous introgression levels using old exotic material already established in the field. A grant was obtained from the Canadian Regulatory System of Biotechnology to estimate the level of spontaneous hybridization between exotics and their native counterparts to develop an alternative method for measuring the impact of the introduction of genetically modified trees on the integrity of local populations. The study is being conducted by one of us and a postdoctoral fellow (P. Meirmans, University of Amsterdam), and also a M.Sc. student (G. Guigou) supervised by D. Khasa and J. Bousquet. For larches, species-specific markers already developed by us (Gros-Louis et al. 2005) were used to monitor the rate of gene flow from plantations of exotic species of *Larix* into natural populations of their respective native congeners. For *Larix*, we use Primer Extension and CAPS techniques to genotype paternally inherited chloroplast markers and maternally inherited mitochondrial markers, allowing for a rapid identification of first-generation hybrids. For *Populus*, we use SNP stream technology to simultaneously genotype 12 nuclear, species-specific, SNPs (Meirmans et al., in preparation) allowing for a high power of identification of first- and second-generation hybrids. Preliminary results for *Larix* show that the rate of hybridization is quite low: genotyping 1,779 offspring produced by 98 trees of native *L. laricina*, we found 2.2% hybrids. The funding has just been renewed and the next phase of the project will focus on population genetics and hybridization of poplar species across Canada in collaboration with B. Schroeder (AgriFoodCan, Saskatchewan) and B. Thomas (University of Alberta).

ÉPINETTE DE NORVÈGE

Les efforts concertés du Service canadien des forêts (SCF) et du MRNF ont permis de poursuivre le programme conjoint d'amélioration génétique. Au cours de la dernière période, les efforts du SCF ont surtout été consacrés à l'entretien et au mesurage des dispositifs mis en place au cours des dernières années. Par la suite, plusieurs dispositifs établis avec des espacements restreints ont fait l'objet d'éclaircie systématique. Le parc d'hybridation de Valcartier, regroupant plus de 350 clones, a été éclairci afin de permettre un meilleur développement des cimes et une plus grande production de cônes à long terme. Ce parc d'hybridation est utilisé pour la production de semences et de croisements dirigés devant servir au programme d'amélioration et au programme de reboisement et de production de boutures du MRNF.

L'étude entreprise pour étudier l'impact du charançon sur la productivité et la qualité du bois a été complétée et sera publiée sous peu (Daoust et Mottet 2006). Plusieurs présentations de cette étude ont été faites auprès des aménagistes et des utilisateurs de cette ressource. L'étude a permis de faire ressortir, malgré l'impact du charançon, le potentiel de cette espèce lorsqu'on la compare à l'épinette blanche tant pour la productivité que pour les caractéristiques du bois.

PIN BLANC ET PINS HYBRIDES

Le programme d'amélioration du pin blanc s'est poursuivi au cours de la dernière période. La majorité des ressources disponibles a été consacrée à l'établissement et au maintien des dispositifs expérimentaux en région et à Valcartier. Des efforts soutenus pour lutter contre le charançon du pin blanc et la rouille vésiculeuse dans les jeunes dispositifs expérimentaux ont été maintenus.

Les tests de descendances issues de pollinisations libres entre les clones de notre population d'élevage ont été mesurés après 5 ans. Les analyses préliminaires ont permis de formuler des recommandations à la Division des semences et des plants (DPSP) du MRNF afin de mieux cibler les descendances les plus performantes à récolter dans leurs vergers à graines ayant du matériel commun avec notre population d'amélioration et débuter des éclaircies dans ces derniers.

Soixante-quinze clones d'un verger à graines de l'Outaouais, constitué de sélection locale, ont été échantillonnés en 2004, une excellente année semencière. Un test de descendances a été démarré à partir

de ce matériel et les semis seront repiqués en pépinière sous forme de dispositif expérimental en vue d'une évaluation à trois ans. Par la suite, les résultats seront transmis à la DPSP pour la récolte sélective et l'éclaircie du verger. Éventuellement, le dispositif mis en place à la pépinière sera utilisé pour l'établissement de tests de descendances dans la région ciblée pour ce verger à graines.

Plusieurs croisements dirigés et libres entre le *P. wallichiana* et *strobos* ont été ensemencés en 2005 en vue de vérifier la résistance à la rouille vésiculeuse du pin blanc. Les tests d'inoculation artificielle seront réalisés en 2006.

IF DU CANADA

Les tests clonaux mis en place dans Charlevoix et à Valcartier par la Société d'aide au développement de la collectivité de Charlevoix et le SCF ont été échantillonnés pour des analyses de taxanes. La croissance relativement lente des plants n'a pas permis de faire un échantillonnage complet tel que planifié au départ. Les analyses chimiques des contenus en taxanes ont été réalisées. Ces résultats seront comparés avec les données des populations de départ et devraient permettre de valider si le contenu en taxanes est sous contrôle génétique. Une affiche scientifique portant sur ce projet a été présentée à la première conférence internationale portant sur les « Crop Wild Relative Conservation and Use » tenue en Italie à l'automne 2005.

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