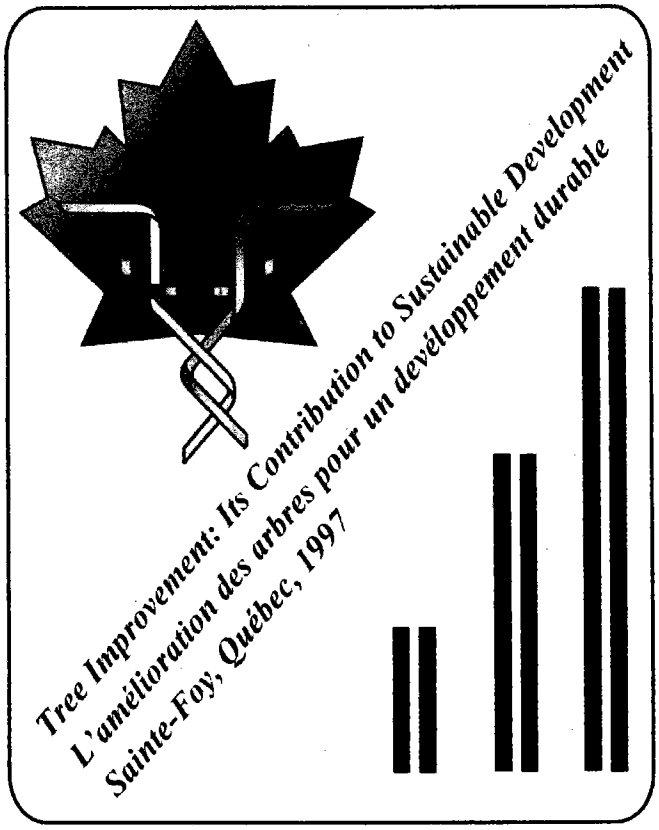
PROCEEDINGS OF THE TWENTY-SIXTH MEETING

OF THE

CANADIAN TREE IMPROVEMENT ASSOCIATION

PART 1 Minutes and Members' Reports
PART 2 Symposium



COMPTES RENDUS DU VINGT-SIXIÈME CONGRÈS

DE

L'ASSOCIATION CANADIENNE POUR

L'AMÉLIORATION DES ARBRES

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

PROCEEDINGS

OF THE

TWENTY-SIXTH MEETING

OF THE

CANADIAN TREE IMPROVEMENT ASSOCIATION

PART 1

Minutes and Members' Reports

TREE IMPROVEMENT: ITS CONTRIBUTION TO SUSTAINABLE DEVELOPMENT

Sainte-Foy, Québec
August 18-21, 1997

Editor
J.D. Simpson

Additional copies of this
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COMPTES RENDUS

DU

VINGT-SIXIÈME CONGRÈS

DE

L' ASSOCIATION CANADIENNE POUR L' AMÉLIORATION DES ARBRES

1ere PARTIE

Procès-verbaux et rapports des membres

L' AMÉLIORATION DES ARBRES POUR UN DÉVELOPPEMENT DURABLE

Sainte-Foy, Québec
18-21 août 1997

Rédacteur
J.D. Simpson

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PROCEEDINGS OF THE TWENTY-SIXTH MEETING OF THE CANADIAN TREE IMPROVEMENT ASSOCIATION

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CTIA/ACAA 26th BUSINESS

MEETING MINUTES

C.T.I.A./A.C.A.A
26th Business Meeting
Minutes

Michel Villeneuve chaired the 26th Business Meeting of the CTIA/ACAA held in the Multimedia Room, Pavillon Desjardin, Québec City, Québec on Wednesday August 20, 1997. Twenty-two members were present.

296 Minutes of the 25th Meeting
(as printed in the proceedings from the 25th meeting, Part I)

Motion: The minutes of the 25th Business Meeting be approved as published.
Moved by: Kathy Tosh
Seconded by: Jerry Klein
Carried.

297 Membership

297.1 Honourary Membership

Motion: That the following members of the CTIA/ACAA be nominated for "honourary" membership for the outstanding contributions to the field of genetics and tree improvement in Canada during their career.

Jim Coles	Consultant
Alan Gordon	Ontario Ministry of Natural Resources
Gyula Kiss	BC Ministry of Forests
Jerry Klein	Consultant
Yves Lamontagne	Ministère des Ressources naturelle du Québec
Gordon Murray	Natural Resources Canada-Petawawa
Louis Parrot	Université Laval
Hugh Schooley	Natural Resources Canada-Petawawa
Gilles Vallée	Ministère des Ressources naturelle du Québec

Moved by: Jean Beaulieu
Seconded by: Gaétan Daoust
Carried.

297.2 Active Membership

The names of nominated active members were presented as follows:

Tannis Beardmore	Canadian Forest Service Fredericton, NB
Francine Bigras	Service canadien des forêts Sainte-Foy, Qc

Anne-Christine Bonfils	Canadian Forest Service Ottawa, ON
Peter Copis	Canadian Forest Service Chalk River, ON
Nathalie Isabel	Service canadien des forêts Sainte-Foy, Qc
Sylvie Laliberté	Université du Québec à Montréal Montréal, Qc
Wade MacKinnon	Dept. Agriculture and Forestry Charlottetown, PEI
Bryce MacInnis	New Brunswick Tree Improvement Council Fredericton, NB
Jon Sweeney	Canadian Forest Service Fredericton, NB
Rong-cai Yang	University of Alberta Edmonton, AB
John Quinn	Alberta Land and Forest Service Smoky Lake, AB

Motion: That the nominated active members be duly elected.
Moved by: Jean Beaulieu
Seconded by: John Dojack
Carried.

298 Chair's Report

Organizing the 26th meeting of the association was a challenge that could not be met without the support of numerous people from the Ministère des Ressources naturelles, Ressources naturelles Canada, Université Laval, Forintek Canada Corporation, and I.U.F.R.O.-S5.01(wood quality). The 1995/1997 executive committee (Jean Beaulieu, Jean Bousquet, André Rainville and myself) particularly appreciated the efforts of Pierre Bélanger (logistics coordinator) and François Larochelle (on-site arrangements).

Two excellent workshops were presented by the Tree Seed Working Group (chaired by Stéphan Mercier) and the Wood Quality Working Group (chaired by Tony Zhang, with the contribution of IUFRO-S5.01). Proceedings of the first will be published in the Forestry Chronicle; for the latter, they were included in the registration package (copies may be obtained by contacting Forintek Canada Corp.).

Thanks to the voluntary contribution of Jean Ménétrier, the logo of the CTIA/ACAA is now available in digitized formats for use on letterheads and other official documents. Designer of the cover page

on the program and the proceedings, Jean is a biologist, tree lover and artist who works as a researcher for the Ministère des Ressources naturelles du Québec.

299 Treasurer's Report

The financial statement for the period of July 30, 1995 to July 30, 1997 was prepared by Treasurer Linda DeVerno and tabled for information and acceptance by the membership (see Attachment #1). The statement shows a balance of $4,539.99 in the Association's account and GIC's totalling $23,000.00.

Motion: That the financial statement be accepted as presented.
Moved by: Jean Bousquet
Seconded by: Alvin Yanchuk
Carried.

300 Financial Contributions

Avenor Inc.
Campbell Scientific (Canada) Corp.
Harnois Inc.
Industries James Maclaren Ltée
IPL Inc.
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Quebec Balsam Export Inc.
an anonymous contributor

Motion: That the CTIA/ACAA executive of the 26th meeting express our sincere appreciation to these contributors.
Moved by: Om Rajora
Seconded by: Gilles Vallée
Carried.

301 Editor's Report

The proceedings were printed and distributed during April 1996 to all active members, honourary members, Canadian universities and libraries, and all participants of the 25th meeting. Three hundred and forty-six proceedings were mailed.

A form letter advising the theme of the 25th biennial meeting, the proceeding context, and a request for a twenty dollar donation to obtain a copy was sent to all corresponding members, USA addresses and international addresses. There were 11 donations from Canadian addresses, 13 from USA and 19 international.

302 Education Committee

To promote students' knowledge and understanding in tree improvement activities and forest genetics research, the forestry faculties of Canadian universities were encouraged to nominate a student to attend the 26th CTIA/ACAA meeting. Sponsorship of these students is paid by the executive committee of each biennial meeting through registration fees. Students received an autographed copy

of Kris Morgenstern's book "Geographic Variation in Forest Trees." Jean Beaulieu reported that the following students received the award which provided all meeting costs including registration, accommodation, tours, and travel:

Andy Benowicz	University of British Columbia
Changxi Li	University of Alberta
Daniel Rouillard	Lakehead University
Christina Idziak	University of Toronto
Sauphie Senneville	Université Laval
Frédéric Poirier	Université de Moncton
Yijun Xu	University of New Brunswick

303 Working Group Reports

303.1 Tree Seed Working Group

André Rainville moderated the Tree Seed Working Group's (TSWG) morning workshop "Artificial Pollination in Seed Orchards" on August 18, 1997. The workshop was dedicated to Dr. Guy-Etienne Caron, chairperson of the TSWG (1991-1997).

Immediately following the workshop, the Tree Seed Working Group held its regular Biennial Business Meeting. Three issues of the TSWG News Bulletin (Nos. 24-26) were produced since our last meeting on August 28, 1995 in Victoria. Editor Ron Smith (CFS - Atlantic) reiterated that he intends to maintain the traditional two issues per year, but due to 'technical difficulties' there was one fewer issue printed over the last two-year period. A suggestion was forthcoming that due to more and more people now able to use electronic mail that this avenue should be explored. Ron is in the process of updating addresses and is also expecting to have a web site for the TSWG at CFS - Atlantic in the near future. So, the News Bulletin will be available to the Discussion Group on the Internet soon.

Thanks go to Ron for editing, printing, and mailing of our News Bulletin. There were no further changes noted with respect to working parties; Dave Kolotelo remains as Coordinator of the Tree Seed Processing and Testing Working Party and Peter de Groot stays on as Coordinator of the Cone and Seed Insects Working Party. Howard Frame agreed to take on the role of Chairperson for the TSWG.

The question of when and where our next meeting will be, was left unanswered. The CTIA business meeting on Wednesday may shed some light on this topic, but the News Bulletin will keep everyone apprised on this subject.

Howard M. Frame
NS Dept. Natural Resources
Chair, CTIA Tree Seed Working Group

303.2 Wood Quality Working Group

No formal workshop was specifically organized for the 1997 CTIA Wood Quality Working Group (WQWG) due to an excellent wood quality meeting organized by Forintek Canada Corporation and sponsored by the IUFRO and CTIA. Tony Zhang and I discussed several approaches and it was decided that the wood quality papers related to tree improvement could be incorporated into one session and that would represent the objectives of the WQWG more than adequately. On behalf of

CTIA members who are involved in wood quality research, I'd like to thank IUFRO and Forintek for an excellent meeting and the 14 papers published in Chapter IV of the proceedings "Timber Management Toward Wood Quality and End-Product Value" are an excellent contribution to the literature on this topic. The effort far exceeded that which could have been organized by the WQWG alone, and much of this credit goes to Tony. The excellent international participation and the papers by Drs. Zobel and van Buijtenen provided for a truly world class meeting.

No business meeting was held. It would be appropriate, considering the renewed interest in wood properties breeding in many programs across Canada, to review and update the objectives and mandate of the WQWG at the next CTIA meeting. Renewing our interest in the newsletter would also be important. I will coordinate this matter with the executive of the CTIA for the next meeting.

Alvin Yanchuk
BC Forest Service, Victoria
Chair, CTIA Wood Quality Working Group

304 Business Arising from Previous Meetings

304.1 Funding for History of Forest Genetics in North America

Motion 292.4: That the CTIA/ACAA support Lauren Fins with two thousand Canadian dollars, for the proposed manuscript "A History of Forest Genetics and Tree Improvement in North America" on the condition, that L. Fins receive five supporting offers from other sectors.

In a letter dated March 29, 1996 Lauren Fins confirmed she was able to secure funding from two other sources. However, she stated she intended to continue her efforts to raise the additional funds. As such, until she receives five supporting offers the CTIA/ACAA will not advance any funds.

305 New Business

305.1 University of Northern British Columbia

Chairman Michel apologized that the university had not been contacted to send a student to the CTIA/ACAA meeting. It was realized too late that it might have been appropriate. He asked why there was no student from this university at the Victoria meeting. Alvin Yanchuk stated that the forestry program is new and it was an oversight in not inviting them to send a student to the last meeting. Concern was raised that with more universities offering forestry/environmental courses it may be difficult to draw the line on which ones to invite to send students. Kathy Tosh suggested that only universities offering an accredited forestry degree program should be contacted.

305.2 Constitution and Bylaws

Last summer, Narinder Dhir brought to the attention of Chairman Michel that several amendments have been made to the Constitution and Bylaws since they were last published. Dale agreed to re-publish them and distribute them to Active members with the copy of the Proceedings from this meeting.

305.3 Registration of CTIA/ACAA

The CTIA/ACAA is currently registered in Ontario as a society. Since the business affairs have been transferred to New Brunswick it is logical to become registered there. Jim Richardson pointed out that

the Poplar Council of Canada is registered nationally and such a registration status may also be appropriate for CTIA/ACAA. The Executive Secretary will examine the situation and register the organization where most appropriate.

305.4 Bursary

Chairman Michel suggested the Association should consider a recognition award to students who write a paper or essay on their research results or any tree improvement/genetics topic. Honorary members could be selected to review the papers and recommend up to three names for awards. The awards would be presented at the CTIA/ACAA meeting. Michel agreed to form a small committee to develop a terms of reference for this award program.

306 Future Meetings

306.1 Location of 1999 Meeting

Weyerhaeuser Canada of Saskatchewan is unable to host the 1999 CTIA/ACAA meeting. Ontario Ministry of Natural Resources suggested they were willing to host the next meeting in Sault Ste. Marie. Dennis Joyce pointed out this is a good location because of its close proximity to the USA allowing the option for Americans to obtain accommodation in Sault Ste. Marie, Michigan and travel to Canada daily to attend the meeting. He further suggested teaming up with the Southern Forest Tree Improvement Conference (SFTIC) and Western Forest Genetics Association (WFGA) in order to have a larger, more dynamic meeting. It was pointed out that SFTIC and WFGA have already determined where they will meet in 1999. Dennis suggested to delay a year and have it in 2000. It was pointed out that the Constitution and Bylaws state the CTIA/ACAA must meet every two years. After considerable discussion the following motion to **amend** "Article VI, Section a" of the Constitution was tabled.

Motion: Meetings of the Association shall be held at least once every third year.
Moved by: Jerry Klein
Seconded by: Jean Beaulieu
Carried

This allowed a motion on when the next meeting should be held.

Motion: CTIA/ACAA hold its next meeting in Sault Ste. Marie, Ontario in the year 2000.
Moved by: Jerry Klein
Seconded by: Kathy Tosh
Carried.

306.2 Location of the Next Meeting

Motion: Alberta will explore the feasibility of hosting a meeting in 2002 or 2003.
Moved by: Om Rajora
Seconded by: Jean Beaulieu
Carried.

306.3 Location of Subsequent Meeting

No suggestions were forth coming.

307 Election of New Executive

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

Chairperson:	Dennis Joyce Ministry of Natural Resources
Vice-Chairperson: Symposium	to be confirmed
Vice-Chairperson: Local arrangements	to be confirmed
Treasurer:	Linda DeVerno Canadian Forest Service
Editor:	Dale Simpson Canadian Forest Service
Executive Secretary:	Dale Simpson Canadian Forest Service

308 Adjournment

Motion: That the members of the CTIA/ACAA thank the executive for their efforts over the past two years for an exciting and successful meeting.

Motion: That the 26th business meeting of the CTIA/ACAA be adjourned
Moved by: Om Rajora

Attachment # 1

CTIA/ACAA
Financial Statement
July 30, 1995 to July 30, 1997

Cash Balance July 30, 1995	**$7 175.53**
Credit:	
Interest earnings (GIC, Account)	3 058.34
Purchase of 25th Meeting Proceedings	940.85
Donation for the 26th Meeting Proceedings from CFS Headquarters	4 817.84
Total Credit:	**$8 817.03**
Guaranteed Investment Certificates: (as of July 30, 1995)	
GIC Principal	10 000.00
GIC Principal	7 000.00
GIC Principal	6 000.00
Total GICs	**$23 000.00**
Guaranteed Investment Certificates: (as of July 30, 1997)	
GIC Principal	10 000.00
GIC Principal	8 000.00
GIC Principal	5 000.00
Total GICs	**$23 000.00**
Debit:	
Labels	36.74
Bank Service Charge	20.00
26th CTIA Meeting advance	5 000.00
Film Processing	17.46
Envelopes & Letterhead	495.83
Envelopes	58.20
Student Recognition Awards	1 000.00
Money Order Fees	6.50
26th CTIA Meeting Proceedings Advance	4 817.84
Total Debit:	**$11 452.57**
Cash Balance July 30, 1997	**$4 539.99**
Invested GIC Balance	**$23 000.00**
Total Holdings	**$27 539.99**

ACTIVITY REPORTS

FROM ACTIVE CTIA/ACAA

MEMBERS

THE NEWFOUNDLAND AND LABRADOR TREE IMPROVEMENT PROGRAM: AN UPDATE

B. J. English

Dept. of Forest Resources & Agrifoods
P. O. Box 2006, Fortis Building
Corner Brook, NF
A2H 6J8

The Newfoundland Forest Service has in place tree improvement programs for three native conifers: black spruce, white spruce, and eastern larch. Six first generation seed orchards have been established, two per species. One orchard per species will serve the reforestation needs of the Northern Peninsula/Labrador breeding zone. The second orchard will produce seed for the southern part of insular Newfoundland (i.e., the Main Island breeding zone).

In 1997, emphasis shifted from plus tree selection/first generation establishment to preparations for first generation roguing and second generation plus tree selection. In the case of black spruce, family tests have been in place since 1993. Remeasurement of these at ages 10 and 15 will serve as a basis for roguing the existing black spruce orchards and making selections for the second generation orchards and breeding program. To augment the limited number of trees in the current program, in 1997 we made an additional 40 selections (20 per breeding zone) from several 1970's era Canadian Forest Service all-range black spruce provenance trials. These 40 new selections will advance into the second generation program. In preparation for the breeding work/progeny testing required in the second generation program, black spruce polycross sources were also identified. These also came from the all-range provenance trials mentioned above. Scion collection and grafting will be carried out during the winter of 1998 to establish these polycross pollen donors and additional plus trees in our clone banks.

In 1998, we will also be selecting polycross pollen donors in our white spruce program. Although we will probably have to collect pollen directly from these selections for several years to come, we also intend to establish a polycross clone bank at our main tree improvement facility, Wooddale Provincial Tree Nursery. Scion collection and grafting will commence the winter of 1998. It is our expectation that many of the clones in the first generation white spruce orchards will begin to bear substantial numbers of female flowers over the next several years. We hope to begin specific crossing and polycrossing as soon as possible. In preparation for this, several staff involved with the Tree Improvement Program toured Provincial tree improvement facilities in the Maritimes earlier this year. The focus of the tour was pollen monitoring, collection, handling, storage, testing, and application (i.e., controlled crossing).

Due to a limited demand for eastern larch planting stock and a need to focus our limited tree improvement resources on the more economically important spruces, we have made a decision this year to put our eastern larch tree improvement program on hold. Basic maintenance activities will continue but otherwise we have no plans to move this program forward at this time.

A growing interest in white pine reforestation in recent years, coupled with a chronic shortage of local seed, has resulted in a decision to establish a clonal white pine seed orchard. Beginning in 1998 up to 200 well formed, disease-free pine will be selected from across the Island. Scions will be collected and grafting will commence. The orchard will serve the dual purpose of providing seed for reforestation and preserving the local white pine gene pool.

After a somewhat shaky start in the 1980's, Newfoundland's tree improvement program is now well established. We feel we have the proper infrastructure in place to move foward, making substantial genetic gains in the process.

COOPERATIVE TREE BREEDING IN NOVA SCOTIA

Howard Frame and David Steeves

Department of Natural Resources
Tree Breeding Centre
P. O. Box 190
Debert, NS
B0M 1G0

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

The Tree Improvement Working Group (TIWG) was established in 1977 and is the coordinating body for tree improvement in Nova Scotia. Active members include the Provincial Government, Bowater Mersey Paper Company Limited, Kimberly-Clark Nova Scotia Inc., Stora Forest Industries, and J.D. Irving, Limited. Meetings are held in the spring and fall of each year to review progress and plans, while day-to-day activities are coordinated by the Department of Natural Resources. Species of interest include *Picea mariana, P. glauca, P. rubens, P. abies and Pinus strobus.*

SEED ORCHARD ESTABLISHMENT

First generation orchard establishment was completed in 1993. The summer of 1996 saw the first planting of second generation black spruce orchards, with additional material established in 1997. Total second generation black spruce orchard area stands at 1.5 ha.

SEED ORCHARD PRODUCTION

White spruce clonal orchards have produced so many cones that they were left unharvested in 1996. Two red spruce clonal orchards have been able to supply all seed requirements for the reforestation effort in the province. Sufficient seed is produced within the black spruce seedling seed orchards to meet reforestation requirements with rogued orchards providing a large portion of this seed. In 1996, renewed interest in the planting of white pine has tightened the supply of orchard seed, with some wild collections augmenting the supply of orchard seed. Clonal Norway spruce orchards supplied 60 percent of the 1997 seed requirement at the provincial government nursery, yet seed production is generally disappointing in the Norway spruce orchards.

ORCHARD MAINTENANCE

Additional roguing in two black spruce first generation seedling seed orchards occurred during the spring of 1997. Crown management by 'topping' was carried out in black, red, white and Norway spruce orchards, with 11 576 trees topped for the first time and 7 141 trees receiving a repeat topping.

BREEDING

Second generation selection (family plus within-family) of black spruce continued on the basis of 10-year measurements made in a 1988 series of family tests. Just over 200 selections have now been made. The

remaining 155 needed to complete the F_2 breeding population will come from a 1990 test series which is scheduled for measurement in 1999.

As for our white, red, and Norway spruce programs, following is a summary of breeding progress to date (excluding spring 1997 breeding effort).

Species	Mating	Total Crosses Required	Crosses Completed
White Spruce	Pair Mate[1]	930	783 (84%)
White Spruce	Polycross	465	441 (95%)
Red Spruce	Pair Mate	497	451 (91%)
Red Spruce	Polycross	497	471 (95%)
Norway Spruce	Polycross	295	56 (19%)

[1] two crosses per clone

Flowering in the spring of 1997 was poor among red and white spruce clones at Debert, particularly among those that have yet to be crossed. To facilitate completion of the first generation red and white spruce breeding and to ensure continued Norway spruce flower production for 1998, over 500 grafts received a stem injection of $GA_{4/7}$ during spring 1997.

PROGENY TESTING

In 1996, a polycross progeny test series was established for each of white, red, and Norway spruce. In 1997, cooperators are outplanting a series of white and one of red spruce polycross tests. A Norway spruce trial designed to compare performance among ten seed sources was planted at 13 locations in 1997. In these, sources are planted in large, unreplicated contiguous blocks.

A back-log of 15 year data collected in 1996 from three 1978 test series, a white spruce stand test series, a white spruce seed orchard progeny test series, and a black spruce series comparing stand progeny from Nova Scotia, New Brunswick, and Quebec, were analysed. Ten-year (1995) heights in seven 1986 test series were taken and assessed. These series tested half-sib white, red, and Norway spruce progeny from a clonal orchard in Lawrencetown which is used as the polycross pollen source. Ten-year (1996) heights from a Cape Breton Highland black spruce outcrossing trial were also assessed.

Nine year heights taken in 1996 from a 1988 series of black spruce family tests provided roguing information for seedling seed orchards at Debert and East Mines. They were also the basis for the second generation selections mentioned above.

TREE IMPROVEMENT ON PRINCE EDWARD ISLAND

W.J. MacKinnon, W.M. Glen, and M.N. Myers

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Charlottetown, PE
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Keywords: tree improvement, seed orchard, progeny tests, provenance trials, gene conservation

SEED ORCHARD

The major objective of the PEI Forestry Division Tree Improvement Program is to reverse the impact that two centuries of forest exploitation has had on the resource. Only remnants of the original Acadian Forest cover-type can be found. Agriculture as well as ship building at the turn of the century depleted the Island of its very best genetic base. To reverse this trend, a tree improvement program was initiated for black spruce (*Picea mariana* (Mill.) B.S.P.), white spruce (*Picea glauca* (Moench) Voss), red spruce (*Picea rubens* Sarg.) eastern white pine (*Pinus strobus* L.), and eastern larch (*Larix laricina* (Du Roi) K. Koch). Research plantings of hardwood to develop establishment guidelines as well as gene conservation for yellow birch (*Betula alleghaniensis* Britton.), red oak (*Quercus ruba* L.) white ash (*Fraxinus americana* L.) black ash (*Fraxinus nigra* Marsh), and ironwood (*Ostrya virginiana* (Mill.)K. Koch) have been initiated.

The tree improvement program on PEI began in 1974. The development years included testing progeny from open pollinated plus tree selections, general stand collections, and species range-wide provenance tests. Species included were white spruce, red spruce, and yellow birch. In 1980, a black spruce seedling seed orchard was established using material from the New Brunswick Tree Improvement Council which has subsequently been found to be superior to Island sources. In the late 1980's, selections were made from primarily natural stands for the establishment of clonal seed orchards of white spruce, eastern larch, eastern white pine, and red spruce and later balsam fir (*Abies balsamea* (L.) Mill.) for the Christmas tree industry.

ORCHARD ESTABLISHMENT

First generation orchard establishment was completed in 1995 with the exception of red spruce. A second generation black spruce orchard has been established at the Upton Road Nursery. All black spruce reforestation stock on PEI has come from the first generation seedling seed orchard since 1988. A 30% roguing of the 1980 orchard has improved genetic gain until the second generation orchard comes online. White spruce improved stock was available in 1997 while eastern larch improved material has been available since 1995. At present, white pine and red spruce stock are grown from Nova Scotia sources. Plans to graft a hybrid larch orchard with twenty European larch (*Larix decidua* Mill.) and twenty Japanese larch (*Larix kaempferi* (Lamb.) Carrière) are underway for 1998.

ORCHARD MANAGEMENT

The Province's primary tree seed orchard area in Dover was established on abandoned farm land in the late 1980's. Soil type and a wide window of frost free days make this an excellent location. Orchard management

includes fertilizing, mowing, irrigation, pest management, topping and fall herbicide treatments. The formerly wooded portions of the property are being used for provenance testing of non-native tree species and establishment of hardwood research plots.

PROGENY TRIALS

In the spring of 1997 there was a white spruce polycross test established as well as an open-pollinated eastern larch test (both used three sites). Fifteen-year diameter and height measurements of a black spruce progeny trial have been collected. These measurements will be compared to the ten year results to see if family ranking is constant.

PROVENANCE TESTING

The main area of research includes Norway spruce and *Larix* species. Several tests of each have been established and measurements have been made on both five and ten year tests. PEI established a Norway spruce provenance trial, in cooperation with the Nova Scotia Tree Improvement Working Group in 1997, containing 11 sources. A Corsican Pine (*Pinus maritima*) provenance trial (3 sites) as well as a white pine provenance trial (3 sites) will be established in 1998.

HARDWOOD RESEARCH/GENE POOL CONSERVATION

Five-year heights and survivals have been completed for yellow birch and red oak planting density trials. White ash five year measurements will begin in 1998. Survival and height have been encouraging to date.

Tree improvement efforts towards gene pool conservation for black ash and ironwood have been very slow. Earlier plantations show poor survival and growth. Additional research is required on germination techniques and establishment requirements. There were poor seed crops the past two years. Additional areas containing these rare species have been identified for future collection sites.

The Forestry Division is currently conducting research on *Taxus canandensis* (Ground Hemlock). Fruit collections were made and germination techniques will be developed. Mapping and plant indicators have been developed for PEI as well as clipping trials and rooted cutting research.

FRASER PAPERS INC. TREE IMPROVEMENT PROGRAM

Paul Roussel

Fraser Papers Inc.
27 Rice Street
Edmundston, NB
E3V 1S9

Keywords: Supplemental mass pollination, clonal seed orchard, Gibberellin $A_{4/7}$, paclobutrazol, polycross matings, progeny tests

This report summarizes 1996 and 1997 tree improvement activities at Fraser Papers Inc. Information on cone production and collection, test establishment, nursery production, and new developments is included.

SUPPLEMENTAL MASS POLLINATION

Due to insufficient quantities of male cones, half of the second generation black spruce (*Picea mariana* (Mill.) B.S.P.) clonal seed orchard (BSCSO) received a supplemental mass pollination early spring 1996. The pollen used came from 30 phenotypically superior trees chosen amongst 11 families in the Second Falls black spruce seedling seed orchard (BSSSO).

CONE PRODUCTION AND COLLECTION

Clonal Orchards

The first generation white spruce (*Picea glauca* (Moench)Voss) clonal seed orchard (WSCSO), covering 3.6 ha, contributed 41.36 kg of viable seed in 1996 (+/- 17 247 120 seed). At the same time, cones were collected from the 1991 second generation BSCSO which yielded 0.6446 kg of viable seed.

Nearly 25% of the WSCSO grafts (484) received Gibberellin $A_{4/7}$ ($GA_{4/7}$) injections in 1997 for cone induction. At the same time, a combined $GA_{4/7}$ and Paclobutrazol trial initiated by Ron Smith from CFS-Atlantic was undertaken in the BSCSO on 18 ramets of 10 clones for a total of 180 injected grafts.

Due to very low cone production, no cone collection is expected in the WSCSO in 1997. Small quantities of cones will be collected from the BSCSO. Total seed yield from the BSCSO in 1997 is expected to be lower than in 1996.

Natural Stands

On top of clonal seed orchard collections, red pine (*Pinus resinosa* Ait.) and Norway spruce (*Picea abies* [L.] Karst.) seed was collected from a natural stand and an old plantation, respectively, yielding close to 2 775 000 seed all together.

SEEDLING PRODUCTION

For the first time in 1997, seed from Fraser Papers Inc. second generation BSCSO was sowed at the nursery. All of the 1996 seed collected in the BSCSO was used. Seed from Fraser Papers BSSSO was also used to fulfill the 1997 black spruce seeding requirements (3 021 298).

For the first time in 1997, the total white spruce seedling production (4 975 822) came from first generation seed collected from Fraser Papers Inc. WSCSO. Other species grown at the nursery in 1997 were red pine (205 690) and Norway spruce (1 300 336) for a total of 9 503 146 seeded cavities reflecting a 10.5 per cent increase over 1996.

TESTING

As initiated in the mid-seventies, Fraser Papers Inc. continued to assist the New Brunswick Tree Improvement Council by performing polycross matings on second generation black spruce selections to obtain seed for progeny testing.

In 1996 and 1997, a total of 1.84 ha of tests was planted. This total includes one white spruce and two black spruce progeny tests. The total area covered with tree improvement related tests is now over 52 ha.

SPECIAL PROJECTS

A collaborative project with Dr. G.-É. Caron regarding the crossing of various Norway spruce provenances was initiated in 1996 to familiarize both parties with this foreign species. In 1997, with the guidance of both the Atlantic and Laurentian Centres of the Canadian Forest Service, Fraser Papers initiated its Norway spruce improvement program by grafting nearly 1000 scions from both CFS-Atlantic and CFS-Laurentian clone banks. These grafts, along with others to be grafted in early 1998, will be outplanted in 1998 at the Ste. Anne clonal orchard complex. Site preparation for this new orchard has been initiated.

J.D. IRVING, LIMITED - TREE IMPROVEMENT SUMMARY

Greg Adams

J.D. Irving, Limited
Sussex Tree Nursery
RR #4
Sussex, NB
E0E 1P0

Keywords: spruce species, jack pine, white pine, hardwood species, tree breeding, seed orchards, clonal propagation, somatic embryogenesis

STATUS OF BREEDING PROGRAMS AND ORCHARD PRODUCTION

J.D. Irving, Limited (JDI) participates in breeding and testing programs with the New Brunswick Tree Improvement Council (NBTIC) and the Nova Scotia Tree Improvement Working Group (NSTIWG) as well as operating independent testing programs. The company manages a total of 80 ha of clonal seed orchards including white spruce, black spruce (first and second generation), Norway spruce, red spruce, jack pine (first and second generation) and eastern larch. Breeding and progeny testing associated with first generation orchards of white spruce, black spruce and jack pine is completed and genetic roguing is in progress. Breeding and testing of red spruce and Norway spruce is still underway and work with eastern larch is not a priority at present. Second generation orchards of black spruce and jack pine were established from selections made in NBTIC open-pollinated family tests and the breeding and testing work for these orchards is nearly completed.

Annual reforestation stock production for the company is 15 million seedlings and seed orchards have been filling this requirement since the early 1990's for all species with the exceptions of Norway spruce and red spruce. Difficulty is still being experienced in obtaining good cone crops of Norway Spruce and flower induction is being used routinely. Vegetative propagation of controlled crosses of this species has also been started. A seed orchard of red spruce was not established until 1992 and cone production should begin in the next two years. Second generation orchard seed production for black spruce and jack pine increases each year and in 1996, sufficient jack pine seed was harvested to satisfy nursery stock production requirements in 1997.

In the last five years, JDI has been conducting operational trials on white pine management because of the high value of this species. Demand for reforestation stock has risen to a high enough level that a tree improvement program was initiated by the company in 1997. Plus tree selection is progressing and grafting for seed orchard and clone bank establishment will begin in 1998. Cone crops on plus trees will be assessed and open-pollinated family tests will be established if seed can be obtained from enough of the selections.

VEGETATIVE PROPAGATION AND CLONAL TESTING

Controlled crossing among tested second generation black spruce selections is conducted each spring using the most current field test information to produce crosses with high average breeding values. The resulting full-sib families are grown and potted as hedge stock for cutting production. Production of rooted cuttings is now at a level of one million per year and further expansion is planned. Norway spruce hedges have also been established. Controlled crossing was started in 1997 among the best tested first generation white spruce selections for hedging. The cuttings are being operationally planted on better quality planting sites across the

company districts.

Clonal tests of 256 black spruce clones from 30 families were established at two field sites in 1996. The clones are being serially hedged on a four year cycle at Sussex Tree Nursery so that selected clones will be available for bulking up in 5 to 10 years based on performance in the field tests. A similar sized clonal testing effort is underway using clones produced by somatic embryogenesis. Somatic seedlings have been produced from close to 300 clones again originating from 30 families. These seedlings are being hedged for intermediate bulking up by rooted cuttings prior to field testing. Embryogenic tissue from all clones is being preserved in liquid nitrogen during the lengthy field testing process. Further expansion of this work to include Norway spruce and white spruce is planned.

PUBLICATIONS

Adams, G.W.; Kunze, H.A. 1996. Clonal variation in cone and seed production in black spruce and white spruce seed orchards and management implications. For. Chron. 72: 475-480.

Park, Y.S.; Adams, G.W.; Mullin, T.J. 1997. Incorporation of new information and technology in breeding and deployment strategies for black spruce in the Maritimes, Canada. *in* S. Puri, ed. Tree Improvement: Applied Research and Technology Transfer, Oxford and IBH Co. Publishers.

NEW BRUNSWICK TREE IMPROVEMENT COUNCIL UPDATE

K.J. Tosh

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Kingsclear Provincial Forest Nursery
3732 Route 2 Hwy
Islandview, NB
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Keywords: Breeding, second generation, seed orchard, black spruce, jack pine, white spruce, tamarack

The New Brunswick Tree Improvement Council (NBTIC) completed its 20th year of operation in 1996. Technical coordination and direction of NBTIC operations is now provided through the NB Department of Natural Resources and Energy (NBDNRE). Funding cuts at the Canadian Forest Service resulted in withdrawal from managing this program. The full-time data analyst is still funded by members, however the position is staffed out of the Kingsclear Forest Nursery. This important function is being conducted by Bryce McInnis who replaced Victor Steel following his resignation in late 1995. The program is focusing on completing breeding and testing of first generation white spruce (*Picea glauca* [Moench] Voss) and tamarack (*Larix laricina* [Mill.] Karst.) and selection and testing of second generation black spruce (*Picea mariana* [Mill.] B.S.P.) and jack pine (*Pinus banksiana* Lamb.). First generation orchards are providing enough seed for all reforestation and production in second generation orchards continues to increase.

SEED ORCHARDS AND SEED PRODUCTION

Since 1978, seed orchards have been established by the industrial members of NBTIC who operate reforestation programs on freehold land, as well as by the NBDNRE who is responsible for planting programs on Crown land. Over 130 ha of black spruce and jack pine seedling seed orchards were planted over a 10-year period ending in 1987. Clonal seed orchards, primarily of white spruce and tamarack, were also established over this time period, with over 60 ha planted. Second generation orchard establishment began in 1989, with three agencies participating. To date over 29 ha of second generation black spruce and jack pine orchard have been established.

As expected, after the record breaking cone crop in 1994, seed production was down in 1995. Seed production was up once again in 1996, however, many Council members had sufficient seed in storage and large collections were not made. The only exception to this was a large collection of white spruce made in a clonal seed orchard. The real good news in 1996 was the increased seed production in second generation orchards. Substantial quantities of seed are starting to be produced in these orchards and it is anticipated that within the next 4 years all of the black spruce and jack pine seed will originate from these second generation orchards.

BREEDING

The Council conducts a complimentary breeding program which began in 1987 with white spruce and tamarack. A polycross, consisting of a mix of 20 unrelated pollens, is used to estimate breeding values. Pair-mating, involving specific crosses, is conducted to produce material from which selections will be made for the next generation. Polycrossing of second generation black spruce and jack pine has made tremendous progress since the start of breeding. This is partly due to polycrossing *in situ* on the selections in the family

tests as well as the considerable experience that Council members have gained over the 10 years of breeding work. Pair-mating of black spruce and jack pine commenced in 1994 and 1996, respectively, and will produce material for third cycle selections. Table 1 summarizes breeding progress for all species. Pair-mating of tamarack was discontinued due to reduced interest in planting this species.

Table 1. Summary of breeding progress.

	Polycross		Pair-mate	
Species	No. Completed	% completed	No. Completed	% completed
White spruce	362	87	467	90
Tamarack	209	78	60	-
Black spruce	213	53	89	18
Jack pine	243	60	104	22

The Council continues to follow the breeding strategy for black spruce that was adopted in 1993. Clones have been uniformly deployed to breeding groups and breeding is conducted in a positive assortative manner. A total of 6 sub-lines has been established for black spruce and 8 for jack pine. Breeding continues in the elite sub-line of black spruce and a clonal test from elite crosses was initiated in 1997 and will be planted in 1999.

SELECTION PROGRESS

Trees are selected in family tests for inclusion into the second-generation breeding population. Top performing families are identified based on 10-year height for black spruce and 7-year height for jack pine. Candidate trees are initially identified based on height growth, phenotypically graded in the family tests, and the final selection made from each family based on a combination of superior phenotypic and metric traits. The goal is to select 400 trees for these breeding populations. Selection work began in 1988 and is complete for jack pine with a total of 402 selections. The total number of black spruce selections to date is 347 or 87% of the total. Second-generation selection will be completed in 1997.

TESTING AND DATA ANALYSIS

Testing continues to be an important component of the NBTIC program. Over the past 20 years, 235 tests were planted on over 274 ha. Over the past 8 years, progeny tests have been established to assess the performance of white spruce and tamarack plus trees and second generation black spruce and jack pine selections. It is anticipated that progeny testing of white spruce and tamarack will be completed by 1999 while the establishment of white spruce selection plantations from pair-mate breeding will continue until 2000. Establishment of polycross tests of second generation black spruce and jack pine will be completed in the year 2000.

Realized gain tests of black spruce and jack pine have also been planted to quantify the actual gains of using improved seed. The test design used for both species was the same consisting of six seedlots planted in four replicates using 64-tree plots at five locations. Seedlots consisted of rogued and unrogued orchard and unimproved stand checklots. These tests were measured when 5 years old. The unrogued black spruce orchard seedlot was the same height as the average checklot, however, the rogued orchard seedlot was over 6% taller which can translate into a potential volume gain of 18% to 20%.

For jack pine, the unrogued seed orchard seedlot was 3% taller than the checklots, while the seedlot from the same orchard following a second roguing was over 4% taller. This has the potential for a 12 to 15% gain in volume. Stem straightness is an important quality trait for improvement in jack pine. Selection is made for this trait when seed orchards are rogued. The seedlot from the orchard twice rogued was 28% straighter than the checklots. Significant areas of jack pine plantations from seed orchards have been established since 1988 and trees in these areas are straighter with narrower crowns than those in unimproved plantations.

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TREE IMPROVEMENT PROGRESS BY NEW BRUNSWICK DEPARTMENT OF NATURAL RESOURCES AND ENERGY

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The tree improvement program in N.B. is celebrating over twenty years of operation. Our efforts continue to be focused on 4 major reforestation species, black spruce (*Picea mariana* (Mill.) B.S.P.), jack pine (*Pinus banksiana* Lamb.), white spruce (*Picea glauca* (Moench) Voss) and tamarack (*Larix laricina* (Du Roi) K. Koch.). Secondary species for tree improvement include balsam fir (*Abies balsamea* (L.) Mill.), and Norway spruce (*Picea abies* (L.) Karst).

All first generation selection and seed orchard establishment has been completed for the four major tree species. Second generation selections for black spruce and jack pine, cross-pollinations, and orchard establishment are well underway, or in some cases complete. The following report highlights some of our major accomplishments over the past twenty years:

TREE BREEDING/TESTING

The New Brunswick Dept.of Natural Resources and Energy (NBDNRE) is a member of the New Brunswick Tree Improvement Council (NBTIC) , a group of co-operators including NBDNRE, the federal government and eight large induatrial companies located in New Brunswick. All tree improvement work in the province is co-ordinated by the NBDNRE and all co-operators share in the workload. The 1995 and 1996 jack pine polycrosses done on second generation selections provided sufficient seedlots to grow and outplant the third and fourth series of jack pine progeny tests. The polycross seedlots totaled 131 families. All crosses on jack pine are done in the breeding garden. Black spruce crosses, however, are still being carried out in family tests, as well as in the breeding garden. Table 1 gives a brief summary of the ten years of our breeding effort.

In 1995 and 1996, white spruce, black spruce, jack pine and tamarack progeny tests were outplanted. Polycross breeding on both white spruce and tamarack is over 90% complete. Pair-mate breeding continues with the white spruce and is now 74% complete. Sub-lining of the second generation black spruce and jack pine is well underway with a full-sib clonal test of the elite group begun in 1997. It will be ready for outplanting in 1999.

Table 1. Summary of ten years of tree breeding

Species	Tamarack	White spruce	Black spruce	Jack pine
Number of bags	1 928	3 378	451	2 941
No. Females bagged	43 854	110 146	22 596	18 753
Number of crosses	444	941	321	734
Mean no. female/bag	21	29	34	5
Mean full seed/cone	5.0	10.9	8.9	17.2

SEED ORCHARDS

The best individuals selected from the best families in NBTIC family tests are being identified for second-generation material. To date, we are 87% complete for black spruce selections and 100% complete for jack pine. By the fall of 1997, all 2nd generation black spruce selections will be identified. A total of 11 ha of second generation black spruce orchard has been established and should be completely filled by 1998. The jack pine second generation orchard consists of 6 ha of the total of 7.5 ha to be planted. It is anticipated that the orchards will be fully stocked in 2-3 years.

Roguing has started in first generation clonal orchards of larch and white spruce based on five-year progeny test results. The bottom 4 and 18 families were removed, respectively; a conservative roguing for now.

CONE COLLECTION IN SEED ORCHARDS

The second generation clonal orchards have started to produce seed (Table 2). In 1995, jack pine was the only 1st generation species we collected cones from as we had an ample seed supply for the three other species. Second generation orchards are coming on line, enabling us to harvest cones in 1995 and 1996. We also collected from some of the top performing clones in our white spruce orchard (based on 5 year progeny test results).

Table 2. Cone collection and seed yield from orchards in 1995 and 1996

		Quantity of cones (l)		Amount of seed (kg)	
Species	Seed orchard	1995	1996	1995	1996
Jack pine	Otter Brook	3 938	-	45.8	-
Jack pine	2nd gen KCL	26	136	0.09	0.6
Black spruce	2nd gen KCL	22	192	0.02	0.45
White spruce	Queensbury	-	915	-	14.5

MINI ORCHARD PROJECT

In co-operation with the Canadian Forest Service and with funding under the Canada/NB CO-OPERATION Agreement, a miniaturized seed orchard project was initiated in 1993. The NBDNRE selected jack pine as the species to use for this study. The objective of the project is to develop a prototype mini orchard and to examine various seed orchard management techniques.

A total of 25 second generation jack pine clones were selected, grafted, and grown in 1993. Growth of the grafts was accelerated during the winter of 1994. The grafts were out-planted at the Kingsclear Nursery in 25 ramet clonal row plots. In conjunction with this project a research trial was conducted in the spring of 1994 on other older grafts to investigate the low seed set often obtained from jack pine controlled crosses. The three factors that were investigated were: 1) timing of pollen application, 2) amount of pollen applied per pollination bag, and 3) number of pollen applications.

Results indicated that: 1) timing of pollen application made no significant difference (am vs. pm), 2) three puffs of pollen applied weresufficient, especially if receptivity was maximum, and 3) one application was as effective as 2 or 3, if receptivity was maximum.

STUDIES RELATED TO TREE IMPROVEMENT AT THE UNIVERSITY OF NEW BRUNSWICK

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In June 1995, our Faculty's long-standing research emphasis on tree-crown and reproductive development, pollen and seed production, seed germination, and seedling development suffered a setback with the retirement of Graham R. Powell. Graham continues to support the Faculty's undergraduate teaching effort, however, his research program is winding down.

SEEDS AND SEEDLING DEVELOPMENT

Dormancy of seeds of *Bauhinia malabarica* Roxb. and *Acacia auriculiformis* A. Cunn. ex. Benth., both caused by impermeability of the seed coats to water, was overcome by treatment with sulphuric acid (30 min was best) and by nicking of individual seeds (Magsambol 1995; Welgas-Briz 1995). Treatments with hot or boiling water elicited much slower responses. For *B. malabarica*, studies of seed-coat morphology by scanning-electron microscopy and of rates of water uptake led to the proposition that the impermeability resided in the palisade layer of the seed coat (Magsambol 1995).

Seedlings of *A. auriculiformis* grown for their first 60 days under full light had significantly higher oven-dry weights than did seedlings grown under 70 or 45% of full light, though seedling heights did not differ (Welgas-Briz 1995). The seedlings typically produced, above the cotyledons, one pinnately compound leaf, then two bi-pinnately compound leaves, then one leaf with a basal phyllode and terminal bi-pinnately compound lamina, and then only phyllodes from leaf five onward. This changeover to phyllode production was more rapid than that in *Acacia mangium* Willd. Seedlings of *A. mangium* typically produced one pinnately compound leaf and then six or seven bi-pinnately compound leaves, the later ones with lengthening basal segments, and then through leaves eight to ten the basal segments broadened into phyllodes, but bi-pinnately compound segments persisted terminally. Phyllodes only, were formed thereafter (Mahdan 1995).

TREE PHYSIOLOGY

Genetic and environmental regulation of xylogenesis biochemistry continues to be a major focus of the UNB physiology research program (Savidge 1996). Chemical, immunological, and kinetic investigations into coniferyl alcohol oxidase (CAO), a new enzyme spatio-temporally associated with lignification in conifers, confirmed the novel nature of this cell-wall-bound enzyme and led to the conclusion that CAO is not a laccase and can best be regarded as a catechol oxidase (Udagama-Randeniya and Savidge 1995). Phenological

investigations into uridine 5'-diphosphoglucose:coniferyl alcohol glucosyltransferase (CAGT), the enzyme catalyzing biosynthesis of *E*-coniferin (coniferin, the 4-*O*-β-D-glucopyranoside of *trans*-coniferyl alcohol), revealed CAGT to be active specifically in the cambium and only during active cambial growth, in agreement with coniferin itself being localized to the cambium and associated specifically with seasonal growth (Förster and Savidge 1995, 1996). Of all cambial metabolites so far investigated, coniferin is the only one to be qualitatively associated precisely with the period of cambial activity in temperate-zone conifers (Savidge 1996). Continuing investigation into CAGT should provide needed insight into the genetic control of seasonal cambial growth and dormancy.

In planta cambial growth of *Larix laricina* (Du Roi) K. Koch was simulated *in vitro* using explants from eight-year-old stem regions onto solid media containing varied concentrations of the auxin 1-naphthalene acetic acid (NAA). Subsequent microscopy revealed that NAA concentration was a factor determining bordered-pit numbers, diameter, and whether bordered pits were positioned in radial or tangential walls during differentiation of cambial derivatives into tracheids (Leitch 1995; Leitch and Savidge 1995; Savidge and Leitch 1995). *In vitro* research to determine the hormonal and nutriment requirements for cambial growth and xylogenesis in *Eucalyptus globulus* Labill. have been initiated (Leitch *et al.* 1996).

Using one- and twenty-year-old stem cuttings of *Fraxinus americana* L., woods induced to form *in vitro* in response to varied concentrations of exogenous auxin and gibberellic acid were investigated anatomically and chemically. Auxin promoted formation of a vessel-rich wood having a high guaiacyl:syringyl lignin ratio, whereas gibberellic acid promoted a fibre-rich wood having a high syringyl:guaiacyl lignin ratio (Zhong and Savidge 1995a, 1995b; Zhong 1996).

In addition to the evident role of auxin in determining wood-quality, as described above, evidence has been found for the existence of a direct correlation between cambial auxin content and growth rate by investigating three-month-old and 18-year-old members of three half-sib families of *Pinus banksiana* Lamb. of known growth capacity (slow, intermediate and fast). As reported at this conference, a method for screening seedlings rapidly and non-destructively for their cambial auxin content has been developed (Xu and Savidge 1997).

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FOREST GENETICS RESEARCH AT THE CANADIAN FOREST SERVICE - ATLANTIC FORESTRY CENTRE

R.F. Smith, J.M. Bonga, S.I. Cameron, K. Forbes, G. Gesner, M. Grant, R.D. Hallett, C.H.A. Little, I. MacEacheran, Y.S. Park, S. Pond, J. Sweeney, S. Whitney, and L.D. Yeates

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One of the outcomes of the reorganization of the Canadian Forest Service in 1995, was that research programs were partitioned into a series of ten National Networks. Research activities in the area of forest genetics and tree improvement now fall under three networks: 1) Tree Biotechnology and Advanced Genetics, 2) Biodiversity, and 3) Forest Health. The report below represents research and development efforts within the Tree Biotechnology and Advanced Genetics, and Forest Health Networks.

GENETIC STUDIES OF TREE POPULATIONS

by Y.S. Park

Nursery performance of white spruce progeny in a clonally replicated test has been evaluated. The test included a total of 300 clones derived by rooting of cuttings from progenies of a 20-parent disconnected diallel mating. The purpose of this experiment was to explore possibilities for implementing high-value clonal forestry and to examine potential genetic gains from clonal forestry. The nursery test was laid out using ten blocks of single tree plots. At age five, the mean height of the ten best clones was 54% taller than the average of all the clones in the test. As expected, the test exhibited a consistent within-clone uniformity while showing a large among-clone variability. The data on this 5-year nursery test will be correlated with the field test already in place at a regular intervals for long-term evaluation based on the same clones.

Diversity of managed tree populations in plantations can be an important factor for successful establishment and subsequent wood production. A study on genetic diversity of various seedlots of black spruce populations has been initiated to develop a relative diversity index, as well as to identify traits that best indicate genetic differences. The study included 16 seedlots: three open-pollinated plus tree collections from New Brunswick, four open-pollinated collections from Nova Scotia, three stand collections from New Brunswick, three full-sib controlled crossings among first generation selections, one seedlot from an unrogued seedling seed orchard, and two seedlots from two rogued seedling seed orchards. To date, detailed measurements on greenhouse and 3-year nursery experiments have been collected, including germination, survival, growth, phenology, and other morphological observations.

In collaboration with Dr. T.J. Mullin, a revision of POPSIM, a computer program for a stochastic tree population simulator (Mullin and Park 1995), has begun to include additional features of tree breeding components such as "multiple-population breeding" and optimization of parental contribution. Also, a computer program, called "DISCLONE" for analysis of clonally replicated disconnected diallel mating system, has been revised and compiled under Microsoft FORTRAN Power Station. The use and theoretical background of both programs were presented at the North American Quantitative Forest Genetics Group meeting in Orlando, FL, USA (Park and Mullin 1997, Mullin and Park 1997). Both programs are available for downloading from an internet FTP site at this centre (FTP server: **fcmr.forestry.ca**).

SOMATIC EMBRYOGENESIS IN JACK PINE, WHITE SPRUCE AND MATURE LARCH, AND STABILITY OF CRYOPRESERVED CLONES

by J.M. Bonga, S.I. Cameron, K. Forbes, M. Grant, I. MacEacheran, Y.S. Park, and S. Pond

Work on somatic embryogenesis (SE) of jack pine (*Pinus banksiana* Lamb) was initiated in 1995, and was given main emphasis in the past 3 years. The work on SE of white spruce (*Picea glauca* (Moench) Voss), which was initiated in 1991, continued. Also, the work to induce SE from mature European (*Larix decidua* Mill) and hybrid (*L.* x *eurolepis*) larches continued.

Jack pine is a commercially important species in eastern Canada, but is recalcitrant for SE induction. In the spring of 1995, immature cones from 20 open-pollinated families were collected at four weekly collection dates, i.e., July 4th, 11th, 17th, and 24th, and subjected to SE induction using two induction media. Of the 1083 cell lines produced, we obtained four embryogenic lines from the July 4th collection. The embryogenic cell lines were subsequently matured and produced plants (Park et al 1997). Thus, the induction frequency of SE was very low; however, this is the first such success in jack pine. The initial limited success in jack pine SE is considered a primary step for further detailed work.

Three major barriers to efficient jack pine SE culture still exist: 1) the inability to use stored and/or mature seed as a source of explant material, thereby removing the limitation of one two-week window during the year during which successful SE initiation can occur, 2) rapid ageing of SE tissue, requiring frequent cryopreservation to rejuvenate the callus, and 3) development of only a small number of acceptable quality embryos during maturation. Experimentation is currently focused on improving initiation protocols, using both immature and mature seedlots to investigate which combinations of different storage pretreatments and tissue culture media are able to stimulate early-stage SE tissue development.

A 2-year study of the effect of callus age on embryo production has been done. Samples of the same white spruce SE tissue line, either kept in continuous production for over 3 years or newly regrown from cryopreserved callus, were used as "old" and "new" variants, respectively, and their growth was compared in a series of experiments. It was confirmed that embryo yield decreases over time, and that cryopreservation, but not re-initiation from an existing embryo, can rejuvenate production in old SE callus. Production may also be stimulated by withdrawing either auxin, cytokinin, or both phytohormones from the proliferation medium, which may have operational benefits, but the effects differ depending on tissue age. Partial results were presented as a poster (Cameron and Grant 1997) at two meetings.

Genetic stability of cryopreserved white spruce embryogenic clone lines was studied by thawing a set of clones at two different dates, i.e., after 3 and 4 years of freezing, and by comparing developmental and morphological characters during the subsequent *in vitro* and *ex vitro* procedures. This work resulted in a MScF thesis at University of New Brunswick by D. Barrett under supervision of Dr. Y.S. Park. The results indicated that the clones were highly consistent with respect to *ex vitro* morphological characters. However, with respect

to *in vitro* characters, clones were less consistent between the two thawing dates because inconsistent frequencies were found for morphological maturation categories. However, clones were consistent for eight germination categories examined. This indicates that tissue culture characteristics are influenced by the laboratory procedures but, once plants are produced, the growth and morphological traits are governed by genetics in a consistent manner.

The effects of glutamine-based dipeptides, glutamine and casein hydrolysate, as well as deletion of organic nitrogen, were investigated during white spruce somatic embryogenesis (Barrett *et al.* 1997). The results indicated that, without organic nitrogen, fresh weight increase was significantly lower than with organic nitrogen on both initiation and proliferation media. However, there were no differences in the total number of mature somatic embryos produced in cultures grown with various organic nitrogen combinations or without organic nitrogen.

Buds from 36-year-old *Larix decidua* and L. x *eurolepis* trees were used for culture immediately after collection in the field and after 3 months of storage in a freezer (-5 and -10°C). Storage in the freezer strongly stimulated the formation of somatic embryos. Subsequently it was found that the same stimulatory effect could be obtained by starving the explants (on agar with water; no nutrients) for 3 weeks.

REPRODUCTIVE BIOLOGY

by R.F. Smith, S. Whitney, and L.D. Yeates

Research trials focusing on reproductive development continued, primarily on two areas: 1) cone stimulation and 2) the molecular biology of cone development.

Although the induction of seed cones in many conifers has been successfully achieved through applications of gibberellins A_4 and A_7 (GA), pollen production remains more problematic. Work continued on trials to evaluate if a stem injection of Paclobutrazol (2RS,3RS)-1-(4-chloro-phenyl)-4,4-dimethyl-2-(lH-1,2,4-triazol-1-yl) (PAC) could be used as an adjunct treatment to increase the efficacy of GA in increasing flowering in black spruce. Results over 2 years indicate a dose-dependent, but non-linear increase in the production of cones of both sexes in response to stem injections of either GA or PAC. The optimum rate of GA for stimulating pollen production was 3.3 mg whereas the most seedcones were induced on trees receiving 11 mg. The sex ratio (number of seed cones/number of pollen cones) increased with the rate of GA applied. Injecting PAC also promoted cones of both sexes, equally, resulting in sex ratios comparable to that of the control trees. The use of PAC as an adjunct to GA treatments in black spruce seedling seed orchards appears effective, practical and safe. Both the mechanisms whereby PAC affects flowering in black spruce and the potential for increasing flowering in clonal seed orchards and in other conifer species remains to be determined.

Although considerable research has been directed at understanding the factors that control bud differentiation in conifers, efforts have been limited because, until recently, it has not been possible to either visualize or quantify the changes in gene expression that precede morphological differentiation. In 1995, a study was initiated in conjunction with Dr. Bob Rutledge and colleagues at CFS in Quebec on isolating and identifying expression patterns of flowering genes in black spruce. Our efforts focused on the latter component of the project and comprised primarily the use of the technique of *in situ* hybridization. Although preliminary, our findings indicated that the AGAMOUS-like gene, from which probes were synthesized in this study, exhibits a pattern of expression consistent with that of AGAMOUS. Work is ongoing to determine a course for expression of this gene with future studies to focus on testing additional genes that have been isolated and in comparing gene expression patterns between male and female strobili.

PHYSIOLOGY AND GENETIC ENGINEERING OF WOOD FORMATION

by C.H.A. Little

Research on the hormonal control of wood formation and the enhancement of wood quantity and quality using genetic engineering was continued in collaboration with personnel at 1) Swedish University of Agricultural Sciences, Umeå, Sweden, 2) Växjö University, Växjö, Sweden, and 3) University of New Brunswick, Fredericton.

A series of experiments was performed to investigate the interaction of ethylene and gibberellins (GAs) with indole-3-acetic acid (IAA) in the control of tracheid production in shoots of *Abies balsamea* and *Pinus sylvestris*. It was demonstrated that ethylene cannot mimic the promoting effect of IAA on cambial growth; however, when applied in a ring around the stem at unphysiologically high concentration in the form of Ethrel, ethylene does stimulate tracheid production, but indirectly, by locally increasing the IAA concentration in the cambial region (Eklund and Little 1995,1996). That GAs play a role in the control of cambial growth in conifer shoots, and act directly, rather than indirectly by raising the cambial region IAA level, is indicated by the findings that: 1) GAs 1, 3, 4, 9, 12 and 20 occur naturally in the shoot, 2) labelled GA4, GA9 and GA20 applied in a ring around the stem were absorbed into the cambial region, then translocated and metabolized, 3) provided an IAA source was present, ringing with GA4/7 promoted xylem and phloem production without elevating the IAA concentration, and 4) ringing with prohexadione, an inhibitor of GA biosynthesis, decreased cambial growth and the levels of GA1, GA3 and GA4 and increased the GA9 level, but did not alter the IAA level (Wang *et al.* 1995a, 1995b, 1996). The results also indicated that the GA9 to GA4 to GA1 pathway is a major route of GA biosynthesis in conifer shoots. IAA was observed to promote the formation of callus and ensuing differentiation of the vascular cambium that occurs in a bark-peeled portion of *Betula pubescens* stems, provided the girdled region is wrapped in transparent plastic to prevent dessication (Cui *et al.* 1995). Recent evidence implicating plant hormones in the regulation of radial and longitudinal growth in the stem of woody species was reviewed (Little and Pharis 1995).

Feulgen microspectrophotometry, flow cytometry and image analysis were used to measure the nuclear DNA content in ray cells obtained from the vascular cambium of *Fraxinus americana* shoots at intervals during the annual cycle of cambial activity and dormancy (Zhong *et al.* 1995). The results support the view that there is an annual oscillation in the nuclear genome size in shoot meristematic cells in tree species native to the northern temperate zone.

The relationship between nuclear genome size, measured cytophotometrically, and relative ribosomal RNA gene (rDNA) content, determined as the ratio of the hybridization signals from a 25S rRNA gene probe and a randomly labelled total genomic DNA probe, was investigated in cambial region cells of *Abies balsamea* shoots during the onset of dormancy and the transition between the dormancy stages of rest and quiescence (Lloyd *et al.* 1996). The data suggest that the increase in nuclear genome size associated with the rest-quiescence transition is caused by amplification of a fraction that is not rDNA but is recognized by our genomic probe.

The activities of two model heterologous promoters, the *Agrobacterium rhizogenes rolC* and the cauliflower mosaic virus (CaMV) *35S*, both fused to the *uidA* ß-glucuronidase (GUS) reporter gene, were observed to vary in very different, unpredictable ways during the annual cycle of growth and dormancy in stems of hybrid *Populus* (Nilsson *et al.* 1996). The spatial and temporal variation in *rolC* promoter activity reflected cellular and seasonal changes in sucrose content.

To investigate how the superior shoot growth of *Pinus contorta* compared with *Pinus sylvestris* is manifested, the patterns of current-year shoot, needle and terminal bud elongation, as well as mitotic activity in the apical

meristem of the terminal bud, were compared in seedlings of three provenances per species during the third and fourth growing seasons after planting (Norgren *et al.* 1996). The greater final shoot, needle and bud lengths in *Pinus contorta* were attributable more to a faster rate than a longer duration of elongation. The longer final shoot length in *Pinus contorta* reflected more stem units rather than greater stem unit length.

SEED ORCHARD INSECT PEST MANAGEMENT RESEARCH

by J. Sweeney and G. Gesner

Most of our work has focused on the ecology and management of the major cone pest in white spruce seed orchards in Canada, the cone maggot, *Strobilomyia neanthracina* Michelsen. We have studied the population dynamics of the cone maggot at two seed orchards in New Brunswick since 1992, using cohort sampling for life table analysis and manipulative studies to estimate the impact of natural enemies such as parasites and predators. Results suggest that size of the cone crop and its year-to-year fluctuation is a big factor affecting cone maggot population dynamics. Light cone crops in 1995 and 1997 were heavily infested (>95%) and produced little, if any, filled seed; most maggot mortality was due to intraspecific competition. Heavier cone crops usually suffered less than 10% maggot infestation and intraspecific competition was a minor factor affecting maggot survival. The message for seed orchard managers is to concentrate on collecting large volumes of seeds in heavy cone crop years, and either ignore or abort light cone crops. Decisions to apply direct control measures will be more critical in moderate cone crop years, and when managers do not have a surplus of seeds in storage.

A greater proportion of cone maggots remained in extended diapause in light cone crop years, apparently opting to wait for a year when competition for food was less fierce, but we do not know how the maggots "predict" cone crop size. Results of an experiment in which we injected trees with gibberellic acid did not support the hypothesis that extended diapause in *S. neanthracina* was influenced by the perception of chemical cues by larvae feeding in the cones.

In collaboration with Dan Quiring (UNB) and graduate student, Laura Fidgen, we have looked at how certain host attributes, such as cone size, affect the foraging and survival of *S. neanthracina,* and its cousin on black spruce, *S. appalachensis.* Both oviposition and maggot survival tend to increase with cone size. Since maggots prefer to feed in larger cones that have more seeds, their impact on seed production is somewhat greater than if attack was independent of cone size. With support from Forest Renewal B.C., in collaboration with Robb Bennett (BC Min. of Forests), we also determined that the female *S. neanthracina* deposits a host-marking pheromone on cones after ovipositing, and that this pheromone deters oviposition by subsequent females. Manuscripts on both of these topics have been submitted to journals, and are currently in review.

We continued to field test entomopathogenic nematodes as a biological control for direct suppression of cone maggot populations in 1996 and 1997, with support from the Forest Renewal Program of B.C. Attempts to extend nematode efficacy and persistence through the application of bark or hay mulches had inconsistent results. Adequate suppression of cone maggots with nematodes would probably require 2-3 applications per season, and would not be as effective as applying a systemic insecticide. If use of systemics is restricted in the future, however, nematodes may become a viable part of an orchard pest management program.

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BIODIVERSITY RESEARCH AT CANADIAN FOREST SERVICE - ATLANTIC FORESTRY CENTRE

Tannis Beardmore, Bernard Daigle, Moira Campbell, Linda DeVerno, Kurt Johnsen, Judy Loo, John Major, Donnie McPhee, Alex Mosseler, Garry Scheer, and Dale Simpson

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With the closing of the Petawawa National Forestry Institute, researchers and facilities were transferred to the Atlantic Forestry Centre to join staff there as part of the new Biodiversity Network. Although the Biodiversity Project at Fredericton includes speciesand ecosystem diversity work, the group specializes in genetic aspects of biodiversity science including: population genetics, molecular genetics, physiological genetics, seed research, and germplasm storage.

RED SPRUCE ECOSYSTEM BIODIVERSITY PROJECT

by Linda DeVerno, Judy Loo, John Major, and Alex Mosseler

The Biodiversity Project at the Atlantic Forestry Centre, part of the Canadian Forest Service Biodiversity Network, has initiated a long-term study centered around the species red spruce. A workshop was held at CFS-Atlantic, October 22-24, 1996, in which scientists, foresters, and interested biologists from, government, university, and industry sectors of Ontario, Quebec, all Maritime provinces, and the USA were invited to discuss the status and issues surrounding red spruce. Red spruce is a shade tolerant, valuable timber species which is an important component of late successional forests. However, because of past forest practices, red spruce has been in decline over the majority of its range. In the United States, it has been estimated that there are now currently 1/5 the number of red spruce compared to pre-European colonization. This new project is a multi-faceted, multi-partner, collaborative research effort which intends to address a myriad of important forestry issues including the need to rigorously assess alternative silviculture, the impact, extent and role of hybridization, indicators of old growth forests, the sensitivity to climate change factors, and species and ecosystem restoration.

Red spruce and ecosystems containing red spruce represent a particularly useful "model" system for study. Genetic variation in red spruce appears low relative to other conifers. Red spruce also hybridizes with black spruce and clearcut logging appears to have increased the number of hybrids across the landscape; however, the extent of hybridization is hotly debated. In addition, red spruce is particularly interesting in regard to potential responses to climate change. In the United States, red spruce "decline" has been studied extensively and there are strong indications that red spruce responds negatively to winter climate variability. Such variability may increase dramatically in Canada with climate change over the next century. If monitored closely, red spruce, may act as a "bio-indicator" of climate change in Canada. Thus, by intensively studying red spruce, lessons and approaches learned regarding uneven-aged forest management, restoration, high-grading, species hybridization, and climate change, can be applied to other species and systems.

Newly initiated studies are progressing on several fronts. Individual-tree seed collections were made in Ontario, New Brunswick and Nova Scotia, mostly from "old-growth" stands. These will serve to assess the reproductive capacity of the species and also to serve as a "genetic benchmark" for future work in managed and disturbed ecosystems. In addition, the Project is taking advantage of older, pedigreed genetic plantations to study the physiology and molecular genetics of red spruce × black spruce hybrids. One paper currently in press, re-examines a previous hypothesis of physiological depression in F1 hybrids as a physiological barrier between species. Molecular markers are being developed to differentiate between red and black spruce and to identify hybrids. We also wish to establish how species-level biodiversity in managed red spruce stands compares to natural stands and what the impacts of various silvicultural regimes are on biodiversity, both temporally and spatially.

RED SPRUCE RESTORATION

by Alex Mosseler

Only remnant populations of red spruce remain in central Canada. Red spruce decline can be directly linked to human activities such as pollution and clearcutting. However, a growing interest in restoring this species comes from two sources: 1) timber management of conifers, using silvicultural alternatives to clearcutting (e.g. shelterwood and selection harvesting), and 2) habitat management for wildlife. It is anticipated that creation of red spruce deer yards may relieve adverse browsing pressure on hemlock regeneration. Such browsing pressure currently threatens hemlock across central and eastern Canada.

Red spruce restoration must be based on proper seed source selection for artificial regeneration. Unfortunately, there are few remaining viable populations of red spruce from which to collect seed adapted across much of its former range in central Canada. The few extant stands occur in parks and ecological reserves and are thus largely unavailable for operational seed collection. Furthermore, very little is known about the genetic and reproductive status of local remnants of red spruce. The CFS Atlantic Forestry Centre organized seed collections across much of the Canadian range during the good cone crop of 1996 to assess the reproductive status and health of the disjunct Ontario population which consists of small, isolated stands. In collaboration with the Ontario Ministry of Natural Resources, four sites were selected in 1997 for underplanting a range-wide sample of the red spruce gene pool aimed at: 1) assessing the genetic status of the Ontario gene pool both for molecular genetic diversity and for adaptive trait variation, 2) conservation of genetic resources, 3) assessing silvicultural protocols based on nurse crop testing, and 4) establishment of seed sources for eventual species restoration. These sites will be planted to red spruce in spring 1999. Following an analysis of provenances for growth performance, the range-wide red spruce tests established across eastern and central Canada by the CFS 40 years ago will also be rehabilitated to prepare them as seed sources for future restoration purposes.

Other partners in this red spruce restoration effort include companies holding Sustainable Forest Management

Licences in Ontario, the Eastern Ontario Model Forest, and the Forest Gene Conservation Association of Ontario. The strategic objectives of this restoration effort are to foster sustainable forest management practices by conserving the genetic diversity of one of Canada's major tree species by maintaining the wildlife habitat associated with red spruce dominated forests and by providing commercial wood supply based on naturally regenerating silvicultural systems.

GENETIC DIVERSITY OF BUR OAK

by Donnie McPhee and Judy Loo

Bur oak exists in New Brunswick as a few small isolated populations, near the species' northern limit. Historical accounts indicate that the New Brunswick range was once more extensive but the combined effects of harvesting, conversion for agriculture and cottage development throughout much of the species' habitat (river floodplain and lakefront) has greatly reduced the frequency of the species in this province. Threats to the remaining populations continue, prompting concern for gene conservation.

All six of the poulations in New Brunswick, meeting size and distributional requirements, have been sampled for genetic analysis. In addition, three isolated populations in New England, four populations on the fringe of the species' continuous range and six populations from within the continuous range have been sampled. Bud tissue is used for isozyme analysis to estimate levels of genetic diversity and gene flow among populations. In particular, these genetic analyses will be used to determine whether the New Brunswick populations differ from other isolated populations, fringe populations or populations within the continous range of the species, and if so, in what way. The results will be used to develop a gene conservation strategy for the New Brunswick populations.

SEED SOURCES FOR RESTORATION OF ACADIAN FOREST ON PRINCE EDWARD ISLAND

by Judy Loo

A study was completed to evaluate the genetic diversity of populations of sugar maple, red oak, white pine, and white spruce on Prince Edward Island (PEI) in comparison with populations from the mainland. For all but one of the species, four populations were sampled on PEI and three populations were sampled in each of the two Maritime mainland provinces.

Sugar maple was found to have the highest total genetic diversity among the four species and white pine had the lowest levels. Sugar maple also had the highest among population component of isozyme diversity, with the lowerst estimateed gene flow among populations. White spruce and white pine showed very little genetic structuring among regions and only a small proportion of teh total diversity was at teh population level. PEI populations of red oak had significantly less alleles per locus than the mainland populations. Alleles were detected in all mainland populations that were missing in the Island populations, but the Island populations did not have any alleles that were different from mainland populations. Two sugar maple alleles, on the other hand, were found only on the Island.

There is evidence that red oak populations on PEI are genetically isolated from mainland ones and that they have become genetically impoversihed, probably as a result of small population size. Thus restoration efforts should include material from coastal mainland populations. Sugar maple seed should be obtained on the island for planting on the island because of evidence of poplation differentiation and the possibility of specific adaptation.

PROTECTED AREAS AND GENE CONSERVATION STRATEGIES

by Judy Loo

For a short period of time, there was an ecological areas program within CFS funded by Green Plan. At the termination of Green Plan funding the protected areas mandate was transferred to the Biodiversity Network. The main CFS strategy for influencing protected areas was to work with the Model Forest program. Gap analysis projects were initiated in four of the model forests, primarily funded initially, by the Green Plan Ecological Reserves program. Each of these projects has either ended or changed direction at this point. Three of the projects have resulted in one or more new areas recommended for protection. In New Brunswick's Fundy Model Forest, the focus was on identifaction of small-scale plant community types, followed by an assessment of the degree to which the areas are protected or are threatened with land use which would lead to loss of biodiversity.

Approximately 60 sites were identified in the Fundy Model Forest to be in need of formalized protection. Most of these sites are small, but many include species which require conservation measures owing to their risk level and relative rarity in the province. Protection of the largest of these sites from resource extraction would significantly contribute to *in situ* gene conservation. The largest of the identifed sites, totalling more than 600 ha, is in the process of being protected through a land swap agreement. Another smaller site has been donated to the Nature Conservancy and securement of other areas is being pursued.

Recently, a new initiative was begun here to develop gene conservation strategies for tree species which are declining, or are perceived to be at risk. The first steps are to draw up a set of criteria by which tree species may be judged to require special conservation attention. The species are then rated according to the seriousness of the problem. The next step will be to develop conservation strategies for those species for which sufficient data are available and to determine how to obtain the information if it is not readily available.

GENETIC VARIATION IN RED PINE

by Linda DeVerno and Alex Mosseler

The RAPD technique was used in conjunction with high resolution agarose gel electrophoresis to detect genetic variation among individuals within and between seed sources in *Pinus resinosa*. A total of 57 primers that gave consistent, strongly amplified fragments were selected for further screening with DNA from four mutant trees and a rangewide sample of 21 normal trees. Only three of these primers generated amplification fragment patterns that were polymorphic. Restriction endonuclease digestion of RAPD reaction products (RAPD-RFLP analysis), using enzymes with four base-pair recognition sequences, was used to determine if fragments of identical electrophoretic mobility were the result of priming at either single or multiple genomic locations. Out of 64 primer/enzyme combinations tested, one primer/enzyme combination created reproducible polymorphic banding patterns in rangewide red pine DNA samples. Therefore, digestion of RAPD reaction products can be used as a method to increase the probability of detecting genetic variation between highly conserved genomes.

SEED RESEARCH

by Tannis Beardmore and Garry Scheer

Our work focuses on the study of tree seeds which are difficult to store. These 'hard to store' seeds are

classified as recalcitrant. Many of Canada's native tree species, primarily hardwoods, produce seed which deteriorates very quickly in storage. Our main goal is to develop strategies and treatments for storing these tree seeds of a recalcitrant nature.

Low and Ultra Low Temperature Tolerance of Butternut Embryonic Axes

Butternut (*Juglans cinerea* L.) survival is being threatened in North America by the fungus *Sirococcus clavigignenti-Juglandacearum*. To date, control for this fungal disease does not exist and long-term seed storage is not a viable option for this species. Low (0°C, -5°C, -10°C, -15°C, -40°C) and ultra low (-196°C, cryopreservation) storage of butternut embryonic axes has been examined as a method of *ex situ* conservation. Embryonic axes with approximately 3 mm of cotyledonary tissue attached to the hypocotyl area germinated after exposure to 0°C, -5°C, -10°C, -15°C, -40°C and at -196° C (temperature reduced at a rate of -0.33°C/min from 0°C). Percent germination after exposure to 0°C and -5°C was 87 and 82%, respectively and after -10°C, and -15°C was 29, and 27%, respectively. Thirty-two percent of axes germinated after -40°C while 36% germinated after exposure to -196°C. Significant tree to tree variation was found in the embryonic axes tolerance to low temperature. This variation corresponded with the embryonic axes water content; the lower the embryonic axes water content, the greater the tolerance to -196°C. Reducing embryonic axes' water content by slow desiccation to 4.8 % or less resulted in an increased tolerance to -196°C. These results suggest that cryopreservation may be a viable means for *ex situ* conservation of butternut.

Silver and Red Maple Seed Development

In this project, silver maple (*Acer saccharinum*), which produces a recalcitrant seed and red maple (*Acer rubrum*) which produces an orthodox seed are used as a model system for studying biochemical processes that occur during seed development (e.g., the ability of the seed to tolerate desiccation), which are associated with the seed's ability to tolerate storage. *Acer rubrum* can be crossed to *Acer saccharinum* and these hybrid trees, taxonomically recognized as *Acer x freemanii*, produce seeds with a phenotype intermediate to that of the parentals. These three *Acer* species create an excellent experimental system for elucidating biochemical mechanisms that are involved in recalcitrant and orthodox seed behaviour. Results of this work have shown that desiccation tolerance of *Acer saccharinum* embryos can be induced and these embryos can then be stored in the dry state.

Long-Term Storage of Black Spruce, White Spruce, and Lodgepole Pine Seeds

This project initiated in 1994 is an on-going seed storage experiment which is examining the effect of seed moisture content (i.e., 3%, 5%, 15% and 20% moisture content for each species) and storage temperatures (i.e., +4°C, -20°C, -85°C, -195°C) on the germination of black spruce, white spruce, and lodgepole pine seeds. This experiment is on-going and will be completed in 2001.

NATIONAL FOREST GENETIC RESOURCES CENTRE

by Dale Simpson and Bernard Daigle

The Centre is comprised of four sections: National Tree Seed Centre, cryogenic facility, OECD seed certification, and genetics experiment database.

The mandate of the Seed Centre will focus *ex situ* gene conservation by aquiring and maintaining an inventory

of species native to Canada. Collections of native species already in storage will be expanded and seed will be obtained from additional tree and shrub species. Samples will be obtained from throughout the natural ranges of the species in Canada. The Centre will continue to provide seed for research. Facilities for the Seed Centre consist of one walk-in cooler maintained at +4°C and two walk-in freezers set for -20°C, a cone drying room, and equipment for cleaning and testing the seed.

A cryogenic facility was established to provide support for research on alternative means for long-term storage of genetic material and to store somatic embryogenesis material. Facilities consist on two programmable and two stainless steel freezers.

Canada is a signatory member of the OECD Scheme for the Control of Forest Reproductive Material Moving in International Trade. The responsibility for implementing the scheme was directed to the Canadian Forest Service (CFS). Historically, all certification has been conducted in British Columbia. As a result of program review, management of the OECD Scheme was transferred to the Genetic Resources Centre while certification will continue to be conducted from the CFS lab in Victoria. The current scheme, which was implemented in 1974, will soon be replaced by a new scheme following its approval by OECD member countries.

A database of all genetics experiments established by the Petawawa National Forestry Institute was created over a ten-year period. This, as well as all experiment files, were also transferred with the Genetic Resources Centre. Work has begun on creating a similar database for genetic experiments planted in the Maritimes. Over time, databases can be developed for experiments established by other CFS laboratories.

PHYSIOLOGICAL GENETICS

by Kurt Johnsen, John Major and Moira Campbell

Results from intensive drought tolerance research conducted on four black spruce full-sib families at the Petawawa Research Forest have continued to be analyzed and written up. In addition to the photosynthetic responses reported previously, the families have been shown to differ in shoot water relations. Drought tolerant families consistently maintained higher turgor than intolerant families. Thus, it appears that stable families possess both a higher source of carbon from higher P_n and a higher sink for carbon via the influence of higher turgor on growth. It is unclear still if higher P_n and higher turgor are genetically independent or not. Across all three study years, genetic differences in carbon isotope discrimination, net photosynthesis, and water relations were remarkably consistent. Some of the measured traits were highly correlated with growth. For example, the relationship between mean family predawn turgor and mean family growth results in a 0.818 correlation coefficient. The genetic differences in the various traits were always small to moderate in magnitude. However, it is our contention that, due to compounding over time, these small differences in physiological function have contributed to large growth differences among the families.

In collaboration with Dr. Larry Flanagan (Carleton University) , samples were collected from 25 families (all crosses of a diallel without reciprocal crosses), measured for carbon isotope discrimination and subjected to quantitative genetic analyses (Dudly Huber, U. of Florida). Approximations of genetic correlations are -0.94 between height and discrimination and -0.82 between diameter and discrimination. Selfs deviate greatly from the strong growth-discrimination relationships indicating self pollination results in differential deleterious effects on photosynthetic properties and other mechanisms related to growth. Individual-tree heritability for carbon isotope discrimination was 0.66, higher than that calculated for height (0.45) or diameter (0.14), indicating carbon isotope discrimination in this population is under a high degree of genetic control.

In addition, experiments have been established with the main aim to assess within-stand variation to both drought and elevated CO_2. In cooperation with Dennis Joyce of the Ontario Ministry of Natural Resources, seed was collected from four stands in Ontario. Clones were serially propagated using rooted cuttings. Three clones from each of eight mother trees per stand were established in both a CO_2 and a drought interaction study. The studies will be three years in duration and will assess growth, phenological, and physiological traits.

Work conducted in the early 1970's indicated that seedlings resulting from the hybridization of black and red spruce exhibited negative heterosis in regard to both growth and photosynthesis. At that time, two field plantations were established with the same genetic material. Analysis of height growth to date indicates that in both plantations height growth decreases with the increased proportion of red spruce ancestry (based on morphological index of parents). A preliminary examination of gas exchange over a week in 1994 indicated no differences among progenies ranging in quartile increments from pure black spruce to pure red spruce. Gas exchange was examined more intensely in 1996 on the parent species as well as both putative F_1 and F_2 hybrids which again revealed no photosynthetic hybrid inferiority . These results all suggest negative heterosis appears to be of little importance as an isolating barrier between red and black spruce. Crossability and ecological barriers appear more important in maintaining the segregation of the species.

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Keywords: China, computer simulation, genetic engineering, group coancestry, *Picea glauca*, *Picea mariana*, *Pinus banksiana, Populus* spp., population-merit selection, Sweden, transgenic forest trees

Genesis has offered forestry consulting services for the past 7 years, specializing in aspects of forest renewal. This report summarizes the major contracts, completed or in-progress over the past 2 years, which are related to tree improvement and forest genetics.

GANSU FOREST TREE NURSERY PROJECT, CHINA

Technical services have been provided since 1992 to Roche ltée, Canadian Executing Agency for the Gansu Forest Tree Nursery Project, funded by the Canadian International Development Agency. In 1995, a 7-month overseas mission was completed as "Nursery Systems Specialist" to design and implement a program of technology transfer on all aspects of containerized and bareroot nursery production and to lead a program of applied research with the aim of adapting Canadian reforestation and tree improvement technology to local conditions (Mullin 1995a).

REGULATION OF GENETIC ENGINEERING FOR FOREST TREES

Canada is participating with the Organization of Economic Cooperation and Development (OECD) in efforts to harmonize regulatory control of products derived from biotechnology, including forest trees. An OECD committee adopted a plan to produce consensus documents on the biology of various tree species, as a first step in the regulatory process. Genesis was contracted by the Canadian Forest Service to draft consensus documents for white spruce (*Picea glauca*) and poplars (*Populus* spp.), as part of Canada's contribution to the OECD effort (Mullin 1997a, 1997b). While the release of transgenic materials in natural ecosystems must be done in an environmentally responsible manner, the issues surrounding the potential benefits and biosafety of this technology have not been well evaluated by Canadian stakeholders. The Canadian Forest Service thus contracted Genesis to prepare an overview document to facilitate a discussion within the forestry community, leading to improved direction of research and contributing to the harmonization of regulatory oversight of

genetically engineered forest trees (Mullin and Bertrand 1997).

ANALYSIS OF STODDART BLACK SPRUCE FAMILY TEST DATA

Member agencies of the Northeast Seed Management Association established a black spruce (*Picea mariana*) seedling seed orchard for the Island Lake Tree Improvement Area and 2 associated family tests in Stoddart Township, Ontario in 1986. Field data collected from the 400 families after 5 and 9 growing seasons were analyzed and families ranked according to Best Linear Prediction (BLP) of breeding value, as a guide to orchard roguing. Estimated average gain in breeding value if the top 20% of the families are retained and mate as an ideal seed orchard population averaged 4.5% (Mullin 1996).

ANALYSIS OF LAKE NIPIGON WEST JACK PINE FAMILY TEST DATA

In 1987, members of the Ontario Tree Improvement Board established a jack pine (*Pinus banksiana*) seedling seed orchard at Kakabeka Falls, Ontario, and associated family tests at four locations in the Nipigon West Breeding Zone. The 400 families were ranked at 10 years according to BLP estimates of breeding value, as a guide to orchard roguing. If the top 20% of the families are retained and mate as an ideal population, volume gain from the roguing is estimated to average 8.7% (Mullin 1997c). Individual breeding values were calculated by BLP for over 32,000 trees growing in the family tests and seed orchard, as candidates for selection into the second-generation breeding population. Expected gain in volume from selecting the top 1.5% by the selection index was estimated as 18.6% (Mullin 1997d).

SIMULATION OF GENETIC CHANGE IN MANAGED POPULATIONS

The simulation techniques developed earlier in a computer program known as POPSIM were further developed and made available on the Internet (Mullin 1997e; Mullin and Park 1997; Mullin *et al.* 1995). A collaboration was formed with Professor Dag Lindgren (Swedish University of Agricultural Sciences, Umeå) to promote the application of simulation techniques to evaluate genetic change. During two visits to Sweden as an invited scientist, funded by the Jacob Wallenberg Foundation, the writer collaborated with graduate students and scientists at the Centre for Prediction of Genetic Change (Swedish University of Agricultural Sciences) and the Swedish Forest Research Institute (SkogForsk), and applied POPSIM in an evaluation of genetic diversity and potential for future gains from Sweden's tree improvement program (Mullin *et al.* 1996). This collaboration will continue under a two-year research grant, awarded to Genesis by the Swedish Tree Breeding Foundation (Föreningen Skogsträdsförädling).

COMPARING SELECTION METHODS AND THEIR IMPACT ON GAIN AND RELATEDNESS

When selecting breeding populations, tree breeders face the paradox of how to achieve gain while controlling relatedness so that genetic diversity is conserved. Selecting related individuals results in rapid loss of genetic diversity, and lost potential for future gain. The consequences of different selection approaches, in terms of both gain and relatedness, were examined in a series of simulation studies (Andersson *et al.* 1997a, 1997b; Lindgren *et al.* 1997b). The concept of "group coancestry" was incorporated in a new selection method that considers both gain and relatedness simultaneously (Lindgren and Mullin 1997a; Lindgren *et al.* 1997a; Mullin

and Lindgren 1997). The new selection method, now known as "population-merit selection", was applied to the Lake Nipigon jack pine data described earlier and found to result in a more optimal balance of gain and relatedness in a real-life selection operation for advanced-generation breeding (Mullin 1997d).

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DETERMINATION OF WOOD QUALITY AND END-PRODUCT POTENTIAL FOR A GENETICALLY IMPROVED RESOURCE

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With the decreasing supply of softwood resource, poplar has become an important source of fibre supply in many eastern mills. Although great strides have been made in utilizing the poplar resource for various products there are still many problems to address and opportunities for value-added products. The development of fast-growing hybrid poplar clones, over nearly 30 years in Québec, has produced promising clones that are recommended for reforestation. While these clones have shown excellent growth performance and resistance to diseases and pests, there is, however, very limited knowledge on the wood quality and end-product potential of these clones. In an attempt to provide needed information and guidance to industry and governments, we initiated a multi-year project to address wood quality and end-product potential for balsam poplar and hybrid poplar clones recommended for reforestation in Québec.

In collaboration with Drs. N. Isabel, J. Beaulieu, and J. Bousquet, we also carried out a study on wood density of black spruce (*Picea mariana* (Mill.) B.S.P.) in Québec. The objectives of this study were to: 1) determine the general trend in growth and wood density profiles, 2) estimate the age of transition from juvenile to mature wood, 3) examine the phenotypic correlations among some intra-ring characteristics in juvenile and mature wood zones, 4) determine the age at which wood density and ring width can be predicted, and 5) evaluate the possibility of predicting wood density and growth from individual rings. Such a study is useful to both wood scientists and tree breeders. Wood density is highly correlated with wood mechanical and physical properties. Thus, the knowledge of density variation and its radial patterns are essential to efficient wood processing and end uses. For tree breeders, this study will provide valuable information on the variation in wood density and ring width.

In addition, we also carried out other research projects dealing with the impact of silviculture on log and wood characteristics, product quality, and value.

ESTABLISHING POTENTIAL END-USE OF HYBRID POPLAR CLONES

In collaboration with Dr. Gilles Vallée from the Québec Ministry of Natural Resources, les Industries Mégantic, Domtar, and University of Québec, we initiated a multi-year and multi-disciplinary project on end-use wood

characteristics and utilization of hybrid poplar clones. The project is divided into two parts. The main objective of Part I of the study is to compare important wood characteristics of 21 three-year-old hybrid poplar clones growing on a fertile clay soil and on a poorly drained low quality site, for early selection of better performing clones. The objective of Part II is to evaluate key wood quality characteristics which determine end-use and product quality of 5 fast-growing hybrid poplar clones recommended for reforestation in southern Québec. These clones have reached rotation age (15 year-old).

Part I: Wood quality of 21 three-year-old hybrid poplar clones

This study revealed that site conditions affect the diameter and height growth of nearly all three-year-old hybrid poplar clones. The average DBH for the best quality site (St-Ours) was approximately 55 mm compared to 24 mm for the site in Windsor, Québec. The average fibre length in such young trees does not differ appreciably from site to site (< 2%). However, approximately $^2/_3$ of the clones recorded higher average fibre lengths on the most productive site.

Results also indicate that average wood density is lower on the best site for the overwhelming majority of the clones. On average, wood density for trees from St-Ours and Windsor is 351 kg/m^3 and 401 kg/m^3, respectively. Several clones did well on both sites. Among them are three *Populus interamericana* (*P. trichocarpa x P. deltoides*) clones from Belgium, that are recommended for reforestation in southern Québec. They were among the best 4 clones on each site.

It is difficult to determine if all wood characteristics of mature clones can be predicted at such an early age. However, as an example we noted that clone 3308, which ranked in the top 5 out of 21 clones on both sites for average fibre length, was first (see below) among 5 fifteen-year-old clones recommended for reforestation in southern Québec.

Part II: Wood characteristics and end uses of 5 fifteen-year-old clones recommended for Québec

Wood characteristics of approximately 45 fifteen-year-old trees from five clones recommended for reforestation in southern Québec and two reference clones were evaluated. Trees from this site were pruned and thinned at age 5. Three *Populus interamericana* clones (*P. trichocarpa x P. deltoides*) from Belgium had the best radial growth rate. Although preliminary mill trials showed that all poplars on this site can produce very good veneer, we believe that these 3 clones have the potential for producing above average veneer bolts because of their excellent growth rate and short rotation, which will maximize yield and practically eliminate any decay formation before harvesting. Pruning also helped produce veneer bolts with a very low incidence of knots in the lower parts of the stem. These clones can also be considered for lumber production, even if their wood density and bending properties are somewhat lower than those of the reference clones. Wood from these clones generally has shorter fibers which could limit their application in pulp and papermaking.

Clone 3308, a *Populus euramericana* (*P. deltoides x P. nigra*) from France, on the other hand, has the best average fibre length of all the recommended clones for southern Québec. It should thus have superior pulp and paper properties. A study by Dr. Jacques Valade and his associates from University of Québec, using the same material, concluded that this clone was the only selected clone for southern Québec that produced chemithermomechanical pulps having properties similar to aspen. Another clone (reference clone 3005), *P. euramericana* from France, also has excellent fibre length values (slightly superior to clone 3008). Clone 3008 may also produce high-quality logs for veneer production.

Wood properties of clone 131, a *Populus euramericana* from Québec, are among the lowest for most product applications. This clone has the lowest diameter growth, the lowest wood density and the shortest average fibre length. It is also at the lower end of the clones for bending properties. Its use in reforestation should be limited.

In conclusion, 4 of the 5 clones recommended for reforestation in southern Québec have shown good potential for producing quality fibre for the manufacture of composites and other end products (e.g., pulp and paper, lumber). It should, however, be kept in mind that volume growth comes at the expense of wood density and mechanical property. Nevertheless, large proportion of high quality clear wood will help industry produce high-quality and value-added products commanding top prices.

WOOD QUALITY AND END-USE POTENTIAL OF BALSAM POPLAR IN RELATION TO SITE, AGE, AND HEIGHT

In collaboration with the Québec Ministry of Natural Resources and the Société d'exploitation des ressources de la Neigette, we initiated a study on wood quality and end-product potential of balsam poplar because this species is almost completely underutilized in the Lower-Saint-Lawrence and little information is available on the effect of site and age on wood quality. Approximately 25 trees representing 5 diameter classes were selected from each of 3 typical sites where balsam poplar grows in the region. Discs were collected at several height levels from each tree for the determination of major wood characteristics (density, growth rate, age, stain, rot, ring shakes, etc.) that affect product quality and value. Additionally, a one-meter long bolt was collected from one tree per diameter class on each site, for the determination of bending properties.

The results indicate that balsam poplar grows almost twice as fast on the best type of site as on the worst site. Growth rate is extremely important in this species since trees are often severely affected by decay at a very young age. In fact, Zhang and Chauret (1996) found that balsam poplar should ideally be harvested at approximately 60 years of age to prevent severe occurrence of decay. The average DBH at this age will vary from site to site (20 cm to 50 cm). On the downside, both wood density and mechanical properties of the wood from trees grown on better sites are significantly lower. This study also revealed that wood density increases with position along stem height. It is unknown whether the mechanical properties of the wood from bolts located higher in the tree will be greater or not.

In conclusion, this study failed to identify any serious utilization problem in this species providing that trees are harvested at the recommended ages for each type of site. In fact, we found that the wood characteristics of this species seem to be adequate for the production of a number of products (e.g., pulp and paper, panel products, lumber). To take advantage of the variation in wood quality with height and to maximize the value of each tree, we recommend that each log (height levels) be sorted and transformed into products of the highest possible value. For example, large clear logs should be used for veneer, while smaller logs from tops or smaller trees could be converted into pulp and paper or panel products.

VARIATIONS AND CORRELATIONS OF WOOD DENSITY AND GROWTH IN BLACK SPRUCE

Part I: Transition age from juvenile wood to mature wood based on wood density

Two black spruce plantations from two different locations were sampled. At the end of the 1995 growing season, 1 032 trees from 86 provenances were sampled from a provenance test at Mont-Laurier, Québec. In addition, at the end of the 1996 growing season, 1 000 trees were sampled from a mature commercial plantation in Victorialle, Québec. From a constant compass direction, 6-mm increment cores were sampled from each tree at breast height. Each core was sawn into a 1.57-mm-thick (longitudinal) x 6-mm wide strip for X-ray densitometric analysis. This analysis provides various density and growth data on individual rings. These include earlywood width, latewood width, ring width, earlywood density, latewood density, and average wood density of the ring. Based on the data from individual rings, weighted averages were computed for all measured traits.

The intra-ring characteristics of all sampled trees in both plantations were studied. The radial trends were similar to those reported previously for *Picea* species. The ring density in juvenile wood is high near the pith, then decreases rapidly to a minimum in the transition zone, and finally shows a steady increase thereafter. Latewood density increases constantly to a maximum at 12 years of age, then decreases outwards.

Part II: Correlations in wood density and growth between juvenile wood and mature wood

Juvenile-mature correlations in black spruce were carried out based on increment core samples taken from the two plantations. For all measured characteristics, correlations between juvenile and mature wood are highly significant. Thus, selection for these wood characteristics appears feasible during the juvenile period. Twelve growth rings from the pith serve as a good predictor for the wood characteristics of both mature wood and the whole tree. In addition, individual growth rings from the juvenile-mature wood transition zone can be used to predict wood density of mature wood or of the whole tree. Moreover, ring density components are significantly correlated with their respective ring width components. Earlywood density and ring density are negatively correlated with ring width and earlywood width while ring density and latewood density are positively correlated with latewood width. These hold true in both juvenile wood and mature wood. However, the correlations are lower in mature wood. In fact, the correlations between these traits and ring density tend to decrease with increasing age.

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AMÉLIORATION DES ARBRES FORESTIERS À LA DIRECTION DE LA RECHERCHE FORESTIÈRE DU MINISTÈRE DES RESSOURCES NATURELLES

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AMÉLIORATION GÉNÉTIQUE DU PIN GRIS, DU PIN LODGEPOLE ET DU PIN SYLVESTRE

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Mots-clés : Test de descendances, test de provenances, éclaircie génétique, récolte dirigée.

L'éclaircie génétique des vergers à graines de semis de pin gris (*Pinus banksiana*) s'est poursuivie en 1995 et 1996. Le calcul du gain génétique pour 11 options de récolte de cônes (récoltes dirigées) dans sept vergers déjà éclaircis a été effectué. Des prescriptions d'éclaircie ont également été faites dans quatre tests de descendances de pin gris.

L'éclaircie génétique (sélection de provenances et sélection individuelle) a été faite en 1995 dans six tests de provenances de pin lodgepole (*P. contorta*). En 1995, un mémoire a été publié sur les résultats de 10 ans de 11 tests, de pin lodgepole établis en 1980 et 1981.

L'éclaircie génétique (sélection de provenances et sélection individuelle) a été faite en 1995 dans sept tests de provenances de pin sylvestre (*P. sylvestris*) et en 1996 dans deux tests de provenances et un verger à graines. En 1996, un mémoire a été publié sur les résultats de 10 ans de 12 tests de provenances de pin sylvestre d'Europe et d'Asie et de quelques sources issues de plantations du Québec. Ce mémoire comprend l'évaluation de la coloration automnale des aiguilles, pour les arbres de Noël.

En 1996, une note de recherche forestière a été publiée sur la performance (croissance et coloration des aiguilles) de 42 provenances d'épinette du Colorado (*Picea pungens*) et de deux provenances d'épinette d'Engelmann (*P. engelmannii*) en plantation sur un site au Québec.

RECHERCHE ET DÉVELOPPEMENT SUR LES SEMENCES ET LE POLLEN

par Stéphan Mercier

Mots-clés: Pollen, semence, pollinisation, verger sous abri, stratification, criblage.

Vergers à Graines Sous Abri

Ces vergers sont axés sur la production de graines améliorées d'épinette noire (*P. mariana*), d'épinette blanche (*P. glauca*) et de mélèze hybride (*Larix* sp). Les efforts sont mis principalement sur l'établissement d'une seconde génération de verger à graines, sur l'accélération de la croissance des greffes et sur les travaux de R-D concernant la pollinisation dirigée et de masse et l'induction florale du mélèze. Depuis cette année, nous travaillons à la mise en place d'un nouveau concept de verger de 2^{e} génération qui consiste à disposer les arbres à l'intérieur de brise-vent espacés de 60 à 80 m afin d'augmenter la température du site (accélération du développement des cônes mâles et femelles) et pour faciliter la gestion du vent lors des travaux de récolte massive de pollen et de pollinisation de masse. Par ailleurs, nous mettons au point un abri économique qui permet d'accueillir des arbres de 6 m de haut.

Production et Utilisation de Semences

Notre Direction, en collaboration avec le Centre des semences forestières de Berthier, a développé un système de stratification dit « en lasagne » qui permet d'accélérer, d'uniformiser et d'augmenter la germination des graines d'épinette blanche. Cette technique s'avère très supérieure par rapport à celles utilisées conventionnellement, dont la technique de *priming*. Par ailleurs, nous avons suivi l'effet du criblage des graines d'épinette blanche sur la qualité des plants produits en pépinière. Nous tentons de faire actuellement la démonstration que le criblage de ces graines ne réduit en rien la diversité génétique du matériel produit.

SÉLECTION D'ARBRES FEUILLUS POUR LEUR RÉSISTANCE AUX MALADIES

par Marie-Josée Mottet

Mots-clés : *Populus, Betula, Septoria musiva, Entoleuca mammata, Hypoxylon mammatum, Nectria galligena.*

Depuis 1986, la méthode employée pour évaluer la sensibilité des clones et semis de peuplier au chancre septorien (*Septoria musiva*) consiste à inoculer artificiellement le pathogène en pépinière. Plus de 200 clones et plusieurs milliers de semis de peuplier ont été sélectionnés pour leur résistance ou leur faible sensibilité. La sensibilité et la résistance des plants sélectionnés est suivie en plantation.

Les inoculations en serre et en pépinière nous ont permis de noter des différences de sensibilité à *Entoleuca mammata* (*Hypoxylon mammatum*) entre certains clones de peuplier. Quelque 135 semis sélectionnés pour leur résistance, ont été clonés et sont évalués sur différents sites. La plupart des semis résistants sont des hybrides avec *Populus alba*. Sur une centaine d'autres clones - principalement des peupliers faux-trembles - testés en 1993 dans la région de l'Abitibi, les résultats de 5 ans confirment la résistance de 30 clones d'hybrides de baumier. Sur 80 clones de la section Leuce, seulement six clones dont trois *P. tremuloides*, montrent une plus grande résistance.

Une méthode d'inoculation artificielle en serre et en pépinière a été utilisée sur différents clones et provenances de bouleau jaune afin d'étudier la variabilité génétique de la sensibilité de cette essence à *Nectria galligena*, champignon responsable du chancre. Un dispositif a été établi au champ pour un suivi à

plus long terme. L'analyse des résultats des tests précoces a permis d'obtenir des clones et une provenance de bouleau jaune montrant jusqu'à maintenant, une meilleure résistance. Ce type de sélection pourra s'appliquer à d'autres maladies rencontrées en plantation.

AMÉLIORATION GÉNÉTIQUE DE L'ÉPINETTE BLANCHE

par Marie-Josée Mottet at André Rainville

La responsabilité de l'amélioration génétique de l'épinette blanche a été transférée du SCF (Service canadien des forêts) au MRN en 1996. Au programme de recherche initié par le SCF, qui a maintenant été repris par M André Rainville, le MRN a greffé un volet visant à tirer profit du matériel présent dans les vergers à graines clonaux de première génération en orientant les récoltes de cônes sur les meilleurs clones ; c'est Mme Marie-Josée Mottet qui en est responsable.

Quelque 3 000 greffes représentant 460 arbres sélectionnés dans des tests génétiques ont été repiquées en pépinière à la Station forestière de Duchesnay en 1996 pour culture intensive. Des observations sur la floraison et sur la densité du bois seront prises pour permettre de réduire cette population à 240 arbres ; ils serviront de géniteurs pour la prochaine génération.

Au printemps de 1996, deux plantations de sélection ont aussi été réalisées au Québec ; elles sont constituées de descendances issues de croisements dirigés réalisés entre les 100 premiers arbres sélectionnés par le SCF. En 1997, le MRN a fait la plantation de cinq tests de descendances issues de croisements dirigés polycross, réalisés à partir de la même population d'arbres sélectionnés.

Dans le cadre du second volet initié par le MRN en 1996, des tests de descendances seront réalisés à partir des 17 vergers à graines clonaux de première génération. Les graines issues de pollinisation libre, ont été récoltées en moyenne sur six ramets de chaque clone. En 1997, deux tests de descendances reliés à un verger ont été établis. Quatre autres tests ont été réalisés pour étudier les effets du type de pollinisation et du nombre de ramets sur le classement des clones. La production de plants pour 21 autres tests a été amorcée.

AMÉLIORATION GÉNÉTIQUE DES FEUILLUS À BOIS NOBLE

par André Rainville

Mots-clés : Amélioration génétique, croisements dirigés, feuillus nobles, sélection d'arbres, greffage.

Pour le chêne rouge (*Quercus rubra*) et le frêne d'Amérique (*Fraxinus americana*), le programme d'amélioration génétique est orienté vers une stratégie à long terme ayant pour base les tests de provenances-descendances. Les 5 tests de chêne rouge établis en 1993 devaient être complétés en 1995 et 1996, mais le gel des plants nous oblige maintenant à recommencer le travail de récolte. En 1997, deux vergers à graines clonaux constitués d'une centaine d'arbres-plus ont été plantés au Québec. Pour le frêne d'Amérique, un verger à graines a été planté en 1994. La constitution de tests de provenances-descendances est retardée depuis quatre ans en raison de la production erratique de samares de l'espèce.

Pour le bouleau jaune (*Betula alleghaniensis*), plus de 120 arbres-plus ont été établis dans 4 vergers à graines clonaux en 1996. Ces greffes serviront à faire des croisements dirigés interspécifiques. En 1996, deux tests de provenances-descendances ont été plantés en milieu forestier. Ces tests sont constitués de demi-fratries

récoltées sur des arbres sélectionnés et sur des arbres-témoins ; en plus de valider l'efficacité de la sélection phénotypique, ces tests serviront à évaluer le comportement de l'espèce (qualité des tiges et croissance) lorsque plantée en milieu forestier.

Le programme d'amélioration du bouleau à papier (*B. papyrifera*) comporte une voie exploratoire basée sur les croisements inter-spécifiques, et une stratégie basée sur l'amélioration simultanée du bouleau à papier et de bouleaux d'espèces exotiques. Des greffes de bouleau à papier sélectionnés ont été plantées en 1996 et doivent servir pour effectuer les croisements interspécifiques à partir de 1998. Des bouleaux d'espèces exotiques seront greffés en 1997-98 dans cet objectif. Dans le second volet, la récolte de semences provenant de toute l'aire de distribution de l'espèce au Québec est prévue.

Finalement, 3 vergers à graines clonaux de noyer noir (*Juglans nigra*) ont été établis en 1996 et 1997 avec du matériel ayant démontré une bonne résistance au froid et une croissance intéressante au Québec. Les semences récoltées sur les ortets ont aussi permis d'établir 2 tests de descendances en 1997 (ensemencement direct sous paillis). Pour le noyer cendré (*J. cinerea*), trois plantations conservatoires constituées de provenances de tout le Québec ont été réalisées en 1996, hors de l'aire de distribution naturelle, dans le but de protéger l'espèce contre une maladie dévastatrice, le chancre du noyer cendré (*Sirrococcus clavigignenti-juglandacearum*).

AMÉLIORATION GÉNÉTIQUE DES MÉLÈZES (*LARIX* SP.) ET DE L'ÉPINETTE DE NORVÈGE (*PICEA ABIES* KARST.)

par Ante Stipanicic

Mots-clés : Éclaircie génétique, croisement dirigé, tests de descendances, tests de provenances.

Nous avons continué les éclaircies génétiques dans les tests de descendances ou de provenances de mélèze d'Europe (*L. decidua*), de mélèze du Japon (*L. kaempferi*), de mélèze hybride et de mélèze laricin (*L. laricina*) qui ont atteint l'âge de 15 ans. Le but est de transformer ces tests en sources de graines génétiquement améliorées. Nous avons profité de la bonne fructification de 1996 pour récolter les cônes dans un des tests éclaircis. La qualité génétique des arbres semenciers sera évaluée. La sélection des arbres était basée sur la hauteur et la flexuosité du tronc. Actuellement, 30 plantations d'une superficie totale de 40 ha ont été traitées.

Nous avons effectué 23 croisements entre des arbres d'élite de mélèze d'Europe, de mélèze du Japon et de mélèze laricin. Nous avons obtenu quelques hybrides triples (*decidua* X hybride *kaempferi* X *siberica* ou *decidua* X hybride *laricina*X *kaempferi*). En 1996, nous avons commencé une série de croisements dans notre parc à clones sous abri composé de 25 clones de mélèze du Japon et 25 clones de mélèze d'Europe sélectionnés. Les ramets sont cultivés dans des contenants de 50 l, et la première série de pollinisation nous a permis d'obtenir 10 croisements biparentaux et huit croisements issus de mélange de pollen (polycross).

Deux nouvelles plantations d'introduction sont composées de quelques provenances de mélèze d'Europe et d'hybride x *eurolepis*. Quatre nouveaux tests de descendances englobent 30 hybrides obtenus par nos croisements dirigés. Soixante descendances de mélèze de Sibérie font partie d'un autre test dans la forêt boréale. Un nouveau verger à graines de mélèze hybride, composé de 36 clones de mélèze d'Europe et de 190 de mélèze du Japon, est installé en partie sur trois sites. Parmi nos résultats, il faut mentionner l'accroissement remarquable de quelques hybrides dans un test de descendances âgé de 4 ans situé dans l'arboretum de Verchères : l'hybride x *eurolepis* « Lola 1 » d'Allemagne a atteint 4,19 m de hauteur moyenne

et un de nos croisements, S10743, 3,99 m.

Pour l'épinette de Norvège, nous avons actuellement 14 dispositifs éclaircis (superficie totale de 11,6 ha), dans lesquels on peut récolter des graines améliorées. Les croisements dirigés et l'installation de nouveaux dispositifs expérimentaux sont faits en collaboration avec le Service canadien des forêts. En 1996, nous avons effectué huit croisements dans notre parc à clones de Duchesnay, en vue de compléter deux plans factoriels pour deux zones d'amélioration; 120 arbres sélectionnés dans 12 provenances recommandées seront utilisés pour obtenir des hybrides inter et intraprovenances. Une série de neuf tests de provenances-descendances pour la zone du Saint-Laurent a été mise en marche en février 1995. On veut délimiter avec plus de précision les zones d'amélioration de l'épinette de Norvège ainsi qu'évaluer plusieurs descendances de provenances québécoises et polonaises.

Deux tests de descendances en pépinière sont inclus dans une étude sur la biologie du charançon du pin blanc (*Pissodes strobi*) menée par l'équipe du Service canadien des forêts. Pour une autre étude dirigée par les chercheurs de Direction de la conservation des forêts du Ministère des Ressources naturelles du Québec, deux tests de provenances servent à évaluer l'impact des attaques de charançon sur différentes provenances d'épinette de Norvège.

SÉLECTION DE CLONES ET AMÉLIORATION GÉNÉTIQUE DU PEUPLIER

par Gilles Vallée

Mots-clés : *Populus* Michx., croisement, test clonal, test de descendances, test de provenances.

Durant les deux dernières années, l'intérêt de l'industrie forestière québécoise pour la plantation de peuplier hybride a continué de s'amplifier en même temps que la demande de bois de peuplier. Une nouvelle usine de panneaux à particules orientées s'est ajoutée aux cinq usines déjà en fonction et les usines de pâtes et papiers, de sciages et de déroulage consomment plus de bois de peuplier. L'un des problèmes rencontré est le manque de billes de qualité pour le déroulage et le sciage dans les peuplements naturels de peuplier faux-tremble (*Populus tremuloides* Michx.). La plantation de peuplier hybride peut pallier ce problème. La récolte d'arbres de 15 ans dans un test clonal de la région de Montréal a permis de faire un essai de déroulage en usine. Cet essai, fait à partir d'un échantillonnage des cinq meilleurs clones, a été très concluant de sorte que la compagnie qui a fait l'essai a réservé toutes les billes de déroulage du test.

En 1996, un plan de croisements a été mené en collaboration avec Panneaux Chambord inc. et la Direction régionale du Ministère, afin de développer une population d'hybrides pour le domaine écologique 8 de la région du Saguenay - Lac-Saint-Jean. Les croisements ont été faits en utilisant des espèces et hybrides autochtones (*P. deltoides* Marsh., *P. balsamifera* L., *P. tremuloides* Michx., *P. grandidentata* Michx., *P.* x *Jackii* Sarg., *P.* x Rollandii Rouleau, *P.* x *euramericana* Dode) et des espèces et hybrides de la collection du Ministère (*P. alba* L., *P.* x *canescens* Smith, *P. maximowiczii* Henry, *P. nigra* Dode, *P. tremula* L., *P. trichocarpa* Torr. & Gray, etc.). Quelque 741 croisements de la section Leuce ont été faits dont 140 ont donné 12 420 semis et 227 croisements des sections Aigeiros et Tacamahaca sur 1 215 réalisés ont donné 25 241 semis. Les plants ont été cultivés en 1996 et seront plantés en tests en 1997.

Onze tests clonaux et trois tests de descendances d'hybrides ont été établis en 1995 et 1996. Depuis 1969, 3 707 clones ont été évalués ; 575 ont été sélectionnés dans les peuplements naturels, 2 373 dans des plantations comparatives et des pépinières et 759 autres clones ont été introduits surtout d'Europe et d'Ontario. De plus, 93 tests clonaux, 28 plantations de collections de clones et 41 tests de provenances et

descendances ont été mis en place. Quelque 6 260 croisements ont été faits dont 1 041 ont donné des semis. Ajoutons l'obtention de 257 lots de semences de pays étrangers et la récolte de 601 lots de semences du Québec sur 34 espèces ou hybrides.

AMÉLIORATION GÉNÉTIQUE DE L'ÉPINETTE NOIRE

par Michel Villeneuve

Mots-clés : Bouturage, embryogenèse somatique, gains réels, test précoce, variété multifamiliale, préconditionnement.

Depuis 1995, 15 nouveaux dispositifs ont été implantés sur 9 sites : 200 clones produits par bouturage (2 sites), 81 descendances biparentales (3 sites), 32 clones produits par embryogenèse somatique (2 sites), 32 croisements réalisés selon un plan factoriel (2 sites), 16 descendances produites par croisements d'arbres à cime étroite et à cime large (2 sites), et 4 plantations d'évaluation des gains réels.

Onze prescriptions d'éclaircies génétiques de vergers à graines ont été produites. Les gains en hauteur calculés varient de 2,3 à 8,0 %. L'intensité de l'éclaircie est souvent limitée par la capacité de production minimum à atteindre. On a calculé que les 100 clones d'élite qui serviront à établir un verger de 2e génération donneront un gain de croissance en hauteur de 20 % à 10 ans.

Le testage précoce en pépinière s'est avéré très efficace. L'héritabilité familiale de la croissance en hauteur est élevée et stable (> 0,8). Après trois saisons de croissance, les familles sont supérieures de 12 à 16 % en hauteur par rapport aux six provenances témoins. Les meilleures familles ont une croissance jusqu'à 35 % supérieure. Il est possible d'identifier les familles les moins intéressantes dès la troisième saison de testage en pépinière.

Le suivi de 360 boutures pour chacune de 21 familles, depuis l'enracinement jusqu'au tri des plants avant livraison, a permis de déterminer que le nombre de pieds-mères et la productivité de ceux-ci (en boutures), variables entre les familles, détermine la diversité génétique effective du mélange. On doit viser à obtenir un nombre égal de boutures par famille à chaque nouveau bouturage, afin de préserver la diversité génétique anticipée dans la variété multifamiliale.

Afin d'évaluer l'importance du préconditionnement environnemental sur l'expression des gènes, des ramets de six clones ont été plantés sur cinq sites en 1994. Les mêmes croisements seront réalisés à chaque endroit. Ces combinaisons « croisement x site » seront testées dans un environnement commun.

Nous avons installé quatre plantations de comparaison de plants issus de variétés améliorées (3 lots) et de plants issus de semences tout venant (3 lots), afin d'évaluer les gains réels en croissance et en rendement (volume/ha) engendrés par le premier cycle d'amélioration génétique. Le dispositif utilisé est constitué de parcelles carrées de 196 arbres.

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FOREST GENETICS AND SOMATIC EMBRYOGENESIS

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GRADUATE STUDENTS AND POSTDOCTORAL FELLOWS

As a university research group, one of our main goals is to train graduate and postdoctoral research associates. We have witnessed, over the years, a large amount of high quality research work realized by these young scientists and their important contributions to various areas of forest genetics and evolutionary biology must be underlined. Dr. Nathalie Isabel completed her PhD in 1995 in molecular population genetics and its applications to somatic embryogenesis of spruce species. She is now a research scientist at the Canadian Forest Service and adjunct professor to Laval University. Dr. Sylvain Jeandroz, who was a postdoctoral fellow from the University of Besançon, returned to France in 1997 as associate professor at the University of Nancy after completing several studies on the molecular population and evolutionary genetics of ashes. Dr. Rodolphe Boivin also completed his postdoctoral studies in 1996 on the molecular genetics of somaclonal variation. Dr. Ahmed Koubaa also left us in 1997 for a position as research scientist at Forintek-BC, after completing postdoctoral studies on wood density in black spruce.

Among the new postdoctoral fellows, Dr. Daniel J. Perry joined us in 1996 from the University of Minnesota and is actively working on developing new codominant DNA markers for spruce species. Since 1997, Dr. Yvan L'Homme from McGill University has been pursuing work initiated by Drs. Isabel and Boivin on the molecular genetics of somaclonal variation. Dr. Zhou Yu Ping from Bejing University also joined us in 1997 to develop genetic maps in black spruce. Dr. Edwidge Cazaux from University of Montpellier has been working for the past year on spruce somatic embryo encapsulation while Dr. Marika Delalonde, from the same university, is currently involved in the characterization of endogenous growth regulators in the spruce somatic embryogenesis systems.

Several PhD students are expected to complete their degree in the following months. Mr. Martin Perron is pursuing his PhD studies on the molecular ecology of the black spruce - red spruce complex and he is expected to complete his work by the beginning of 1998. Mr. Jérome Laroche is also expected to complete his Ph.D. by the end of 1997 in the area of DNA sequence analysis and the study of plant and conifer

mitochondrial genes. Mr. Stéphane Plante shall complete his PhD in 1998 on the biosystematics and conservation genetics of endangered plant species. Mr. Claude Bomal should also complete a PhD in 1998 on the dehydration of spruce somatic embryos while Mr. Abdelmalek El Meskaoui is expected to complete his PhD on the environmental control of spruce somatic embryogenesis. More recently, Mr. Driss Iraki initiated a PhD on the effect and biochemistry of carbohydrates during the different stages of somatic embryogenesis.

At the MSc level, Mr. Éric Forget, who was working on methods of mass selection for sap sugar content in maple, completed his degree in 1996 and is now working for the BC Ministry of Forests. Three new MSc students, Mrs. Sauphie Senneville, Marie Bouillé, and Mr. Ricardo Morin initiated several studies in 1997 related to the conservation genetics of endangered plant species and the population genetics of forest trees, in collaboration with scientists from the Canadian Forest Service (G. Daoust and J. Beaulieu).

MOLECULAR POPULATION AND CONSERVATION GENETICS

Over the past two years, much of the work in molecular population genetics has focused on estimating population genetics parameters from RAPD markers in black spruce (Isabel *et al.* 1995a) and white pine (Isabel, in prep.), and comparing these estimates with those derived from allozyme markers (supported by FCAR of Québec and in collaboration with J. Beaulieu from the Canadian Forest Service). The various estimates were quite congruent when derived from genotypic data but biases were observed when using dominant RAPD fingerprint data from diploid tissues. New codominant markers from polymorphic expressed gene sequences (PEGs) (Perry and Bousquet 1997) were also developed for spruce species and they are being applied to study the effects of different management regimes and natural disturbances on the diversity of black spruce (supported by the Network of Centers of Excellence on Sustainable Forest Management).

RAPD species-specific markers were used to estimate the incidence of natural hybridization and introgression between black spruce and red spruce (Perron *et al.* 1995; Perron and Bousquet 1997), and estimating levels of genetic diversity in red spruce (M. Perron, in prep.). These last studies, supported by the Québec Ministry of Natural Resources, have shown that natural hybridization between the two spruces is extensive in the area of sympatry in the St. Lawrence Valley while introgression has been detected in both zones of allopatry. Preliminary results also indicate that allopatric red spruce is genetically depauperated at the DNA level, as compared to allopatric black spruce. With the support of a France-Québec cooperation program, species-specific RFLP and RAPD markers have also been developed for the French ashes (Jeandroz *et al.* 1995, 1996) and they are currently being used to estimate the levels of natural interspecific hybridization and the population structure in collaboration with researchers at the University of Paris-Orsay (N. Frascaria-Lacoste *et al.*).

EVOLUTIONARY GENETICS

Studies conducted during the past two years in the area of molecular biosystematics and evolutionary genetics have been supported by grants from NSERC and FCAR of Québec and have encompassed a very large spectrum of subjects. They reflect the many collaborations we have had recently in that field of research. The phylogeny of the main groups of land plants has been estimated and compared between *rbc*L gene sequences and the newly isolated chloroplast gene *chl*B. Notably, the position of the main groups of gymnosperms was in agreement between the two gene phylogenies (Boivin *et al.* 1996). *Rbc*L gene sequences have also been used to estimate the phylogeny of over 100 angiosperm taxa; the results demonstrate that the nine families of nitrogen-fixing actinorhizal shrubs and trees have an unexpected large phylogenetic proximity in spite of being taxonomically unrelated (Roy and Bousquet 1997).

Our studies aimed at estimating the modes and tempos of evolution of mitochondrial introns and exons of angiosperms have also been completed, with a sampling of over 170 distinct DNA sequences distributed among 15 genes (Laroche *et al.* 1995, 1997). Notably, intron sequences have been shown to vary as much as synonymous sites of exon sequences, and mitochondrial *Cox*I genes of poplar, birch and other woody angiosperms were shown to evolve much slower than similar gene sequences in annual plants, paralleling such a contrast observed for the chloroplast gene rbcL from gymnosperms and angiosperms (Bousquet *et al.* 1992; Savard *et al.* 1994). Therefore, on the geological scale, woody taxa such as trees are evolving more slowly than annuals, and this is likely attributable to longer generation time, larger population sizes and slower speciation rate due to archaic reproductive isolation mechanisms.

At a much finer scale, the phylogeny and phylogeography of the genus *Fraxinus* was established using internal transcribed spacer sequences (ITS1 & ITS2) of nuclear ribosomal DNA for about 30 taxa (Jeandroz *et al.* 1997). This is the first complete phylogeny deduced from DNA sequences for a tree genus. Intraspecific variation was minimal and closely related hybridizing taxa showed a small divergence at the DNA level. Much higher diversity was observed among sections. The origin of the genus was determined to be in North America, with two latter events of intercontinental migration towards Asia. Biosystematic studies conducted with fungi and bacteria were aimed at describing chromosome length polymorphisms and determining the phylogenetic position of new species using 16S ribosomal DNA sequences (Dufresne *et al.* 1996; Shooner *et al.* 1996).

MOLECULAR TREE IMPROVEMENT AND QUANTITATIVE GENETICS

A collaborative project involving the development of marker-aided selection for mature wood density in eastern spruces was pursued and further supported by grants from NSERC and the Québec Ministry of Natural Resources, and active collaborations from the Canadian Forest Service (N. Isabel & J. Beaulieu), Forintek-Canada (S.Y. Zhang), the Québec Ministry of Natural Resources (M. Villeneuve), J.D. Irving (G. Adams), Fraser Papers Inc., and the Biotechnology Laboratory at UBC (J.E. Carlson). Over a five-year period, the goals of the project are to develop genetic markers for mature wood density in black spruce and white spruce, map these markers, and develop strategies for using these markers in tandem selection for growth and mature wood density. A similar study supported by the Québec Ministry of Natural Resources on marker-aided selection for sap sugar content in sugar maple and the development of first generation selections is also underway.

The studies conducted in collaboration with the Canadian Forest Service (J. Beaulieu) and P. Li (now at BC Research) on the development of multivariate approaches for the risk assessment of seed source movements in eastern white spruce have been completed (Li *et al.* 1997). Briefly, two large breeding zones have been delineated and validated for Québec. Latitude appeared as the main factor of population differentiation, and more geographic differentiation was observed for phenological traits than for growth characters. We have also completed our collaborative study supported by the Canadian Forest Service on the selection of superior white spruce seed sources for the eastern regions of Québec (Beaulieu *et al.* 1996). The publication of results from the provenance studies on Acacias and other tropical species has also been completed (Khasa *et al.* 1995a-e, now at University of Alberta). The early evaluation of intraspecific and interspecific crosses between black spruce and red spruce under various regimes of light conditions is underway, with the families completing their third year of growth (M. Perron). This project involves financial contributions and the active collaboration of the Québec Ministry of Natural Resources (M. Villeneuve).

SOMACLONAL VARIATION

Populations of white spruce raised *in vitro* following somatic embryogenesis typically show a low incidence of somaclonal variation. One of the few spontaneous changes observed, the *variegata* phenotype, was shown through ultrastructural studies to harbour extensively modified cells with immature chloroplasts (Isabel *et al.* 1995b). These mutants shared a single RAPD marker out of hundreds screened, but the sequence of the DNA fragment did not show any homology to known genes or intergenic regions. The presence of the fragment could be related to the relative abundance of nuclear and chloroplast genomes in the modified *variegata* phenotypes (Isabel *et al.* 1996).

SOMATIC EMBRYOGENESIS AND GENE TRANSFORMATION

With the financial support of the Québec Ministry of Natural Resources and the Québec Ministry of Trading, Industry, Science and Technology in partnership with three industrial seedling producers CPPFQ Enr., PAMPEV Inc., and BECHEDOR Inc. various research projects have continued to focus on white spruce and black spruce somatic embryogenesis.

Different components of the culture medium were studied to improve embryo production and quality. The results showed that glutamine could be used as the sole nitrogen source to support embryo maturation (Khlifi and Tremblay 1995). The beneficial effect of an increased sucrose concentration in the maturation medium was also shown to be caused by its complete and rapid hydrolysis under the enzymatic action of the cells (Tremblay and Tremblay 1995a). Furthermore, the experiments showed that sucrose, and later on glucose and fructose, were not utilized by the cells during maturation but seemed to act on maturation mainly through an increase in the osmotic potential of the medium. Other investigations conducted on the dessication of black spruce and white spruce somatic embryos have shown that they can survive dessiccation but survival was dependent on the relative humidity treatment (Bomal and Tremblay 1995a, b, 1996). An adequate control of the water loss was also shown to be necessary to subsequent embryo germination. When water loss was well controlled, desiccated embryos germinated in a comparable way to fresh embryos.

With the financial support of the Québec Ministry of Natural Resources, investigations were also conducted to understand the factors involved in the genetic transformation of white spruce. Using embryogenic suspension culture as study system, different factors such as the culture conditions of the cells prior to bombardment and the age of the suspension have been tested (unpublished). Transgenic tissues were obtained (Belles-Isles *et al.* 1995) and plants were regenerated. They are currently growing under greenhouse conditions for further testing.

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GÉNÉTIQUE ET AMÉLIORATION DES ARBRES AU SERVICE CANADIEN DES FORÊTS - QUÉBEC

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Mots-clés : *Picea glauca, Picea abies, Pinus strobus,* amélioration génétique, induction florale, culture *in vitro*, rouille vésiculeuse, charançon du pin blanc, marqueurs alloenzymatiques et RAPD, selection assistée

La présente période a été très fertile en changements. Nous sommes maintenant intégrés à des réseaux de recherche pan-canadiens plutôt qu'à des programmes régionaux. Nous avons de plus recentré nos efforts de recherche au niveau de la génétique forestière et transféré nos responsabilités en amélioration au ministère des Ressources naturelles du Québec. Notre consoeur, la D^re^ Ariane Plourde est devenue directrice du programme de biologie forestière au Centre de foresterie des Laurentides (CFL) et gestionnaire du réseau de biotechnologie des arbres et de génétique de pointe. Nous lui souhaitons un franc succès dans ses nouvelles fonctions et lui assurons notre support. Deux nouvelles chercheures font maintenant partie de notre groupe de recherche, soit la D^re^ Francine Bigras, spécialiste de la physiologie des arbres, et la D^re^ Nathalie Isabel, généticienne forestière spécialisée en biologie moléculaire. Le D^r^ Peng Li, qui était avec nous depuis deux ans à titre de chercheur post-doctoral, nous a quitté et poursuit maintenant sa carrière chez BCRI. La D^re^ Élisabeth Garin, spécialiste de la culture *in vitro*, est également avec nous depuis plus d'un an grâce à une bourse post-doctorale.

Toute une équipe de gens dévoués est venue appuyer nos efforts. Ainsi, en culture *in vitro*, nous avons eu le support de Julie Dubé, cadre technique, Fabrice Lantheaume (MSc, Université Laval) et Gervais Pelletier (MSc, UQUAM). L'équipe travaillant sur le marquage moléculaire a pu compter sur l'aide de Magella Gauthier, cadre technique, et Hugo Laplante (B. Sc. Université de Montréal). Tous nos progrès en génétique de pointe n'auraient pu être accomplis sans l'implication continue de René Pâquet, Serge Légaré, Roger Gagné et Yves Dubuc, cadres techniques, et de Chantal Ferland (MSc, Université Laval) et Elise Dubuc (agronome), assistés d'étudiants d'été.

Nous participons également à la formation d'une main-d'oeuvre hautement qualifiée en supervisant les travaux d'étudiants gradués. Ceux-ci sont Philippe Thériault, Sauphie Senneville, Ricardo Morin et Bruno Girard.

GÉNÉTIQUE ET AMÉLIORATION DE L'ÉPINETTE BLANCHE

par Jean Beaulieu

Le Service canadien des forêts conduisait depuis les années 1970 le programme d'amélioration génétique de l'épinette blanche (*Picea glauca* Moench Voss) au Québec. Toutefois, en 1995, le gouvernement du Canada décidait de revoir l'ensemble de ses programmes. Ainsi, à la suite de cette revue, le Service canadien des forêts recentrait son action et se retirait du champ de l'amélioration génétique pour concentrer ses efforts dans les recherches plus fondamentales portant sur la génétique des espèces forestières. Depuis, une entente a été conclue avec le ministère des Ressources naturelles du Québec et le transfert du matériel et des connaissances acquises est en cours. Afin de faciliter le transfert des responsabilités, un rapport d'information décrivant la stratégie d'amélioration suivie et le matériel constituant les populations d'amélioration a d'abord été produit. Ainsi, les nouveaux améliorateurs pourront poursuivre le travail initié en suivant une même ligne de pensée. Les croisements dirigés nécessaires à l'évaluation des géniteurs de la première génération ont été réalisés au cours des deux derniers printemps.

Des croisements dirigés ont été réalisés pour évaluer les aptitudes générale et spécifique à la combinaison des géniteurs. De plus, des variétés clonales et multifamiliales ont aussi été développées pour déterminer la valeur de l'approche clonale pour la production de matériel amélioré. Les semences issues de croisements dirigés avec mélange de pollen (polymixe) devant servir à l'évaluation de l'aptitude générale à la combinaison des géniteurs ont été mises en terre et les semis ont été produits. Un test en champ et un test avec espacements classiques ont été installés en 1996. Cinq autres tests en champ ont été installés au printemps 1997 par le ministère des Ressources naturelles du Québec. Les semences de descendances bi-parentales générées pour évaluer l'aptitude spécifique à la combinaison des géniteurs et de matériel pour la sélection de la seconde génération ont été mise en terre au cours de l'hiver 1997. Les semis seront cultivés en récipients pendant deux ans avant d'être utilisés pour établir quatre tests dans autant de régions écologiques différentes. Quatre tests en champ ont été installés en 1996 en vue de comparer les valeurs familiales obtenues à l'aide des semis et de variétés multifamiliales. La durée de vie prévue de ces tests est de huit à 10 ans. Les résultats permettront d'identifier les croisements à faire en priorité pour approvisionner le Centre de bouturage de Saint-Modeste. Des ramets de quelque 700 clones issus de familles non-apparentées ont aussi été produits au cours de la période 95-97 en partie grâce à la collaboration établie par le Centre de bouturage de Saint-Modeste. Cinq tests ont été établis au printemps 1997 dans le but d'évaluer les variances additive et non-additive des caractères de vigueur et d'identifier les familles fournissant les clones les plus performants.

À la suite de l'analyse des données des tests génécologiques établis au cours des années 1970 et 1980, des recommandations ont été faites pour la création de nouveaux vergers à graines plus performants. Ces vergers sont actuellement en phase de développement et aménagés par le ministère des Ressources naturelles du Québec. De même, des zones de déplacement des semences ont été proposées (Li *et al.* 1997).

Une étude d'impact de la pollution génique sur les complexes géniques adaptés a été initiée. Des semences issues de pollinisation libre dans deux peuplements naturels et dans un essai de provenances comprenant des individus représentant ces deux populations naturelles ont été récoltées. Des croisements de type polycross réalisés sur des arbres des mêmes provenances ont aussi été réalisés. Les semences ont été mises en terre et un dispositif expérimental sera établi dans chacun des sites des populations naturelles. Un suivi à long terme sera assuré pour détecter le cas échéant des différences en terme d'adaptation des divers matériels.

Les semences de familles issues de deux plans de croisement diallèle réalisés pour l'estimation des paramètres génétiques de caractères de croissance ont été mises en terre à l'hiver 1997. Les semis seront cultivés pendant deux ans avant d'être transférés dans quatre sites différents. De même, les croisements

dirigés nécessaires à la recherche de marqueurs génétiques associés à la densité du bois mature chez l'épinette blanche ont été réalisés et les graines ont été ensemencées en 1996 et en 1997. De plus, une partie des semis est soumise à une croissance accélérée pour pouvoir réaliser une F_2 le plus rapidement possible. Des croisements dirigés ont également été réalisés suivant un plan diallèle dans le cadre du programme de recherche de marqueurs associés à la capacité embryogène chez l'épinette blanche.

GÉNÉTIQUE DES POPULATIONS ET RECHERCHE DE MARQUEURS

par Nathalie Isabel, Jean Beaulieu et Marie Deslauriers

Ce secteur d'activités de recherche est en pleine expansion au CFL puisqu'en plus de faire de la diversité génétique nous avons débuté un programme axé sur le développement de marqueurs associés à des caractères d'intérêt économique tel que la densité du bois mature et la capacité embryogène. Initialement, un projet de cartographie génomique et de sélection assistée par marqueurs pour la densité du bois mature avait été amorcé chez l'épinette blanche en collaboration avec les D^{rs} Jean Bousquet (U. Laval) et John Carlson (UBC). Depuis deux ans, ce projet s'est élargi et inclut maintenant l'épinette noire grâce à une subvention CRSNG-stratégique (obtenue par les D^{rs} J. Bousquet, J. Beaulieu et N. Isabel) dont la mission est l'amélioration des caractères de qualité du bois chez l'épinette blanche et l'épinette noire par la mise en place de systèmes de sélection assistée par marqueurs. Ce projet se fait en collaboration avec les D^{rs} Tony Zhang, Ahmed Koubaa (Forintek Canada Corp.), J. Carlson (UBC), MM. Michel Villeneuve (MRNQ-Québec) et Greg Adams (J.D. Irving Ltd).

Un autre projet semblable a également été initié chez l'épinette blanche mais cette fois en vue de disséquer au niveau génétique la capacité embryogène. Ce projet se fait en collaboration avec les D^{rs} Jean Bousquet (U. Laval) et Yill Sung Park (SCF-Region de l'Atlantique). Une partie des travaux s'effectue dans le nouveau laboratoire de culture *in vitro* du CFL qui s'est agrandi depuis l'arrivée de l'équipe des D^{rs} Bob Ruthledge et Armand Séguin de l'Institut forestier national de Petawawa en juin 1996. Les principales espèces étudiées dans ce laboratoire sont l'épinette blanche et le pin blanc. En ce qui concerne l'épinette blanche, la majorité des travaux porte sur l'induction et fait partie intégrante du projet sur la recherche de marqueurs associés à la capacité embryogène. Les travaux sur le pin blanc visent quant à eux l'optimisation de l'étape de la maturation. Ce travail est effectué par la D D^{re} Élisabeth Garin (Univ. Compiègne) en collaboration avec la D^{re} Kristina Klimaszewska (SCF-BCRI).

Plusieurs projets portant sur la diversité génétique sont en cours. Ainsi, nous visons à estimer les niveaux de diversité génétique existant dans les populations nordiques de noyer cendré avant que le chancre du noyer qui fait des ravages au sud de la frontière n'attaque sérieusement nos populations. Ce travail est réalisé par Ricardo Morin, étudiant à la maîtrise. De plus, des recherches au niveau de la culture des tissus et de la cryoconservation devraient être entreprises sous peu. Sauphie Senneville, étudiante à la maîtrise, réalise quant à elle une étude sur la génétique des populations d'if du Canada. Les aiguilles de cette essence renferme des taxanes utiles à la lutte contre certains cancers. Le but visé est de déterminer le niveau de diversité génétique chez cette essence avant que des récoltes viennent l'altérer. Ces deux étudiants sont co-dirigés par M. Gaétan Daoust et le D^{r} Jean Bousquet. Une étude du comportement au séchage du bois d'épinette blanche produit en plantation a été entreprise par Bruno Girard, étudiant à la maîtrise, co-dirigé par le D^{r} Yves Fortin. L'étude est réalisée grâce à la collaboration de la compagnie Avenor inc. qui a effectué une éclaircie dans un de nos essais de provenances et mis les grumes à notre disposition. L'étude portant sur le pin blanc et initiée par Philippe Thériault en collaboration avec le D^{r} J. Bousquet (U. Laval) est maintenant complétée (Isabel et coll. 1997). Elle visait la comparaison des alloenzymes et des marqueurs RAPDs dans des populations provenant de la Vallée du St-Laurent et de l'Outaouais qui démontraient des niveaux de diversité différents au niveau alloenzymatique (Beaulieu et Simon 1994). Une étude de la structure génétique des populations d'épinette blanche à partir de matériel récolté dans un essai de provenances a également

été réalisée. Celle-ci l'a été dans le cadre d'un projet visant à mesurer l'impact de source étrangère de pollen sur les caractéristiques adaptatives de populations locales.

GÉNÉTIQUE DE L'ÉPINETTE DE NORVÈGE ET DU PIN BLANC

par Gaétan Daoust

Au cours de la dernière période, le projet (Série E390) portant sur l'amélioration génétique de l'épinette de Norvège, entrepris conjointement avec Ante Stipanicic de la direction de la recherche du ministère des Ressources naturelles du Québec, s'est poursuivi. Les 3 tests de descendances, établis dans la zone d'amélioration des Appalaches et âgés de 8 ans, ont été mesurés et des analyses préliminaires ont été effectuées. Les résultats nous ont permis d'identifier des descendances performantes présentant une grande stabilité phénotypique dans les 3 régions écologiques testées. Ces descendances présentent une supériorité de croissance en hauteur de 10,5 % par rapport aux témoins et représentent des sources issues de matériel recommandé, de provenances commerciales sélectionnées et de sources importées de Pologne.

En collaboration avec le Dr Robert Lavallée, entomologiste au SCF, près de 70 clones d'épinette de Norvège, représentatifs de provenances recommandées, ont fait l'objet d'une évaluation pour leur susceptibilité au charançon du pin blanc. L'étude qui a été répétée à deux reprises (1994 et 1996) a permis de démontrer une grande variation interclonale et d'identifier des clones tolérants à l'insecte. Des affiches et des conférences ont également été présentées afin de ramener à un juste niveau l'impact réel du charançon sur l'épinette de Norvège et ainsi promouvoir l'utilisation de cette espèce à fort potentiel pour le Québec.

En mars 1997, un test comprenant près de 200 descendances bi-parentales a été ensemencé dans les serres du CFL. Ce test qui vise à étudier les aptitudes spécifiques à la combinaison et à l'identification de croisements prometteurs en vue de la production de variétés synthétiques par bouturage a été rendu possible par la mise en commun des lots de semences provenant de la Division des semences et des plants et de la Direction de la recherche du MRNQ et du SCF. Ces tests seront établis ultérieurement dans 3 régions écologiques. La majorité de ces croisements ont pu être réalisés grâce aux traitements d'induction florale à base d'$AG_{4/7}$ appliqués préalablement aux géniteurs.

Un suivi floristique de 260 clones a été effectué en 1994 et 1996 dans le parc d'hybridation de Valcartier. Ce relevé a permis de démontrer la contribution disproportionnée de certains clones dans la production de pollen et de cônes. Parallèlement, une étude de stimulation de la floraison a permis de démontrer que l'on pouvait éviter cette disporportion en augmentant le nombre de clones florifères, le nombre de fleurs mâles et femelles et la production totale de semences.

Concernant la génétique du pin blanc, la dernière période a surtout été consacrée à la publication des travaux portant sur l'estimation de paramètres génétiques reliés à la structure génétique et aux patrons de variation. Ces travaux ont été réalisés principalement par le Dr Peng Li, chercheur post-doctoral supporté par le Fonds de recherche en sciences et technologie du SCF. Une étude portant sur la comparaison des paramètres génétiques du pin blanc estimés à partir de tests établis à découvert et sous couvert feuillu et sur le nombre de familles nécessaires pour bien représenter une provenance a été entreprise par le Dr J. Beaulieu en partenariat avec Louise Gonthier de la compagnie Tembec.

PHYSIOLOGIE GÉNÉTIQUE

par Francine Bigras

Des études associant la génétique et la physiologie ont débuté en 1996. L'étude de la tolérance au gel de familles d'épinette blanche issues de croisements dirigés effectués en serre et dans un verger à graines a été entreprise en collaboration avec le D[r] Jean Beaulieu. Une étude similaire sur des familles d'épinette de Norvège produites en serre et à l'extérieur, avec ou sans traitement d'induction florale, a été démarrrée en collaboration avec M. Gaétan Daoust. Des différences de rusticité ont été observées entre les traitements et ces expériences se poursuivront pour une période de trois ans afin de déterminer leur persistance.

Un projet sur la sélection précoce de l'épinette blanche a été initié en 1997 avec la collaboration du D[r] Jean Beaulieu. Douze familles ont été identifiées quant à leur croissance en hauteur à partir de tests établis sur trois sites. Quatre familles de croissance supérieure, quatre de croissance intermédiaire et quatre de croissance inférieure ont été sélectionnées et des semis issus de ces familles ont été produits. Des expériences portant sur des critères morphologiques juvéniles en cycle de croissance régulier et en cycle de croissance accéléré ont débuté. D'autre part, une série d'expériences sera entreprise afin de déterminer la plasticité de ces familles face à l'exposition à un certain nombre de stress, soit les stress de gel, de chaleur, de sécheresse. Les familles démontrant une tolérance à un grand nombre de stress pourraient montrer une croissance supérieure. Des études de biologie moléculaire viendront compléter ce projet.

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POLLEN DISPERSAL AND POLLEN CONTAMINATION

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Keywords: airborne pollen, pollen contamination, software, pollen release, long-distance

Work on modelling contaminant pollen influx into seed orchards was completed in 1994. The end-product was computer software which models the effect of differing anti-contamination scenarios at specific, open-pollinated seed orchards. Details were given in (Di-Giovanni 1996). Since then, two scientific publications, relating to the pollen contamination work have been produced and are described below.

ESTIMATING THE TIMING OF MAXIMUM POLLEN RELEASE FROM JACK PINE (*Pinus banksiana* Lamb.) IN NORTHERN ONTARIO

A practical heat sum method was validated for estimating the date of maximal pollen release of jack pine in northern Ontario. The base temperature and start date that minimized differences between estimated and observed maximum pollen release dates were sought. Heat sums were calculated for all data sets (n = 26) for a range of base temperatures (1 to 20°C) and start dates (January 1 to maximum pollen release). The best combination was a start date at Julian day 107 and base temperature at 4°C. The heat sum to maximum pollen release was 288.58 degree days and the average difference between estimated and observed was 2.75 days. Recommendations for operational testing are made (For. Chron. 72: 166-169).

LOWER PLANETARY BOUNDARY LAYER PROFILES OF ATMOSPHERIC CONIFER POLLEN ABOVE A SEED ORCHARD IN NORTHERN ONTARIO, CANADA

Atmospheric concentration profiles of pollen from jack pine and black spruce (*Picea mariana* (Mill.) B.S.P.) were measured up to 300 m above ground level at the edge of a seed orchard in northern Ontario. Large amounts of pollen were found up to 300 m showing the occurrence of meso-scale transport of coniferous pollen and also possible long distance transport. The profile shapes were found to be a function of source characteristics and meteorological conditions. Changes in profile shape were in general agreement to those found in previous work. If meso- or synoptic-scale transport of conifer pollen occurs, then the use of isolation zones would not be useful to stop this component of the contaminant pollen cloud entering a seed orchard, especially if pollen longevity in the atmosphere is long. However, further work is required to determine if this component of the contamination pollen cloud is large and whether or not the pollen is viable (For. Ecol. Manage. 83: 87-97).

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A final publication is also planned on an examination of pollen dispersal patterns at the Island Lake field site. Organizations interested in the pollen contamination software are invited to contact the author.

ECOSYSTEM BIOTECHNOLOGIES AND MANAGEMENT

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Keywords: marketing, ecosystem biotechnologies, woodlands management, tree improvement

ECOLOGY PRODUCTS AND SERVICES operates commercial and technology transfer activities: 1) HERITAGE TREES AND SEEDS produces and wholesales trees for landscaping, reclamation and reforestation, 2) ECOSYSTEM BIOTECHNOLOGIES AND MANAGEMENT develops, produces and markets plant and ecosystem control products and wood products for the landscape trades, manages woodlands, and consults professionally, and 3) LANDSCAPE ECOLOGISTS retails products and services for landscaping, vegetation management, and land reclamation.

RED OAK (*Quercus rubra* L.) FOR LANDSCAPING

Slow growth and tap root development hinders the use of northern red oak (*Q. rubra* L.) for landscaping purposes. Growth of seedling trees can be accelerated in plastic tubular tree shelters (Ponder 1995) and tap root development can be controlled using in-ground fabric containers with vinyl bottoms (Ingram *et al.* 1987). A field trial was conducted to test the response of red oaks to shelters. Six seed sources from the upper Ottawa Valley were tested at six sites with 15 to 30 seedlings per source at each site. Growth was highly variable after two years; 60- to 75-cm tall shelters stimulated growth of some seedlings to heights of up to 1.5 m while others responded poorly. The response was most uniform on fertile sandy loam compared to clays or sands. In-ground fabric containers were tested on 25 randomly selected seedlings at each of two sites. After 5 years, trees are readily transplantable.

TREE IMPROVEMENT OPPORTUNITIES FOR LANDSCAPE TIMBER

A search for cedar (*Thuja occidentalis* L.) logs, rough-cut and dressed lumber in the Upper Ottawa and Madawaska Valleys revealed shortages for poles and landscaping lumber for decks, fencing, and outdoor furniture. With suitable management and cutting regimes to facilitate natural regeneration, the supply problem might be alleviated. There is little opportunity for genetic improvemnt because plantation establishment is not practised (Fowells 1965).

Tamarack (*Larix laricina* [Du Roi] K. Koch) may be a suitable replacement for cedar for some landscape timber

purposes because of its resistance to rot and superior strength, especially for poles, posts and decking. Natural seeding is a common method of regeneration (Davis *et al.* 1996) but abundant seed fall is rare because of losses to cone-feeding insects and many dispersed seeds are lost to mice, fungi or bacteria (Fowells 1965). Plantation establishment on upland sites where highest growth rates are attained may be practical. Mature stands are highly susceptible to the larch sawfly but it may be possible to develop and deploy resistant varieties. *Larix* species respond to selection in a tree improvement program (Boyle 1989) and insect resistance genes could conceivably be incorporated into the genome if technologies for large-scale micropropagation from cell cultures become economically feasible. Stimulating cone production and controlling cone insects in managed seed orchards and seed production areas are required and may be possible, as in other conifers (Fogal and Plowman 1989, Fogal *et al.*1996).

CONE PRODUCTION AS A BIO-INDICATOR OF FOREST HEALTH

Professional consulting with Dendron Resource Surveys required a contribution of technical and scientific information on factors that influence the reproductive biology of commercial forest trees (Fogal *et al.*1997). Employment of conifer cone and deciduous flower production as remote-sensing detectable bio-indicators of forest health and regeneration potential was proposed.

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FOREST GENETICS RESEARCH AT THE FACULTY OF FORESTRY, UNIVERSITY OF TORONTO

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Research at the Forest Genetics Laboratory (FGL) is coordinated by L. Zsuffa, Professor Emeritus of Forest Genetics. Also contributing to studies are graduate students, a Research Associate and Adjunct Professor (R.L. Gambles), a Research Associate (D. Lin), Assistant Professor (W.A. Kenney), and a Research Technician (B.J. Vanstone).

Current research at the FGL has involved: 1) genetic characterization of Salicaceae, 2) genetic variation in cold hardiness of *Salix* species, 3) genetic markers for growth, 4) drought tolerance and response to antioxidant action in black spruce, 5) progeny and clonal testing, 6) biomass quality studies, and 7) assessment of species and clonal variation in resistance to pests and diseases.

DOCUMENTATION AND EVALUATION OF ONTARIO'S POPLAR AND WILLOW GENETIC TRIALS

Recently, the demand for new sources of wood fibre has increased dramatically. The Salicaceae, especially poplar, are gaining in prominence because of their fast-growing character and high quality product. Research began in 1996 at the FGL to complete and update documentation of Ontario's poplar and willow inventories and to evaluate genetic stock.

In Ontario, a significant poplar improvement program, started in the 1960's, associated with an industry-supported short rotation poplar planting program, resulted in good genetic material and new clonal selections. Unfortunately, due to changes in priorities of the government of Ontario, this program was discontinued, and by the late 1980's most of the trials were abandoned. There are over 100 poplar and willow genetic trials that were established consisting of family, progeny, and clonal tests and ranging from Niagara in south-western Ontario to Thunder Bay in the north west to Cornwall in the south east. These represent the culmination of years of valuable genetic research. Stock is in imminent danger of being lost since: 1) the trials have not been maintained since the mid-1980's and 2) the land that most of the collections and trials are planted on has been sold by the Ontario Ministry of Natural Resources. As recommended in "A Strategy for Genetic Improvement of Salicaceae" prepared by Louis Zsuffa (1995) for the Energy from the Forest (ENFOR) program of the Department of Natural Resources, Canada, through the Poplar Council of Canada (PCC), genetically

improved stock is available and could be easily and economically evaluated and used for the next step of an improvement program. This research will allow tree improvement in the Salicaceae to continue, based on the vast amount of work that has already been done. The alternative would be to begin again from square one if a new improvement program was to be started.

All trials were visited initially for general evaluation to determine which were worth more in-depth assessments. Documentation on each trial was evaluated for its integrity and a data base was established containing information on the type of trial, availability of maps, and history of evaluations. These steps led to a determination of which trials should be assessed in detail. Information assessed includes height, diameter, disease resistance/susceptibility, and form, as well as a determination of the viability of each experiment including whether the trees are vigorous, crowded or suppressed, and a recommendation for further action. Where possible, cuttings will be taken from sites which may be destroyed in the near future. The results of this evaluation will lead to important recommendations on: 1) further breeding and hybridization, 2) family and clonal selection and development, 3) deployment of families and clones (site recommendation), 4) rotation length, 5) and yields.

POPLAR AND WILLOW PLANTING STOCK IMPROVEMENT

Planting stock improvement, characterization, and certification for short rotation plantations of poplars and willows was becoming urgent and critical. Planting stock improvement and characterization are carried out on a provincial and regional basis. In Ontario, the FGL has carried out a project to establish the clonal base for short rotation industrial poplar plantations in Northwestern Ontario and Southeastern Manitoba (Internal report, 1996).

Genetic Characterization of *Salix* and *Populus*

Clonal identification in willows represented a larger problem than in poplars, because of the apparent phenotypical similarity of many willow clones. Chong *et al.* (1995a) developed a method for rapid clonal identification in willows by polymerase chain reaction in micro-capillary tubes, which can alleviate this problem.

Studies were completed on differentiation of poplar and willow clones using RAPD fingerprints (Chong *et al.* 1995b; Lin *et al.* 1994, 1997). Random Amplified Polymorphic DNA fingerprints were used to differentiate species and hybrids of the genera *Populus* and *Salix* as well to identify individual clones. Fifty-five poplar clones and 81 willow clones were included in this study.

GENETIC STUDIES IN *SALIX* AND *POPULUS*

Poplar and willow genetic studies were active under the cooperative umbrella of the graduate program at the Faculty of Forestry, University of Toronto.

Genetic Variation in Frost Resistance in Salicaceae

Genetic variation in frost resistance of some *Populus* and *Salix* species was studied at the FGL and a MScF thesis was defended by Tsarouhas (1996). The intention of this study was to evaluate interspecific and intraspecific variation in cold hardiness in some *Salix* species. The analyses of variance for frost injury were conducted at different temperatures during different seasons. The goal of this study was not only to identify species or genotypes which showed high vitality after freezing temperatures but also to search for clones

whose axillary buds sprouted quickly after freezing damage, thereby keeping the loss of biomass to a minimum. In this study, during winter dormancy and early spring, when freezing resistance was the greatest, no significant differences in clonal variation were detected. However, significant clonal variation in freezing resistance was detected during the spring, flushing of terminal buds, axillary new growth, and early fall stages.

The genetic base of *Salix* clonal material selected for intensive forestry plantations was discussed by Aravanopoulos *et al.* (1997). The clonal nature of willow plantations raises concerns over their stability and genetic diversity. In this study, the genetic diversity of 114 willow clones was examined by starch gel electrophoresis. Results indicated that genetic diversity was within the values generally obtained from natural populations of willows. Interspecific hybrid clones contained high amounts of genetic diversity.

The associations of isoenzyme heterozygosity and biomass production were examined in two reports by Aravanopoulos and Zsuffa (1997c, 1997d). Relations were investigated by comparing the performance of heterozygotes with that of corresponding homozygotes. In *S. eriocephala* evidence indicated the presence of positive associations, while in *S. exigua* these associations were absent. The biological and evolutionary differences between these two species may explain this difference. Thus detecting biomass productivity by heterozygosity could be species and hybrid specific.

Chong *et al.* (1995b) examined the genetic relationship between *S. exigua* and other North American willows on the basis of allozyme variation. Several studies examined the inheritance, linkage, and joint segregation of isoenzyme loci. Thorsen *et al.* (1997) examined the inheritance and linkage of isozyme loci in *S. viminalis*. They found Mendelian segregation patterns and the presence of four weakly linked groups. Genetic linkage was investigated in two reports by Aravanopoulos and Zsuffa (1997a, 1997b). The first was a study of joint segregation in *S. eriocephala*, and the second an analysis of genetic linkage in *S. exigua*. Both studies were based on full sib progenies in a 2x2 balanced factorial mating design.

GENETIC MARKERS FOR GROWTH, DROUGHT TOLERANCE AND RESPONSE TO ANTIOXIDANT ACTION IN BLACK SPRUCE

The objectives of this Ph.D. research, by K. Vishnevetskaia, are: 1) to determine fast growing and drought tolerant genotypes of black spruce, 2) to investigate the relationship between heterozygosity and growth, and heterozygosity and drought tolerance, 3) to determine genotypes with the most significant increase in growth and drought tolerance as a result of antioxidant application, and 4) to use the information derived from the above to indicate the potential use of heterozygosity as an early selection criterion and as a marker for antioxidant response.

BIOENERGY RESEARCH

The advent of short rotation, intensively managed biomass plantations has resulted in an interest in the potential of willows to supply part of the world's future demand for wood fibre, energy, chemicals, and food. The FGL is developing high yielding and environmentally acceptable stock for short rotation forestry biomass production systems.

This research begun in the early 1980's has resulted in the identification of willow clones superior in such traits as biomass production, feedstock quality (moisture content and specific gravity), growth habit, disease resistance, and frost hardiness.

These clones are currently being tested in large-scale pilot plantations by the FGL and colleagues at the State

University of New York at Syracuse (SUNY). SUNY has received longer-term funding from the United States Department of Energy (DOE) for the establishment of operational-sized demonstration farms. These plantations demonstrate the concept of short rotation intensive culture (SRIC), provide information for financial analyses, determine overall performance in growth and disease resistance/susceptibility, provide a venue for mechanization of trials, etc.

It is essential that continued breeding be carried out to introduce new varieties into the production population to ensure continued gains in yields, quality and pest resistance, and to develop clones for specific site conditions. SUNY has sub-contracted the FGL to create and carry out a breeding scheme for advanced generation crosses.

DEVELOPING TREE-FORM WILLOWS FOR BIOMASS PLANTATIONS AND FIBRE PRODUCTION

Tree-form willows are fast-growing and promising for a variety of industrial products. They are easily harvested and processed using current industrial technology. Demonstration trials of cloned plus trees from a natural stand and superior trees selected from arboreta were established in 1991 (Iroquois) and 1992 (Berwick). These plantations are being monitored for growth yield, form, and disease resistance. The clones are also being characterized genetically, using DNA analysis. One of the trials also contains, poplar, silver maple and alder, along with the willows, which permits growth comparisons within and between the genera. Both trials were fully assessed at the end of 1993. In the fall of 1996, the Iroquois trial was measured at its mid-rotation age (5-6 years). Surprisingly, the estimated biomass of the willows approaches that of the poplars, which are still growing rapidly in this trial. The Berwick trial is to be assessed in the fall of 1997, at its mid-rotation age.

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ONTARIO'S GENETIC RESOURCE MANAGEMENT PROGRAM

Barb Boysen, Paul Charrette, Randy Ford, Cathy Nielsen, and Dennis Joyce

OVERVIEW

Seed management, tree improvement, and gene conservation are carried out cooperatively through the Ontario Tree Improvement Board (OTIB). Programs are developed locally within 6 autonomous administrative zones with science, technical and analytical support being coordinated at the provincial level. Over the past two years, program development has been overshadowed by the re-organization of the Ministry of Natural Resources (MNR). According to a new partnership agreement between MNR and the members of the OTIB, the operational aspects of the program are now the responsibility of the non-government members, while the MNR covers the costs of scientific and technical support. Most of the 50 first generation seed orchards have been rogued at least once and significant quantities of improved seed are being produced. Activities that are more conservation oriented are still being developed, primarily in southern Ontario. However, genetic diversity is receiving increasing attention throughout the province, especially in the Great Lakes/St. Lawrence Forest.

ZONE 1: LAKE OF THE WOODS - ENGLISH RIVER SEED MANAGEMENT ASSOCIATION

The Association made progress in its jack pine, black spruce and white spruce tree improvement programs. In the last year, three of the 12 black spruce seed orchards were rogued for the first time, and cones were collected from several orchards. Analyses of data from one of the six jack pine programs have been completed, and measurements in a second program are underway. In some of the white spruce orchards the last of the grafts have been established.

Like many aspects of forestry in Ontario, tree improvement is undergoing a transition from MNR to the forest industry. It is expected that there will be a slight slow down in tree improvement activities in Zone 1 during the transition in 1997, but activities in many of the programs will increase again in 1998.

ZONE 2: LAKE NIPIGON WEST ZONAL TREE IMPROVEMENT ASSOCIATION

The cooperative jack pine and black spruce programs of the Association made excellent progress during the past two years. In addition, one of the Association members has initiated an aspen program in conjunction with the Aspen and Larch Genetics Cooperative in Grand Rapids, Minnesota. The range and number of activities are increasing as programs mature, which includes the start of second generation activities. The following are highlights from the tree improvement programs.

Black Spruce

There are two active breeding programs within the Association. Data analyses and the initial roguing of the oldest (Abitibi-Consolidated) black spruce orchard was completed in 1997. Since the roguing, management activities have focused on increasing cone production. The last of the breeding orchards was established in

the summer of 1996. Active management of genetic tests continues to focus on reducing competition and minimizing damage by white pine weevil.

Jack Pine

During the fall of 1996, three family tests and the associated seed orchard were measured for the second time. Avenor completed the second roguing of the Kakabeka Falls orchard in the spring of 1997. Analyses for selecting the second generation population has been completed by Genesis Forest Science and planning for second generation activities will be completed this summer.

Aspen

Avenor has started two projects in an aspen program: 1) the collection of seed for a regional provenance trial, and 2) additional collections from plus trees. In the summer of 1996, Dr. Bill Parker (Lakehead University) led the collection with 27 seed sources (five trees per source) from the Thunder bay region, and additional collections have been obtained from the Aspen and Larch Genetics Cooperative. A poor seed year in 1997 has prevented additional collections from the Dryden area. Stock for the trials is being grown and three test sites are being prepared this summer.

ZONE 3: LAKE NIPIGON EAST SEED MANAGEMENT ASSOCIATION

The Association focuses on operational seed and stock deployment issues. During the summer of 1996, members measured and analyzed two jack pine provenance tests. Jack pine and white spruce clone banks were also established, but many of the jack pine clones had poor survival. Kimberly Clark Forest Products collected cones from their McPherson black spruce and white spruce clonal seed orchards. They are planning to expand the black spruce program and will be making plus tree selections in the summer of 1997 as well as taking over management of another seed orchard complex.

ZONE 4: NORTHEAST SEED MANAGEMENT ASSOCIATION

The Northeast Seed Management Association (NESMA) has been working cooperatively in a tree improvement and seed management program for black spruce and jack pine since 1984. The forest industry members are: Abitibi-Consolidated Inc., E.B. Eddy Forest Products Ltd., Hearst Forest Management Inc., J.E. Martel & Sons Lumber Ltd., Malette Inc., McChesney Lumber, and Superior Forest Management Ltd.

General highlights include: 1) completion of the process to revise the seed zone boundaries, 2) completion of the black spruce and jack pine genecology studies, 3) expansion of the NESMA membership, and 4) the continued development of the "SeedWhere" system for facilitating decisions about seed and nursery stock collection and deployment. The following are highlights from the tree improvement programs.

Black Spruce

The 13 hectare Aidie Creek seed orchard was rogued for the first time with 21,600 trees (40%) being removed at a cost of $0.56/tree. The topping of the remaining trees has resulted in as many as five new cone-bearing

tops in 1997. Ammonium nitrate was applied in part of the orchard in June 1996 to determine the usefulness of this practice for flower induction. The assessment has been done but the results are not yet available.

Approximately 4,000 trees were thinned in the Breeding Zone (BZ) #2 Farm-Field Test planted in 1991. A second thinning is planned for 1998 and another in 1999 for the BZ #5 test planted in 1992. Results from these test are being used to guide the development of testing procedures for advanced generation efforts.

Seed Orchards for BZ #1 and #4, located at the Island Lake Tree Improvement Area (ILTIA), are being rogued in the summer of 1997. Approximately 48,000 trees are expected to be removed in this first roguing. The sister BZ #4 orchard, outside Timmins, will also be rogued for the first time (approx. 13,000 trees from the 12 hectare orchard). The BZ #1 orchard is the first to be rogued on the basis of data analysis done under contract.

Jack Pine

The BZ #1 and #3 seed orchards, at ILTIA, were rogued for the final time in the fall of 1996. After removal of a total of 47,000 trees, only 20 percent of the original trees remain to provide improved seed.

At the Ramore orchard, 35 % of the trees were removed in the first roguing. The crowns of the remaining trees are being managed to facilitate cone collection (10 % of the trees are left untreated as controls). In 1996, cone collection costs were significantly higher for cones collected from control trees. In conjunction with the Canadian Forest Service, we are determining the cost effectiveness of crown management over time. The 30 hectoliters of cones collected in 1996 yielded an average of 34 viable seed per cone with a germination rate of 97%.

The BZ #1 population has been selected as the pilot advanced generation program for guiding the implementation of the breeding strategy presented by Dennis Joyce at the 1993 CTIA meeting. To date, 300 infusion and 49 elite selections have been made and seedlings from the selections are growing in containers. Three planting sites have been selected and test establishment is planned for spring 1998.

ZONE 5: NORTH SHORE TREE IMPROVEMENT ASSOCIATION

The North Shore Tree Improvement Association (NSTIA) has been working cooperatively since 1985 in a tree improvement and seed management program primarily with black spruce and jack pine. The forest industry members are: Domtar Forest Products, Dubreuil Forest Products Ltd., E.B. Eddy Forest Products Ltd., and St. Mary's Paper Limited. Administratively, the geographic area encompassed by the NSTIA has been amalgamated with the Northeast Seed Management Association for MNR support.

Two member companies are now cooperating in seed zone 21 to satisfy their mutual demand for seed. The remaining members are in the process of sorting out their obligations under the new Sustainable Forest Licenses and are still in the process of defining their long-term tree improvement goals. As part of this process, all program components are being reviewed; particularly the orchard and tests for all species. These are being assessed for their present and future value to both the current and potential membership. Black spruce and jack pine continue to be the species of interest. The long term interest in species such as white pine and white spruce is part of the review process.

In the summer of 1997, half of the Durban Township jack pine seed orchard was rogued for the final time. Approximately 7,000 trees were culled from this 13 ha orchard. The remainder will be rogued in 1998. The Hambleton Township jack pine seed orchard will be rogued for the first time in the fall of 1997. Approximately 9,000 trees will be removed from the 13.5 ha orchard. The family tests associated with the Chenard Township and Firstbrook Township jack pine orchards will be assessed in 1997 in preparation for the 1998 final and first roguing respectively of these orchards.

ZONE 6: FOREST GENE CONSERVATION ASSOCIATION

The Forest Gene Conservation Association (FGCA) was established in 1994 and is comprised of government and non-government organizations who operate in southern Ontario. The FGCA currently has 24 member organizations including Domtar Forest Products, Avenor Inc., Townsend Lumber, The Arboretum at University of Guelph, Mohawk Council of Akwesasne, Six Nation of the Grand River, Ontario Forestry Association, Association of Conservation Authorities of Ontario, Society of Ecological Restoration (Ontario Chapter), Eastern Ontario Model Forest, Canadian Chestnut Council, Canadian Forest Service, Society of Ontario Nut Growers, Seed Source, Ontario Ministry of Natural Resources, Nation Capital Commission, Alfred College, St. Lawrence Islands National Park, Parks and Recreation (City of Toronto), Somerville Nurseries, Ecological Services for Planning, Landscape Ontario, Grand River Conservation Association, and New Leaf Forest Services.

The goals of the FGCA are: 1) to promote the maintenance and restoration of the genetic base of woody plant species, 2) to increase the economic benefits of planting through planning and implementation of tree breeding programs for selected woody plant species, and 3) to ensure the use of biologically appropriate seed sources in support of reforestation and restoration programs.

Over the last two years the FGCA has completed a strategy, developed a business plan, and became incorporated as a non-profit corporation. The business plan is focused on revenue generating activities that are consistent with the goals and priorities as described in the FGCA strategy.

Biologically Appropriate Seed in Southern Ontario

Stock Certification The Canadian Forest Service requested that the FGCA act as a steering committee for a study designed to examine and document the availability of plant material of appropriate seed source for restoration programs in southern Ontario. The methods used included a literature review and an issue analysis survey. The study was completed and recommended the establishment of a seed certification system for southern Ontario. The ongoing withdrawal of the OMNR from production of reforestation stock has increased the need for a seed certification system in southern Ontario to ensure that biologically appropriate planting material is used in planting projects on private and public land in southern Ontario. The FGCA has amalgamated background information on seed certification systems used by other jurisdictions and has issued an invitation to seed collectors, seed processors, stock producers and stock consumers to participate in the development of a stock certification system. This will be the highest priority for the FGCA over the next 2 years.

Genecology Studies Demonstration Genecology studies have been initiated for white spruce, white pine and red oak. Results of the genecology tests allow forest managers to make decisions about biologically appropriate seed sources for their planting sites. The information is also used to delineate breeding zones in tree improvement programs and to determine the number and location of gene pool reserves needed in a conservation program. Over the last four years members of the FGCA have participated in genecology studies

in cooperation with Dr. Dennis Joyce of the Ontario Forest Research Institute.

In 1996, several of FGCA members planted demonstration areas to show differences in seedlings that originate in geographic areas of different climatic conditions in Ontario. Domtar (McKinnon Forest Interpretation Center) and The Arboretum, University of Guelph have planted white pine genecology demonstration areas. The Eastern Ontario Model Forest has participated in the establishment of a red oak genecology demonstration area located on the G. Howard Ferguson Forest Nursery property.

Butternut Conservation

Search for Resistant Butternut The search for butternut canker resistant trees was initiated in 1994 and continued in 1995 and 1996. Over 500 landowners sent in survey forms describing the location and condition of butternut trees on their property. Teams of summer students completed field surveys. To date 20 potentially resistant trees have been located and grafts have been produced. The search for more potentially resistant trees continued in 1997.

Butternut Literature Review The Eastern Ontario Model Forest and the MNR pooled resources to complete "A Literature Review of Butternut and The Butternut Canker". The review is an excellent summary of current literature and information obtained through interviews of scientists in the US and Canada who are working on butternut canker. The review includes a report on the status of butternut conservation programs in the US and Canada. This document will serve as an important base of information as the FGCA proceeds with the development of a conservation strategy for butternut in Ontario.

Communications A fold-out pamphlet on butternut canker was produced through the FGCA. FGCA members, Eastern Ontario Model Forest and the MNR sponsored the production of the pamphlet. The pamphlet contains information informing landowners of the severity of butternut canker, symptoms, recommendations for management and a call to landowners to contact the FGCA to identify potentially resistant trees on their property. An extension note on butternut was produced to describe how to grow butternut seedlings from seed, and how to plant and care for the seedlings. A display about butternut canker has also been produced. The display is a mix of text and pictures explaining butternut canker, symptoms, and information on the search for resistant trees.

Pitch Pine Conservation

Pitch pine in Canada is restricted to Leeds County in Ontario and two populations in Quebec. In Ontario, pitch pine exists as isolated populations that range in size from 1 to 5000 trees. A status report for pitch pine was completed by examining and comparing 1991 aerial photography to surveys completed in 1981 and 1966. A literature review on the genetics, reproductive, and silvicultural management of pitch pine was also completed.

A pitch pine genetics study was initiated in 1995 in cooperation with Dr. Alex Mosseler of the Canadian Forest Service, Fredericton, New Brunswick. Dr. Mosseler designed a study to examine the minimum viable population size of pitch pine in Ontario. Cones were collected from 18 stands that cover a range of sizes. Dr. Mosseler will examine number of viable seeds, germination rates and growth of the seedlings to determine the reproductive success of the populations of different sizes. Seedling growth will be measured in field tests, one of which has been established at the G. Howard Ferguson Nursery property. The Eastern Ontario Model Forest, MNR, Ted Cormier of The Seed Source and landowners who allowed seed to be collected from pitch pine on their land have all contributed to the study.

The status report, literature review and genetics study will form a base of information for the development of

a strategy for the conservation of pitch pine in Ontario.

Education - Forest Gene Conservation

In March, 1995 the FGCA in partnership with Canadian Forest Service, Eastern Ontario Model Forest and Science and Technology Transfer Unit, MNR organized a two day workshop called "Forest Gene Conservation: Principles to Practice". The proceedings of this workshop have been published and provide excellent documentation of the interesting and informative papers presented.

GENETIC RESOURCE MANAGEMENT PROGRAM - ONTARIO FOREST RESEARCH INSTITUTE

As in the operational program, scientific and analytical support provided by the Genetic Resource Management Program at the Ontario Forest Research Institute has been overshadowed by the re-organization of the MNR. Staff reductions and turnover have left several projects in a partially completed state. However, a Genetic Resource Management Policy is under development, the climatically-based seed zone system is being used operationally, and will become official policy in the near future, two genecology studies have been completed and the results are being used to develop advanced generation breeding zones, three genecology studies are still in progress, and analytical procedures have been developed to guide final roguing decisions in first generation orchards and to guide the selection of trees for advanced generation programs.

TREE GENETICS RESOURCES - OPPORTUNITIES AT THE PETAWAWA RESEARCH FOREST

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RESOURCE OPPORTUNITIES

The Canadian Forest Service has occupied the 100^2 km Petawawa research forest since 1918. With the closing of the Petawawa National Forestry Institute (PNFI) in September of 1996, the Petawawa Research Forest (PRF) was kept active but with significantly reduced on-site resources. Restructuring within the Canadian Forest Service has orphaned decades of tree genetic plantations at the PRF, many of published significance and having great potential for study in current themes related particularly to biodiversity and genetic resource conservation. The future of these resources will be determined by the requirements of the forest research community. This report provides a brief history of the tree genetic resources at the PRF, current status of operations, and potential resource opportunities.

TREE GENETIC RESOURCES AT THE PRF - A BRIEF HISTORY

Tree genetics plantations were first initiated at the PRF in 1922. They are primarily of pine, spruce and larch, native and exotic and occupy an area of 200 ha. Included are replicated provenance and progeny experiments, diallel experiments leading to second generation advancements, orchards and archives, and simple observation plantations. In total, there are 37 plantation areas containing 318 tree genetics experiments. Studies related to transport of seed sources for planting, the effects of climate change, early predictions for growth and yield, breeding to enhance growth performance or resistance to insect and disease are but a few contained within this research forest. A key component to the genetic trials at the PRF, is the management of pedigree records (identification for sources of seed or vegetative materials used in experiments) and the meticulous documentation of measurement data.

CURRENT STATUS

With the closing of the PNFI, resident scientists in the Biodiversity and Biotechnology programs were relocated to Fredericton and Quebec City. It was determined, however, that the research forest would remain active and open to scientific research and forest management. A small staff was retained and operate out of the former nursery location in renovated facilities which include offices, lab space, and workshop. It is the function of this group to promote the PRF and to facilitate and participate in research activities as well as to

apply ethical silvicultural practices in the management of the forest without compromising research potential.

RESOURCE OPPORTUNITIES

As previously mentioned, there are numerous tree genetic experiments dating back decades. The stark reality is that in order to maintain them under the current levels, at least two key conditions must occur: 1) a demonstrated requirement by the research community, particularly the CFS Networks and specifically the Biodiversity network, and 2) an evaluation of genetic resources to focus efforts and money on experiments of greatest current value. On this point, a set of decision criteria were drawn up and an evaluation process initiated.

It became evident that the evaluation of the genetics experiments would be dictated by theme, as follows, and that the level of current or potential scientific importance will be an important factor: 1) native species take priority over exotics, 2) tests of broad genetic base have higher priority than narrow genetic base tests, and 3) genetic resources conservation potential .

As a preliminary application of these decision criteria, a group of core experiments are noted. They include all range and regional experiments of native species established between 1950 and 1980 and include jack pine, red pine, white pine, white spruce, black spruce, red spruce and tamarack all having application to current interest in biodiversity, climate change and genetic resource conservation. Other groups of experiments of native and exotic species are identified and can be categorized into: orchards and archives (of particular note in white and Norway spruce); first and second generation plantations in jack pine, white, black and Norway spruce (breeding for resistance to frost and weevil) and others as well as provenance hybrids; rare genotypes which include virescent white spruce and fastigiate red pine. As well, there are 11 species of pine, 13 species of spruce, 6 species of larch and 12 species of fir planted on site and having world wide origins. Further information can be made available on request.

SUMMARY

Genetic resources of the magnitude which are in existence at the PRF are rare. Decades-old plantations, well documented and maintained, are available to the forest research community. It is the will of that community to determine the potential of this resource and to use it in their research activities. Only in this way will the genetic resources at the PRF continue to exist as a resource for tree genetic investigations for future generations.

MANITOBA'S TREE IMPROVEMENT PROGRAM

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Keywords: Jack pine, black spruce, white spruce, co-operative

This report highlights the progress which occurred from 1995 to 1997. Tree Improvement programs have been established for jack pine (*Pinus banksianna* Lamb.), black spruce (*Picea mariana* (Mill) B.S.P.) and white spruce (*Picea glauca* (Moench) Voss). Co-operatives have been established with Repap Manitoba and Pine Falls Paper Company.

JACK PINE

A second cone crop was harvested from the Hillside pedigree orchard with 3.45 hectoliters of cones harvested.

The third 50 per cent roguing was undertaken at the Interlake mass selection seed orchard. Repap Manitoba harvested 7 hectolitres of cones from the rogued trees.

A 1.5 ha site has been prepared for the establishment of a clonal orchard in 1998 in the Mountain breeding zone. The clonal orchard is based upon the work of Dr. J. I. Klein.

A crown management program is underway at the Hillside pedigree orchard and Cranberry mass selection seed orchard.

BLACK SPRUCE

A co-operative with Repap Manitoba was established for the Highrock breeding zone. In June-July 1997 three family tests were established and a 7.0 ha seed orchard site selected. The orchard site will be prepared for planting in 1998.

The plus tree selection program continued in the Mountain breeding zone with a total of 204 selections made to date.

WHITE SPRUCE

A container breeding garden is being managed in co-operation with Repap Manitoba for the Saskatchewan River breeding zone. Polycross controlled breeding programs were carried out in 1996 and 1997. A cold frame structure is presently being constructed at the Birds Hill Tree Improvement facility to enhance the breeding program.

SUMMARY

The four co-operatives with Repap Manitoba and the co-operative with Pine Falls Paper Company are working very well. Presently co-operatives for a further 3 breeding zones are being prepared. Currently there are 44.7 ha of black spruce family tests, 22.3 ha of seedling seed orchards and 3.8 ha of mass selection seed orchards representing seven breeding zones. There are 9 ha of white spruce family tests and 6.4 ha of clonal seed orchards representing 2 breeding zones. There are jack pine programs in 3 breeding zones with a total of 4.5 ha of family tests, 9.7 ha of seedling seed orchards and 10.1 ha of mass selection seed orchards.

AGRICULTURE AND AGRI-FOOD CANADA, MORDEN RESEARCH CENTRE TREE IMPROVEMENT REPORT

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Keywords: *Populus, Acer, Tilia, Fraxinus, Malus, Betula,* shrubs, genetic resources

The primary goal of the Morden tree improvement program is the development of superior hardy plants for the landscape industry in prairie Canada. A diversity of species of both trees and shrubs are involved in the various projects. Most Morden introductions to the industry are handled by the Canadian Ornamental Plant Foundation (COPF). This agency acts as a royalty collection centre for our introductions.

ASH (*Fraxinus)*

Goal

Development of new hardy cultivars for the landscape industry.

Progress

Two new hybrids between black and Manchurian ash have been introduced to the nursery industry: 'Northern Treasure' and 'Northern Gem'. The former is an upright tree, while the latter is more oval. Both are very hardy and have superior growth rates and form. Both hybrids are perfect flowered, unlike either parent. A population of white ash seedlings is being screened using an ultra-low temperature freezer with the goal of identifying individuals with good cold tolerance. Green ash seedling populations are being evaluated for growth and resistance to ash plant bug.

POPLAR (*Populus)*

Goal

Development of superior, fast growing, cold tolerant, and disease resistant clones.

Progress

Approximately 10 new selections have been made over the last two years. These include both inter-specific and intra-specific hybrids. Selected populations were approximately 20 years old and near mature size. A new crossing program was initiated among hybrid aspen clones. This project is funded by the Manitoba Hybrid Poplar Team and the AAFC-Matching Investment Initiative (MII).

BIRCH (*Betula)*

Goal

To identify sources of resistance to bronze birch borer.

Progress

A second generation of seedlings derived from putatively resistant parental material was planted in a replicated trial. The planting site is drought-prone to induce early infestation by the borers. Parental lines have also been propagated and will be evaluated further under different environmental conditions

LINDEN (*Tilia)*

Goal

To develop superior inter-specific hybrids of lindens adapted to prairie growing conditions.

Progress

A little leaf linden has been selected as a maternal parent based on seed germination testing. Controlled crossing using a diversity of other *Tilia* species is underway. To date, putative hybrids between *Tilia cordata* and *T. mongolica* have been germinated. Plants will be field planted for further evaluation. All other hybrid combinations have not produced seed or seed has failed to germinate.

MAPLE (*Acer)*

Goal

To select superior red maple and hybrids between red and silver maple for prairie growing conditions.

Progress

Controlled crossing between *Acer* species is underway with *in vitro* embryo rescue techniques being deployed. A putative backcross hybrid between a red/silver hybrid female and a silver male have been obtained. Plants

have been field planted for further evaluation of hardiness and adaptability to prairie growing conditions.

APPLE (*Malus)*

Goal

To develop superior clones of flowering crabs and fruiting types adapted to prairie growing conditions.

Progress

A series of selections have been made among a population of upright growing plants. These will be propagated for further testing. A new crossing program combining columnar growth patterns (non-hardy) with hardy prairie apples was completed at Summerland, BC under the direction of Dr. Harvey Quamme. The seed has been germinated and seedlings will be field screened at Morden for hardiness and growth form characteristics. Seed from the USDA apple *(Malus seversii)* germplasm collection trip to Kazakstan (former USSR) has been obtained and sown. Plants will be screened for hardiness and fruit and ornamental characteristics.

OTHER SPECIES

A wide range of shrubby species including *Rosa, Potentilla, Ribes, Amelanchier, Philadelphus,* and *Weigela* are being developed for landscape potential. A wide range of characteristics is being evaluated, but cold tolerance is a principal issue in all studies.

GENETIC RESOURCES

The Morden Research Centre has been identified as a "node" on the Agriculture and Agri-Food Canada genetic conservation network. Hardy landscape plants in the Morden arboretum are part of this program. A new informational database concerning hardy ornamentals is under preparation. Visual images of the plants as well as descriptive and evaluation information will be included in the final product (CD-ROM).

MANAGEMENT RELATED STUDIES

Goal

Asexual propagation of jack pine for application in tree improvement programs.

Progress

Research activities over the past 5 years have focussed on developing procedures for operational asexual propagation of jack pine (*Pinus banksiana* Lamb.). A series of scientific publications (Browne et al., 1997a, b) regarding various aspects of the study were published. An operational manual outlining key features for asexual propagation of jack pine is under preparation. Financial support from the Canada-Manitoba Partnership Agreements in Forestry is gratefully acknowledged. The authors also appreciate cooperation and

continuing support provided by Dr. J. Klein (formerly CFS), Mr. J. Dojack (Man. Dept. of Natural Resources) and Ms. D. Roddy (Weyerhaeuser Canada Ltd.).

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WEYERHAEUSER CANADA - TREE IMPROVEMENT IN SASKATCHEWAN

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Keywords: *Pinus banksiana, Picea glauca, Populus tremuloides*

JACK PINE PROGRAM

A grafted first generation seed orchard has been producing seed to meet all our reforestation requirements since 1993 and there is now a surplus of seed.

The focus for the next three to four years will be on cross pollinating 160 selected trees from open pollinated progeny trials and from old Canadian Forest Service trials in the Western Breeding District.

A planned roguing of the jack pine orchard has been replaced by top grafting (replacing parts of the crown of the rogued trees in the orchard with grafts of new selections).

WHITE SPRUCE PROGRAM

The year 1996 proved to be an exceptional one for the white spruce orchard due to the record amount of cones collected to produce 67.2 kg of seed. All 40 clones in the orchard contributed to the crop in varying degrees. There is enough improved seed to meet our reforestation needs for the next five years.

Breeding work for a series of cross pollinated tests of orchard parents was completed with the final tests being established in 1997. Maintenance of these and all other trials is becoming a major project.

ASPEN PROGRAM

Weyerhaeuser Canada is a member of the Aspen/Larch Cooperative at the North Central Experimental Station, University of Minnesota. Current efforts by the Cooperative are mostly assisting Weyerhaeuser in Alberta by supplying 26 selected trees for a provenance test being established through the Cooperative. Scions from eleven of these trees were collected in February 1997 for grafting.

Approximately 30 ha of farmland on the seed orchard property will be converted to various aspen plantations. One replication of the provenance test mentioned above will be established at the seed orchard. It will include trees from Minnesota, Alberta, and Saskatchewan. In addition, the PFRA (Prairie Farm Rehabilitation Administration) will be establishing plots of hybrid poplars as part of a study to test the growth performance of several hybrid poplars for commercial woodlot owners. The remainder of the farmland will be planted with local aspen to provide woodlot demonstrations.

OTHER PROJECTS AND DEVELOPMENTS

Weyerhaeuser Saskatchewan Ltd. is undertaking an Environmental Impact Assessment (EIA). For the EIA, information about the impacts of planting material from the jack pine tree improvement program on the genetic diversity of the resulting forest was collected. This was done in four stages: 1) a literature review, conducted by Dr. Jerome I. Klein, 2) a lab analysis done by Dr. Jim Hamrick, University of Georgia to compare samples from each clone in the jack pine seed orchard with samples from five geographically distributed wild populations, 3) flower counts were done on three to five trees per clone in the orchard to provide an indication of the parental balance in the orchard, and 4) an independent peer review, combining the results of the studies listed above with an evaluation of the program's long term strategy, was done by Dr. Jerome I. Klein.

PFRA SHELTERBELT CENTRE - TREE IMPROVEMENT SUMMARY

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Keywords: shelterbelt, windbreak, agroforestry, tree improvement

The objective of the tree improvement program at the PFRA Shelterbelt Centre is to develop genetically superior trees and shrubs for shelterbelt planting in the prairie provinces of western Canada. The programme has focused on poplar (*Populus* spp.), Scots pine (*Pinus sylvestris* L.), Siberian larch (*Larix sibirica* Ledeb.), green ash (*Fraxinus pennsylvanica* Marsh. var. *subintegerrima* [Vahl.] Fern.), bur oak (*Quercus macrocarpa* Michx.), sea buckthorn (*Hippophae rhamnoides* L.) and choke cherry (*Prunus virginiana* var. *melanocarpa* [A. Nells.] Sarg.).

SCOTS PINE

A major collection of Mongolian Scots pine(*Pinus sylvestris* L. var. *mongolica* Litvin.) from its native range in Northeast China (Heilongjiang province and Inner Mongolia) was completed. This collection, in co-operation with the Northeast Forestry University, Harbin, China includes 34 individual trees from 24 locations. Provenance tests were established at two sites in 1997. The Scots pine improvement program initiated in 1960 has concluded. The project has involved provenance and full and half-sib progeny testing. All Scots pine seed grown for shelterbelt plantings in Western Canada originates from a one hectare clonal seed orchard.

SIBERIAN LARCH

Improvement of Siberian larch has been under way since 1981. The program has included provenance testing and evaluation of half-sib progeny throughout the Canadian prairies. This has resulted in the release of the seed-propagated cultivar 'Lindquist' which has consistently shown superior performance when planted in prairie shelterbelts.

GREEN ASH

Provenance testing of green ash has identified several superior seed sources for shelterbelt planting. The best

sources originate from Willowbunch, SK and Somerset, MB. In 1994, green ash seed was collected from xeric sites throughout the Great Plains. These sources have been propagated and were planted at three sites in southern Saskatchewan in 1997. The objective of the project is to identify drought tolerant seed sources adaptable to semi-arid planting sites.

POPLAR

A clonal field test of 100 *Populus* hybrids developed at the Shelterbelt Centre was evaluated after five growing seasons. Several hybrids with superior growth, hardiness, and insect and disease resistance were identified. The best performing clones will be used to make up a clonal package, the objective being to increase the diversity of poplar shelterbelts which will help to maintain healthy populations that have the genetics to accommodate the movement of a disease or virus through the shelterbelt. In 1995, a new male poplar *P.* x 'Hill' selected from an open pollinated population of *P. deltoides* Marsh var. *occidentalis* Rydb. was released. 'Hill' is a vigorous female tree resistant to the major insects and diseases found on the prairies. It is most suitable for agroforestry and woodlot plantings.

BUR OAK

In 1993, bur oak provenance tests were established at four locations, two in Saskatchewan and one each in Alberta and Manitoba. The trees will be evaluated in the fall of 1997. To date survival at the test sites ranges from 95 to 100 percent.

CHOKE CHERRY

The Shelterbelt Centre is actively involved in a choke cherry improvement project. The project involved selection of superior native choke cherry trees in the Canadian prairies. A population of 17,000 individuals representing 175 sources has been assembled, propagated, and planted in tests in Saskatchewan and Manitoba. The project will provide valuable information on the genetic diversity of choke cherry in the prairies. In addition, 20 vegetatively propagated cultivars with superior fruit production have been selected. The best cultivars will be released for orchard planting.

SEA BUCKTHORN

Sea buckthorn has been widely used for shelterbelt planting in the Canadian prairies. In 1995, a project designed to select and develop sea buckthorn cultivars for shelterbelts and fruit orchards was initiated. Genetic diversity among sea buckthorn populations in Canada is considered to be low, but there is great variability in yield and morphological characteristics of the fruit, as well as phenological characteristics among individual trees. As a first step toward improvement and cultivar development, phenotypically superior female and male plants are being selected from planted shelterbelt populations. To broaden the genetic base for long term breeding sea buckthorn species from China, Russia, Finland and Germany have been introduced. A total of 100 new accessions have been propagated and planted in a germplasm nursery. Genetic diversity of local populations and the introduced plants is being quantified by isolating DNA for RAPD analysis. Preliminary data shows that the local population has a narrow genetic base compared to the introduced accessions.

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CANADIAN FOREST SERVICE TREE IMPROVEMENT IN THE PRAIRIE PROVINCES

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From the summer of 1995 until the closing of the Manitoba District Office at the end of March 1996, Northwest Region tree improvement staff were principally occupied with disposition of tree improvement assets. The Canadian Forest Service forest genetics program in Northwest Region, which was largely tree improvement for Saskatchewan and Manitoba, ran from about 1960 to 1996. Although the work included participation in several cooperative provenance experiments with some plantations in Alberta, the major part of the resources were expended on breeding programs for jack pine in Saskatchewan and Manitoba. Recently, projects were initiated in mass selection jack pine seed orchards for northern Manitoba and in seed orchard management research in jack pine and black spruce. Subsequent to closing of the Manitoba District Office, Tree Improvement Specialist Paul Chapman has been employed as a forester with responsibilities in silviculture with Repap Manitoba in The Pas, and Forest Geneticist Jerome Klein retired and is operating a forest science consulting practice in Edmonton.

JACK PINE SEED ORCHARDS

Completing the transfer of ownership of Manitoba seed orchards to Manitoba Forestry Branch involved little more than providing documentation including data files in an accessible format. Data files for Saskatchewan family-test plantations were provided to Weyerhaeuser Saskatchewan Ltd. Scions from family test trees in western Manitoba and eastern Saskatchewan plantations, selected by Best Linear Prediction based on 17-year measurements, were provided to Manitoba Forestry Branch for seed orchard grafting.

APPLIED TREE IMPROVEMENT RESEARCH

Reports were produced and distributed on the applied research trials that were ongoing until discontinuance of the program, although the results were generally too preliminary to support refereed publication. Reports, in some instances in letter form, were produced on flowering response to seed orchard management practice trials, a retrospective progeny test, a realized gain test, and a demonstration test of superior and average progenies. Data files from black spruce provenance test plantations were provided to Dr. Alex Mosseler, Natural Resources Canada, Atlantic Forestry Centre.

SECOND GENERATION BREEDING OF JACK PINE

The plan for second generation breeding for eastern Manitoba was revised based on additional literature

review. Number of parents and number of first-generation families providing parents was increased beyond the number proposed in earlier versions to reduce restriction of options in later generations. The plan identifies family-test trees selected as parents of second-generation families and suggests a pairing scheme for matings of these trees. Parents at the lower end of the select group are mated among themselves rather than with high-ranking parents to avoid relatedness between advanced-generation families.

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FOREST GENETICS, BIOTECHNOLOGY AND TREE IMPROVEMENT RESEARCH AT THE UNIVERSITY OF ALBERTA

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Keywords: Molecular, population, evolutionary and conservation genetics; disease resistance, quantitative traits and genome mapping; speciation, biosystematics, phylogenetics and molecular evolution; RAPD, RFLP, microsatellite DNA, and allozyme markers; genetic implications of silvicultural practices and conservation and sustainable management of forest genetic resources; tree improvement; mycorrhizae.

During the past two years, our research activities continued primarily in the areas of molecular, population and conservation genetics, molecular evolution, speciation, biosystematics, phylogenetics, genetic implications of silvicultural management, and molecular linkage mapping of disease resistance and quantitative traits, and ectomycorrhizae. We also initiated projects on identifying and mapping DNA markers for *Melampsora medusae* Thum. leaf rust resistance and sex determination in *Populus* L.

Since the last report, two graduate students completed their M.Sc. degree program in forest genetics.

MOLECULAR, POPULATION AND EVOLUTIONARY GENETICS, MOLECULAR EVOLUTION, SPECIATION AND BIOSYSTEMATICS

Allozyme Studies

The study on genetic diversity, population structure, and spatial genetic structure of eastern white pine (*Pinus strobus* L.) in virgin old-growth stands in Ontario was completed by using 54 allozyme loci (Buchert *et al.* 1997; Rajora *et al.* in preparation). Manuscript preparation is nearing completion (Rajora and Dancik 1997) for the study on population genetic structure, variation, and evolution on Engelmann spruce (*Picea engelmannii* Parry), white spruce (*P. glauca* (Moench) Voss), and their putative natural hybrid complex in Alberta. The study on genetic diversity of two closely related tropical species *Racosperma auriculiforme* (Cunn. Ex Benth.) Pedley and *R. mangium* (Willd.) Pedley using multivariate analysis of allozymes and morphometric traits was completed (Khasa *et al.* 1997a). Other papers on *Racosperma* genetics have already been published (Khasa *et al.* 1995a, b, c). Endah Suwarni started her MSc program aimed at estimating the levels of pollen contamination in a *Pinus merkusii* Jungh & de Vriese seed orchard.

Molecular Genetics

Om Rajora, in collaboration with Dr. S. Dayanandan of the University of Massachusetts, developed and characterized microsatellite DNA markers for trembling aspen (*P. tremuloides* Michx.) and other poplar species (*P. deltoides* Marsh., *P. nigra* L., and *P. maxmimowiczii* Henry). Work on development of additional microsatellite markers and examination of microsatellite DNA variation is progressing well. The study on examination of random amplified polymorphic DNA (RAPD) variation in trembling aspen selections from Alberta and in *Populus* x *canadensis* Moench cultivars was completed by Rajora and associates. Data analysis for variation in cpDNA and nuclear genes in trembling aspen is in progress. The study on genetic diversity of white spruce populations in Saskatchewan using RAPD markers was completed by Rajora. Manuscript preparation for the poplar and white spruce molecular genetic studies is progressing well.

Heather Cobban completed her MSc program aimed at assessing genetic diversity in naturally and artificially regenerated populations of white spruce using microsatellite markers. The study on population genetic structure and evolution of the *P. engelmannii* and *P. glauca* complex using RAPD analysis is progressing well. A few species-specific RAPD fragments have been identified to differentiate these sister species (Khasa and Dancik 1996). We are starting a population survey of both species using microsatellite markers. A study on population genetic diversity and breeding systems in *Larix lyallii* Parl. using microsatellites is underway in collaboration with Barry Jaquish of the B.C. Ministry of Forests. Microsatellite markers have been developed for larch using both database searches and cloning techniques (Khasa *et al.* 1997b). Chris Todd is starting a PhD study on molecular genetics of maturation in white spruce.

MOLECULAR MARKERS FOR DISEASE RESISTANCE, SEX DETERMINATION AND OTHER QUANTITATIVE TRAITS, AND GENOME MAPPING

Rajora initiated and has been conducting projects on identifying and mapping markers for *Melampsora medusae* leaf rust resistance in *Populus deltoides* and its interspecific hybrids with *P. nigra,* and *P. maximowiczii* and markers for sex determination in *P. tremuloides*. The study on wood decay and stain and genetic fingerprinting of trembling aspen clones was completed by Rajora, Dancik, and associates. DNA variation related to natural wood decay resistance in aspen has been identified and data analysis and preparation of manuscripts are in progress.

The study on identifying molecular genetic markers for resistance to western gall rust (WGR), caused by *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka, in jack pine (*Pinus banksiana* Lamb.) was completed by Rajora and Dancik. About a dozen potential DNA markers for WGR resistance were identified. Reciprocal controlled crosses were made within and between WGR-resistance and WGR-susceptible genotypes. The F_1 progeny will be raised and analyzed for genetic linkage mapping of WGR resistance, when funding is available.

GENETIC IMPLICATIONS OF SILVICULTURAL PRACTICES, CONSERVATION AND SUSTAINABLE MANAGEMENT OF FOREST GENETIC RESOURCES

The studies on determining the impacts of silvicultural practices on genetic diversity in white spruce and on genetic diversity of natural white spruce populations from ecologically-distinct sites in the Prince Albert Model Forest were completed using RAPD analysis (Rajora 1997). Inheritance of RAPD markers was determined in F_1 progeny of white spruce controlled crosses. Ecological site-related RAPD variation was found to exist

in white spruce. However, this work needs to be expanded to additional sites.

The project on genetic implications of silvicultural management and conservation of eastern white pine in Ontario progressed well (Rajora, Dancik, and George P. Buchert - Ontario Forest Research Institute). Rajora and associates have completed the genetic diversity analysis of the old-growth preharvest and postharvest residual stands of white pine using allozymes, RAPD and microsatellites DNA markers, and the mating system analysis of the preharvest old-growth white pine populations using allozymes. The first manuscript from the study has been published (Buchert *et al.* 1997) and several other manuscripts are in preparation. A user's workshop was organized in North Bay, Ontario in May 1997 by Rajora in collaboration with Andree Morneault and Cathy Nielsen of the Ontario Ministry of Natural Resources and Dr. Alex Mosseler of the Canadian Forest Service. Rajora is actively collaborating with Alex Mosseler in genetic studies aimed at biodiversity, effective population size, conservation, restoration, and sustainable management of eastern white pine, white spruce, red spruce (*Picea rubens* Sarg.) and other forest genetic resources.

Our group is also deeply involved in issues of biodiversity and sustainability of tropical forests (Khasa *et al.* 1995d, Khasa and Dancik 1997a,b).

TREE IMPROVEMENT

Rajora has been actively involved in improvement and breeding programs for aspen and other poplars. The parents and F_1 progeny of intraspecific *P. deltoides* and interspecifc *P. deltoides* x *P. nigra*, and *P. deltoides* x *P. maximowiczii* controlled crosses, made in 1982 by Rajora and field tested in Ontario, were procured and propagated. These have been planted in nursery clone banks at the University of Lethbridge and Weyerhaeuser Canada - Drayton Valley and will be used for clonal tests. A survey of the cottonwoods of southern Alberta and selection of the breeding stocks was completed.

Ruichan Zhang completed his PhD program on biochemical and genetic approaches to very early selection of *Pinus radiata* D. Don. under the supervision of Dancik and Dr. Richard P. Pharis (U. of Calgary). Deogratias Rweyongeza also completed his study on assessment of growth performance of Scots pine (*Pinus sylvestris* L.) in Alberta while being supervised by Dancik and Francis Yeh.

MYCORRHIZAL RESEARCH

Khasa and Dancik have initiated a project with Alberta Environmental Protection on development of biofertilisers for commercial forest tree species . From the first-year greenhouse results, it was possible to select the best ectomycorrhizal co-partner for each of the five conifer species studied: white spruce, black spruce (*Picea mariana* (Mill.) BSP), engelmann spruce, lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) and jack pine. Fungal metabolites have been isolated from ectomycorrhizal fungi which have potentially antagonistic effects on pathogenic fungi such as *Fusarium oxysporum* Schlecht. Molecular characterization of the different ectomycorrhizal strains has been accomplished successfully by using PCR-RFLP. This work is being conducted in collaboration with U of A colleagues W. Ayer (Chemistry) and Lyne Sigler (Devonian Botanic Garden).

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QUANTITATIVE AND POPULATION GENETICS, APPLIED TREE BREEDING AND IMPROVEMENT AT THE UNIVERSITY OF ALBERTA

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Keywords: Quantitative and population genetics, conservation, early selection, applied tree breeding and improvement, balancing gain and diversity, introgression, genomic mapping and QTL

During the past two years, our research continued with: 1) theoretical and experimental investigations of population genetic structure in lodgepole pine (*Pinus contorta* Dougl.), jack pine (*P. banksiana* Lamb) and *Pterocarpus macrocarpus* Kurz. from Thailand, 2) retrospective early genetic evaluation and selection in lodgepole pine, white spruce (*Picea glauca*) and Scot pine (*P. sylvestris* L.), and 3) investigation of geographic variation, resistance breeding, host-fungus interaction and QTL of western gall rust (*Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka) in lodgepole and jack pine hosts. We also initiated new studies on the genetic effects of forest fragmentation in Costa Rica and the theoretical evaluation of balancing genetic gain and diversity in breeding.

GENETIC STRUCTURE AS REVEALED BY ISOZYMES, RAPDS AND QUANTITATIVE TRAITS

We published a comparative study of population differentiation in lodgepole pine using quantitative traits and isozymes (Yang *et al.* 1996). All 19 isozymes tested neutral. In contrast, *F*-statistics indicated 2 of 6 quantitative traits were neutral and the remaining 4 were under divergent selection. This would suggest that it is important to choose judiciously among the available genetic markers when assessing genetic diversity and population differentiation.

Doctoral student Chaiyasit Liengsiri studied the mating system in eleven natural populations of *Pterocarpus macrocarpus* Kurz in Thailand based on the mixed mating model using 16 isozyme markers. Single-locus outcrossing rates (t_s) averaged from 0.620 to 0.931 among populations while multilocus outcrossing rates (t_m) ranged from 0.719 to 0.959. Both t_s and t_m revealed a geographic pattern with western populations exhibiting higher outcrossing than eastern populations. The low estimates of outcrossing rates in eastern populations were attributed to habitat characteristics, degree of disturbance, density, and distribution of flowering mature trees. Individual tree outcrossing rates (t_{mi}) indicated that reduced population density associated with disturbance could result in low t_m in some eastern populations.

Effective management of a species hinges on knowledge of its biology and genetic diversity. MSc student Aron Fazekas is determining the genetic relationships of Alberta lodgepole pine to populations outside the

province and the migration history of Alberta populations using RAPD markers. This study will identify potential centers of diversity which is important for conservation of genetic resources and tree improvement (Yang and Yeh 1995). As well, it will improve our understanding of the postglacial movement of the species, indicating whether Alberta lodgepole pine populations migrated from northern or southern refugia or, perhaps both.

A considerable amount of effort and time was spent to develop POPGENE, the Microsoft Window-based user-friendly freeware for population genetic analysis. POPGENE is a joint project between the University of Alberta (Francis Yeh and Rong-Cai Yang) and Center for International Forestry Research (Tim Boyle). Current version (POPGENE 1.20) is distributed on the World Wide Web, http://www.ualberta.ca/~fyeh/.

RETROSPECTIVE EARLY TESTING AND SELECTION

We have an on-going collaborative early selection research project in Scots pine with Drs. Gosta Eriksson and Alena Jonsson of the Department of Forest Genetics, Swedish University of Agricultural Sciences in Uppsala. One study examined the effects of wide and narrow spacing on genetic variation and juvenile-mature genetic correlations. Seedlings from open-pollinated families already field tested since 1964 were cultivated for three growth periods in the phytotron. Compared to other studies, their phytotron-field genetic correlation estimates under narrow spacing were the highest reported. The results were real, significant, and favorable to early genetic selection.

CONSTRUCTION OF HIGH DENSITY GENOMIC MAP AND IDENTIFICATION OF QTLS FOR WESTERN GALL RUST RESISTANCE IN LODGEPOLE PINE

We constructed a high density linkage map which identified three quantitative trait loci (QTLs) that infer resistance to western gall rust (WGR) in Alberta lodgepole pine. Genomic DNAs from haploid megagametophytes of a half-sib family with large phenotypic variance in resistance to WGR were amplified with 850 random decamer oligonucleotide primers by the polymerase chain reaction. One hundred and ten decamer oligonucleotide primers were segregating and they generated 245 sharp and reproducible Random Amplified Polymorphic DNA (RAPD) markers. Each megagametophyte was obtained by removal from the extending cotyledonary needles from germinating seed prior to natural abscission. The seedlings were inoculated separately with two WGR fungal isolates sampled in Alberta and Manitoba, and evaluated for WGR resistance. Two hundred of 245 RAPDs were mapped onto 16 linkage groups covering a distance of 3663.2 cM. Two putative QTLs accounted for 65% of the total phenotypic variance in resistance to WGR fungal isolates from Alberta. A third putative QTL accounted for 23% of the total phenotypic variance in resistance to WGR fungal isolates from Manitoba. This is part of Mr. Li Changxi's PhD research.

POPULATION BIOLOGY AND EVOLUTIONARY GENETICS OF WGR RUST RESISTANCE IN LODGEPOLE-JACK PINE HYBRID ZONE

Rong-Cai Yang completed the greenhouse portion of his long-term NSERC-funded study on the population biology and evolutionary genetics of resistance in lodgepole pine, jack pine and their hybrids. The study consisted of 40 populations sampled across the entire hybrid zone in Alberta and British Columbia. Two sources of inoculum, one from lodgepole pine and the other from jack pine, were used for the greenhouse inoculation. Two greenhouse assessments have been made on rust incidence and severity and growth. After one-year of greenhouse growth, inoculated trees were outplanted to a nursery bed for further monitoring on

rust development and impact on tree growth. Results from this study will provide factual evidence to help resolve contentious issues such as: 1) levels of rust infection on lodgepole vs. jack pine, 2) patterns of interspecific response to rust infection and their relation to geo-climatic variation, and 3) importance of jack pine introgression on rust resistance in lodgepole pine.

In collaboration with Alberta Land and Forest Service, we have carried out several field and greenhouse studies to assess geographic and genetic variation of WGR resistance in lodgepole pine (e.g., Yang *et al.* 1997). A theoretical and computer simulation study has been carried out to investigate the effect of discontinuity due to dichotomous or polychotomous responses of pines to WGR on the estimation of genetic parameters (Yang *et al.* 1997). Analysis of several field trials on WGR is in progress.

APPLIED TREE BREEDING AND IMPROVEMENT

We collaborated with the Alberta Land and Forest Service on the genetics and breeding of lodgepole pine and white spruce. Retrospective early testing and selection, geographic variation patterns, heritabilities and genetic correlations, genetic gain predictions, deployment of genotypes to balance genetic gain and diversity (Wei *et al.*, 1997), geographic variation and host-fungus interaction in WGR incidence, and mapping for WGR resistant genes have been major components of our studies.

Deployment of genotypes to a production population decisively depends on the measure of diversity, a consideration that parallels genetic gain in the management of genotype mixtures. Wei Rung-peng and Francis Yeh have now developed the optimal diversity-dependent deployment for a family of diversity indices in the genetical and ecological context. Furthermore, a selection model has been derived to find a set of optimal family contributions that maximizes gain at restricted selection proportion and coancestry, or minimizes coancestry at restricted selection proportion and gain.

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GENETICS AND TREE IMPROVEMENT PROGRAM, 1995-97 ALBERTA LAND AND FOREST SERVICE

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Keywords: Tree breeding, genetic improvement, provenance studies, species testing, seed orchards, progeny testing, white spruce, lodgepole pine

This report summarizes the progress of the Alberta Land and Forest Service's (L.F.S.) genetics and tree improvement program for the period 1995-1997.

PROGRAM DEVELOPMENT

Pine Ridge Forest Nursery (P.R.F.N.) seedling production and seed processing operations were privatized. This required several adjustments as the main facilities for the tree improvement program are located at the Pine Ridge site. Reforestation seed and several other functions of P.R.F.N. were merged with the provincial Tree Improvement Centre which is being renamed as the Forest Genetics and Seed Centre.

GENETIC IMPROVEMENT

Assembly of Breeding Stock

Selection of superior parent trees to provide material for L.F.S and L.F.S./Industry cooperative projects continued. L.F.S. wild stand tree selections included six lodgepole - jack pine hybrids *(Pinus contorta x banksiana)* and six jack pine trees. Nine white spruce *(Picea glauca)* trees were selected from existing provenance and open-pollinated progeny trials. Forty progeny tested superior lodgepole pine and 46 progeny tested white spruce parents were acquired from the interior British Columbia region from B.C. Ministry of Forests. Seven young red pine *(Pinus resinosa)* from a promising Ontario source were also selected and propagated from a field test in central Alberta. Selections made as part of L.F.S./Industry cooperative projects included 181 white spruce, 85 lodgepole pine, nine lodgepole-jack pine hybrids and 10 black spruce *(Picea*

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mariana) trees. Companies participating in these projects included Alberta-Pacific Forest Industries Inc, ANC Timber Ltd., Blue Ridge Lumber (1981) Ltd., Canadian Forest Products Ltd. (Grande Prairie and Hines Creek Divisions), Manning Diversified Forest Products Ltd., Millar Western Industries Ltd. Sunpine Forest Products Ltd., Vanderwell Contractors (1971) Ltd., Weldwood of Canada Ltd. (Hinton Division) and Weyerhaeuser Canada Ltd. (Grande Prairie and Drayton Valley Divisions). One new company, Northland Forest Products Ltd. joined the cooperative tree improvement program in 1996-97.

Genetic Testing

White spruce progeny trials for Breeding Region 'G' were measured in 1995 at 11 years from seed. Seventy-one half-sib families were assessed at two sites. Ten- and 11-year heights were measured and plant vigour and white pine weevil damage were assessed. For both sites combined, heritabilities for 11-year height were 0.23 (individual) and 0.68 (family). Family differences in weevil susceptibility were significant at one site with individual and family heritabilites of 0.03 and 0.61, respectively.

Three U.S.S.R. Scots pine (*Pinus sylvestris*) progeny trials were assessed in 1995 as part of a MSc thesis research project by Deogratias Rweyongeza at the University of Alberta at 6 years from seed. Significant family differences and family x site interactions were found for 4, 5 and 6 year heights. For the three sites combined, heritabilities for 6-year height were 0.07 (individual) and 0.34 (family).

Three lodgepole pine/jack pine hybrid and outlier population trials were measured in 1995 at 10 years from seed. Three trials in the same series were measured in 1996 at 7 years from seed. A total of 9 field trial of this experiment are established across Alberta.

Northern areas white spruce provenance/progeny trials were measured in 1996 at 12 years from seed. One hundred and twenty-five half-sib families were assessed at three sites located in northwestern Alberta. Eleven- and 12-year heights were measured and plant vigour and white pine weevil damage were assessed. For all sites combined, individual and family heritabilities for 12-year height were 0.09 and 0.40, respectively. Family differences in weevil susceptibility were significant at two sites with individual heritabilities of 0.09 and 0.03 and family heritabilities of 0.75 and 0.50.

A series of Siberian larch (*Larix siberica*) provenance/progeny trials was established in 1996. Fifty-eight single tree seedlots originating from southern Siberia were outplanted on five sites in a 5-replicate, randomized complete block design. Seed for these trials was obtained from the National Tree Seed Centre, formerly at Petawawa National Forestry Institute.

In 1996, lodgepole pine open pollinated half-sib progeny trials were established for breeding region 'K1'. One hundred and two superior tree selections are represented on two sites planted in a 6-replicate, randomized complete block design.

Data analyses were completed for two lodgepole pine half-sib family tests for breeding region 'B2' measured in 1994 at 6 years from seed. The tests were assessed for height, western gall rust (WGR) infection, survival, and climatic injury. For the two sites combined, heritabilities for 6-year height were 0.04 (individual) and 0.11 (family); heritabilities for WGR incidence were 0.09 (individual) and 0.21 (family). Significant site x family interactions were found for height growth as well as WGR incidence. Interactions for height growth were accounted for primarily by changes in genotype ranks, but the interactions for WGR incidence were due mainly to heterogeneity of family variances between sites.

Seed Orchards

In 1996, as part of the L.F.S./Sunpine Forest Products Ltd. Cooperative Tree Improvement Program, the Region 'K1' (southern east slopes)lodgepole pine clonal orchard was established at the Alberta Crop Diversification Centre - South located near Brooks. The orchard is designed for 500 ramets and will contain clones of 60 superior parent trees. Approximately 50% of the orchard was planted in 1996.

Two new cooperative tree improvement projects were initiated for white spruce during the report period. The Region 'E1' white spruce project will be complimentary to the existing Region 'E' (northeastern boreal) cooperative project between Alberta-Pacific Forest Industries Inc. and the L.F.S. Northland Forest Products Ltd. is a partner in this project. The breeding population will consist of new parent tree selections from north eastern Alberta provenances andprevious selections from the northern area of Region 'E'. A clonal orchard is planned for this project and will be established at Pine Ridge Forest Nursery in 2001.

A cooperative white spruce tree improvement project for Region 'G2' (Peace region) was initiated between Manning Diversified Forest Products Ltd., Canadian Forest Products Ltd., and the L.F.S. in cooperation with Fairview College. A 5 ha area on campus was allocated by Fairview College for orchard establishment. Graft production is ongoing for a clonal orchard to be established in 1999.

Also planned for establishment at the Fairview College site is the Region 'J' (Peace region) lodgepole pine clonal orchard. The Region 'J' tree improvement project, initiated in 1995, is a cooperative venture between Manning Diversified Forest Products Ltd. and the L.F.S. Graft production is underway with orchard establishment projected for 1999.

The Region 'P1' (north eastern boreal) jack pine project is being developed as a cooperative partnership between Northland Forest Products, Alberta-Pacific and the L.F.S. Superior tree selections are ongoing and establishment of a clonal orchard is planned for 2001.

The first improvement thinning of the Region 'D1' white spruce seedling seed orchard was completed in 1995. Thinning of the 15-year old orchard was based on 10-year performance results from four half-sib family field trials. The objective of the thinning was to remove one-half of the poorest families within each block of 150 families. Consideration was given to orchard spacing and the condition of corresponding trees. Families were ranked by height superiority with consideration to white pine weevil resistance. A total of 1 598 trees was removed leaving 1 637 trees for seed production. In 1996, the orchard produced its first good cone crop yielding 8.1 kg of seed.

Site management and seed orchard operations at the Huallen Seed Orchards site near Grande Prairie were carried out during 1995 and 1996 on a cost recovery basis on behalf of Huallen Seed Orchard Company. Three seed orchards are currently established on the site, (Region 'G1' clonal white spruce, Region 'B2' clonal lodgepole pine and Region 'B1' seedling lodgepole pine) and establishment of two new clonal orchards commenced in 1996. The new orchards are being developed for the Region 'I' (west central Alberta) white spruce project and the Region 'L2' (west central Alberta) black spruce project.

Cone and Seed Insects and Diseases

In 1996, surveys of the spruce cone maggot (*Strobilomyia neanthracina*) were initiated in white spruce seed orchards to evaluate a sequential sampling plan that uses incidence of cone maggot egg and young larval infestation levels to predict percentage of filled seeds destroyed. In the two orchards surveyed, estimates of the total reduction in seed yield due to the cone maggot were about 36-38%. The study is continuing in 1997 in

collaboration with Dr. Herb Cerezke and is being extended to a third white spruce orchard and to a black spruce orchard, all located at P.R.F.N. and its satellite locations

A study of the spruce cone rust (*Chrysomyxa pirolata*) in white spruce orchards was initiated in 1997 by Pat Crane, a PhD candidate at the University of Alberta. The goal of the study is to gain a better understanding of the disease cycle and its relationship to the reproductive cycle of white spruce. The study will explore questions such as the length of time that rust producing inoculum is available on the alternate hosts (*Pyrola* sp), the correlation between basidiospore production on *Pyrola* and cone development on spruce and early signs and symptoms of infection.

TREE IMPROVEMENT RESEARCH

Provenance Studies

Eighteen-year performance assessment of three Alberta-wide white spruce provenance trials was done in 1995. Twenty-eight seed sources were evaluated for survival , plant vigour, height, dbh, white pine weevil susceptibility, and stem straightness. Local provenances were among the best seed sources at all test sites in terms of survival and height performance. Variation among seed sources was significant and the ratio of among- and within-provenance variation tended to increase with age. The relationship between growth and survival performance and geographic origin of seed sources was best described by a polynomial regression. Over 75% of total variation in height growth was explained by a quadratic polynomial of elevation at the Prairie Creek test site, 64% at the Chinchaga River test site and 54% at the Sexsmith test site. Similar results were found for plant vigour. Survival performance was not as responsive as growth and vigour to the change of elevation.

In 1996, 21-year assessment of the Alberta-wide white spruce provenance trial was completed at the Hay River Genetics site in northern Alberta. Survival, height, dbh, vigour, stem straightness and weevil damage were measured on 26 seed sources. Significant variation was found among provenances with northern and lower elevation seed sources performing better than southern and high elevation seed sources. A quadratic polynomial of elevation of seed source origin was found to explain 38% of total variation in tree height. Comprehensive analysis and summary of Alberta-wide white spruce provenance trials is planned to be completed in 4-5 years after all the trials have been measured for 21 year performance.

Data analyses were completed on a greenhouse inoculation study of western gall rust infection of a total of 291 lodgepole pine half-sib families from west central Alberta. A general east-west trend was noted in family infection levels, with western and high elevation families found to be more susceptible to the disease.

PLANT PROPAGATION, WOOD AND SEED TECHNOLOGY

Plant Propagation

During the report period, stock production consisted of 8 275 seedlings for rootstock, 14 310 coniferous seedlings for experimental trials and 7 217 grafts.

Grafting of new superior tree selections accounted for about 70% of graft production. The majority of grafts produced were white spruce (65%) followed by lodgepole pine (25%) and black spruce (10%). A small number of jack pine and tamarack (*Larix laricina*) was also grafted. About 20% of graft production was secondary

grafting to increase ramet numbers of previously established clones. White spruce comprised 75% of secondary grafting followed by lodgepole pine at about 15%. The remaining 10% included black spruce, tamarack, Douglas-fir (*Pseudotsuga menziesii*), western larch (*Larix occidentalis*) and Scots pine. In addition, 241 white spruce and 205 lodgepole pine grafts were produced from scion material provided by the B.C. Ministry of Forests as part of a cooperative project development involving northwestern Alberta and northeastern boreal region of British Columbia.

Wood Technology

Relative density and fibre length measurements were completed on 215 superior parent trees during the report period. Of these trees, 114 were white spruce, 81 were lodgepole pine, 11 were black spruce, and 5 were jack pine. In February 1996, a wood information management system (WIMS) for wood characteristics was developed. The data generated is now being stored under this system.

In March 1997, a wood imagery system was purchased in consultation with Dr. Mathew Koshy, Research Associate, University of British Columbia. This electronic system will store and calculate the individual superior tree measurements for age, relative density, and fibre length and will allow cross references to the seed data base and other superior tree data bases.

Seed Technology

A total of 283 seedlots was added to the genetics seed bank over the report period. Of these seedlots, 34 were white spruce, 229 were lodgepole pine, 11 were black spruce and 4 were jack pine. The seed bank presently contains 4 016 seedlots.

Quality of seed bank seedlots continues to be monitored annually by testing a set of reference seedlots representing about 2% of seed bank entries. In the seventeen years since monitoring began, lodgepole pine seedlots have remained stable with a mean germination in 1981 of 87% and, in 1997, of 88%. Mean germination of white spruce seedlots has declined slightly from 91% in 1981 to 86% in 1996. Mean germination of aspen (*Populus tremuloides*), plains cottonwood (*Populus deltoides*), and the balsam poplar (*Populus balsamifera*) has declined 16-24% after 7 years of testing.

In February 1996, a seed information management system (SIMS) for seed data was developed. The new system is linked to other data bases allowing professional staff to track and cross-reference lineage, traits and inventories through WIMS, SIMS, the grafting inventory information system, the parent tree data base, and the geographic data base.

PROGRESS REPORT FOR CANADIAN TREE IMPROVEMENT ASSOCIATION, 1997

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The following principal scientists direct research areas related to tree improvement at the Forest Biotechnology Centre: Dr. Steve Grossnickle, ecophysiology; Dr. David Ellis and Dr. Craig Newton molecular biology; Dr. David Cyr, somatic embryogenesis. There are numerous other scientists who are involved in this work some of whom are referenced in this report. Recently, I was appointed President of Silvagen Inc., a company formed by B.C. Research Inc. (BCRI) and others, to commercialize somatic embryogenesis and related technologies in forest biotechnology.

This summary covers projects conducted and in progress in the last two years at B.C. Research. More detailed summaries of some of these projects, including figures, have been published in the Tree Improvement Council of British Columbia 1996 Progress Report available from the B.C. Ministry of Forests.

SOMATIC EMBRYOGENESIS RESEARCH

Dr. David Cyr, Dr. Kirsten Finstad,
Ms. Sheila Binnie and Coworkers

Work has continued on the establishment and deployment of embryogenic clones of interior spruce. The process involved placing embryos from full-sib seed on tissue culture medium to form an embryogenic culture. These cultures are stored in cryopreservation and also used to produce mature embryos and plants for field trials. The establishment of clonal field tests of interior has been ongoing since 1994 and will be completed in 1998. The program is focused on families which are high ranked for height and resistant to weevil. Approximately 700 clones were established on three field sites over the period 1994-1997 by the Ministry of Forests. A further 500 are currently in the nursery for planting in 1998, bringing the total to 1,300. The first results will be used to make selections after five seasons of growth.

A similar, although smaller program is being carried out with Sitka spruce with selected families which have shown high levels of weevil resistance in short term field tests conducted by the Ministry of Forests. In this case about 300 clones have been produced for nursery production in 1996 and 1997 and field tests have begun.

In 1996 the group began work with Douglas-fir embryogenesis. This has resulted in a bank of 350 embryogenic clones being established and stored in cryopreservation. Work is ongoing in the optimization of Douglas-fir embryo maturation and germination. The results appear encouraging and somatic seedlings have

been produced.

Over the last two years great emphasis has also been placed on pine embryogenesis. A project to develop somatic embryogenesis of western white pine (*Pinus monticola*) began in 1995. We have been able to compare the success with somatic embryogenesis of eastern white pine in collaboration with the Canadian Forest Service. So far a small numbers of embryogenic lines have been permanently established frozen. Embryos and germinants have also been produced. White pine remains a challenging species to do this work with. Interestingly, there are quite significant differences in *in vitro* behaviour between eastern white pine and western white pine although they are closely related species.

OPERATIONAL PRODUCTION OF INTERIOR SPRUCE

Silvagen Inc., Dr. Dan Polonenko and Coworkers

During the past year, pilot operational production of interior spruce somatic embryos and somatic seedlings has been carried out from 46 lines selected from 9 full-sib families from the Prince George selection unit. The parents of these families were tested in open pollinated progeny trials by the Ministry of Forests (Gyula Kiss et al.). These data indicate ten year growth is about 10% greater for these families and weevil damage at 15 years is reduced by approximately 60% when compared to the average for all parent trees tested. Families chosen for operational production are designed to meet genetic diversity guidelines with a minimum of ten parent trees (effective population size of ten) contributing to not less than 30 lines.

Approximately 400,000 interior spruce somatic seedlings have been produced for the Ministry of Forests trials and industry customers as 1+0 and 2+0 stock during 1996/97. Sitka spruce families carrying weevil resistance were introduced into production.

PHYSIOLOGICAL GENETICS

Dr. Steve Grossnickle, Dr. Shihe Fan and Coworkers

Previous work on the physiological variation of Sitka interior spruce hybrids was completed and papers published. Two continuing projects in this area are summarized below.

Adaptive Physiology of Western Redcedar

This program is investigating the phenotypic plasticity and genetic variation of western redcedar seedlings/rooted cuttings originating from a series of elevational transects and moisture gradients. Studies have been ongoing to determine population differences in physiological and morphological responses to both field and controlled environment conditions. Studies have examined performance under controlled soil moisture conditions, while field trials have examined performance to both temperature conditions, and in relation to water use efficiency patterns. Brief highlights from these studies are described below. Details will be given in the presentation.

Response to soil moisture Genetic variation was detected in the gas exchange capability of seedlings exposed to drought. Provenances from drier locations had greater gas exchange capability during an initial drought cycle. All populations showed a fairly similar rate of morphological development in response to repeated drought cycles. Drought tolerance, as measured by shoot water relations parameters, indicated little genetic variation between populations.

Response to temperature There was genetic variation in seasonal freezing tolerance patterns between high, mid and low elevation populations. During fall acclimation and spring deacclimation, there is a general trend of greater freezing tolerance as the elevation of the source population increases. No genetic variation was detected in the gas exchange capability of seedlings exposed to fall, winter and early spring time conditions.

Water use efficiency There was genetic variation detected among the western red cedar populations in the rates of all measured gas exchange parameters in response to the range of vapor pressure deficits normally found on reforestation sites. Initial analysis indicates that there does not seem to be a segregation of provenances based on dry or wet seed source locations.

Clonal Variation in Interior Spruce

Work has continued on various aspects of physiological variation among interior spruce clones produced by somatic embrygoenesis. The ability to recapitulate individuals from embryogenesis over many years is a powerful tool and allows a large array of data to be collected. These data have included variation in photosynthetic rates under optimum and limiting soil moisture and soil temperature, frost hardiness and others. In addition, extensive morphological characterization of clones has been carried out and much data remain to be analyzed. One study was conducted to assess water use efficiency instantaneously using CO_2 poromoter measurements versus carbon isotope ratio. Using four clones a very strong correlation was evident. ($r = 0.987$).

Chloroplast Markers for Pollen Monitoring

A number of intergenic regions in the chloroplast of lodgepole pine have been amplified by PCR and assessed for polymorphism. Generally, several alleles can be identified at each locus. This allows trees to be conveniently genotyped using simultaneous amplification using 7 primer pairs (so called multiplexing). A comprehensive survey of seed orchard parents and a range-wide provenance sampling have been carried out. While these data are still being analyzed, because of the paternal inheritance of chloroplasts, it is clear that this tool can be used to assess pollen contributions and pollen contamination in seed orchards on a routine basis. This adds to work conducted previously at BCRI in which a single hypervariable chloroplast region from Douglas-fir (not present in other species) was used in a similar application.

Analysis of Selfing in Western Redcedar Using DNA Markers

Western Redcedar is generally thought to have lower levels of genetic variation than most conifers. In order to increase the chances of finding polymorphism for studies of self-pollination frequency, a highly polymorphic intergenic region was cloned and used as a probe. Frequencies of specific alleles were found to vary from 0.00 to 0.21 among 50 clones. Analysis of seed from seven open-pollinated families in the breeding program showed that mean self-fertilization varied from 0.184 to 0.329 depending on the assumptions of random mating. Thus outcrossing is 0.67 to 0.82 which is twofold higher than previous estimates and more consistent with other conifers.

Genetic Mapping of Blister Rust Resistance in Western White Pine

A newer project is underway in which families of western white pine segregating for blister rust resistance are being screened for DNA marker associations. Part of this work was conducted with RAPD markers which tended to give a low yield of useful markers in the diploid seedling tissue. A great deal of effort is being devoted to the generation of microsatellite markers. Several hundred will be screened and we anticipate that at least 200 will be used in bulk segregant analysis. Dr. Peng Li, formerly with John Carlson at UBC, joined us in December 1996 and collaboration with Dr. Carlson's lab continues.

Genetic Engineering Projects

Three projects involving genetic marker development have been active in the last two years. The molecular biology group is also involved in a number of genetic engineering projects which will be reported in due course. These projects involve: 1) engineering reproductive sterility, 2) altering lignin composition, and 3) enhancing cellulose production and productivity through over production of UDP-glucose.

Most of these projects are at an early stage. However, the ability to increase productivity and cellulose production has been proved in tobacco plants through the addition of a bacterial UDP-pyrophosphorylase gene. This enzyme is responsible for the synthesis of UDP-glucose. The effects of introducing the gene are now being evaluated in Interior spruce.

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PACIFIC FOREST PRODUCTS LIMITED
SAANICH FORESTRY CENTRE
TREE IMPROVEMENT PROGRAM AND FOREST GENETICS ACTIVITIES

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Keywords: seed orchards, seed production, genetics research

SEED ORCHARDS

The consolidation plan for Pacific's two seed orchard complexes (Sannichton and Nootka) has been completed. At present, Pacific manages 10 seed orchards for six different species: 1) Douglas-fir (low elevation, 2nd generation with backward and forward selections). The original low-elevation, 1st generation Douglas-fir seed orchard has been upgraded to a 2nd generation, as well as a 1st generation with full-sib families, 2) western hemlock (low elevation advanced generation clonal-row and 1st generation high elevation), 3) western white pine (advanced generation clonal-row), 4) redcedar (clonal hedge), 5) yellow-cedar (1st generation), and 6) Sitka spruce (advanced generation clonal-row).

Seed production for this reporting period is as follows: Douglas-fir 50 kg, western hemlock 4 kg, Sitka spruce 0.5 kg, yellow-cedar 0.3 kg, Pacific silver fir 1 kg, and western redcedar 0.2 kg.

Pacific Forest Products Ltd. and the University of British Columbia, Faculty of Forestry, Department of Forest Sciences have reached an agreement for a part-time (25%) academic appointment for Yousry El-Kassaby as a professor in Forest Genetics. During this period, I have participated in teaching and graduate student supervision.

RESEARCH

Several research projects, as follows, have been initiated and funded by Forest Renewal British Columbia, Pacific Forest Products Ltd., and the British Columbia Ministry of Forests.

Genetics of Mountain Hemlock

This project is designed to develop a utilization/conservation strategy for mountain hemlock. The species represents an important component of the sub-alpine forest. Information generated will be of assistance in regenerating the species, *ex-situ/in-situ* conservation, and for seed transfer rules.

Genetic Consequences of Alternative Silvicultural Systems for Conifer Species in Coastal Montane Forests

This project will determine the genetic costs of silvicultural systems that are alternatives to clear-cut. Genetic quality, as determined by levels of diversity and inbreeding of both natural regeneration and parent trees' seed crops, are being evaluated in the Montane Alternative Silvicultural Systems (MASS) project. The hypothesis being tested is that, for three conifer species, there is no change in genetic diversity or levels of inbreeding in an experimental series of treatments including control (old growth) that ensure natural regeneration for range of canopy densities (shelterwood, green tree, and patch cut, and clear-cut) and levels of parental selection. Collaborative Organizations: MB.

Genetic Diversity in Minor BC Species

This project investigates BC's "minor" woody species for which there are no tree improvement or genetic studies. The ultimate goal of this project is to generate data on the level and distribution of variation among and within populations for several physiological and metric attributes and to utilize this information in detectng genetically unique populations for conservation purposes. It is expected that the information generated will form base line data for alder, Garry oak, ponderosa pine, and paper birch. Collaborative Organizations: University of B.C.

The Impact of Commercial Thinning on Genetic/Species Diversity

This project investigates the dynamic changes in species/genetic diversity as affected by commercial thinning treatments. The level of forest tree structure (social status and spatial distribution), understory plant community changes and genetic variation before and after thinning are being assessed. Several empirical and theoretical thinning scenarios are being analyzed and compared. The results are expected to assist foresters in modifying their methods to maximize species and genetic diversity.

Conservation of Biodiversity Through *Ex-situ* (Seed Bank) Gene Conservation

This project aims at developing molecular markers that can be used to test the germinative capacity of seeds stored in seed banks. Changes in seed germinative capacity (i.e., loss of viability) of stored seeds is associated with genetic changes that could undermine the concept of long-term seed storge for *ex-situ* gene conservation. The development of these markers will provide seed banks with a much needed tool (i.e. detecting method) for monitoring stored seedlots. Collaborative Organizations: University of Victoria.

Evaluation of Container-Nursery Management Practices on the Genetic Diversity of Seedling Crops

This research is designed to genetically evaluate the impact of seed production in seed orchards, as well as their seed biology, and their interaction with container nursery management practices. Evaluation of biological processes (seed) and common management practices of seed orchards and seedling nurseries are essential

to ensure that the genetic integrity of seed and seedling crops is being maintained.

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GENETIC IMPROVEMENT OF INTERIOR DOUGLAS-FIR AND WESTERN LARCH

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Keywords: Interior Douglas-fir, western larch, tree breeding, genetic testing, wood density

INTERIOR DOUGLAS-FIR

The Interior Douglas-fir breeding program began in 1982 and has advanced to where second-generation breeding is about to begin. In total, more than 1 700 first-generation parent trees have been selected from wild stands and established in breeding orchards at Vernon or clone banks near Enderby. Between 1984 and 1992, more than 1 660 wind-pollinated families of these selected trees were established in progeny tests on 36 sites in nine breeding zones. More than 250 000 trees are included in these tests.

Six-year test results are presently available for 31 sites in eight of the nine breeding zones. Six-year measurement of the East Kootenay test series is scheduled for fall, 1997. Mean 6-year survival across the 31 test sites was 89 percent with individual site survival ranging from 66 percent at Spiker's Creek, near Revelstoke, to 99 percent at the Skimikin nursery. Small mammals were largely responsible for the mortality at Spiker's Creek. Mean plantation survival was lowest (81 percent) in the Central Plateau zone, which is at the northern limit of Douglas-fir's natural range. Mean plantation height ranged from 72 cm at Leo Creek, north of Ft. St. James, to 212 cm at Duncan Lake, north of Kaslo. Family mean height on these sites ranged from 49 - 96 cm at Leo Creek and from 148 - 284 cm at Duncan Lake. Additive genetic variances and heritabilities were lowest in the Central Plateau zone and highest in the West Kootenay low elevation zone. Average individual, within-family, and family heritabilities for six-year height across all 31 sites were .32, .27 and .58, respectively. These moderate heritabilities combined with abundant variation at all levels of Interior Douglas-fir's genetic system portend genetic gains of about 20 percent for 6-year height. Predicted gains in 6-year height varied from 15 percent in the Central Plateau and Mt. Robson breeding zones to 30 percent in the West Kootenay low elevation zone.

These six-year results have been used to establish 1.5 generation seed orchards at three Interior seed orchard sites: 1) for the Central Plateau, Cariboo Transition and Quesnel Lakes zones at the Vernon Seed Orchard Company in Vernon, 2) for the Shuswap Adams low elevation zone at the Weyerhaeuser Canada nursery near Armstrong, and 3) for the West Kootenay low elevation zone at the Ministry of Forest's Bailey seed orchard site near Vernon. Six-year results will also be used to establish second-generation breeding populations in high priority seed planning zones.

WESTERN LARCH

The objective of B.C.'s western larch tree improvement program is to produce sufficient quantities of genetically improved seed to meet the rapidly increasing demand (4.4 million seedlings in 1996). The program is based on recurrent selection for general combining ability, first-generation seed orchards, and wind-pollinated progeny testing (Jaquish *et al.* 1995). The traits to be improved are tree height, stem diameter, and stem volume.

In total, 608 parent trees have been selected in wild stands in two breeding zones (East Kootenay and West Kootenay/Shuswap Adams) and established in breeding orchards/clone banks at Vernon. Since 1991, four series of wind-pollinated progeny tests have been established to estimate breeding values of all selected parents. Table 1 provides an establishment summary of the western larch progeny testing program.

Table 1. Summary of the B.C. western larch wind-pollinated progeny testing program.

Test Series	Planning Zone [a]	Number of sites	Year Planted	Number of local families	Number of non-local families and operational checks	Number of test seedlings planted
One	EK	4	1991	140	62	32,320
Two	WK/SA	5	1993	192	32	35,840
Three	EK	4	1995	110	50	20,480
Four	WK/SA	4	1996	165	45	26,880
Total		17		607	189	115,520

[a] EK represents East Kootenay seed planning zone; WK/SA represents West Kootenay/Shuswap Adams seed planning zone.

In fall 1996, six-year tree height and condition were recorded for three sites (Lamb Creek, Sawmill Creek, and Semlin Creek) in the East Kootenay Series One tests. The fourth site in this series (Lussier Creek) was abandoned because of poor survival. Mean survival, damage,and tree height for the three sites were 93.4 percent, 6.3 percent, and 178.4 cm, respectively. Mean individual, family and within family heritabilities for six-year height were .18, .53 and .15, respectively. Parental breeding values were estimated by Best Linear Prediction (BLP) and were used to rogue the East Kootenay seed orchard. Seed production from the two first-generation western larch seed orchards is expected to begin by year 2000.

SUPPORTIVE RESEARCH

Several research projects have been initiated to support the Douglas-fir and western larch tree breeding programs. Studies in Douglas-fir include intervarietal hybridization, elevational displacement of seedlots, and correlated responses to selection for wood density. Studies in western and subalpine larch include estimation of mating system parameters in natural and seed tree stands, genetic variation within and among natural BC populations, and interspecific hybridization.

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GENETIC IMPROVEMENT OF WHITE AND ENGELMANN SPRUCE

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Keywords: white spruce, engelmann spruce, tree breeding, genetic testing

PERSONNEL

January 31, 1997 saw the retirement of Gyula Kiss, a true pioneer of tree improvement in the BC Interior. Gyula joined the Research Branch in June 1967 and spent nearly thirty years studying and breeding interior spruce. His contributions to the scientific community and to forestry in BC have been significant.

Gyula developed the original breeding plan for interior spruce and successfully implemented the plan to where large second-generation progeny tests have been established. Much of the genetic material that forms the base of BC's improved interior spruce seed orchards originates from Gyula's program. It was also through his astute observation and early research that a genetic basis for weevil (*Pissodes strobi*) resistance was recognized in interior spruce. Based on his preliminary weevil research, large and truly multidisciplinary research programs evolved. These programs included entomologists, molecular geneticists, tree breeders, and biotechnologists from the provincial government, federal agencies, universities, and the private sector. Gyula also had the foresight and determination to guide the establishment of the Kalamalka Forestry Centre in Vernon, the centre from which all interior tree improvement research and development is based. Above all, Gyula was a respected and trusted friend of all who had the pleasure of working with him. He will be missed and we wish him well in all of his future endeavours.

SECOND-GENERATION PROGENY TESTING

Over the past two years, second-generation progeny tests were established in two (Prince George and East Kootenay) of the three original interior spruce breeding zones. Second-generation tests for the Prince Rupert breeding zone will be established in year 2000. These tests represent the results of nearly thirty years of interior spruce tree improvement in the BC Interior. Initially, between 125-200 parents were selected from wild stands in each breeding zone. Parental breeding values of these initial selections were estimated through wind-pollinated progeny testing on multiple sites within the breeding zone. The second-generation breeding population consisted of the top 50 percent of the parents in each zone. A disconnected partial diallel mating design with four parents randomly assigned to breeding groups was used for each breeding zone. In 1996 and 1997, full-sib family tests were established on agricultural land at three sites in the Prince George and East Kootenay breeding zones, respectively. These field tests were established in a randomized complete-block design with four replicate blocks of 5 x 5 tree square plots. Individual trees were planted at 1 x 1 m spacing.

SEED PRODUCTION RESEARCH - BC MINISTRY OF FORESTS

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Keywords: supplemental mass pollination, seed orchard management, alternate seed orchard design, flower induction, pollen management, genetic diversity, seed orchard after effects.

SEED PRODUCTION RESEARCH

Our seed production research program continues to emphasize the genetic quality of orchard production through studies of the reproductive biology and orchard dynamics for all tree improvement species. We have expanded our research scope into wild stand seed production studies because British Columbia will continue to rely on natural stand seed production for the establishment of plantations on 50% of the areas planted and seed orchard seed production for the other 50%. Our seed orchard management studies are now emphasizing the operational application of flower induction and pollen management including cone induction, pollen storage, and supplemental pollination techniques. We have also begun studies on the effect of environment on progeny performance including seed orchard after effects. This information forms the basis for seed orchard management research which is currently emphasizing the genetic quality of broadly adapted seed from mature orchards as well as the establishment of new orchards with designs specifically to facilitate full-sibling family production. Protocols for rating annual orchard seed lots are now in practice for coastal species (Woods *et al.* 1996) and are being modified for interior species as well. Included in all of these three topic research areas are new studies on genetic diversity from seed orchards, plantations, and natural stands.

Our research in wild stand production is currently emphasizing *Abies* species, including amabilis and subalpine fir. Our initial efforts include a categorical estimate of spring seed cone flowering, abortion and pollen cloud capture. The relationship between pollen capture and actual cone yields will be tested according to Sorensen and Webber (1997) to determine if pollen cloud density can be used to estimate fall seed yields at the stand level.

SEED ORCHARD AFTER EFFECTS

The effect of seed orchard environment has been tested in two white spruce orchards on contrasting sites (Prince George and Vernon). One-year old progeny from identical females and pollen poly-mix showed significant differences in height growth and adaptive traits, such as stress-enhanced electrolyte leakage (SEEL, an indication of frost hardiness) (Stoehr *et al.*, in prep.). Experiments to determine if site effects are a result of temperature during seed development are underway (Webber, Stoehr, L'Hirondelle, Binder). Progeny derived from identical crosses but reared under two different temperature regimes are being

compared for first year height growth and adaptive response (controlled freezing tests). The effect of temperature on pollen meiosis, and early/late embryo development are also being compared.

SEED ORCHARD STUDIES

Lodgepole pine

Seed yields in the lodgepole pine orchards in the interior of BC at Kalamalka have traditionally been very low with an average of 5.5 filled seed per cone (FSPC) over the last five years. The hot and dry climate at this orchard site may be the reason for the low seed set, possibly due to the absence of a pollination drop. In 1996 and 1997, liquid pollinations and dry pollination followed by misting were conducted (without isolation bags) to mimic a pollination drop. In an earlier study (1995 pollinations) seed yields were considerably higher when insect bags were used during the second year of cone development, pointing to a possible seed predation problem. All 1996 and 1997 pollinations were made with pollen from male parents that can be identified using PCR amplified chloroplast markers. Thus, we will be able to determine if the male parent was due to applied pollen or due to wind pollinations.

Douglas fir

A pollen competition study was carried out to evaluate the effects of pollen viability on paternal success. Paternal analysis was carried out with our recently developed male-parent specific chloroplast DNA marker, amplified by PCR (Stoehr *et al.*, submitted). Success of low-viability pollen can be increased by raising its amount in a polymix, without reducing the number of filled seed per cone.

Using the same chloroplast marker system for determining the origin of male parentage, we are currently conducting a study to test the efficacy of single-parent control-pollinations of individual female cones prior to and at peak receptivity without isolation bags for the operational production of high gain seed.

DIVERSITY STUDIES

Levels of genetic diversity, using isozyme markers, were evaluated at different stages of tree improvement in BC's interior spruce (Stoehr and El-Kassaby 1997). The 100 seed orchard clones were found to represent natural populations in the Shuswap-Adams low elevation (SAL) breeding zone very well, having very similar genetic diversity parameters (number of alleles per locus, percent polymorphic loci and heterozygosity). A bulked seedlot from the SAL orchard also showed very high genetic diversity, although some outside orchard pollen contamination was detected. In a plantation established from the same seedlot, genetic diversity dropped somewhat compared to the seedlot diversity levels, possibly due to culling in the nursery.

Genetic diversity evaluations in quantitative traits using common garden studies are in progress for interior-coastal transition zone Douglas-fir and interior spruce. In the Douglas-fir study, effects of seed tree and shelterwood systems on mating systems (using isozymes) and morphological traits of progeny are evaluated. In spruce, year-to-year variation in seed orchard seedlots is measured by comparing family variances in progeny performance.

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PROVENANCE RESEARCH IN BRITISH COLUMBIA

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In the past two years, much effort has been directed to two research projects: 1) analyzing lodgepole pine pest data for incorporation of the results into operational tree improvement and reforestation seed transfer guidelines, and 2) developing predictive models assessing the effect of climate change on managed forests based on long-term provenance testing results. Both projects are funded by Forest Renewal B.C.

More lodgepole pine, than any other species, is being planted in British Columbia (over 100 million seedlings in 1995-96). Lodgepole pine is fast-growing and can grow well on a wide range of site conditions. However, many insect, disease, and wildlife pests can cause severe damage to plantation lodgepole pine. We may not be able to realize the gain expected from intensive silviculture without effectively reducing the impact of pest damage.

B.C. Forest Service Research Branch established an extensive network of long-term provenance tests in the early 1970's which offer rare experimental materials to assess the effects of site environment, genetic resistance, and their joint effect on pest incidence. Beginning in 1992, pest incidence in stem rust, needle cast, pine terminal weevil and rodent damage were surveyed in all test sites. We have completed most of the analyses and published the results. Provenances from the species' northeast edge adjacent to the jack pine-lodgepole pine hybridization zone showed a high level of resistance to western gall rust while low elevation provenances from southern B.C. interior were highly resistant to needle cast. Their resistance was very stable across diverse site environments. The results also indicate selection for genetic resistance can be effective. We are now analyzing the correlation between growth and pest incidence in an attempt to quantify pest effect on growth.

Establishment, maintenance, and data collection of long-term provenance tests represent a large resource investment. In addition to the traditional objective of provenance performance and genecological information, we are also using the data and the tests for other scientific purposes. Using the data to construct empirical models predicting climate change effect is one of them. The study, also funded by Forest Renewal B.C., was started in 1995. We have successfully completed the first phase of this project in cooperation with Dr. G. Rehfeldt of USDA Forest Service.

The results provide empirical evidence that lodgepole pine populations, particularly those at the northern margin of the species' natural distribution, can potentially occupy a physical environment (fundamental niche) much broader than its current range (realized niche). Density-dependent selection fuelled by gene flow relegated most populations to the suboptimal environments they currently occupy. The models predict severe maladaptation of natural populations if temperature increases by 3° to 5°C, particularly, in central and

northern B.C. interior. We are continuing to explore more efficient modelling approaches, e.g. multi-dimensional, and extend the application to other species.

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REPORT OF FOREST GENETIC RESEARCH AT UNIVERSITY OF BRITISH COLUMBIA

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MANAGING FOREST TREE GENETIC VARIANCE FOR CLIMATIC CHANGE

by Mathew P. Koshy, Jiwei Zhi, and Gene Namkoong

A method for genetically pre-conditioning populations for the uncertain futures presaged by climatic change is to generate populations with high genetic variance in traits that condition response to climatic variables. Either by selective breeding for population divergence, or using natural provenance differences, F2 and advanced generation hybrids can be developed. These can provide high variances and, depending on the population differences in traits that have diverged, the correlations in the advanced segregating generations can also be different from that which exists in one presently available population. Basic equations for relating the increase in genetic variance to the population divergence for long term selection models on mutation-selection balance are available and can predict the rate of increase in genetic variance as a function of the mutation-selection balance in finite populations.

Current research is to extend the predictions to conditions of replicate populations as well as partial inter-crossing between replicate and divergent selection populations, and to include pleiotropic and linkage effects. Experimental research is to create 20 sets of non-inbred crosses, each with F1 and F2 families of the same grand-parental genotypes but from widely separated provenances of Douglas fir from coastal and inland sources in British Columbia, Washington, Oregon and California. These are to be grown and tested for growth rhythm and drought response traits and estimates derived for the predicted genetic variance. The crosses for this study have been already completed. Theoretical models for F2 genetic variance have also been developed. Seeds from these crosses were collected in September. Nursery growth trials were conducted at Cowichan Lake Research Station during March-December. The seedlings are now ready for multiple-site field trials which will be laid out in March 1997. Seedling data have indicated higher levels of within family genetic variance in F2 full-sib families raised from parents which are from more distant sources compared to parents from closer sources. These results conform to theoretical predictions. However, the results need further confirmation based on long-term field trials.

DYNAMICS OF GENETIC VARIATION IN RESOURCE USE EFFICIENCY IN LODGEPOLE PINE

by Pia Smets and Gene Namkoong

The response of trees to limitations of soil moisture and nitrogen is considered to be a major factor affecting fitness. Therefore, water and nutrient use efficiencies are expected to be major determinants of fitness in the

fluctuating environments of forests. Techniques for measuring these efficiencies have been developed such that genetic variations have been detected in efficiency indices in various tree species including conifers. However, two dimensional norms of reaction to simultaneous water and nitrogen variables have not been estimated, and the consequences of derived genotype by environment interactions on the population genetics have not been explored. Therefore, the mechanism for the maintenance of genetic variation in water and nitrogen use efficiencies and the effects of management on the conservation of genetic variation in naturally and artificially regenerated stands remains unknown.

This experimental work consists of estimating water and nitrogen use efficiencies on full and half-sib families of lodgepole pine to obtain two dimensional response surface norms of reaction for families, and to test for differences among them. Models of these derived norms of reaction will then be used in analyses of population genetic dynamics. The domains of attraction for genetic polymorphism will be examined for several models of the joint effects of soil moisture and nitrogen availability.

Nursery trials were set up at UBC Forest Nursery. A factorial experiment was conducted with three water levels and three nitrogen levels. Five provenances, chosen along a moisture gradient, with 20 half-sib families per provenance were used for the trial. There were 4 replications, which yielded a total of 3528 plants. Measurements were taken after the growing season had ended (October). Samples are being prepared for carbon isotope analysis and C/N ratio, and first results should be available by the end of March, 1997. An augmented study will follow the current preliminary study being completed.

GENECOLOGY OF DOUGLAS-FIR IN THE SUB-MARITIME SEED-ZONE: TESTING SEED TRANSFER GUIDELINES

by Francisco Luna-Lopez J., Gene Namkoong, and Mathew P. Koshy

This study forms part of a comprehensive study on the genecology of Douglas-fir in the sub-maritime (SM) zone undertaken by the Ministry of Forests to determine the patterns of genetic variation within the SM zone, from 49 degrees to 53 degrees latitude and altitudes from 50 to 1 200 meters above sea level. This part of the study focuses on seedling shoot and root growth, and physiological traits like water use efficiency and frost hardiness. We will also look into the comparative dynamics of evolution of genetic variance in fitness related traits of Douglas-fir in the coastal, interior and transition zone.

A pilot study which included 44 families from nine different sources within the SM zone plus five families from the coastal zone evaluated seedling growth and physiological traits and assessed the genetic variability in about 2 700 seedlings. During this phase, seedling height, root collar diameter, and shoot and root ratio were measured in the nursery. Physiological traits like relative water use efficiency using carbon isotope discrimination, relative water content of leaves, seedling water potential and cold hardiness were also measured.

Preliminary results showed significant variability between families and between provenances for growth traits. Physiological traits showed significant variability among provenances. In general, provenances from lower altitudes and southern latitudes showed better growth compared to the other provenances. Based on these results an augmented design for the first phase of the study was developed. The augmented design included 77 families from 17 provenances from the SM zone plus families from coastal and interior zones. The nursery study is completed and samples are being processed for carbon isotope studies. A farm field test which follows the nursery trial will evaluate the early growth stages.

The information generated in this study will be useful for the definition of boundaries in seed and breeding zones. Subsequent observations in diverse environments will help to establish age trends in genotype x environment interactions and norms of reaction for growth and water use efficiency traits. This study will also

help to understand the comparative dynamics of evolution of genetic variance in Douglas-fir in the coastal, interior and transition zones.

UTILIZATION AND MANAGEMENT OF RED ALDER GENETIC RESOURCES IN BRITISH COLUMBIA

by Andreas Hamann, Mathew P. Koshy, and Gene Namkoong

This project aims to study the genecology and genetics of growth and adaptive traits, estimate their heritabilities and genetic correlations, and assemble multiple breeding populations with different trait combinations. This study will also generate sufficient genetic resources in red alder for future breeding and for future theoretical and applied genetic studies. It is also intended to develop a framework for testing fundamental breeding strategies in the light of evolution of genetic variance of populations and provide basic material for ongoing theoretical and applied genetic studies in forest trees.

The development of an effective breeding strategy for red alder primarily depends on knowledge of the genetic variability of certain traits and on spatial patterns of genetic variation. Data from 290 half-sib families from 58 BC provenances, established by the Ministry of Forests in 1992, have been used to estimate genetic parameters for economic and adaptive traits.

At the site of the existing trial, a new test with provenances from different sources has been established to broaden the genetic base for a breeding program. The new material includes 29 half-sib families from isolated populations in Idaho, and 71 half-sib families from three provenances in British Columbia. The progeny test shall allow for selection and breeding from age 5 and shall be evaluated up to age 15.

Further, several small experiments were conducted in preparation for breeding in 1997. They comprise tests to determine suitable techniques for controlled pollination, harvesting of pollen and short term storage of pollen, timing of maximum receptivity of female flowers and flower induction treatments.

GENETIC EFFECTS ON FIBER AND STRUCTURAL WOOD PROPERTIES IN WESTERN HEMLOCK

by Mathew P. Koshy, Awadel Karim Salah, Gene Namkoong, and John King

This project will estimate genetic variance, heritability, and genetic correlations of fiber characters and structural properties of western hemlock. Also, the effect of silvicultural treatments on genetic effects in fiber and wood properties will be studied. Screening of the breeding population for fiber and wood properties and derivation of economic weights for wood and fiber properties are the other objectives of the project.

Fifteen-year old progeny trials at Jordan River and Carmanah are being used for the estimation of genetic variance and genetic parameters for the different wood and fiber properties. A 15-year old trial at Bonanza was selected for studying the interaction of thinning regimes and genetic effects of wood and fiber properties. Another trial at Sombrio is being used for the fertilizer and genetic effect interaction studies. Wood samples (discs) from progeny of 30 families at Carmanah and Jordan River were measured for early and late wood width using a WinDendro scanning device. An image analyzing system is being used for measurement of density and fiber properties. Cell wall area, lumen size, coarseness, relative density, fiber length, and fibril angle are currently being assessed on collected samples.

At the 15-year-old Bonanza trial (near Port Hardy), three treatments are laid out with four replications. These three treatments include: 1 000 stems/ ha, 700 stems/ha, and no thinning (1 841 stems/ha). Thinning and sample collection were completed in collaboration with Ministry of Forests.

RAPID BREEDING FOR TRACHEID CHARACTERS IN INTERIOR SPRUCE

by Milosh Ivkovich, Mathew P. Koshy, and Gene Namkoong

This study will estimate genetic variance in tracheid characters, length, wall thickness, cellulose content, fibril angle, coarseness etc., in the breeding population in BC. Genetic correlations for these traits with other wood and growth traits will also be estimated. Subsequently, multiple breeding populations for different combinations of traits of economic importance will be developed. This project will also aim at developing an accelerated breeding strategy for tracheid traits.

One hundred-sixty families of Prince George and East Kootenay provenances were sampled from 4 test sites, with each family replicated in 6 blocks within each site. Altogether more than 2 000 large size (10 mm) increment cores were taken. The sampled trees are located in common plantations at Red Rock Tree Improvement Station, Quesnell, Invermere (Jumbo Creek), and Cranbrook (Perry Creek). Data on height and dbh of selected trees were collected in the field. Using WinDendro, early and late wood width were measured. Comparison between 180 ring profiles obtained from the X-ray densitometer and reflective light scanning machine show that reflective light intensity is indeed a precise indicator of late- and earlywood within growth rings. The correlation of the density profiles from X-ray densitometry and the profiles based on the reflective light scanning were typically highly statistically significant (R^2>85%). Ring width measurements in the samples were completed. Various methods to measure relative density of wood was compared to use a cheaper but efficient method for the study. Photometry, gravimetry, morphometry, and x-ray densitometry are being compared.

IMPACT OF FOREST OPERATIONS ON GENETIC DIVERSITY OF UNDERSTOREY SPECIES

by Kendrick Marr and Gene Namkoong

This study is intended to estimate the demographics and genetic structure of indicator understorey species that are likely to have their selection and migration environments changed by forest practices. Further, this study will evaluate the impact of different forest management systems on selection and migration parameters and the levels and structure of the genetic variance and development of genetic monitoring techniques so that early warnings can be issued for a broad class of species that are likely to fall into the threatened or endangered category.

Vaccinium alaskaense, Blechnum spicant, Cornus unalaskensis, Trillium ovatum, Streptopus amplexifolius, Vaccinium ovalifolium, Cornus unalaskensis, Aralia nudicaulis, and *Streptopus amplexifolius* are the species targeted for the study. The sample plots are located at old growth, logged, burned and planted and natural regeneration sites. Leaf samples and seeds were collected from approximately 11 individuals from three areas of each stand, for a total of 33 individuals per stand. Electrophoresis for isoenzymes is currently going on. Enzymes that will be screened include the following: Malate dehydrogenase, Isocitrate dehydrogenase, Phosphogluconisomerase, Amino asparate transferase, Glyceraldehyd-3-phospate dehydrogenase, Glutamate dehydrogenase, Triose phosphate isomerase, 6-phosphoglucodehydrogenase, Malic enzyme, Menadione reductase, Diaphorase, Alcohol dehydrogenase, and Aconitase, Leucine amino peptidase.

For each species in each stand the density, coverage, frequency, relative density, relative coverage, relative frequency, and important value (the sum of relative density, relative coverage and relative frequency) have been calculated. These are the basic data of vegetational characteristics of forests. Based on these data, the species diversity of each stand and the population distribution patterns of species will be analyzed, and also some comparison study of effects of forest practices on forest understorey species will be conducted.

EXAMINATION OF UNBALANCED DESIGNS FOR GENETIC TESTING OF FOREST TREES

by Yong Bi Fu, Gene Namkoong, and Alvin Yanchuk

Field experiments are a vital part of forest research but time-consuming and expensive, thus requiring efficient experiment designs. Traditional field layouts (e.g., complete block designs) are of great limits in the capacity to control the errors imposed by heterogeneous testing sites when a large number of families (e.g., 500 families) are required, and thus less efficient. Incomplete block designs, which allow the tests of a large number of families with small block size and the control of within-block variability, would be a great option for genetic testing of forest trees, but often require efficient layout schemes and sophisticated analysis packages. With modern computing capacity and the ability to control error rates, much higher power of estimation and greater cost efficiencies can be made available for forest genetics experiments. To this end, we examined several unbalanced designs such as partially overlapping, disconnected, randomized with constraints, and alpha designs for estimations of family means and genetic components. For the designs examined so far for estimating family means, increasing block number tends to reduce the variances of family mean estimates and increasing block size can reduce the variances of estimates of a contrast, while increasing family size at the expense of family number will reduce the variances of estimates of both means and contrasts. Variations in block size are not so important in considering the efficiencies of these designs. Variations in family size greatly affect the estimates of family means as well as the contrasts. The results on estimations of genetic components are being analyzed.

ANALYSIS OF DNA DIVERSITY IN WESTERN RED CEDAR

by John Carlson and Associates

We have completed an extensive study on genetic variation in western red cedar (*Thuja plicata*) using DNA markers for all three genomes, i.e. chloroplast, mitochondrial, and nuclear. Populations were sampled from across the natural range of western red cedar, including coastal BC, interior BC, the Charlotte Islands, Utah, Washington, Oregon, and northern California. Previous results with isozyme markers and terpenes uncovered very little genetic diversity in this species. Our estimate of species-wide genetic diversity using RFLP markers (expected heterozygosity of 6%) closely agreed with those reported in earlier isozyme studies, although we observed higher levels of genetic polymorphisms.

MOLECULAR KARYOTYPES FOR CONIFERS

by John Carlson and Associates

In conifers, chromosome identification is hindered by uniformity in chromosome size and morphology. Homeologous chromosomes of related species are also usually indistinguishable. *In situ* hybridization augments conventional cytogenetics, permitting the visualization of defined DNA sequences along metaphase chromosomes and interphase chromatin. Using the fluorescence *in situ* hybridization (FISH) technique we have developed karyotypes for Sitka spruce, white spruce, and Douglas-fir. The chromosomal locations were determined for the genes encoding the 18S-5.8S-26S ribosomal RNA, the 5S ribosomal RNA, and a centromeric satellite DNA, from which we could produce karyotypes for white spruce, Sitka spruce and Douglas-fir. Comparison of the white spruce cytogenetic map to that of Sitka spruce revealed that despite the overall uniformity in appearance among homeologous chromosomes, the chromosome sites for theses genes differ enough to distinguish most of the homeologous chromosome pairs between the two species. This capability should be very useful in characterizing hybrids.

ENVIRONMENTAL BIOTECHNOLOGY WITH TREES

by John Carlson and Associates

We have established protocols for the genetic engineering of hybrid poplar, Douglas-fir, and interior spruce, to transfer of unique, valuable traits to these species. At this time, we are using gene transfer and gene regulation techniques to address environmental problems using trees. One of our long-term goals is to improve lignin quantity and quality better suited to chemical free pulp and paper production. We have cloned and characterized a gene specific to the production of the conifer-type lignin monomer which we are now testing by gene transfer for its ability to depress synthesis of that specific lignin type. We are also interested in transferring genes for heavy metal uptake and accumulation to develop hybrid poplar for both remediation of contaminated sites and the production of a valuable tree or fiber crop. Such research should lead to poplar hybrids that can accommodate heavy metals in sludge treated soils and for the clean up of industrial sites.

MICROSATELLITE DNA MARKERS FOR TREE GENETICS

by John Carlson and Associates

We are developing improved molecular tools for assessing diversity and for identifying DNA markers genetically associated with important traits in forest trees. Although RAPD markers continue to play an important role in forest biotechnology, microsatellite markers (or SSRs) are more powerful for reproducibly detecting a great number of alleles per locus. We are in the process of developing as many SSR markers (PCR primer pairs that amplify multiple alleles at specific microsatellite loci) as possible for Douglas-fir, spruces, and western hemlock. With these SSR markers we will develop marker-assisted selection (MAS) assays for important quantitative traits such as volume, dbh and height increments, wood quality, weevil resistance, etc. Such simple, indirect assays should greatly assist tree improvement and seed orchard programs. Our SSR markers will also be of general interest for use in population genetics, DNA fingerprinting, and other tree genetics research in which high resolution genetic markers are required.

THE MATING SYSTEM IN WILD POPULATIONS OF WESTERN RED CEDAR

by Frederique Viard and Kermit Ritland

Western red cedar is among the few conifers that exhibit low genetic (isozyme) diversity (Yeh 1988). It also has among the highest selfing rates recorded for a conifer -- a seed orchard exhibited 68% selfing based on isozymes (El-Kassaby *et al.* 1994) and ca. 30% selfing based on DNA markers (Newton and Vo 1996, BC Research). To document natural levels of selfing, we collected seed progeny from two natural populations in British Columbia. In these populations, 243 and 138 seedlings were genotyped, respectively, and allocated among 9 and 5 families respectively. Only one enzyme locus, G6PDH, was sufficiently variable to provide information about selfing. Population and family selfing-rates were estimated using the method of Ritland and Jain (1981). The selfing rate estimates in the two populations were 0.70 (s.d. 0.10) and 0.17 (s.d. 0.08), respectively. Also, either large among-family variation of selfing, or extreme heterogeneity of the pollen pool, was apparently occurring. Evidently, the mating system is extremely labile in this species and probably depends on environmental factors, such as density and phenology, since this species probably has reduced genetic variability for quantitative traits. More extensive study is needed to elucidate the true level of variation for selfing, as well as factors responsible for this variation. This species also offers a good opportunity to estimate inbreeding depression, since the inbreeding coefficient of parents is ca. 0-10%, (Glaubitz and Carlson, unpub.), yet that of seedling progeny is ca. 20-50%, indicating selection against selfing takes place during the life cycle of this tree. Sufficiently polymorphic co-dominant PCR markers, if they can be found, can be used to estimate this selection using the methods in Ritland (1990, Evolution 44: 1230-1241).

LOCAL POPULATION STRUCTURE AND QUANTITATIVE TRAIT VARIATION IN BRITISH COLUMBIA *ABIES* AND *TSUGA* SPECIES

by Steve Travis and Kermit Ritland

We have initiated a study of local population structure in four BC conifer species. In our first year, 100 individuals from each of 10 populations across four taxa -- *Abies lasiocarpa, Abies amabilis, Tsuga heterophylla, Tsuga mertensiana* -- were sampled along a 100-200 m transect and genotyped for isozymes using needle tissue. We found the level of fine-scale structuring, as measured by Wright's coefficient of relatedness, to be highly variable among populations, with clear negative associations of relatedness with distance in 6 of 10 populations. Among these six cases, relatedness within 30 m was about equivalent to half-sibs for *Abies* spp. and to first-cousins for *Tsuga* species. These cases also tended to be populations with more patchy distributions of individuals. In our study, two pairs of transects involved old-growth and adjacent natural regeneration (at the seedling stage). In both cases, regeneration showed more pronounced local structure than did adults. This might be due to age-heterogeneity within old-growth stands, over which episodes of local founding dampen local population structure (indeed, gene frequencies sometimes differed between age (size) classes within old-growth). We are currently re-sampling two populations with the greatest local structure (200-400 additional individuals) and also developing more informative DNA markers, with the ultimate purpose of correlating relatedness with quantitative traits, to infer heritabilities in wild species using natural relatedness using the method of Ritland (1996, Evolution 50: 1062-1073).

GENETIC RELATIONSHIPS AMONG WOOD QUALITY, GROWTH RATES AND SEEDLING PHYSIOLOGY IN INTERIOR LODGEPOLE PINE

by Sally Aitken, Tongli Wang, Michael Carlson, and Philippe Rosenberg

Relationships between wood density and its components (xylem tracheid anatomy and proportions of earlywood and latewood) and seedling growth and water-use physiology (including growth phenology and hydraulic architecture) are being investigated in lodgepole pine (*Pinus contorta*) in this project funded by Forest Renewal BC. Open-pollinated families representing all combinations of growth rate (fast and slow height growth) and wood density (high and low Pilodyn readings) have been selected from sapling-aged progeny tests for inclusion in a seedling study. Wood anatomy and density is being assessed on wood disks from sapling-aged trees using x-ray densitometry and microscopic image analysis. Seedlings are being grown under well-watered and drought treatments, and assessed for growth rate, shoot growth phenology, biomass allocation, hydraulic architecture and vulnerability to xylem cavitation under drought stress. The results of this research will identify any potential indirect effects of genetic selection for growth rate or wood density on adaptive physiology.

PHYSIOLOGICAL GENETICS OF COASTAL DOUGLAS-FIR GENOTYPES EXHIBITING HIGH AND LOW STABILITY OF PERFORMANCE IN FIELD TESTS

by Sally Aitken, Sonya Budge, Joanne Tuytel, and Jack Woods

The objective of this study is to determine if genotypes that are relatively stable in growth performance across a large number of progeny test sites (i.e., contribute little to genotype-by-environment variance for growth traits) can be distinguished from those that are relatively unstable when grown in seedling tests under different temperature and moisture regimes. Ten pairs of Douglas-fir (*Pseudotsuga menziesii*) parent trees were selected for inclusion in the study based on field performance across 11 test sites. In each pair, the breeding value of the two parent trees is similar, but the stability of performance is very different. Offspring of these parents are being grown in both outdoor raised nursery beds and greenhouse environments. In year two, temperature and moisture treatments will be applied. We will then assess whether 'stable' and 'unstable' genotypes differ significantly in their norms of reaction across different seedling growth environments, or if

they differ significantly in growth, drought response, and cold hardiness in a single seedling environment. The results will be used to assess the feasibility of early testing for stability of performance. This project is being funded by Forest Renewal BC.

GENECOLOGY AND NATURAL SELECTION DYNAMICS IN THE SITKA X INTERIOR SPRUCE INTROGRESSION ZONE

by Sally Aitken, Sampson Yaw Bennuah, John King, John Carlson, Rene Alfaro, Kermit Ritland, and Steve Grossnickle

Hybrid zones offer unique opportunities to investigate selection dynamics in natural populations. They are also important from a genetic management standpoint as seed transfers over relatively short geographic distances within introgression zones can be problematic. We are investigating relationships between adaptive traits (including growth rate, cold hardiness, and weevil resistance) and fitness across environmental gradients in the spruce introgression zone along the Nass and Skeena Rivers in northern British Columbia. In this area, Sitka (*Picea sitchensis*), white (*P. glauca*) and Engelmann spruce (*P. engelmanii*) naturally hybridize. Environmental gradients are complex, with evidence of strong selection for growth rate in the highly competitive environments near the coast, and both cold hardiness and shoot weevil (*Pissodes strobi*) resistance in inland areas. Populations from across this hybrid zone are being characterized for both microsatellite genetic markers and for adaptive traits related to growth, cold hardiness, and weevil resistance. Genetic markers will indicate the proportion of hybrid individuals in different populations as well as the degree of introgression (first generation only or repeated hybridization or backcrossing). They will also provide a means of assigning a hybrid index (percent of genome derived from different parent species) that can be used to investigate concordance or discordance of genetic clines for neutral and adaptive traits. This project is funded by Forest Renewal BC.

ACCUMULATION OF MUTATIONAL VARIANCE FOR QUANTITATIVE TRAITS IN CONIFERS

by Sally Aitken and Michael Lynch

Studies of accumulation of mutational variance in model species (e.g., *Drosophila*) over many generations among lines starting from genetically identical individuals have changed scientific perspectives on effective mutation rates for quantitative traits. Due to long generation lengths and severe inbreeding depression, these experiments involving repeated meiotic events are impossible to conduct with conifers. However, trees undergo many mitotic divisions between generations, and somatic mutations may contribute to the considerable genetic variation found in most conifers. Efforts to quantify somatic mutation rates in trees using single-locus genetic markers have largely failed due to the low mutation rates at individual loci. We are investigating the accumulation of somatic mutational variance for quantitative traits, which have a much higher effective mutation rate than single genes through the evaluation of large numbers of progeny produced by controlled crosses of members of clones. Due to its high levels of genetic variance, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) has been selected for this investigation. This study is the first step in understanding the relative contribution of accumulated somatic mutations to total genetic variance affecting quantitative traits.

CONSERVATION GENETICS AND HABITAT USE IN THREATENED STOCKS OF COHO SALMON

by Diana Dobson, Sally Aitken, and Scott Hinch

Coho salmon populations are declining at a rate faster than any other species of Pacific Salmon. One possible cause of this decline is the availability of suitable overwintering habitat. Previous studies have found that overwintering coho fry populations have a bimodel rather than a normal distribution of fish length, and there

are indications that these phenotypes are associated with habitat usage. It has been suggested that there may be a genetic basis to these phenotypes, and that habitat availability may affect genetic makeup of stocks. MSc student Diana Dobson is investigating the genetics of size and habitat use in coho fry populations by combining microsatellite markers with quantitative traits. The results of her study will be used to evaluate the effects of artificial spawning habitat on the genetic makeup of coho populations.

WESTERN WHITE PINE IMPROVEMENT PROGRAM FOR BRITISH COLUMBIA

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Keywords: *Cronatrium ribicola, Pinus monticola*

"OPERATIONAL" PROGRAM OF SELECTION AND RUST SCREENING

In the course of screening for resistance, there have been seedlings which have not developed cankers 2-4 years after inoculation, and these have been re-inoculated by placing them in a disease (*Ribes*) garden. As a result of this additional inoculation, a few seedlings have 0-3 cankers, while the majority have 30+ cankers; the former have been cloned for seed orchards. Needle spotting from the 1995 inoculation (the final year of screening) was considered to be too low and all seedlings were re-inoculated by placing them in a disease garden.

The first inoculation, done in 1987, had all canker-free seedlings at 16 months post-inoculation planted in transplant beds. Many of these developed cankers and nearby *Ribes*, contributed to additional infection. All surviving trees were examined in 1996 and those displaying resistant reactions were removed and placed into a new seed orchard. Other coastal ortets and clones from later inoculation years have been added to this seed orchard. This makes a total of one Interior and three Coastal orchards established from the programs. Material from ongoing evaluations of progeny will continue until 2002, with selections continually being added to these orchards.

RESEARCH PROGRAM

From the operational program there are now seedlings, and parents which yield seedlings with known phenotypes, that can be used in experiments. Current projects being worked upon are: 1) to determine the stability of "bark reaction" resistance; 45 clones with putative "bark reactions" were inoculated and none consistenty maintained the phenotype, 2) to predict resistance gain; seedlings from controlled matings contrasted with OP seedlings from the same parents are being raised for inoculation, 3) to determine why seedlings with "needle shed" resistance are successful in the Interior but fail at the Coast; seedlings possessing this resistance are being raised for inoculation and for later placing in Coastal verses Interior sites, and 4) to determine if various slow-canker-growth phenotypes are morphologically similar; cytological comparisons are being made.

The cold protein, *Pin m* III, is induced by *Cronartium ribicola*. In inoculated seedlings, the cankered tissue had high levels of cold protein, while outside the canker margin had low levels; but it also was detected in some healthy bark samples of cankered trees. Also the level of cold protein was higher in inoculated foliage of resistant sugar pine seedlings than in susceptible ones, while remaining low in bark samples from both groups of seedlings. The cold-protein gene was sequenced and comparisons revealed significant homology with other known genes of pathogenesis-related proteins. Southern blot analysis indicated that the homologous genes are present in other conifers.

A set of proteins was shown to be at significantly higher levels in inoculated foliage and bark of sugar pine possessing hypersensitive resistance than in susceptible sugar pine. This finding may indicate that the genes encoding these proteins can escape the putative suppressing mechanism of the blister rust fungus. It is also possible that some of these proteins may play roles in the host defense against the rust disease. Monthly foliage samples were collected from western white pine seedlings possessing putative hypersensitive resistance. Once the phenotype is confirmed, the samples will be examined for unique pathogenesis-related proteins.

Cronartium ribicola produces the protein Tr-2. An anti-Tr2 antibody detected a protein in susceptible sugar pine foliage at 30-fold greater quantities than in resistant sugar pine.

Population genetic parameters were estimated for rust populations from BC to western Sierra Nevada and in two outgroups from eastern North America, using isozymes, random amplified DNA (RAPD), and restriction enzyme polymorphism (RFLP) markers. Expected heterozygosity within populations (He) was only 0.025, yet the proportion of genetic diversity attributable to differences among populations (Gst) was 0.205. Both cluster and multivariate analysis indicated differentiation among populations, but the distribution of the variation had no pattern associated with geographic distance, similar to the results of Hamelin *et al.* (1995) for populations of the rust in Quebec, and to our previous results for BC populations. This lack of pattern suggests a species that is not in equilibrium, where founder effects are important. The populations which fell furthest from the others using different clustering algorithms, Happy Camp and Thompson Ridge, come from California.

Polymorphisms in the disease response gene phenylalanine ammonia lyase (PAL), isolated from white pine, were examined in a cross expected to segregate for a putative single gene resistance to blister rust. The polymorphic PAL locus will be placed on a linkage map as part of an FRBC funded project (with B. Sutton and C. Newton, BCRI, and J. Carlson, UBC) to identify genetic loci conferring blister rust resistance in white pine.

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