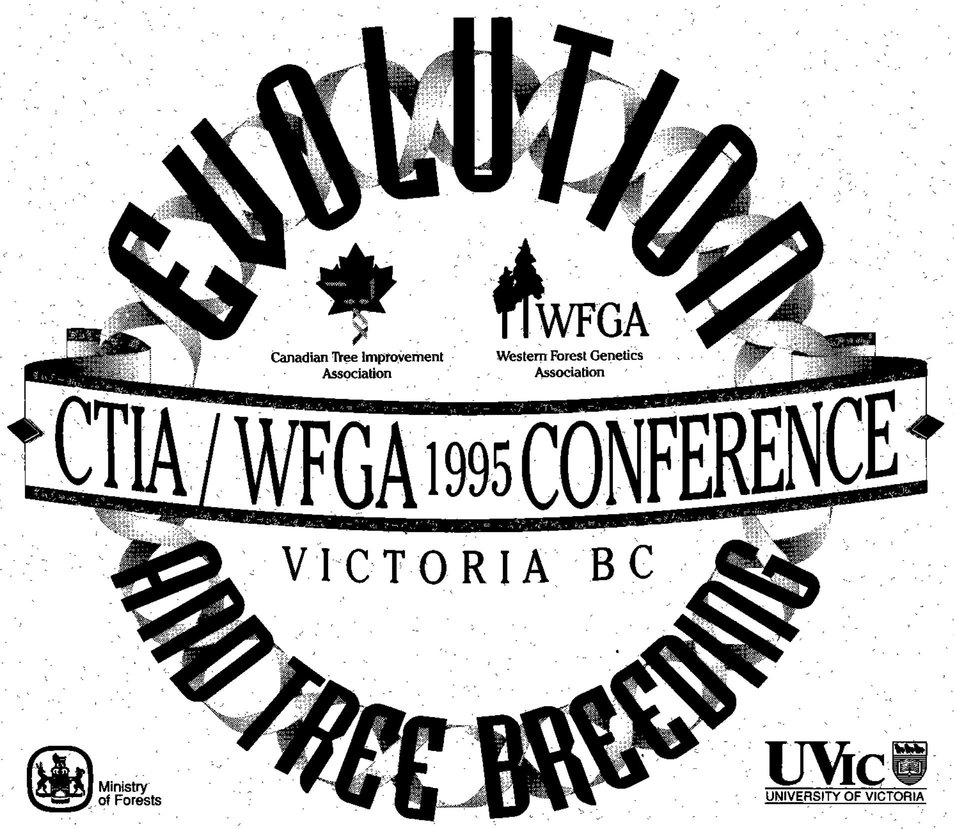
PROCEEDINGS OF THE TWENTY-FIFTH MEETING
OF THE
CANADIAN TREE IMPROVEMENT ASSOCIATION

PART 1	Minutes and members' reports
PART 2	Symposium



COMPTES RENDUS DE LA VINGT- CINQUIÈME CONFÉRENCE
DE
L'ASSOCIATION CANADIENNE POUR
L'AMÉLIORATION DES ARBRES

1^{re} PARTIE	Procès-verbaux et rapports des membres
2^e PARTIE	Colloque

PROCEEDINGS

OF THE

TWENTY-FIFTH MEETING

OF THE

CANADIAN TREE IMPROVEMENT ASSOCIATION

PART 1

Minutes and members' reports

EVOLUTION AND TREE BREEDING

Victoria, British Columbia
August 28 - September 1, 1995

Editor:
J. Lavereau

Additional copies of this
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MacMillan Bloedel

COMPTES RENDUS

DE LA

VINGT- CINQUIÈME CONFÉRENCE

DE

L'ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES

1re PARTIE

Procès-verbaux et rapports des membres

DÉVELOPPEMENT ET L'AMÉLIORATION DES ARBRES

Victoria en Colombie-Britannique
du 28 août au 1 septembre 1995

Rédactrice:
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Victoria, 1995

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Centre de biologie forestière, Universitè de Victoria
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Quebec -- Université Laval

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C.T.I.A./A.C.A.A. 25th BUSINESS MEETING MINUTES

C.T.I.A./A.C.A.A.
25th BUSINESS MEETING
MINUTES

Alvin Yanchuk chaired the 25th Business Meeting of the CTIA/ACAA held in the David Lam Auditorium, MacLauring Building, Victoria, British Columbia on Thursday August 31, 1995.

283. Minutes of the 24th Meeting

(as printed in the proceedings from the 24th meeting (Part I))

Motion: The minutes of the 24th Business Meeting be approved as published.
Moved by: J. Dojack
Seconded by: J. Klein
Carried.

284. Membership

284.1 Honourary Membership

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

Don Fowler	N.R. Canada-Maritimes, N.B.
Chris Heaman	B.C. Ministry of Forests
Kris Morgenstern	University of New Brunswick
Graham Powell	University of New Brunswick
Oscar Sziklai	University of British Columbia
Ben Wang	Petawawa National Forestry Institute
Louis Zsuffa	University of Toronto

Moved by: J. Klein
Seconded by: D. Simpson
Carried.

284.2 New Active Members

The names of nominated new active members were presented as follows:

Linda DeVerno	Natural Resources Canada-Maritime Fredericton, New Brunswick
S. Y. Zhang	Forintek Canada Corp. Sainte-Foy, Quebec
Brian Barber	B.C. Forest Service Victoria, British Columbia
Mathew Koshy	Univ. of British Columbia Vancouver, British Columbia.

Motion: That the nominated new active members be duly elected.
Moved by: M. Stoehr
Seconded by: K. Tosh
Carried.

284.3 New Corresponding Members

The following were recorded:

Chang-Yi Xie	B.C. Ministry of Forests Victoria, British Columbia
Frederick D. Beall	Natural Resources Canada Sault Ste. Marie, Ontario
Tim Gylander	Weyerhaeuser Canada Ltd. Drayton Valley, Alberta

285. Chair's Report

On behalf of the B.C. Forest Service and University of Victoria, I would like to thank you for participating in the 1995 joint meeting of the Canadian Tree Improvement Association and the Western Forest Genetics Association. This is the first joint meeting of these two organizations and we feel the timing could not be better.

I would like to acknowledge and thank: John Russell, Michael Stoehr, and Pat McGuire (Organizing Committee), John Owens (University of Victoria sponsor), Jack Woods (field trips), Michael Carlson (art designs), Sally Aitken (WFGA Chair) and John Barker (Western Forest Products), Yousry El-Kassaby (Pacific Forest Products), Glen Dunsworth (MacMillan Bloedel) for industrial sponsorship of field tours and Joy Lavereau (CTIA Executive Secretary, Editor and Treasurer).

We would like to take this opportunity to acknowledge the pioneering efforts of the late Dr. Alan Orr-Ewing, and dedicate this meeting in his honor.

286. Treasurer's Report

The financial statement for the period of July 30, 1993 to July 30, 1995 was prepared by Treasurer Joy Lavereau and was tabled for membership information and acceptance (see attachment #1). The statement shows a balance of $7,175.53 in the association's account as of July 30, 1995 and G.I.C.'s totalling $23,000.00.

Motion: That the financial statements as presented be accepted.
Moved by: D. Simpson
Seconded by: M. Stoehr
Carried.

287. Financial Contributions

British Columbia Ministry of Forests
Centre for Forestry Biology, University of Victoria
Western Forest Products
Pacific Forest Products
MacMillan Bloedel

Motion: That the CTIA/ACAA of the 25th meeting express our sincere appreciation to these contributors.
Moved by: J. Klein
Seconded by: K. Tosh
Carried.

288. Editor's Report

The proceedings were printed and distributed to all active members, Canadian universities and libraries, all participants of the 24th meeting, sponsoring and honourary members during April 1995. Two hundred and ninety-seven proceedings were mailed.

A form letter advising the theme of the 24th biennial meeting, the proceeding context and a request for a twenty dollar donation to obtain one copy, was sent to all corresponding members, U.S.A. addresses and other foreign countries. There were 23 donations from Canadian addresses, 20 from U.S.A. addresses and other foreign countries obtained 26.

289. Educational committee

289.1 Canadian Tree Improvement Association biennial "**Sponsored Student Awareness**":

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 for the sponsorship to attend the 25th CTIA/ACAA meeting. The following students received the award which provided all the costs of meeting, including registration, accommodation, pre-conference tour and travel:

Susan Boyes	Lakehead University
Denise Donelle	University of Moncton
Peggy Hickey	University of New Brunswick
Martin Perron	Laval University
Pia Smets	University of Brititsh Columbia
Barb Thomas	University of Alberta
Vasilios Tsarouhas	University of Toronto

Sponsorship of these students is paid by the executive committee of each biennial meeting through registration fees.

289.2 Canadian Tree Improvement Association biennial "**Student Recognition Awards**":

The following students received a cash award, for recognition of oral presentations given at the 25th CTIA/ACAA meeting in Victoria, British Columbia.

John Runions	Forest Biology, Univ. of Victoria Victoria, British Columbia	$500.00
Rungna Pattaavibool	Forest Biology, Univ. of Victoria Victoria, British Columbia	$300.00
Martin Perron	Fac. de Foresterie, Univ. Laval Ste. Foy, Quebec	$200.00

Motion: To grant the 1997 executive committee to establish a series of CTIA/ACAA student recognition awards for oral presentations, posters, or other contributions and draw no more than $1000.00 per meeting.

290. Working Groups Reports

Tree Seed Working Group

The Tree Seed Working Group held its regular Biennial Business Meeting on August 28, 1995. Four issues of the TSWG News Bulletin (No. 20-23) were published in the past two years and distributed to all members (about 250) and 32 Institutions. Hugh Schooley, Editor of the TSWG News Bulletin since 1985, retired from

Forestry Canada in 1995. Hugh was instrumental in developing a sound periodical for tree seed managers and scientists throughout Canada and abroad. His continued support will be deeply missed!

Ron Smith (CFS - Maritimes) has accepted to become our next TSWG News Bulletin Editor.
Motion: That the name of Ron Smith be accepted as the new Editor.

Moved by: Dave Kolotelo
Seconded by: Howard Frame
Carried. Thanks Ron for your support!

In 1994, Hugh Schooley organized a Tree Seed Discussion Group on the Internet (see News Bulletin No. 21). It is a great way to correspond with colleagues around the world. Ron Smith has accepted to look after the Discussion Group. The move from Petawawa National Forestry Institute (PNFI) to CFS-Maritimes should occur early this fall. All members of the Discussion Group will be informed of the change in due course.

PNFI has since 1985 covered the printing and mailing cost of the News Bulletin. We would like to acknowledge this continued support over the years. Since PNFI will terminate its mandate as a result of the Federal Government cutbacks, we needed financial support for continuing the publication of the News Bulletin. Dr. Geritt van Raalte, Science Director - Forest Resources, CFS-Maritimes, has agreed to take over the cost related to publishing and mailing the NewsBulletin.

It was suggested that the News Bulletin be available to the Discussion Group on the Internet. I will address Ron Smith on this.

Dave Kolotelo, Peter de Groot, and Guy-É. Caron have accepted to continue as Coordinator of the Tree Seed Processing and Testing Working Party, Coordinator of the Cone and Seed Insects Working Party, and Chairman of TSWG, respectively.

Dave Kolotelo coordinated this year's Workshop in Victoria. The Workshop, like the CTIA/ACAA meeting, was well organized and well attended. Dave also organized two tours to the BC Ministry Tree Seed Center in Surrey on August 27 and September 1. Our sincere thanks to Dave for his dedication towards the success of the Workshop.

For the first time, the TSWG will have its.presentations refereed. Dr. D.B. Burgess, Co-Editor of the Forestry Chronicle, has agreed to the publication of a Special Issue sometime in 1996.

The TSWG will host another Workshop at the 26th CTIA/ACAA meeting to be held in Quebec City, in 1997. Stéphan Mercier, Ministère des Forêts du Québec, will coordinate that workshop and will act as liaison to the organizing committee of the 26th CTIA/ACAA meeting.

Finally, a short discussion was had on the role of CFS on seed research throughout Canada. Seed research will now be headed by CFS-Maritimes. It is hoped by all that the National perspective be respected. As Chairman of the TSWG, I will transmit this information to the Administration at CFS-Maritimes.

This concludes my report!
Guy-É. Caron Chairperson 1991-97

291. Business arising from previous meetings

291.1 Distribution of proceedings

Motion 279.2: Distribution of the 25th CTIA/ACAA proceedings will be determined by the 1995 executive committee.

Motion: Distribution of the 25th CTIA/ACAA proceedings will be "gratuitous" copies be sent to all active members, all participants of the meeting, all Canadian universities and libraries, honourary members and sponsors of the meeting.

Moved by: J. Klein
Seconded by: M. Stoehr
Carried.

291.2 Name change of CTIA/ACAA

Motion 279.3: That an official name change be held off, until members were advised of the proposal and that the 1995 executive committee would be responsible for notification of the motion to all active members.

A questionnaire was sent to all active members in March 1995. The results were as follows:

Canadian Tree Improvement Association	= 26 Votes
Canadian Forest Genetics Association	= 9 Votes
Other	= 11 Votes

The 1995 Executive committee stated the name "Canadian Tree Improvement Association" will not changed.

291.3 Retirement

Motion 289.1: That in light of the pending retirement of Dr. E.K. Morgenstern, that the current CTIA/ACAA executive compose and send a letter on behalf of the CTIA/ACAA to the Faculty of Forestry, University of New Brunswick urging the Faculty to maintain a staff position in forest genetics.

Kathy Tosh advised a letter was sent to the University of New Brunswick. The faculty was uncertain regarding maintaining a position due to University restraints.

292. New Business

292.1 Closure of P.N.F.I.

With the closure of Petawawa National Forestry Institute, the Canadian Tree Improvement Association would ask Natural Resources Canada, Maritime Region to take on the responsibility of Editor, Treasurer, and Executive Secretary of the Association.

Natural Resources - Maritimes, confirmed commitment to maintain all aspects of the Canadian Tree Improvement Association.

292.2 Funding

The Science and Sustainable Development Directorate and the Petawawa National Forestry Institute have commited 5 000 dollars for the printing and distribution of Part I and Part II of the 25th CTIA/ACAA proceedings.

292.3 Guy Caron asked if the Canadian Tree Improvement Association would be interested in paying the publishing costs of "four" Tree Seed Working Group papers. After some discussion the members decided it was too expensive due to page printing costs. However, the Association would be pleased to publish the four papers in the "Proceedings of the twenty-fifth meeting", Victoria, B.C.

292.4 Proposal for Support

Lauren Fins, Professor of Forest Genetics at the University of Idaho sent a propsal to the 1995 CTIA/ACAA Executive Committee requesting support for a project on the "History of Forest Genetics in North America". The funding request of $3500.00 would be used for travel expenses to record and make available the oral history of forest genetics and tree improvement across North America, as remembered and told by the prominent pioneers of the forest genetics and tree improvement in the U.S. and Canada.

Introduction (of the proposal)
In the earliest days of westward expansion in the United States, forest manipulation consisted of a log-and-move on mentality. The best species and individuals were harvested with little thought to regenerating the stands to the same species mix or to the same level of genetic integrity. Later, when planting began to come into vogue, there was still very little understanding that there were critical adaptive differences between populations of the same species. Seed distribution was hardly monitored and often the criterion for purchase was the lowest price. As a result, many poorly adapted trees were planted throughout the west. As forest geneticists began to study and understand population variation, their work has a major influence on the planting practices in operational forest management. Today, no modern forester would consider planting without first matching the seed source to the planting site . . .

After some discussion, it was decided that the documentation of this historical information was important and that the CTIA/ACAA would put $2000.00 aside for the proposal, on the condition that L. Fins receive five supporting offers from other sectors.

Motion: 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. Fin receive five supporting offers from other sectors.

Moved by: M. Meagher
Seconded by: J. Woods
Carried.

293. Future Meeting

293.1 Location of the 1997 meeting

Michel Villeneuve confirmed that the 1997 CTIA/ACAA meeting be jointly hosted by Yves Lamontagne (Ministere des Forêts), Jean Beaulieu (Natural Resources Canada), and Jean Bousquet (Université Laval) and will be held in Quebec City.

293.2 Location of the 1999 meeting

Unfortunately, Weyerhaeuser Canada is unable to jointly host the 1999 CTIA/ACAA meeting with the prairie provinces.

293.3 Location of the 2001 meeting

Tentative support from Northern Ontario to hold the 2001 meeting.

294. Election of New Executive

The following slate of officers for election to the 1995/97 CTIA/ACAA executive:

Chairperson:	M. Michel Villeneuve Min. des Ressources naturelles
Vice-Chairperson:	MM. Jean Beaulieu (symposium) Ressources naturelles Canada Jean Bousquet (organisation locale) Université Laval
Secretary:	Mme. Arianne Plourde Ressources naturelles Canada

295. Adjournment

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

Motion: That the 25th business meeting of the CTIA/ACAA be adjourned.
Moved by: Dennis Joyce

Attachment # 1.

CTIA/ACAA
Financial Statement
July 30, 1993 to July 30, 1995

Cash Balance July 30, 1993		***$ 2,726.78***
Credit:		
Interest earnings (GIC, Account)	$ 2,214.57	
Donations for 24th Meeting Proceedings	1,387.39	
24th Meeting advance return	1,035.51	
Total Credit	$ 4,637.47	***$ 4,637.47***
Guaranteed Investment Certificates: (as of July 30, 1993)		
GIC Principal	$10,000.00	
GIC Principal	8,000.00	
GIC Principal	6,000.00	
Total GIC's	$24,000.00	
Guaranteed Investment Certificates: (as of July 30, 1995)		
GIC Principal	$10,000.00	
GIC Principal	7,000.00	
GIC Principal	6,000.00	
Total GIC's		***$23,000.00***
Deposited back into account (from GIC)		***$ 1,000.00***
Debit:		
Forestry Chronicle	$ 1,118.15	
Film processing	66.37	
Money order fees	3.25	
Service charge	.95	
Total Debit	$ 1,188.72	**$ 1,188.72**

Cash balance July 30, 1995	***$ 7,175.53***
Invested GIC balance	***$23,000.00***
Total Holdings	***$30,175.53***

ACTIVITY REPORTS
FROM ACTIVE CTIA
MEMBERS

WESTERN HEMLOCK BREEDING PROGRAM

John King and Charlie Cartwright

B.C. Ministry of Forests
Research Branch
1450 Government St.
Victoria, B.C.
V8W 3E7

First Generation Testing

Three series of tests evaluating 144 low elevation open-pollinated parent are undergoing 15 year measurements, with data analysis confirming 10 year results. Three more recent polycross series test a further 182 parents, with the first 5 year measurements completed in 1994, and preliminary selection made.

High elevation (over 600 m) trial establishment was concluded this year with the out-planting of 91 polycross families on 2 sites. These augment 33 parents out-planted in 1993, and 10 included in Mike Meagher's 1979 series.

Advanced Generation Testing

Advanced generation breeding is executed in conjunction with the Hemlock Tree Improvement Co-op (Hemtic). The program components are 5 regional units, each comprised of 5 disconnected 6 tree diallels (30 selected parents). Crossing for the 3 northern elements, B.C., Forks and Gray's Harbour, was 80% complete as of '94, with 950 isolations made this spring. The southern 2 elements, Cavenhams and Tilamook, were 50% complete, but similarly strong crossing efforts were made this year. Field test establishment will commence with sowing January '96 and will extend over 2 years, and 12 sites ranging from Northern Vancouver Island to Central Oregon.

Realized Gain Trials

Verification of per hectare gains has been a recent program focus. Realized gain trials provide not only accurate estimates, but also strong demonstration of the value of selected materials to silviculturists. Three series will be established. The first, out-planted in 1992, has blocks representing the advanced generation (Hemtic) orchard, roqued first generation orchards, and a standard comprised of 14 wild stand seedlots. Early results (height age 4) over 3 sites show gains at 30% for the second generation, and 12% for the roqued orchard. (see figure 1).

Other Research

Additional substantive program initiatives entail genecology installations. Specifically, provenances from California to Alaska are represented in 5 coastal trials monitored annually for frost damage and survival. Twelve much smaller sites have been planted throughout B.C., covering all the major vegetation zones in which hemlock occurs. Data bearing on seed transfer in general, (but in particular selected southern [Hemtic] material), adaptability, and value of specific provenances will be garnered from these sites.

To ensure product quality, wood and pulp property testing was initiated in 1993. All parents in B.C.'s first generation tests are assessed for specific gravity. In conjunction with Papercan (Vancouver), further screenings for fibre length and other properties of importance to pulp quality are evaluated.

SITKA SPRUCE BREEDING PROGRAM

Spruce Weevil Studies

Research of appropriate genotypes for deployment to weevil hazard areas concentrates on mode of inheritance of resistance, and screening selections from putatively resistant populations. A diallel mating design, using parents identified by provenance research and the interior spruce breeding program, has been constructed to investigate trait inheritance. To broaden the base of resistance, 3 series of screenings have been established evaluating 218 open-pollinated parents. Each consists of several disease garden sites, as well as a low hazard area trial to assess growth potential. Identical stock was provided to interested American industry co-operators to establish additional tests. Two further series are planned.

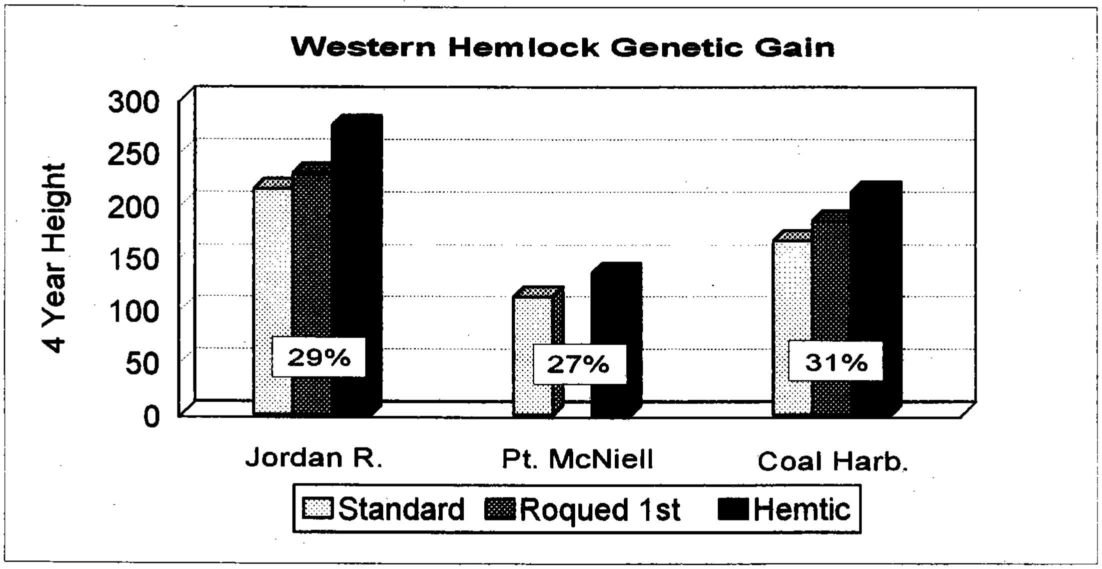
Low Weevil Hazard Spruce

Testing for low hazard lands has proceeded to the point of completion of crossings the Queen Charlotte / North Vancouver Island seed orchard. As well, selections have been made from provenance tests at Holberg and on the Queen Charlotte Islands. Access to tested parents in European programs will also provide advanced generation materials.

Hybrid Spruce

Collections from the wild and from hybrid sources in provenance tests started in 1994, with additional forays planned this year. Field testing is envisioned 3 to 4 years hence. The focus of the information gathering is to understand the dynamics of introgression in the hybrid zone, and identify optimal seed sources for the area.

Figure 1.



LODGEPOLE PINE BREEDING PROJECT EP770

Michael Carlson and John Murphy

B.C. Ministry of Forests
Kalamalka Forestry Centre
3401 Reservoir Road
Vernon, B.C.
V1B 2C7

The British Columbia Lodgepole pine breeding project, EP770, began with breeding zone delineation and parent tree selection in 1975. At that time the annual planting of the species was approximately 10 million seedlings. By 1985 a total of 1,562 trees had been selected for testing in 6 breeding zones. Within a year after selection most of these trees had been grafted into clonebanks and wind pollinated seed collected. Between 1984 and 1988, 36 wind pollinated test plantations were established on 18 sites in 6 breeding zones of the B.C. southern and central interior. Total tree heights were measured after 5 field seasons and a site-weighted selection index computed to rank trees in each zone (~300 trees per zone)

Starting in 1990, the top 15-20 % of the tested parents in each zone were grafted and planted at orchard sites in the dry warm southern interior Okanagan Valley. Today (1995) six of the seven rogued first generation orchards are nearly complete with full seed production expected, on average, for all seven by 2005. The annual planting requirement for the species is now more than 70 million. When full orchard production is achieved some 56 million or about 80% of the required number will come from these orchards. Estimated genetic gains for 6 year height range from 5 to 19%.

Controlled crossing to create second generation selection families began in 1995. The pilodyn was used in 10 year old progeny tests to rank parent trees for wood relative density. This information combined with 10-year height rankings was used to construct breeding populations with subpopulations (group) structure emphasizing relative density in some breeding groups and height growth in others. Small partial factorials ranging from 5x5 to single-pair matings are used starting with top ranked parents and descending to parents at about the 40th percentile in each breeding zone.

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

Yousry A. El-Kassaby

Pacific Forest Products Limited
Saanich Forestry Centre
8067 East Saanich Road
Saanichton, B.C.
V8M 1K1

Keywords: seed orchards, seed production, gene conservation, research.

SEED ORCHARDS

The seed orchard program was reviewed and a consolidation plan for Nootka and Saanichton seed orchard complexes was completed. The number of seed orchards, species, orchard design, and the genetic status of the orchards have changed accordingly. The current state of Pacific's seed orchards is as follows:

1. The Saanichton high-elevation Douglas-fir seed orchard was removed and replaced by three new seed orchards:

a. Western hemlock, clonal-row moving front seed orchard was established with the parent material from the Hw co-op (i.e., advanced generation).
b. Sitka spruces, clonal-row from trees with known resistance history (i.e., advanced generation).
c. White pine, clonal-row with resistance mechanisms as blocks (i.e., advanced generation).

2. The Saanichton low-elevation Douglas-fir seed orchard was upgraded with forward and backward selections, so contamination to the 2nd generation seed orchards is not an issue.

3. New western redcedar seed orchard (hedge) was established.

The seed production for the 1993-95 is as follows:

- 1993-94: 64 kg Douglas-fir, 7 kg western hemlock, 2 kg Pacific silver fir, and 1 kg yellow-cedar.
- 1995 represents the first appreciable cone crop from the Douglas-fir 2nd generation seed orchard.

Gene Conservation

1. Pacific yew (Y.A. El-Kassaby, Pacific Forest Products Ltd. & A.D. Yanchuk, B.C. For. Serv.):

A study was conducted to appraise the level and structure of genetic diversity and differentiation among populations representing several of Pacific yew natural range geographical regions in B.C. The study revealed that the sampled populations were clustered within geographic regions and that geographic regions were highly differentiated from each other. The unique structure of genetic differentiation provided information that was critical for the conservation strategy of this species. The adoption of a regional approach to conservation was recommended.

2. *Ex situ* conservation of tree seeds (D.G.W. Edwards, Canadian Forest Service & Y.A. El-Kassaby, Pacific Forest Products Ltd.):

A research project was initiated to evaluate the effectiveness of *ex situ* gene conservation for B.C. forest tree species. The traditional (crop plant) approach to *ex situ* gene conservation relies on rejuvenation of the genetic resource through frequent replacement with fresh seeds. This rejuvenation can not be accommodated in forest trees due to their long sexual maturation process, so seeds must be stored, often for many years. This project evaluates whether their genetic integrity remains unchanging over long term storage.

Evaluations of viability changes during storage were carried out by means of the accelerating aging concept commonly used by biologist and engineers. The species tested include Douglas-fir, Sitka spruce, white spruce, western hemlock, mountain hemlock, and lodgepole pine. In all species, reductions in seed viability were observed as seeds aged. Without exception, all observed reductions in seed viability were genotype specific (i.e., some genotypes experienced more rapid and more substantial losses than others). This indicates that the original genetic constitution of the seedlots was altered during simulated storage. It is recommended that *ex situ* gene conservation for forest tree species should be implemented on an individual genotype basis so that significant changes to the genetic base can be identified. Changes in the protein matrix of seeds is currently being evaluated to determine if constituents can be used as markers to predict viability losses during storage. The identification of these markers will enhance the use of seed banks as a viable option for forest tree gene conservation efforts.

3. Impact of forest management practices on the genetic diversity (Y.A. El-Kassaby, Pacific Forest Products Ltd.):

The results of a decade of research on the impact of forest management practices were summarized. Figure 1 demonstrates the various steps that might affect the genetic diversity. This study evaluated the effect of selection, breeding, seed orchard genetics and management, seed biology and handling, seedling production, and plantation management on the genetic/species diversity. The results could be summarized in the following points:

a. Information related to forest tree species domestication was mainly devoted to the genetic consequences of domestication rather than evaluation of the process itself.
b. The process of domestication of forest tree species is fragmented and follows the classical horizontal organization of management systems, thus making evaluation difficult.
c. Systematic monitoring of the various steps of the domestication process (selection-breeding methods and strategies and seed and seedling production) has identified cases of unintentional directional selection where genetic diversity could be affected.
d. Phenotypic selection has been proven to be an effective method for capturing the majority of genetic variation existing in natural populations.
e. The progress from breeding population to production populations did not substantially reduce genetic variability, however, the chance for rare, endemic allelic loss increased with the reduction of the number of parental trees in seed orchards.
f. The utilization of a breeding strategy that provides for sampling over several geographical locations and selection for various goals, as well as adaptation to different environments (i.e., Multiple Population Breeding System) was proven to be effective.
g. Understanding the species biology and the role of biology on genetic diversity was proven to be of great importance.
h. The need for change and/or the implementation of management practices was proven to be effective in enhancing the genetic variability.

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GENETIC IMPROVEMENT OF INTERIOR DOUGLAS-FIR AND WESTERN LARCH

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Keywords: *Pseudotsuga menziesii* var. *glauca, Larix occidentalis,* tree breeding, genetic testing.

Tree breeding programmes for Interior Douglas-fir and western larch were initiated in 1982 and 1987, respectively. The objective of both programmes is to develop seed that has been selectively bred to produce trees with improved volume growth and quality, while maintaining acceptable levels of adaptation and genetic diversity. The breeding strategies for both species are based on recurrent selection for general combining ability, wind-pollinated genetic testing, and soil-based clonal seed orchards.

SELECTION OF BASE POPULATIONS, GENETIC TESTING, AND SEED ORCHARDS

In both programmes, the selection of base populations from wild stands has been completed. Over 1700 Douglas-fir trees were selected in eight breeding zones, and 609 western larch trees were selected in two breeding zones. Nearly all of these selected trees have been propagated by field-grafting and established in clone banks and breeding arboreta.

In total, 1661 Douglas-fir families are in field tests across 39 sites in the eight breeding zones. By spring 1995, 608 western larch families will be in test across 17 sites in the two breeding zones. In all of these tests, individual tree height and condition has, or will be, recorded after three, six and ten field seasons. For Douglas-fir, first-phase clonal seed orchards will be established on the basis of family field performance after six growing seasons. Currently, Douglas-fir seed orchards have been established for the Central Plateau, Cariboo Transition, Quesnel Lake, and Shuswap Lake breeding zones. Field-grafting has begun for the low elevation West Kootenay seed orchard. In 1990, two clonal first-generation western larch seed orchards were established at the Kalamalka Forestry Centre. These western larch orchards are expected to come into production by about 1998.

SUPPORTIVE RESEARCH

Several research projects have been recently initiated to support the Douglas-fir and western larch tree breeding programs. Studies in Douglas-fir include intervarietal hybridization, elevational displacement of seedlots, genetic variation in juvenile wood relative density, and genetic relationships among traits associated with shoot elongation and total tree height. Studies in western and subalpine larch include estimation of mating system parameters in natural and seed tree stands, genetic variation within and among natural B.C. populations, and interspecific hybridization.

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GENETIC IMPROVEMENT OF WHITE AND ENGELMANN SPRUCE

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Keywords: Forest genetics, tree breeding, white spruce, Engelmann spruce, insect resistance.

The objective of this project is to produce genetically improved planting stock of white and Engelmann spruce (*Picea glauca* [Moench] Voss and *P. engelmannii* Parry).

Twenty-year height and diameter measurements were carried out for the better performing plantations in the Prince Rupert and East Kootenay Selection Units. A total of nearly 38,000 trees were measured (18,400 in the East Kootenay and 19,500 in the Prince Rupert Selection Units).

In general, trends continued as in previous years. Families that were identified as outstanding performers at age 6 remained outstanding performers at age 20.

Weevil studies were continued. Rory McIntosh is conducting a thorough study of weevil behaviour at the Kalamalka Forestry Centre. He has accumulated a tremendous data base on weevil activities and response by the host.

A national weevil symposium was hosted jointly by the B.C. Forest Service and the Pacific Forestry Centre in Richmond during January 1994. The aim of the symposium was to consolidate scientific knowledge on the white pine weevil and to develop a strategy for future research. The successful symposium had a total of 85 participants. Thirty three papers were presented and the proceedings were published (FRDA report 226).

Wood density assessments continued and continued to surprise as well. While there was no correlation between height traits and pilodyn pin penetration among the PGSU selections, there was a small but significant correlation among the Prince Rupert Selection Unit families (r=0.36) and a strong correlation among the East Kootenay Selection Unit families (r=0.79). In addition, the spread of pin intrusion in the PGSU families was only two units in magnitude, while the PRSU had a spread of about five units and the EKSU families demonstrated a spread of over 8 units. The ramifications of these results are interesting since it demonstrates that in the EKSU selection fast growth will result in reduced wood density.

A number of crosses were made utilizing putatively weevil resistant and susceptible families. Seedlings were established in demonstration plantations and will be evaluated as attacks take place. Some of the seedlings are potted and will be made available for various studies where caging of weevils is required.

Second generation breed material is being raised in containers and will be out planted in 1996. These plantations will provide the basis for second generation selections.

An open pollinated progeny trial will evaluate the genetic potential of eastern Canadian white spruces in our collection. This will enable us to incorporate outstanding eastern Canadian families into our breeding program. As some of these families exhibit fast growth and strong weevil resistance they will be a great asset to the program.

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WESTERN WHITE PINE IMPROVEMENT PROGRAM FOR BRITISH COLUMBIA

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Keywords: *Cronartium ribicola, Pinus monticola,* disease resistance proteins, DNA markers.

"OPERATIONAL" PROGRAM OF SELECTION AND RUST SCREENING

Support for tree selection, seed collection, stock rearing and rust inoculation, plus grafting of selected trees, is provided by the B.C. Forest Service under a Memorandum of Understanding. Following external review of the project in 1994, and in response to other pressures, the decision was made to phase out the program at PFC. Following the 1995 inoculation, our last, we will have screened 291 and 216 trees from the Coastal and Interior areas, respectively. Final examination of stock inoculated in 1995 will require about 6 more years. Seed collections in 1994 were conducted only from trees selected for further study.

Clones of Interior seedlings selected to date will join Idaho selections to form a resistant seed orchard to be established by the BC Forest Service at Vernon for the Interior. This will be the third seed orchard stemming from our program; the other two are on the Coast: one a "forward-selection" orchard composed of seedlings, the other a "backward-selection" orchard based on parents identified by our screenings. Both of these orchards are established by industrial cooperators.

Adequate rust spore density was achieved on 1+1 open-pollination family stock in both 1992 and 1993. Family means of spots per seedling ranged from about 1 to 9. Surviving stock from previous inoculations was examined and seedlings exhibiting "slow canker growth" were cloned. Seedlings remaining uncankered after 2 years are placed in a disease garden for a second inoculation. To the end of 1994, 111 Interior and 219 Coastal clones have been produced.

RESEARCH PROGRAMME

Rust infection on Idaho F2 material planted in 1984 by the B.C. Forest Service was less than on untested local seedlots, while growth vigour was good, indicating potential for this seed in southern B.C. However, inconsistent results from Coastal sites mean that its use on the Coast cannot be recommended. Analyses of data from the field trials assessed in 1992 led to recommendations concerning seed transfer to and within B.C.

Controlled mating of parents judged superior from our operational rust inoculation ended in 1994. Seedlings will be used in further trials and in field "demonstration" plantations. Two plantations containing families from "wide" vs. "narrow" outcrosses were established in 1994 on B. C. Forest Service seed-orchard sites. Results will test the inference that western white pine varies little geographically. Final assessment of a "rust races" test was made in 1994; analyses of results are in draft.

Plantations of unscreened seedlings from selected parents have been established annually, with the last being planted in 1995. More plantations to contrast families differing in needle spotting and cankering incidence were established, bringing the total to four.

The study involving proteins is being approached in three different ways. In one approach, i.e. pathogenesis-related proteins, resistant (Rr) and susceptible (rr) white pine seedlings were inoculated with white pine blister rust (WPBR) fungus and foliar protein patterns were analyzed by 2-dimensional gel electrophoresis in conjunction with densitometry and computer-assisted gel image analysis. In resistant seedlings, both enhancement and suppression of the biosynthesis of proteins were observed. By contrast, only suppression of the biosynthesis of proteins was observed in susceptible seedlings. Two acidic proteins, 36.7 kDa and 28.1 kDa, were detected in large amounts. The 36.7-kDa protein was suppressed in susceptible seedlings at day 3 while the 28.1-kDa protein was enhanced in resistant seedlings at day 9. In another approach, bark proteins of white pine trees displaying slow canker growth - a form of WPBR resistance - were analyzed. A 10.5-kDa protein unique to this resistance type was identified and partially characterized. In the third approach, a series of monoclonal antibodies (Mabs) to WPBR was generated. Although most Mabs were shown to be cross-reactive to western white pine proteins, two Mabs were specific to WPBR. These two Mabs will be used to identify immunocytochemically the pathogen in resistant and susceptible trees during initial infection. In addition, a "cold" protein was shown to be differentially expressed in resistant and susceptible white pine trees, indicating its potential use as a marker for resistance.

DNA markers, isozymes and virulence phenotype were used to examine population structure and breeding system in the rust. Mendelian segregation of alleles was demonstrated, confirming previous results indicating the rust is heterothallic and out-breeding. Changes in virulence may occur as a result of reassortment of virulence determinants during sexual reproduction. This work was carried out in collaboration with the U.S. Forest Service Institute of Forest Genetics, Placerville, California.

No association with geographic region in B.C. was apparent with DNA markers. A relatively low degree of restriction-site variation was observed, consistent with the view that blister rust in B.C. originated from a single introduction of diseased seedlings and has a relatively limited genetic base.

Clones of two disease-response genes (chalcone synthase and phenylalanine ammonia lyase) have been isolated from white pine. Chalcone synthase is coded by a polymorphic multi-gene family. Work is in progress to determine whether any of these polymorphisms correlate with disease resistance.

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FOREST GENETICS ACTIVITIES AT THE UNIVERSITY OF BRITISH COLUMBIA

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Keywords: population genetics, genetic diversity, genome mapping, molecular genetics.

Gene Namkoong, Head of the Forest Sciences Department at UBC, was awarded the prestigious Marcus Wallenberg Award on September 22, 1994, in Stockholm for his contributions to population genetics, quantitative genetics and forest conservation research. His acceptance presentation was entitled "An evolutionary concept in breeding." Gene was also successful in securing funding for two chairs in forest genetics who are expected to join the Faculty of Forestry later this year. These two chairs will make a large impact on teaching and research in population, quantitative, and forest conservation genetics at UBC. During Gene Namkoong's first two years at UBC, his research group at UBC has rapidly expanded and includes many visitors from academic and government institutions around the world. The research being conducted by Gene's group includes three main areas: 1) the evolution and maintenance of genetic variance in natural and artificial populations; 2) the evolution and genetics of norms of reaction in forest species; and 3) biodiversity, particularly the impact of habitat disturbance on species and genetic interactions. In John Carlson's group, research relevant to tree improvement includes genetic linkage mapping, DNA markers for early selection, molecular cytogenetics, DNA fingerprinting, DNA diversity, and genetic engineering of specialty tree genotypes. Specific projects are described below.

MANAGING FOREST TREE GENETIC VARIANCE FOR CLIMATIC CHANGE

Mathew P. Koshy 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 intercrossing 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 Fl 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 also have been developed. Seeds from these crosses will be collected in September. Growth trials will follow in March, 1996.

DYNAMICS OF GENETIC VARIATION IN RESOURCE USE EFFICIENCY IN LODGEPOLE PINE

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.

A COMPARATIVE STUDY OF GENOTYPE BY ENVIRONMENT INTERACTION IN FOREST TREE SPECIES

Jan Svejgaard Jensen and Gene Namkoong

One of the obvious problems in management of genetic resources is the existence of interaction between genotypes and the environments in which they are in. Some of the interactions, i.e. the relation between growth and origin of some tree species, can be very predictable whereas others are unpredictable. There are several different terms and definitions for interaction and the basic assumptions are often very simplified. The phenotypic trait is a combination of genotypic and environmental effects. If the phenotypic trait could be described by an operator that could "convert" genotypic characters into phenotypic, then there is no interaction. However, if this operator is not to be defined, there would be interaction. The relationship between environment and response is non linear for many traits. Many traits could also work interactively in an unpredictable way. The methods are often developed for agricultural practice with large numbers of genotypes and environments. The purposes of these methods have been selection for stable or unstable genotypes. Later it has become important to group the genotypes on basis of their performance in order to define breeding zones and seed zones. Stability can be described by the variability of genotype performance in different environments, as well as by the slope of performance over different environments compared to mean performance of the genotypes. Another way to describe stability is to measure the change of rank of genotypes over different environments. This is called "true interaction" by many authors, and can be described by the correlation between genotypes. In linear statistics the total interaction is easy to calculate, but it is possible to make a break-down of components. True interactions can be separated from interactions due to heterogeneous variance. The total interaction can be caused both by the nature of the genotypes and the environments. Stability statistics can be divided into different groups depending on whether they are based on the deviations from the average genotype effect or on the genotype and whether or not they incorporate a regression model on the environmental index. At least ten different statistic parameters have been proposed by several authors. We are applying these methods on different types of materials to investigate their similarities and interrelationships.

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

Francisco Luna-Lopez J. and Gene Namkoong

This study forms part of a comprehensive study on the genecology of Douglas-fir in the sub-maritime 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 1200 meters above sea level. This part 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 coastal zone evaluated seedling growth and physiological traits and assessed the genetic variability in about 2700 seedlings. During this phase, seedling height, collar diameter, and shoot and root ratio were measured in 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 includes 77 families from 17 provenances from the SM zone plus families from coastal and interior zones. 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.

GENETIC VARIATION IN WESTERN RED CEDAR *(THUJA PLICATA* DONN) SEEDLINGS

Marilyn Cherry and Gene Namkoong

To determine whether the apparent lack of genetic variation in western red cedar *(Thuja plicata* Donn), as previously inferred by isozyme and terpene studies, would hold true for quantitative seedling traits, a provenance study was initiated to investigate patterns of variation in seedling growth and survival characteristics, cold temperature acclimation, and response to inbreeding. Seedlings from ten coastal and ten interior provenances, half with family structure (five families/provenance), were grown for three years at one coastal (Vancouver) and one interior (Salmon Arm) location. Twenty-three potted clones were both self-pollinated and polycrossed at Cowichan Lake; resulting progeny were monitored for growth and frost hardiness. Genetic variation could be detected from the first year, and increased annually. The narrow-sense individual heritability, assuming some inbreeding, of final heights of trees growing in Vancouver was 0.37. Height, root collar diameter, acclimation, and declamation exhibited mainly within-population variation, while variation in dry weight measurements, foliar nutrient content, survival at Salmon Arm, and maximum cold hardiness was evident mainly between populations. Coastal vs. interior differences were noted in first-year heights, root collar diameter, and in height and survival at Salmon Arm following a severe winter in which trees suffered major desiccation damage. In general, adaptive traits appeared to show more between-population differences, while traits under less selective pressure showed mainly within-population variation. Provenances displaying the greatest variation at the family level were those from the southern B. C. coast. Between-population variability appeared to be highest in the B. C. interior, and lowest in northern B. C. populations. Elevation influenced all traits displaying provenance variation. Location effects occurred, but little genotype by environmental interaction was found. Plasticity was evident in timing of growth initiation and cessation, timing of acclimation and declamation, and in degree of maximum hardiness reached per year. Acclimation was apparently triggered by exposure to low temperatures. Early traits showed little evidence of inbreeding depression, but there seemed to be a trend towards gradual expression of inbreeding depression over time, at least in traits under selective pressure. This research showed that western red cedar is much more complex than previously believed, and substantial genetic variation exists in several traits of this species.

BIODIVERSITY MONITORING AND MANAGEMENT: DEVELOPING TOOLS USING PARAMETERS OF DISTURBANCE COMPONENTS

Fredrik von Euler and Gene Namkoong

Understanding the regulation of biodiversity is necessary in order to explain its natural patterns and to predict the effects of human activities. Disturbance agents, both natural such as fires and windblows, and human activities such as logging and pollution affect different arrays of variables of the physical environment. Quantifying disturbance directly in terms of such physical variables is problematic for many reasons: i) Identifying the relevant variables may be difficult, ii) Measuring the variables may not be practicable, and iii) The comparison of disturbance effect of different agents may be impossible. However, regardless of the physical variables, all disturbance agents by definition have some net effect on the abundance of at least some species in a local assemblage, which is expected to affect species packing and species interaction parameters. We are developing practical measures of disturbance based on species interaction and species packing in local species assemblages, and a predictive model for local species richness, based on energy and disturbance information. We are doing this by analyzing species-abundance data from published plot studies. Such data is available from a large number of faunistic and floristic surveys in Canada (e.g. B.C. Ministry of Forests) and around the world. The relationship between species richness, disturbance and energy are being studied for vascular plants, birds, and other classes of organisms. The feasibility of extending methods for species to "higher" taxa such as families will be investigated, and specifically, geographical scale problems will be addressed. Statistical properties of estimators will be evaluated using data generated by simulation models, adapted and extended from published work.

GENETIC ANALYSIS OF THE RISK OF CLONAL PLANTATIONS IN FOREST TREES

Yong-Bi Fu, Gene Namkoong and John E. Carlson

Establishment of commercial populations by use of clonal propagules has been made in several tree species and expected to greatly increase with advanced biotechnology in the future. A great concern for clonal forestry is the probability of catastrophic losses in these clonal plantations with a uniformity of genetic diversity due to an unforeseen disastrous event such as insect or pathogen attacks. One possible alternative is to apply a number of clones in such plantations to ensure genetic diversity and protect against risk of extensive loss. What is the minimum (safe) number of clones in forest clonal plantations is an important, but controversial, question. Some theoretical studies on this question have been made. These studies mainly emphasize the reduction of risk by considering the number of clones and the probability of the event occurrence. We intend in this investigation to address the "effective" genetic diversity for various nature of stochastic events, given a number of clones in a plantation. The effective genetic diversity required to reduce the risk for stochastic events such as forest fire and tropical storm may be low, less depending on the number of clones; for events such as pest and pathogen attacks may be high, thus with the need of using more clones. We examine this issue by specifying various genetic models for the analysis of risk in plantation failure and defining effective genetic diversity for different stochastic events.

PURGING DELETERIOUS GENES IN VARIOUS TREE BREEDING SCHEMES

Yong-Bi Fu and Gene Namkoong

Every initial breeding population of forest trees has a certain level of genetic load (i.e., deleterious genes). Whether these deleterious genes can be purged to a minimum for an increased population fitness by appropriate breeding means presents a great concern for tree breeders as well as conservation geneticists. The answer to this question is less clear and the fate of deleterious genes is expected to depend on both the nature of the genetic load and the level of applied inbreeding. To address this issue, we are currently conducting a theoretical investigation by computer simulations. Three commonly applied tree breeding schemes are examined: selfing, full-sib mating, and half-sib mating. Various genetic models of inbreeding depression are used that take into account selection and dominance levels as well as multiplicative and synergistic interactions. The overall objective is to examine the behavior of purging deleterious genes and the patterns of offspring fitness in a breeding population over 10 generations by three breeding schemes. This is done first by examining the deleterious genes reducing viability and then those genes influencing fecundity traits. Results obtained so far (mainly on viability selection) appear to suggest that it is possible to purge high numbers of deleterious genes in a breeding population. Practical implications of these findings will be explored.

ANALYSIS OF DNA DIVERSITY IN WESTERN RED CEDAR

Jeff Glaubitz and John Carlson

We are engaged in a comprehensive study to estimate genetic variation in western red cedar at the DNA level. We have taken the unique and demanding approach of analyzing all three genomes, i.e. the chloroplast, mitochondrial and nuclear genomes, with RFLP markers. Populations were sampled from across the entire natural range of western red cedar, including coastal B.C., interior B.C., the Charlotte Islands, Utah, Washington, Oregon and northern California. This work will result in an extensive RFLP data base, valid for comparison with previous reports on isozymes which uncovered very little genetic diversity in western red cedar. This study is being conducted in collaboration with Y. El-Kassaby, of Pacific Forest Products, Ben Sutton and Craig Newton of B.C. Research, Inc., and John Russell of the B.C. Ministry of Forests and is supported by the Canadian Forest Service's National Forest Genetic Resources Centre and the B.C. Ministry of Forests.

POPULATION GENETICS OF THE EASTERN WHITE PINE WEEVIL, *PISSODES STROBI*

Kornelia Lewis, John McLean and John Carlson

We have completed an extensive assessment of the extent of genetic variability, population subdivision, and host specificity in weevil populations in British Columbia. We chose RAPD markers, a DNA marker system based on PCR amplification of unassigned loci, with which to obtain data on genetic variability at the DNA level. The data was analyzed by several statistical approaches all of which revealed the same result that weevil populations from the interior of B.C. have greater genetic similarity to populations feeding on Jack Pine in eastern Canada than do B.C. interior weevil populations to those feeding on Sitka spruce in coastal B.C. This information is relevant in the effort to breed for weevil resistance in spruce.

POPULATION GENETICS OF *ENDOCRONARTIUM*:

LiJuan Sun, Bart Van der Kamp, Eleanor White and John Carlson

We have used RAPD markers and RFLPs to assess the extent of variability at the DNA level, the existence of races, the mode of inheritance (life cycle), and the course of the spread of infection for the western gall rust fungus *Endocronartium harknessii.* Spores from individual galls were sampled from forest stands across the province. Preliminary results clearly indicate that western gall rust is not uniform in British Columbia but rather that variability exists both within and between populations. This variability suggests either the presence of a sexual stage in the life cycle of western gall rust or that substantial somatic recombination events occur. Further studies based on inoculations of seedlings with spores from single galls and multiyear sampling schemes are underway to determine how this variability has been generated.

PATERNITY ANALYSIS IN A WHITE SPRUCE OPERATIONAL SEED ORCHARD USING RAPD MARKERS

Yong-Bi Fu, Gene Namkoong and John E. Carlson

Paternity analysis with genetic markers (such as isozymes) has been an important tool for understanding how the magnitude of gene dispersal by pollen influences mating system and the composition of seed crops, and provided critical information for seed orchard management. However, the power of this analysis is limited because of the lack of sufficient genetic markers. Random Amplified Polymorphic DNAs (RAPDs) are a new class of genetic markers with great advantages in terms of unlimited number of loci and easiness in assay. In this investigation, we explore the potential of using RAPDs in paternity analysis. DNA fingerprints for all 95 trees in an operational interior spruce seed production orchard were developed using RAPD marker (accomplished earlier by post-doctoral fellows Yong-Pyo Hong and Robin Davidson). The advantage of DNA fingerprinting was demonstrated in that all clones could be uniquely identified and their gametes followed, as opposed to fingerprinting with isozymes in which rare alleles permit the gametes of only a select few trees in the orchard to be followed. For application of the DNA fingerprints in paternity analysis, we first examined the effectiveness of using RAPDs to evaluate the success of supplemental mass pollination, a widely used technique in seed orchard management. We genotyped seeds of eight maternal trees from 4 and 12 mixed pollen donors in an interior spruce seed orchard, as well as maternal and 12 known paternal trees. By implementing a maximum likelihood method, we aim at determining with more confidence the proportion of seeds of a maternal tree from each of 12 mixed pollen donors. Secondly, we will determine the number of offspring per parental trees using paternity analysis with DNA fingerprints to estimate the effective population size in the seed orchard. The estimate of the effective population size can be made if the number of offspring per parental trees is known. This provides scientific evaluation of effective population size of 20 as presently accepted by the BC Forest Service. We have also recently shown that the RAPD marker system is suitable for DNA fingerprinting and paternity analysis in western hemlock seed orchards. We studied competition among two hemlock pollen sources, and confirmed that fertilization was primarily accomplished by only one superior pollen source, regardless of any timing advantage given to the less viable source. This was assessed using the DNA fingerprints for 6 parental clones. A computer-assisted system was set up during the hemlock seed orchard project for automating the analysis of DNA banding patterns on electrophoretograms for DNA fingerprinting. This project is being conducted in collaboration with Joe Webber and Mike Stoehr of the B.C. Ministry of Forests and Ben Sutton of B.C. Research, Inc.

GENETIC LINKAGE MAPS FOR DOUGLAS-FIR AND MARKERS FOR QTLS:

Hesham Agrema, Jason Broome, and John Carlson

We have completed an NSERC strategic research project to construct genetic linkage maps for Douglas-fir with RAPD markers. Two "single-tree" linkage maps were constructed for parent trees DF60 and DF69 from the B.C. Ministry of Forests coastal Douglas-fir tree improvement program. We also constructed a genetic linkage map for the F_1 progeny from cross of DF60 and DF69, using markers from the maps of parent trees DF60 and DF69. With the F_1 linkage map we were able to identify several QTLs for wood density, volume and height. The phenotypic data were collected by B.C. Ministry of Forests staff during 15 years of progeny trials. Our success in mapping QTLs in full-sib progeny from two elite clones of similar provenance encouraged us to conduct studies for mapping further Quantitative Trait Loci governing important traits in Douglas-fir, and with other important families of coastal Douglas-fir. We pioneered the approach of constructing genetic linkage maps for single-trees using haploid megagametophyte DNA. To increase access to the RAPD technology, we have distributed 700 RAPD primers, at cost, to over 200 research groups around the world. DNA markers permit the early selection of genetically superior seedlings. After a DNA marker has been found that is associated with a given trait, it's use in indirect selection can greatly accelerate breeding programs and ensures that the best genotypes are deployed from seed orchard and nursery production programs. The QTL markers we detected for wood density in Douglas-fir are presently being evaluated in additional families and progeny tests. This project is being conducted in collaboration with Jack Woods and colleagues of the B.C. Ministry of Forests.

GENETIC LINKAGE MAPS FOR WHITE SPRUCE AND DNA MARKERS FOR WEEVIL RESISTANCE

Yong-Pyo Hong, Jeff Glaubitz, and John Carlson

Genetic linkage maps were constructed with RAPD markers for four parent trees in the interior spruce tree improvement program, in collaboration with Gyula Kiss at the B.C. Ministry of Forests Kalamalka Research Station, utilizing megagametophyte DNA and our "single-tree" mapping approach. We have searched for DNA markers from these maps linked to weevil resistance. Destruction of spruce trees by the terminal weevil (*Pissodes strobi*) causes massive loss of spruce trees in B.C. forests, both coastal and interior. We have identified several DNA markers that appear to be associated with weevil resistance based upon preliminary tests with half-sib progeny families in the spruce tree improvement program. Large scale tests with these putative weevil-resistance markers are now underway with over 700 half-sib progeny that have been well characterized for weevil susceptibility and resistance. We also hope to integrate weevil resistance markers into the spruce clonal propagation program of BC Research, Inc.

MOLECULAR KARYOTYPES FOR SPRUCE AND DOUGLAS-FIR

Garth Brown, Vindhya Amarasinghe and John Carlson

We developed a fluorescence *in situ* hybridization (FISH) technique for physically mapping cloned DNA on conifer root-tip chromosomes. With FISH, we have localized the major ribosomal DNA (rDNA) loci to 14 chromosome sites (nucleolar organizers) in white spruce - one rDNA site on each of 7 separate chromosome pairs ($2n = 24$), 12 chromosome sites in Sitka spruce and at least 8 chromosomes in Douglas-fir. We have also mapped two loci for 5S RNA in Douglas-fir, white spruce, and Sitka spruce and a centromeric repeat DNA to several white spruce, and Sitka spruce chromosomes. With these probes, all 24 white spruce and Sitka spruce chromosomes and all 26 Douglas-fir chromosomes can be unambiguously identified in root tip cytological preparations without recourse to morphological characters. This capability should be useful in characterizing spruce hybrids. We are also interested in using FISH to study the organization of the conifer nucleus and for correlating genetic linkage maps with metaphase chromosomes. This requires a FISH protocol with increased sensitivity to permit the reliable mapping of single copy genes on conifer chromosomes, on which we are presently working.

ENGINEERING OF SPECIALTY GENOTYPES OF HYBRID POPLAR AND SPRUCE

P. Dharmawardhana, Monica Schmidt, Madoka Gray-Mitsumune and John Carlson

Plantation forestry provides the opportunity for large scale deployment of "clones" derived from tissue culture that have been engineered for traits necessary to achieve maximum productivity. We have established protocols for genetic engineering of hybrid poplar, Douglas-fir and interior spruce. There are numerous traits that can only be obtained in tree species through gene transfer. At this time, we are focusing on modification of the lignin biosynthesis pathway and uptake of heavy metals. We are collaborating with Brian Ellis in the Plant Science Department and Carl Douglas in the Botany Department at UBC on an NSERC Strategic Research Grant project with a long term target of modifying lignin content in hybrid poplar and conifers. Through this research we hope to learn enough about the genes controlling lignin synthesis and the biochemical process of lignification during wood development to devise a strategy for breeding or genetically engineering trees with altered lignin composition. This research might lead to hybrid poplar more suitable for lumber production by an engineered increase in wood density or in spruce with altered lignin composition requiring less or no chemical bleaching during pulp production. We are also interested in transferring genes for heavy metal tolerance and uptake into poplar so that hybrid poplar could be grown on contaminated sites which would accomplish both remediation of those sites and the production of a tree crop. Such research should also lead to poplar hybrids that can accommodate the build up of heavy metals in sludge treated soils.

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YELLOW-CEDAR, WESTERN REDCEDAR AND PACIFIC YEW GENETIC RESEARCH, CONSERVATION AND IMPROVEMENT

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YELLOW-CEDAR PROVENANCE RESEARCH

Preliminary results from 5 year data from the yellow-cedar range-wide provenance study have shown minimal growth and maladaptation differences related to seed-source elevation among coastal British Columbia populations, when grown at low elevation (<200 m). This has prompted a revision to seed transfer guidelines, with an unlimited downward transfer of seed sources. Frost-hardiness of seed sources when grown at high elevation sites also appears to be unrelated to elevation of source-origin, however, this will need to be further evaluated.

YELLOW-CEDAR BREEDING

Twelve partial diallels from wild-stand phenotypic selections are completed, and the first series of cloned progeny tests have been sown. Four tests will be out-planted in 1997 with one-year-old rooted cuttings from 96 full-sibs (6 diallels), represented by 20 clones per family and 3 ramets per clone per site. The next series will be outplanted in 1998. Twelve more diallels are currently being completed.

REDCEDAR GENETIC STUDIES

Preliminary data from various redcedar genetic studies (Ministry of Forests, University of British Columbia, B.C. Research, Pacific Forest Products) have shown that genetic variation in morphological, physiological and molecular traits exists contrary to earlier isozyme results, and that some of the variation is adaptive. As well, there is evidence of high selfing rate in seed orchards, minimal inbreeding depression in flowering, seed and early growth traits, and delayed inbreeding depression in growth upon out-planting.

PACIFIC YEW GENE CONSERVATION

Five hundred clones from throughout the range of Pacific yew in British Columbia have been planted in the gene archives at Cowichan Lake Research Station.

PROGRESS REPORT FOR CANADIAN TREE IMPROVEMENT ASSOCIATION

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My work involves research direction of the Forest biotechnology Centre. About two thirds of the activities of the Centre are directed towards tree improvement-related technologies. These include: Somatic embryogenesis of conifer species, molecular genetics and physiological genetics. The principal scientists in these areas of research are Dr. David Cyr and Ms. Fiona Webster in somatic embryogenesis, Dr. Craig Newton in molecular genetics and Dr.ÊSteven Grossnickle in ecophysiology.

SOMATIC EMBRYOGENESIS RESEARCH AND DEVELOPMENT

Somatic embryogenesis (SE) work over the past two years has been focused on three principal areas: 1). Large scale establishment and testing of embryogenic clones for the interior spruce tree improvement program in British Columbia (B.C.). 2). Scale up and operational implementation of SE production systems. 3). Application of SE to Sitka spruce weevil resistant families. Progress in each of these is summarized below.

Development of a clonal selection program for interior spruce using somatic embryogenesis

This work was initiated to apply SE to the interior spruce breeding program by establishing a clone bank and deploying plants (somatic seedlings) for clonal selection trials, and in parallel, to address the limitations of this technology. The limitations include: a significant influence of genotype on the success of the process and the need to define and exploit clonal variation for accelerated selection. This follows a project in which 263 genotypes were established from 12 families between 1991 and 1992, 200 of these were delivered to the nursery in 1993 and planted in the field in 1994 and 1995. The strategy for clonal selection using SE has been outlined in a 1993 publication involving BCRI and the B.C. Ministry of Forests (Sutton et al. 1993).

Over the last two years establishment, storage and deployment of embryogenic lines from a range of elite weevil-resistant crosses of interior spruce (Prince George selection unit, Kalamalka arboretum) have been pursued intensively. To date, 656 genotypes from 18 families have been successfully produced and stored in cryopreservation. Approximately 50% of these genotypes, without cultural improvements, produce suitable numbers of embryos for somatic seedling production. For the first round of clonal selection field trials from these crosses (spring 1996), 320 lines from 11 families were delivered to the nursery in March 1995 in sufficient number to be deployed on two to three field sites. BCMoF personnel will subsequently deploy these somatic seedlings in the field trials (Chris Hawkins, Red Rock Res. Stn. B.C.). In the next two years we anticipate bringing the total number of genotypes deployed from these families to 1,000. This will allow selection of the top 5% of the clones within five or six years, based on weevil resistance and growth.

Significant progress has been made on the other objectives of the project including the development of methods to improve induction from recalcitrant families. For maturation, a protocol for improving performance of poor clonal lines has been developed and was assessed using 100 genotypes from the 1994-95 embling production run for clonal selection trials. Selected physiological studies of genotypic variation between clones were initiated during the summer of 1994 and are still in progress. Emphasis has been placed on 6 clones representing high, medium and low growth-ranked families. Preliminary results from these studies indicate very significant clonal differences with respect to seasonal water relations, photosynthetic and transpiration rates, and instantaneous water use efficiency.

Somatic Embryogenesis Development for Weevil Resistant Sitka Spruce

Reforestation with Sitka spruce in B.C. has declined dramatically, largely because of the risk of severe damage of young stands by terminal weevil (*Pissodes strobi*). SE technology represents a significant opportunity to multiply weevil resistant material rapidly and also to select and maintain individual progeny in a juvenile state. During 1991 and 1992, B.C. Research delivered a total of 8,500 seedlings of Sitka spruce from SE. This work showed that, while deployment of plants was possible, SE of this species required further development for routine use. In view of this, a development project was initiated in 1994 with the goal of improving the induction and stabilization of embryogenic cultures, improving embryo production and establishing lines (genotypes) from resistant families for field testing and subsequent operational production.

We have used seed for induction of embryogenic clones from 9 open pollinated families and 3 provenance sources which have substantially increased resistant to weevil. Research on this material indicated that media formulation had a significant impact on induction frequencies. Additional promotive factors have been identified. With the best treatments, initial induction frequencies approached those presently achieved for interior spruce, with values for 8 sources ranging from 23 to 52 %. Currently, approximately 200 genotypes from the 12 sources have been produced and cryopreservation is being completed. BCRI anticipates deploying more extensive trials of somatic seedlings from weevil resistant open pollinated families in 1996 and 1997. This will result in planting stock with an average 30% increase in weevil resistance (based on progeny test results). Selection of individual lines and deployment of full sib families (not currently available) will result in further improvement in next few years.

Large scale production systems for somatic embryogenesis

In order to use SE in operational reforestation, production volumes in the hundred's of thousands to millions, must be achieved. For this reason we have engaged in an intensive effort to implement rapid multiplication of somatic embryos, bulk handling systems and efficient nursery delivery. This has resulted in a system which can be used to produce several hundred thousand embryos, dry them and germinate them in bulk without the need for individual handling. Following bulk germination the small germinants are transferred to nursery containers. Development of the somatic seedlings, while somewhat slower immediately following transplant, allows the production of good quality planting stock.
Using this system approximately 30,000 plants were produced in 1994 and a further 100,000 in 1995. These production runs incorporated about 40 clones from top ranked families of the interior spruce breeding program (Prince George selection unit). Field trials have been deployed by the Ministry of Forests, Fletcher Challenge Canada and Northwood Pulp and Timber in the central interior of B.C..

In the last year we have published data from earlier nursery production and field trials in which somatic seedlings were compared with genetically related seedlings. This data showed that the somatic seedlings reached all stock quality requirements for reforestation and had survival and growth increments in the field which were similar to the seedlings (Grossnickle et al. 1994; Grossnickle and Major 1994a; Grossnickle and Major 1994b).

MOLECULAR GENETICS RESEARCH AND PHYSIOLOGICAL GENETICS

The primary focus of molecular genetics research has been the development of genetic markers for specific applications in tree improvement and seed orchard management. These have included pure population genetics studies in which information about population structure is required in order to provide a basis for designing breeding units and defining seed transfer guidelines. In addition, an intensive effort has been made to identify and develop highly polymorphic markers which can be used reliably in distinguishing seed orchard parents. The latter enables one to measure pollen contributions, pollen contamination and selfing.

Development and application of highly polymorphic markers

Two types of markers have been developed and applied over the past two years: 1). Hyperpolymorphic DNA probes derived from the nuclear genome. 2). PCR primers which are used to detect polymorphisms in the chloroplast genome.

An extensive screening of regions of the spruce genome led to identification and cloning of a highly polymorphic region. This can be used as a probe to generate fingerprints for resolving individual spruce trees and for detecting contributions of unique alleles to the progeny in seed orchards. This probe has recently been used in a study of genetic diversity (with Dr. Michael Stoehr, Ministry of Forests B.C.). The objective of this study was to determine the influence of seed orchard production and reforestation on genetic diversity in interior spruce. For this purpose three stands from each of two watersheds were sampled to represent the native forest. As a comparison seed orchard parents, seed derived from this orchard and a plantation planted with seedlings grown from the orchard seed, were also sampled. The data set consists of DNA fingerprints for a total of about 600 trees. Final analysis of the results of this study will be completed in the near future.

Using an analogous region to that identified as polymorphic in spruce, a study was initiated with western red cedar. In this case, an important issue was to determine the rates of selfing in individual red cedar families. Conventional approaches using isozymes had proved somewhat challenging since very little polymorphism was detected in this species. Using the highly polymorphic region (not homologous with the equivalent region from spruce) it is possible to detect relatively high levels of polymorphism and to use this to estimate selfing. This work is continuing with the Ministry of Forests in association with Dr. John Russell (breeder for western red cedar).

Chloroplast polymorphism has been exploited specifically for studying seed orchard management issues. A report a few years ago by Hipkins and Strauss demonstrated that significant polymorphism exists in a specific region of the Douglas-fir chloroplast genome. We developed PCR primers which amplify this region and used them in a series of tests with an experimental (2nd generation) meadow orchard at Saanich B.C.. Pollen contributions from within the orchard and from outside the orchard were measured. Rates of selfing of the individual parents were also determined. One example of a management practice, supplemental mass pollination (SMP), was studied; in this case the effect of flowering phenology on the receptivity individual clones at the time of SMP was evident. The principal advantages of this method over those available previously is the ability to distinguish most individual trees unambiguously (70%) under operational conditions; this results from the high degree of polymorphism and the uniparental inheritance. Our recent results suggest that, using the same generalized approach, it should be possible to achieve similar, or higher, levels of discrimination in a routine manner with a range of the commercial conifer species.

Genetic and physiological analysis of Sitka / interior spruce hybrids

We recently published a paper showing that ribosomal DNA could be used to determine the extent of hybridization between Sitka and interior spruce . This information is expressed as an index (Si rDNA) which reflects the relative abundance of the ribosomal DNA banding patterns specific to each species. When applied in a survey of spruce in northwestern B.C. a gradation of introgression was found which followed longitudinal and topographical trends (Sutton et al. 1994).

Another study has been conducted in 1993 and early 1994 to determine the relationship between the index (Si rDNA) of a range of hybrids and physiological traits of adaptive significance (Grossnickle et al. 1995). The objective was to determine the physiological significance of hybrid make-up as determined by DNA markers and to use this information in defining the tolerance limits for moving hybrids within the introgression zone. Seed sources ranging from pure Sitka spruce to interior spruce, as well as intermediate types, were used in the study. Clear relationships with the DNA index could be shown for frost hardiness, drought tolerance and gas exchange characteristics. As might be expected, interior spruce types were considerably more hardy with respect to drought and frost but had lower photosynthetic rates under optimal conditions. However, when seedlings were soil-droughted, interior spruce populations maintained higher net photosynthetic rates due to their inherent drought tolerance. We are optimistic that such information can be used to deploy spruce hybrids in an optimal fashion for plantation forestry.

CONCLUDING REMARKS

Studies are continuing in the evaluation of clonal material derived from SE and we are linking up with other researchers using this material. We have observed, as might be predicted, that variation within clones is substantially less than within seedlots or families. It appears there is considerable potential for using embryogenic clones for a range of research studies which can then be integrated to provide a much more precise understanding of plant function and the role of genetic variation. Such studies should lead to a better fundamental understanding of spruce physiology as well as adding to the selection criteria which can be used in the breeding program.

Finally, B.C. Research Inc. gratefully acknowledges the support of the B.C. Ministry of Forests, the National Research Council of Canada (collaborative work with the Plant Biotechnology Institute and direct funding), the Science Council of British Columbia, and industrial clients including: Pelton Reforestation, Western Forest Products, Canadian Forest Products, Pacific Forest Products, MacMillan Bloedel, International Forest Products, Northwood Pulp and Timber and Fletcher Challenge Canada, who supported work in one way or another over the years.

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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.

SEED PRODUCTION RESEARCH

Restructuring of our seed production research program began in 1992 and is now fully implemented. We have three main components to this program. Acquiring basic knowledge of the reproductive biology of all tree improvement species including studies on cone induction, pollen management, supplemental pollination technique, and seed and cone maturation. This information forms the basis for seed orchard management research which is currently emphasizing the genetic quality of orchard production in mature orchards and cultural techniques for the establishment of new orchards (i.e., amabilis fir). The third component of our research is wild stand production, including studies on the factors affecting natural reproduction in wild stands and successful regeneration in partial cuts. Included in all of these three research areas are new studies on genetic diversity from seed orchards, plantations and natural stands.

Clonal Row Douglas-fir Orchard

A Douglas-fir (*Pseudotsuga menziesii)* micro orchard was established in 1991 with the long term objective of determining area based yields and genetic quality of seed produced from parent trees arranged in clonal rows and maintained under 2 crown pruning regimes (tree height kept at 2 and 3 meters). Two of the four blocks were first induced in 1994 using $GA_{4/7}$ only and the remaining two blocks were treated in 1995 (girdling plus $GA_{4/7}$) as part of our biennial induction scheme. The 1995 flowering response to the 1994 induction produced an average of about 20 seed cones and 200 pollen cones per tree. We found no significant flowering response to three dosages levels of $GA_{4/7}$ but variation due to clones was significant.

The evaluation of supplemental mass pollination (SMP) efficacy was enhanced by our ability to determine the male parent of a Douglas-fir embryo or seedling using DNA techniques. The pollen parents are identified by amplifying a hypervariable region of the embryo chloroplast DNA using the Polymerase Chain Reaction (PCR). By pre-screening potential pollen donors for a SMP polymix, easily identifiable male parents can be selected. In addition, if all orchard clones are genotyped this way, estimates of outside orchard pollen contamination can also be obtained. In this study, six clones out of 20 growing in the clonal row orchard received an SMP treatment by directly applying pollen to unbagged individual female strobili. SMP efficacy ranged from 40% to 74% depending on the female parent. Outside orchard pollen contamination was reduced to 9%, a reduction of 9% from untreated, open-pollinated controls (18% outside contamination on average).

To test the hypothesis that natural selfing may be higher in this hedged, clonal row orchards, we used the same genetic marker as described above to determine the paternal parent in open-pollinated seeds collected from individual clones of this orchard. If the hypervariable chloroplast DNA fragment in the embryo is of the same size as the hypervariable chloroplast DNA fragment of the associated megagametophyte, then the embryo is a self. In a study of approximately 86 seeds (on average) from each of six open-pollinated families, natural selfing rates ranged from 0 % to 18%, with an average of 5.5%. Thus, clonal rows and hedged crowns do not seem to contribute to natural selfing in this Douglas-fir clonal row orchard. Both the SMP efficacy and selfing study have been carried out in cooperation with BC Research Inc., Vancouver.

Container Seed Orchard Research

The advantages of a container approach to seed production is fully appreciated but not yet realized in interior spruce. Experience to date with white/engelmann spruce container orchards at Cobble Hill and Skimikin Seed Orchards has shown consistent flowering (pollen and seed cones) resulting from the induction protocol but yields have seldom exceeded 10-15 filled seed per cone (FSPC). In spite of several attempts to improve pollen quality and pollination technique, yields have remained disappointingly low. In the past two years, we have focused on the pre- and post-pollination environmental (temperature) conditions the grafts (in particularly the root) were exposed to. In general, exposing the developing cones to constant temperatures of 24°C during the pre-zygotic cone development results in significantly fewer seeds than lower temperatures. However, even the best yields did not exceed 25-30 FSPC. Last year, we concentrated on pre-pollination conditions and attempted to protect the pots (root development) from direct exposure to sun. The spring of 1994 was relatively cool and seed yields were considerably better (average about 40-45 filled seed per cone). However, comparable yields at the Cobble Hill Container Orchard were still low (12 filled seed per cone). We will continue to use the container approach for seed production research but the operational pilot program has been disbanded.

Pollen Management Manuals

A manual (Webber and Painter 1995) describing the *ex situ* handling and application of Douglas-fir pollen is now in print. This manual details the procedures for collecting, drying, extracting storing, testing and reapplying pollen to meet specific orchard management objectives. A similar manual has been distributed for interior spruce (Webber 1991) and a seed production manual for western hemlock, emphasizing both pollen handling and techniques for crown management and cone induction in both soil-based and container stock, is in preparation.

Western Larch Flower Induction

Seed orchards of western larch (*Larix occidentalis*) have been established but significant production will not be available for a few more years. We are taking two approaches for enhancing western larch seed production. First we are investigating various seed orchard flower induction techniques. Results from a 1994 $GA_{4/7}$ by girdling trial showed that $GA_{4/7}$ alone resulted in a small increase in both seed and pollen cone production but the single treatment of girdling was significantly better and a combination treatment of $GA_{4/7}$ and girdling was best. Timing of these treatments was also significant. Treatments applied at long-shoot (LS) bud flush were significantly better than when applied at about 80% elongation of LS. Our second approach to increasing western larch seed production is to test the feasibility of inducing natural stands. A young natural stand of western larch (12-15-years-old) growing near Invermere, BC was girdled in 1994 but no flowering occurred. A second girdling treatment has been re-applied to the same trees and in 1996 we will compare flowering response to single and repeated girdling.

We are also monitoring pollen cloud density and meteorological factors to help explain crop periodicity, cone abortion and cone yields.

Abies amabilis and *Abies lasiocarpa* Seed Production

Harvesting activity in the mid- to high-elevation areas has increased in the last few years and so has interest in seed procurement of amabilis and sub-alpine fir. Established amabilis fir seed orchards have yet to produce any significant seed (they have produced considerable pollen crops, however). While the grafts seem to be sexually mature, they lack vigour and crown development and are unresponsive to the few induction studies attempted. It is our hypothesis that early cultural treatments of the grafts as well as the warm, dry Saanich peninsula orchard sites were not conducive to establishing vigorous crown development. Cultural studies have begun with the objectives of determining the barriers to establishing vigorous crowns in amabilis fir. This material will also be used for future flower induction studies. We will begin similar studies for sub-alpine fir in 1995.

Pollen Contamination Effects

The long-term effects of out-side seed orchard pollen contamination on growth rate, phenology, growth rhythm and ultimately, survival are being studied in coastal-interior transition Douglas-fir (*Pseudotsuga menziesii*). We initiated a trial that compared seedlings sired by outside orchard (coastal) pollen with seedlings sired by within-orchard pollen. Nursery results indicated that seedlings from the contaminating pollen sources grew significantly faster in a coastal climate (Stoehr et al. 1994). To further evaluate their performance in the field, plantations were established on a coastal, an interior and a transition zone site. Parameters evaluated will be bud flushing, growth differences (early growth, time and amount of maximum growth rate, growth cessation) and survival.

Genetic Diversity and Tree Improvement

We also investigated the effects of domestication on the genetic diversity of interior spruce (*Picea glauca x engelmannii*). The genetic diversity of a total of 360 trees from nine natural stands representing three watersheds in the Shuswap-Adams low elevation breeding zone (SAL) was measured using isozymes. Simultaneously, the 100 clones of the SAL seed orchard were also evaluated. Finally, 120 seeds from a bulked seed lot of the SAL orchard and 120 seedlings raised from the same seed lot and growing in a 2-year- old plantation were also evaluated. Results indicated that the orchard is genetically very similar to the natural stands. The seed lot was genetically very diverse, however, there were some alleles present that were the result of outside-orchard pollen contamination. The diversity in the plantation was still high, but suggests some unintentional directional selection in the nursery. A follow-up to this study will be done on families collected in the orchard and natural stands to assess variation levels in quantitative traits in a common garden study.

Other studies, such as pollen monitoring and the rating of orchard seed lots (Xie et al. 1994), effects of crown pruning (Stoehr et al. 1995) and identification of constraints to seed production in alternate silvicultural systems with western larch as seed tree stands and shelterwoods are ongoing.

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COASTAL DOUGLAS-FIR BREEDING AND GENETIC RESEARCH

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Keywords: Douglas-fir, breeding, advanced generation, inbreeding, selection, genecology.

BACKGROUND

The coastal Douglas-fir breeding program is directed primarily at sites from 0 to 700m elevation west of the coast mountains and south of 51 degrees latitude in coastal B. C. First-generation selection and breeding was completed in the 1960's and early 70's for this zone, and testing proceeded with both diallel and open-pollinated material. Second-generation selections began in 1991.

SECOND-GENERATION BREEDING

Selection for a second-generation breeding population is currently about 80 percent complete. Propagation and early cone induction is a priority. Both potted and soil-based ramets are being used for breeding as we learn more about how to handle grafts from young Douglas-fir trees, and how to induce flowering within 2 to 3 years of grafting. Induction methods used are principally stem injections of $GA_{4/7}$ in combination with either girdling or root pruning.

The breeding strategy entails a complimentary mating design, with a polymix used for GCA testing all clones. The population is divided into small sublines of from 12 to 20 clones, and full-sib mating within sublines is being done so each clone is included in at least two crosses. Inbreeding is avoided in this generation and there is no coancestry between sublines. Breeding is complete for the first three sublines, and nearly complete for an additional five sublines.

REALIZED-GAIN TRIALS

Linking genetic gain predictions with growth and yield information systems is needed when improved material is being extensively used operationally. Realized-gain trails will provide the data to 1. link stem-volume breeding values predicted from progeny test data with actual area-based yield gains, 2. evaluate breeding and selection strategies in a more operational setting, 3. modify growth models (TASS) to account for predicted genetic gain, and 4. demonstrate genetic gains.

A first series of Douglas-fir realized-gain trials was established in 1992 with four genetic levels and a single stand density. Early results are promising, and show height gains to be close to those predicted. Seed from non-tested first generation orchards is showing early height gains of about seven percent over natural-stand seed. A second series of trials will be planted in the spring of 1996 with three genetic levels, four stand densities and three levels of site index.

SUPPORT RESEARCH

Research in support of the breeding program continues, including low-level inbreeding effects, early selection, wood density evaluation and cooperative work on frost and drought hardiness. In addition, testing to evaluate geographic patterns of genetic diversity in the coast-interior transition zone is progressing with a field project and a cooperative project with the University of B. C. (Francisco Luna, Dr. Mathew Koshy and Dr. Gene Namkoong). The UBC work will investigate patterns of early growth, frost hardiness and water-use efficiency.

PROVENANCE RESEARCH

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One major initiation in the past two years was the assessment of pest damage at lodgepole pine provenance trials. We have assessed test plantations at 59 locations throughout interior British Columbia and extending into southern Yukon Territory. The main objectives are to develop predictive models for the assessment of pest hazard of planting sites, and to study patterns of provenance variation in pest tolerance as a supplement to seed transfer guidelines and selection of resistant seed sources for planting and tree improvement.

Our assessments focus on stem rusts, needle diseases, pine terminal weevil and rodent damage, the most common above-ground pests plaguing plantation lodgepole pine. We have nearly completed the field assessment and comprehensive analyses of the data are in progress. Large site differences and provenance variation were evident. Particularly interesting is the high resistance to stem rust, western gall rust in particular, of provenances from the lodgepole and jack pine contact zone. Degree of resistance, expressed as number of infection sites, showed positive correlation with the distance of provenance origin to the boundary of the contact zone. We advance the hypothesis that jack pine introgression may be evolutionarily related to the resistance of lodgepole pine to western gall rust. High resistance of these provenances from the hybridization zone is unequivocal - stable across diverse site environment. Unraveling the genetic and physiological mechanism of resistance requires inter/multi-discipline approach.

Lodgepole pine is now the number one planting species, accounting for about 35% of the total planting in British Columbia. The pest information is not only of commercial value to tree improvement and integrated pest management, it also provides a valuable database for climate modeling to assess the impact of global warming on forest pests.

Because of the potential for short rotation and value-added products, hardwood species are becoming an important component in plantation silviculture. This has created opportunities for studying genetics of hardwood species. On the coast, we have started studies for black cottonwood, red alder, bigleaf maple and Sitka alder since 1992. We have completed the nursery testing and established three long-term field tests of red alder. Provenance sampling of black cottonwood is nearly completed, which covered all the major river drainages on the coast. Nursery testing and isoenzyme studies of Sitka alder are in progress. A study of bigleaf maple will be started this year. All the hardwood studies are cooperative involving scientists inside and outside B.C. Ministry of Forests. In about five years, we will have a solid knowledge base on seed transfer, seed source selection, and genetic potential in selection and breeding of coast hardwood species.

GENETICS AND TREE IMPROVEMENT RESEARCH AT THE UNIVERSITY OF ALBERTA

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Keywords: Molecular, population, evolutionary, and conservation genetics; molecular markers for disease resistance and other quantitative traits; genome mapping; speciation and biosystematics; chloroplast, mitochondrial and nuclear DNA; RAPD, RFLP and microsatellite DNA; isozymes; genetic implications of silvicultural management; tree breeding; mycorrhizae.

During the past two years, our research activities continued in the areas of molecular, population, evolutionary and conservation genetics, speciation, biosystematics, genetic implications of silvicultural management, and molecular markers for disease resistance and other quantitative traits, and genome mapping. We also initiated a project on genetic implications of silvicultural management of eastern white pine (*Pinus strobus* L.) in Ontario, and on ectomycorrhizal inoculation of conifer seedlings.

Since the last report, two graduate students completed their degree programs in forest genetics.

MOLECULAR, POPULATION AND EVOLUTIONARY GENETICS, SPECIATION, AND BIOSYSTEMATICS

Isozyme studies

Data analysis has been completed and manuscript preparation is in progress for a (Rajora and Dancik) study completed earlier on population genetic structure, variation and evolution of Engelmann spruce (*Picea engelmannii* Parry), white spruce (*P. glauca* (Moench) Voss) and their putative natural hybrid complex in Alberta. Phambu. D. Khasa (Ph.D., Laval University), who joined us as a postdoctoral fellow in 1994, and Dancik have completed a genetic diversity study in two closely related tropical species *Racosperma auriculiforme* (Cunn. ex Benth.) Pedley and *R. magnum* (Willd.) Pedley using multivariate analysis of allozymes and morphometric traits. *Populus* isozyme studies with Dr. Burton V. Barnes, University of Michigan, continue.

Molecular Genetics

Om Rajora published his studies on intraspecific and interspecific chloroplast (cp) DNA variation and genome relationships of *Populus* species and hybrids. He also completed examination of variation in cpDNA and nuclear genes in trembling aspen (*P. tremuloides* Michx.) in Alberta, and RAPD variation of *P.* x *canadensis* cultivars. Rajora, with the assistance of research assistant Manjit Sekhon, completed a study on genetic diversity of white spruce populations in Saskatchewan. Khasa is studying the population genetic structure and evolution of the *P.*

engelmannii-P. glauca complex using RAPD analysis and has identified species-specific RAPD markers. Khasa and Dancik have also started a study on population genetic diversity, breeding systems in *Larix lyallii* Parl. using microsatellite DNA analysis, in collaboration with Barry Jaquish of the B.C. Ministry of Forests.

Variation and differentiation

Barbara Thomas is completing a Ph.D. study, under the supervision of Ellen Macdonald and Bruce Dancik, on variation and differentiation of trembling aspen in Alberta. She studied morphology and gas exchange traits. Rob Wright completed a Ph.D. study on the genecology of seedling root growth, stomatal frequency and seed mass in jack pine (*Pinus banksiana* Lamb.) under the supervision of Ross Wein and Bruce Dancik. Christine Hansen has completed an M.Sc. study on genetic variation in early field performance of white spruce in central Alberta. Leonard Barnhardt is completing his M.Sc. study on early performance of Douglas fir (*Psuedotsuga menziesii* (Mirb.) Franco) on five ecologically diverse sites in southwest Alberta.

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

Rajora, and Dancik, with the assistance of research associate Faten Kamel-Aly and research assistant Renato Vitic, completed the study on trembling aspen decay and stain and genetic fingerprinting of clones. DNA variation related to wood decay resistance in aspen has been identified, and data analysis and manuscripts preparations are in progress. We have also developed and initiated a project on molecular markers for quantitative traits and genome mapping in aspen.

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 progressed well. Om Rajora, with the assistance of Renato Vitic, has identified about a dozen potential DNA markers for WGR resistance. F_1 hybrid seeds from the reciprocal controlled crosses within between WGR-resistance and WGR-susceptible genotypes have been collected, and are ready for raising F_1 progeny for genetic linkage mapping analysis.

GENETIC IMPLICATIONS OF SILVICULTURAL MANAGEMENT, AND GENE CONSERVATION

Rajora and Dancik, with the assistance of Manjit Sekhon, partially completed the study on determining the impacts of silvicultural practices on genetic diversity in white spruce using RAPD analysis, and the study is in progress using RFLP analysis. Heather Cobban began a M.Sc. study to examine genetic diversity in white spruce artificial regeneration vs. natural stands using microsatellite DNA analysis.

Rajora, George P. Buchert of the Ontario Forest Research Institute and Dancik conducted a project on genetic implications of silvicultural management of eastern white pine in Ontario. The study was initiated by Buchert and has been conducted by Rajora in partnership with BioGenetica Inc. Rajora has completed the genetic diversity analysis of the preharvest and postharvest and the mating system analysis of preharvest old-growth populations of white pine using allozymes. The data analysis and the first manuscript from the study has been completed. We continue the study by examining genetic diversity of preharvest and postharvest populations and natural regeneration using microsatellite DNA, RAPD and RFLP analyses.

As an expansion of the on-going white spruce study in Saskatchewan, Rajora initiated a project on determining genetic diversity of white spruce populations from ecologically-distinct sites in the Prince Albert Model Forest to develop ecosystem gene inventories and to assist gene conservation strategies.

TREE IMPROVEMENT

Rajora maintained collaboration with the Western Boreal Aspen Cooperative regarding the trembling aspen improvement program. Dancik and Rajora taught a short-course in tree improvement to industrial foresters.

Ruichuan Zhang is continuing his Ph.D. program under the supervision of Dancik and Dick Pharis (U. of Calgary) on biochemical and genetic approaches to very early selection of *Pinus radiata*. Deogratias Rweyongeza began an M.Sc. study (supervised by Dancik and Francis Yeh) on assessment of growth performance of Scots pine (*Pinus sylvestris* L.) on three sites in Alberta.

MYCORRHIZAL RESEARCH

Khasa and Dancik have initiated a project on inoculation techniques for ectomycorrhizal development on conifer seedlings with Alberta Environmental Protection.

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TREE IMPROVEMENT PROGRAMME
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, aspen.

This report summarizes the progress of the Alberta Land and Forest Services (L.F.S.) genetics and tree improvement programme for the period 1993-1995.

PROGRAMME DEVELOPMENT

In order to provide additional resources for the growing programme needs and new projects, a new *revolving fund* function was started in 1994 at the Tree Improvement Centre to raise revenues to supplement regular tree improvement budgets. Programme development during the report period emphasized broadening partnerships with forest industry with particular attention to smaller forestry operators in the province. Three new industries (Manning Diversified Forest Products Ltd., Canadian Forest Products Ltd.- Hines Creek and Vanderwell Contractors (1971) Ltd.) joined the cooperative tree improvement program during 1994-95.

GENETIC IMPROVEMENT

Assembly of Breeding Stock

Field selection of superior parent trees to provide material for L.F.S. and L.F.S./Industry cooperative projects continued. Due to budget constraints and increased industry participation, L.F.S. selections were minimal including only two Scots pine (*Pinus sylvestris*) and two tamarack (*Larix laricina*). Selections made as part of the L.F.S./Industry projects included 131 white spruce (*Picea glauca*), 45 black spruce (*Picea mariana)*, 14 lodgepole pine (*Pinus contorta*) and two jack pine (*Pinus banksiana*). Industry participants in these cooperative projects included Alberta-Pacific Forest Industries, ANC Timber Ltd., Blue Ridge Lumber (1981) Ltd., Canadian Forest Products Ltd., Manning Diversified Forest Products, Millar Western Industries Ltd., Sunpine Forest Products Ltd., Weldwood of Canada Ltd., and Weyerhaeuser Canada Ltd.

Genetic Testing

White spruce progeny trials for breeding Region "D" were measured in 1993 at 10 years from seed. Eight- and 10-year heights were measured and plant vigour and white-pine weevil damage were assessed. Significant family differences were found for all traits. Heritabilities for 10-year height were 0.19 (individual) and 0.74 (family) and gains of 5% in height are predicted relative to the unselected population if the top 50% of families are selected. Progeny trial results will form the basis for the first roguing of the Region "D" seedling seed orchard.

Field testing of selected aspen (*Populus tremuloides*) clones was initiated in 1993. Aspen propagated by root suckers from 9 phenotypically superior clones and from 4 clones selected for divergent wood quality were established in two trials.

Field screening of lodgepole pine half-sib families for resistance to western gall rust (*Endocronartium harknessii*) continued. Of 126 inoculated families from breeding Region "C", 20 resistant, 13 intermediate and 45 susceptible families were outplanted in 1993 on a high infection field site to confirm field resistance and susceptibility. In 1993, 112 families from breeding Region "B1" were greenhouse inoculated and 18 resistant, 13 intermediate and 38 susceptible families were outplanted in 1994 on a high infection field site.

Lodgepole pine half-sib family tests for breeding Region "B2" were measured at age 6 in 1994. Mean survival for the two test plantations was 87% and 85%. Mean incidence of western gall rust was 25% and 1.6%, respectively and mean severity of infection was 0.72 and 0.05, respectively. Severity was assessed on an arbitrary scale of 0 (no WGR symptoms) to 6 (maximum infection as evidenced by multiple stem and branch galls). Mean height of the two plantations was 51 cm and 60.5 cm, respectively.

Two series of white spruce progeny tests were outplanted in 1994. The trials for breeding Region "E" are comprised of 64 open-pollinated half-sib families and those for breeding Region "H" are comprised of 54 families. Three field trials were planted in each of the two regions.

A field trial was established in 1994 in cooperation with B.C. Ministry of Forests to evaluate resistance of a set of lodgepole pine open-pollinated families and seed sources to porcupine and snowshoe hare damage. The test material contained in the trial originate from Alberta and B.C. seed sources found to be less susceptible to porcupine and snowshoe hare damage in a northern latitudinal provenance test series in British Columbia.

In 1995, stock was seeded for Siberian larch (*Larix siberica*) provenance/progeny trials that will be outplanted in 1996. The trials are comprised of 58 open-pollinated half-sib families from Charga, Southern Siberia. The seed was obtained from the National Forest Genetic Resources Centre, Petawawa National Forestry Institute.

Seed Orchards

The first improvement thinning of the Region "B1" seed orchard was completed in 1993. Thinning was based primarily on 11-year field performance results from four half-sib family field trials. In addition, 6-year performance results from the field trials and seed orchard performance results assessed in 1992, were taken into account.

The objective of the thinning was to remove one half of the poorest families in each set of 12 families. Accordingly, family sets were ranked by height, gall rust susceptibility and survival. A total of 8,146 trees were removed leaving 8,354 trees for seed production.

The Region "C" lodgepole pine seedling orchard consisting of 3,400 seedlings from 114 families was established at the Blue Ridge seed orchard site near Whitecourt in August 1994. Outplanting of the Region "D" white spruce clonal orchard at the Blue Ridge seed orchard commenced. These two projects are done cooperatively with Blue Ridge Lumber Ltd. The Region "G" white spruce clonal orchard located at Huallen was expanded in 1994 to meet industry's increased seed needs. This project is done cooperatively with Weyerhaeuser-Grande Prairie and Canadian Forest Products-Grande Prairie. Establishment of the Region "B2" lodgepole pine clonal seed orchard at Huallen commenced. This project is done cooperatively with Weyerhaeuser-Grande Prairie and Weldwood. The Region "F" Douglas-fir (*Pseudotsuga menziesii*) orchard and the Region "Z" western larch (*Larix occidentalis*) clonal seed orchards were established near Brooks. The site proved to be unfavourable and the orchards were relocated to a site at the Crop Diversification Centre-South, also near Brooks, during the spring of 1995.

Production of grafts for the Region "I" white spruce and the Region "B2" lodgepole pine projects is underway. Grafting is also being done for two new black spruce projects.

Work is being done on getting two additional seed orchard sites for new seed orchard developments. A site near Olds and a site near Fairview are being pursued.

The first year that any collectable amount of seed was produced in any seed orchard was 1993. Sufficient seed was collected to meet 2% of the 1994 reforestation requirements. White spruce did not flower in 1994. The only seed orchard crop collected was from the Region "B1" lodgepole pine seedling seed orchard at Huallen. From this orchard, 8.3 kg of seed was collected. A crop twice as large is projected for 1995.

In 1995 the Huallen Seed Orchard Cooperative was formed with all industries having a share in the seed orchards established at Huallen. Management and maintenance of the site has been contracted out to the Tree Improvement Centre - Commercial Partnership Operations.

TREE IMPROVEMENT RESEARCH

Species Testing

The species testing program has been ongoing since 1978. Results from numerous field trials throughout Alberta on various site types have identified Douglas-fir, Scots pine and Siberian larch as promising new forestry species in Alberta. Since the late 1980's species testing has concentrated on identifying appropriate site types and promising seed sources of native and exotic species for forestry use in Alberta.

Sixteen year assessments of two U.S.S.R. Scots pine seed source trials established in central Alberta (54 04' N, 112 12'W) and northern Alberta (59 08'N, 117 34'W) were completed. Survival at both sites was good, averaging 88% at the central Alberta site and 78% at the northern site. The best Scots pine seedlot in the central and northern Alberta test sites outperformed the local lodgepole pine seed sources by 21% and 12%, respectively. Mean heights were 6.1 m and 5.0 m, respectively, for the central and northern Alberta sites. This compares to 4.8 and 4.3 m, respectively, for the control lodgepole pine seed sources. Ten and twelve year results indicated that the top five performing seedlots at each site originated from latitudes similar to those of the test sites. This was also the case for the central Alberta site following the sixteen year assessment. At the northern site, however, this was no longer the case. Four of the top five seedlots at the northern site originated from latitudes 52°23'N to 55°42'N. A southern Siberian seedlot from Kustanaisk Krai, U.S.S.R. (52°80'N, 63°50'E) was ranked third at the central Alberta site and first at the northern site. The Scots pine plantation at central Alberta continues to suffer significant porcupine damage. The lodgepole pine control trees were left untouched.

In 1994 a Douglas-fir planting was established at Pine Ridge Forest Nursery with proven hardy northern seedlots. Test material was grown with seed collected from more northerly stands in Alberta and established in transplant beds for four years at Pine Ridge to screen out less winter hardy trees prior to establishing a field planting. Winter hardy seedlings were established in a replicated planting containing white spruce as a 'check'.

A comprehensive review of 21 Siberian larch demonstration and research plantings and 22 Siberian larch operational reforestation plantings is underway. Objectives of the review are to evaluate the suitability of Siberian larch for large scale reforestation projects; identify appropriate areas and site types in Alberta where Siberian larch can be used; and, develop appropriate reforestation policy regarding the use of exotic tree species.

Provenance Studies

Fifteen year height performance of a Canada range-wide white spruce provenance trial in central Alberta was assessed in 1993. Mean survival and height for the plantation were 80% and 242 cm, respectively. The top ten performing seed sources had mean survival and height of 87% and 298 cm, respectively. The top ten performers included seed sources from Saskatchewan, Manitoba, Ontario and Quebec. Mean survival and height of the two local seed sources were 90% and 258 cm, respectively.

To carry out more detailed field testing of the promising exotic seed sources identified in this trial, three white spruce provenance trials were established in 1993 and 1994. Two additional Canada range-wide seed source trials were also established in 1993 to supplement information gained from the central Alberta trial.

In the early fall of 1993, Interior Douglas-fir *(Pseudotsuga menziesii* var. *glauca)* provenance trials representing eight native Alberta sources established on five ecologically diverse sites were assessed for three year field survival, total height and plant health score. Preliminary results from these trials indicate substantial provenance differences for all three traits. Percent survival and 3-year total height also showed relatively strong clinal relationships with geographic variables. Growth and survival showed a significant ($P=0.05$) inverse correlation. Northern and lower elevation provenances tended to grow faster but exhibited poorer early survival in comparison to southern and higher elevation sources. Site by provenance interactions were significant ($P=0.05$) for percent survival but not for total height or plant health score.

The range of performance for traits by site was also large with the best performance for all three occurring on a site near Rocky Mountain House which was furthest outside the natural range for Douglas-fir. These results indicate significant early provenance and site effects for these traits and the possible opportunity for movement of Douglas-fir outside its natural range, but also the need for careful decisions about deployment on sites both within and outside its natural range.

Seed Production Studies

A study to monitor the effect of relocation using large tree spades on the growth, flowering and seed production of a white spruce seedling seed orchard and a lodgepole pine seedling seed orchard located at Pine Ridge Forest Nursery was continued. Three years after transplanting, trees in the lodgepole pine seed orchard showed significant reductions in height growth and crown width but not in diameter growth when compared to undisturbed trees. Mean height of the transplanted and undisturbed trees was 4.6 m and 5.0 m, respectively. The transplanted lodgepole pine trees produced significantly more cones than the undisturbed trees. Mean number of cones produced was 158 and 56, respectively, for the transplanted and undisturbed trees. Mean number of seeds per cone was 23 and 16 for transplanted and undisturbed trees, respectively. The same trend in cone production and number of seeds per cone was observed four years after treatment. The transplanted trees produced an average of 174 cones per tree and 18 seeds per cone compared to 84 cones per tree and 19 seeds per cone for the undisturbed trees. These trends suggest that the use of large tree spades may be an effective cultural treatment to reduce height growth and promote increased cone production in lodgepole pine seedling seed orchards.

Similar results for growth characteristics were observed in the white spruce seedling seed orchard. Transplanting significantly reduced height, crown and diameter growth of the white spruce. Mean heights, crown widths and diameters for the transplanted trees three years after treatment were 4.8 m, 2.7 m and 7.9 cm, respectively, compared to 4.3 m, 2.2 m and 6.8 cm, respectively, for the undisturbed trees. Three years after treatment the white spruce seed orchard experienced a significant cone crop. The transplanted trees produced on the average 207 cones per tree and 25 seeds per cone compared to 98 cones per tree and 23 seeds per cone for the undisturbed trees. Results suggest that transplanting enhances cone production without affecting seed yield.

Fifteen year results were summarized on flowering and seed production monitoring of white spruce grafts of northern Alberta origin, outplanted at four geographically diverse locations in British Columbia and Alberta. The study corroborated earlier results that indicate that Pine Ridge Forest Nursery and the Grande Prairie seed orchard sites are suitable for establishing grafted white spruce seed orchards. Results indicate that commercial cone crops can be expected at these sites every five to six years.

Grafts from early flowering white spruce trees grown under an accelerated growth rearing regime were established in a seed production study. Forty-eight grafts from three clones were established in the trial. Scions were collected from three white spruce trees in an experimental seed orchard established in 1980 with 1-year old stock grown under 24-hour lights and given high rates of macro-nutrients. These trees began producing significant numbers of female flowers at 4 years of age.

In 1992, work commenced on developing an ocular flower/cone production rating system for white spruce and lodgepole pine seed orchards in Alberta. The objective of the system is to provide seed orchard managers with a reliable tool to predict flower and cone crops. Analysis of data has shown that the ocular system consistently underestimates realized cone crops. The system is being reviewed to decide if it can be modified to become a practical tool for seed orchard managers to reliably estimate flower and cone crops.

Over the past three years there has been a significant increase in interest shown by the forest industry in Alberta for the use of native black spruce for operational reforestation in Alberta. A seed production monitoring program has been set up to develop information on the early flowering and seed production characteristics of black spruce grafts in managed seed orchards. Information will be used to plan and manage black spruce seed orchards being established and/or expanded.

Grafting Program

In 1994, a study was initiated to delineate patterns of graft incompatibility in a Scots pine seed orchard. The grafts are between 5- and 8-years from time of grafting and show needle chlorosis and stunting, differential growth of scion and rootstock at the graft union, abundant flowering and insect infestations. Clonal differences in incompatibility will be investigated.

PLANT PROPAGATION, WOOD AND SEED TECHNOLOGY

Plant Propagation

During the report period stock production consisted of 15,994 seedlings for rootstock utilization, 8,497 coniferous and hardwood seedlings for experimental trials and 5,347 grafts.

A total of 441 white spruce secondary grafts and 842 white spruce grafts from new selections were completed in 1994. A 71% success rate and a 54% success rate respectively were achieved. With an 83% success rate, 337 lodgepole pine grafts were completed. Other species grafted included 419 black spruce and 40 Scots pine. Use of an artificial cold hardening period was implemented on an operational scale in 1994. A reduction of the time required to produce grafts for outplanting is achieved by introduction of a second growth cycle in a 12-month period.

In 1994, a total of 1,297 hardwood seedlings were produced for supply to Alberta Pacific Forest Industries. These seedlings were grown from seed from 27 balsam poplar (*Populus balsamifera*), 7 trembling aspen (*Populus tremuloides*), and 48 cottonwood (*Populus deltoides* var. *occidentalis*) seedlots.

In 1995, a total of 7,200 seedlings were produced for a Siberian larch provenance/progeny trial. These seedlings were produced from seed collected in 1990 in Charga, Southern Siberia.

Wood Technology

Relative density and fibre length measurements of wood samples collected from selected parent trees continued. Species tested were white spruce, lodgepole pine, black spruce, jack pine, tamarack and willow (*Salix*). A total of 356 relative density and 335 fibre length assessments were completed. Based on the recommendation of Dr. Lars Bach from the Alberta Research Council, an additional 461 fibre length assessments were completed on the 77-80 age bracket for wood samples received prior to 1993.

Seed Technology

A total of 120 seedlots were added to the genetics seed bank. Of these seedlots, 51% were white spruce, 18% were lodgepole pine, and 29% were black spruce. The seedbank presently contains 3,735 seedlots.

Quality of seedbank seedlots continues to be monitored annually by testing a set of reference seedlots representing about 2% of seedbank entries. New seedlots are added to the testing program every few years to replace depleted seedlots and sample new seedbank entries. Mean germination of lodgepole pine seedlots in the seedbank has declined from 87 percent in 1981 to 83% in 1995 and mean germination of white spruce seedlots has declined from 91% in 1981 to 85% in 1994. Mean germination of aspen, plains cottonwood, and balsam poplar, has declined 5 to 16% after 4 years of testing.

The ultra-low temperature seed storage study started cooperatively with the National Tree Seed Centre at Petawawa, is now in its 7th year. Average germination for white spruce after 7 years storage at -80°C was found to be 79 percent compared to a baseline mean germination of 82% at commencement. For lodgepole pine, mean germination at -80°C storage was 87% compared to an average baseline germination of 84% at -20°C storage. The study is considered complete after 7 years of testing and results indicate that -80°C storage temperature does not offer any advantage over conventional -20°C storage.

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

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Keywords: Quantitative and population genetics, genomic mapping, early selection, applied tree breeding and improvement, isozyme, RAPD and quantitative trait.

During the past two years, our research continued with theoretical and experimental investigations of population genetic structure in lodgepole pine (*Pinus contorta* Dougl.) and trembling aspen (*Populus tremuloides* Michx.), and retrospective early genetic evaluation and selection in lodgepole pine and jack pine (*P. banksiana* Lamb.). The scope of our research has also extended to include *Cunninghamia lanceolata* Lamb. from People's Republic of China (Yeh *et al.* 1994), *Pterocarpus macrocarpus* Kurz. from Thailand (Liengsiri *et al.* 1995) *and Picea sylvestris* L. from Sweden (Yazdani *et al.* 1995). We also initiated new studies of western gall rust (*Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka) to investigate geographic variation, resistance breeding, host-fungus interaction and QTL.

GENETIC STRUCTURE AS REVEALED BY ISOZYMES, RAPDS AND QUANTITATIVE TRAITS

A major focus was on developing the genetic framework for studying "multilocus genetic structure" at isozyme loci (Yang and Yeh 1993; Yeh *et al.* 1994). We found multilocus genetic structure was extensive in natural populations and the estimates of multilocus population differentiation were on average, more than three times the single-locus estimates at isozyme loci. Results suggests that population subdivision, founder effect, occurrence across diverse environments and historical events were contributing factors in the generation and maintenance of multilocus genetic structure.

The use of random amplified polymorphic DNA (RAPD) in trembling aspen from 8 populations in Alberta proved useful for studying nucleotide divergence between populations (Chong *et al.* 1994) and genetic structure (Yeh *et al.* 1995). We could differentiate among all trees in any population from their multi-band RAPD phenotypes amplified with 5 random primers. Shannon's phenotypic diversity index ranged between 0.58 and 0.69, averaging 0.65. Analysis of molecular variance showed the among-population component accounted for 2.6% of the variation and significantly different from zero at the 2% level of probability. This is identical to the 3% among-population differentiation at isozyme loci (Jelinski and Cheliak (1992). Isolation by distance might have been an important factor in the genetic differentiation of this clonal angiosperm. Agreement between patterns of population structure revealed by isozymes and RAPDs in trembling aspen from Alberta might suggest that neutral forces were likely to be invoked than selection to explain the maintenance of genetic variation.

We developed F-statistics for quantitative traits to estimate population differentiation and completed a comparative study of genetic structure of lodgepole pine using both quantitative traits and isozymes (Yang *et al.* 1995). Two of 6 quantitative traits were neutral and the remaining 4 were under divergent selection. In contrast, all 19 isozymes tested neutral. This suggests that it is important to choose judiciously among the available markers for assessing diversity and genetic structure.

APPLIED TREE BREEDING AND IMPROVEMENT

We conducted collaborative research on genetics and breeding of lodgepole pine with the Alberta Land and Forest Service and the Alberta Tree Improvement Cooperative and on genetics and breeding of jack pine with Northern Forestry Centre, Forestry Canada. Retrospective early testing and selection, multiple trait index selection, geographic variation and host-fungus interaction in western gall rust (WGR) incidence, and mapping for WGR resistant genes have been major components of our studies.

Retrospective Early Testing And Selection

We reported the pattern of genetic variation among seedling traits in the greenhouse for two growth periods (Wu *et al.* 1995a). We completed the theoretical framework and experimental verification on genetic gains due to retrospective early selection under three selection schemes: (1) early selection for tree field traits based on single seedling traits, (2) early selection for tree field traits based on multiple seedling traits and (3) index selection combining early seedling and tree field traits (Wu *et al.* 1995b). We also examined the effect of GE interaction on greenhouse-field correlations in retrospective studies (Wu *et al.* 1995c; Lu 1995). Our results in lodgepole and jack pine suggested that, growing seedlings under carefully designed greenhouse conditions (*e.g.*, large number of families, effective experimental layout on greenhouse benches, seed weight adjustment, selection of target early traits, use of suitable genetic and statistical equations, and the possible mimicking of field [nutrient/moisture] conditions) allowed retrospective test to be an effective tree improvement tool. Collaborative research with Dr. R.P. Pharis showed that gibberrellin class of hormones were useful early traits for indirect selection of field growth.

Early testing also facilitated the study of genetic variation in traits of total biomass partitioning (stem, branch, needle and root) and architecture (harvest index, shoot-root biomass ratio and sturdiness quotient) in a large number lodgepole pine families (Wu and Yeh 1995). Levels of heritability and genetic correlation suggest that selection procedures that exploit additive genetic variance and covariance might be practiced.

Western Gall Rust Resistance

Geographic variation in WGR isolates across British Columbia, Alberta, Saskatchewan, Manitoba and Ontario suggested host (lodgepole and jack pine) specificity as revealed by RAPDs (Li et al. 1995). We also initiated a half-sib QTL analysis using RAPDs to identify host (lodgepole pine) loci involved in WGR resistance. We believe future breeding for WGR resistance will be more efficient using known RAPDs to select and track resistant genotypes because size of breeding population will be made small and pedigree for resistance will advance at a faster rate.

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PFRA SHELTERBELT CENTRE - TREE IMPROVEMENT SUMMARY

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Keywords: shelterbelt, windbreak, progeny test, seed orchard, provenance test.

The primary objectives of the tree improvement programme at the PFRA Shelterbelt Centre is to develop genetically superior trees and shrubs for shelterbelt and agroforestry planting in the prairie provinces of western Canada. From 1991 to 1993 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.) and choke cherry (*Prunus virginiana* var. melanocarpa (A. Nells.) Sarg.

SCOTS PINE

Two full-sib and four half-sib progeny tests were evaluated in 1994. Three superior families with superior form, colour and density were identified. These families are being used for production of Christmas tree in the prairies. Progeny tests were established in multi-row forestbelts. The performance of families for forestry applications will be investigated. All Scots pine seed grown for shelterbelt and agroforestry plantings in 1995 originates from a one hectare seedling seed orchard located at the Shelterbelt Centre. This orchard provides the prairie tree planters with fast growing, drought tolerant Scots pine with minimal winter browning.

SIBERIAN LARCH

Three larch species trials were evaluated in 1994. Data showed that Siberian larch was well adapted to prairie growing conditions. Other larch species, including *Larix eurolepis*, *Larix gmelinii* and *Larix olgensis* were not as well adapted. Roguing of a 1987 clonal seed orchard was completed. This seed source has proven to be superior to other sources tested. This source establishes easily and has superior growth and form.

GREEN ASH

Collections of green ash seed from xeric habitats in the northern Great Plains have been completed. A total of 30 populations have been sampled. Seedlings are being propagated for outplanting in 1996. The sources will be utilized for drought tolerance screening. This is a co-operative project with the USDA Forest Service at Lincoln, Nebraska.

A three hectare first generation clonal seed orchard has been completed. The orchard will be coming into production within the next two to three years. Progeny tests initiated in 1989 were evaluated in 1993. Superior families were identified and data collected is being used to rogue the seed orchard.

POPLAR

The native population of plains cottonwood in southern Saskatchewan was sampled in 1994. Seed and cuttings were collected from 15 populations in the Saskatchewan River Valley. Seed is being propagated for progeny testing and cutting beds of superior trees have been established.

In 1995, a new female poplar *P.* x 'CanAm' selected from an open pollinated population of *P.* x 'Walker' was introduced for planting in the northern Great Plains. 'CanAm' was released co-operatively with the USDA, Agriculture Research Service and the USDA, Natural Resources Conservation Service. 'CanAm' is a vigorous tree with superior drought resistance.

BUR OAK

In 1993, range-wide provenance tests of bur oak were planted at two sites in Saskatchewan and one in Manitoba in 1993. Sixty families are included in the test. This is a co-operative project with the USDA, Forest Service, USDA Agriculture Research Service, and USDA, Soil Conversation Service. Objectives of the project are to: 1.) determine the nature and extent of genetic variation present among open-pollinated progenies of bur oak from selected sources in the Great Plains; 2.) identify best adapted sources of bur oak for planting in the Great Plains; and 3.) provide germplasm that can be used for selection and trait improvement as well as advanced generation breeding.

CHOKE CHERRY

The native range of choke cherry in Manitoba and Saskatchewan was sampled in 1993 and 1994. A total of 175 population were collected. Germplasm is being propagated for provenance testing in 1996. Protocols for micropropagation of superior plants were streamlined.

NATIVE PLANT COLLECTIONS

Assembly of native woody plant germplasm is under way. This project focuses on the collection and genetic improvement of native shrub seed sources for use in conservation tree plantings. Seventeen different species have been collected with emphasis placed on silver buffaloberry, round-leaved hawthorn, snowberry, Wood's rose, and highbush cranberry. In 1993 and 1994 over 350 accessions were collected.

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

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Keywords: *Populus, Acer, Tilia, Fraxinus, Betula,* shrubs, genetic resources, jack pine, *Pinus banksiana,* asexual propagation, rooted cuttings

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.

POPLAR (*Populus*)

Goal

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

Progress

Approximately 30 selections have been made over the last three years. These include both inter-specific and intra-specific hybrids. Selected populations were approximately 20 years old and near mature size.

ASH (*Fraxinus*)

Goal

Development of new inter-specific hybrids and the selection of superior green and white ash clones.

Progress

Two new hybrids between black and Manchurian ash will be introduced to the commercial industry this season. 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 are being screened using a 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.

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 sown and will be field planted for further study. Parental lines have been propagated and will be evaluated further under different environmental conditions.

LINDEN (*Tilia*)

Goal

To develop superior inter-specific hybrids 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.

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. Success to date has been limited.

OTHER SPECIES

A wide range of shrubby species including *Rosa, Potentilla, Ribes, Amelanchier, Philadelphus, Weigela* are being developed for landscape potential. A wide range of characteristics are 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.

MANAGEMENT RELATED STUDIES

Goal

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

Progress

Research activities have focussed on developing procedures for operational asexual propagation of jack pine (*Pinus banksiana* Lamb.). A more detailed summary of results appears elsewhere in this proceeding and two abstracts are presented in the poster section. Methodology for rooting jack pine long shoots cuttings ha been developed which has yielded rooting frequencies of 80-100% for cuttings from plant < 3 years old, 40-60% from plants 4-6 years old and 20-40% from plants 7-10 years old. Investigations into strategies for improving shoot multiplication and rooting frequencies has involved induction of proliferated dwarf shoots through pruning and cytokinin treatments and the *in vitro* culture of cotyledons from germinating seedlings. Using these approaches, targeted goals for operational propagation are expected to be met. Furthermore, field trials are underway to assess performance of rooted cuttings. Additional field trials will be planted in 1996 and 1997. An operational manual outlining key features for asexual propagation of jack pine is under preparation and should be available by April 1996. Financial support from the Canada-Manitoba Partnership Agreements in Forestry is gratefully acknowledged. The authors also appreciate cooperation and support provided by Mr. John Dojack (Manitoba Department of Natural Resources) and Ms. Dianne Roddy (Weyerhaeuser Canada Ltd.).

MANITOBA'S TREE IMPROVEMENT PROGRAMME

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Keywords: Jack pine, black spruce, white spruce, seed orchard, co-operative.

The tree improvement programmes established for jack pine (*Pinus banksiana* Lamb.), black spruce (*Picea mariana* (Mill.) B.S.P.) and white spruce (*Picea glauca* (Moench) Voss) are being managed through the co-operatives with Repap Manitoba, Pine Falls Paper Company and Canadian Forest Service. This report highlights the progress which occurred from 1993-1995.

JACK PINE

The Hillside Pedigree Orchard was expanded in 1994 with the addition of 1,312 seedlings of 18 full sib. families. The first cone crop was harvested from the initial orchard in the fall of 1994 producing 2.9 kg seed.

A second 50% roguing was completed at the Interlake jack pine mass selection seed orchard (MSSO) in 1994. A third 50% roguing was completed at the Cranberry Portage MSSO also in 1994. In 1995 a third 50% roguing is planned for the Root Lake MSSO and a fourth and final roguing at the Simonhouse orchard.

BLACK SPRUCE

In June of 1994 a 7.0 hectare seedling seed orchard was established in the Nelson River breeding zone in co-operation with Repap Manitoba.

An ongoing maintenance programme on existing family tests and seed orchards is being undertaken with members of the co-operatives.

WHITE SPRUCE

Due to budgetary restraints, a change in strategy from a container seed orchard program for the Saskatchewan River Breeding Zone to a soil based clonal orchard planted in June, 1995, was undertaken.

Height measurements were taken, five years after planting, on 2 family tests in the Mountain Breeding Zone.

SUMMARY

The current tree improvement programmes are progressing well. The past experience of working within co-operatives has been very positive. "Memorandums of Understanding" have been prepared to enter into new co-operatives in breeding zones where harvesting will increase.

TREE IMPROVEMENT UNDER THE CANADA–MANITOBA PARTNERSHIP AGREEMENT IN FORESTRY

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Keywords: *Pinus banksiana*, jack pine, seed orchards, mass selection, Best Linear Prediction, stem quality, western gall rust, wood density.

Forest genetics by the Canadian Forest Service in the prairie provinces started about 1960, but the earliest project that is still active is the jack pine (*Pinus banksiana* Lamb.) breeding programme for parts of Manitoba and Saskatchewan, which began in 1967. From 1970, when the former Winnipeg centre closed, until 1991, forest genetics staff were located in Edmonton. Family test and clone bank plantations were established for the breeding programme from 1972 to 1977, as were provenance test plantations for jack pine and black spruce (*Picea mariana* (Mill.) B.S.P.). Breeding program results supported establishment of a jack pine seed orchard programme in Winnipeg under the Canada–Manitoba Forest Renewal Agreement of 1984–89. When the Canada–Manitoba Partnership Agreement in Forestry (CMPAIF) began in 1991, forest genetics staff were relocated to Manitoba District Office.

The CMPAIF tree improvement programme was intended to assist the Province of Manitoba in gaining independent tree improvement capability, while maintaining the ability to respond to opportunities in the other prairie provinces, and to capture forest genetics knowledge available from the region's test plantation assets. Programme activities are intended to (1) continue establishment and development of jack pine seed orchards, (2) develop more efficient delivery of genetic improvement, especially by controlled pollination and vegetative propagation, and (3) initiate a second generation of jack pine breeding.

The Canadian Forest Service forest genetics programme in this region will end in 1996.

JACK PINE SEED ORCHARDS

Development of the Northern and Interlake mass selection seed orchards by Manitoba Forestry Branch continued. By 1998, plots planted with 48 or 25 trees from one stand at 1–m spacing in 1986 and 1987 will have been thinned in four mass selection steps to 1–3 trees, and harvesting of cone crops will begin. Tree pair measurements taken at each thinning are showing a useful amount of genetic variation among the 32 source stands for each seed orchard, and a substantial phenotypic effect of mass selection. A plan has been drafted for genetic quality verification and second–generation breeding following completion of mass selection.

The Hillside Road jack pine seed orchard comprises 6 ha planted in 1988 with first– cousin progenies and a 4–ha expansion area planted in 1994 with full–sib progenies. All of the parent trees were in family test plantations, mated in 1985 after selection on 10–year measurements (Klein 1986), or in 1992 after selection on 15–year measurements (Klein 1992). Both sets of progenies were rescored based on breeding values of height, dbh, stem quality, resistance to western gall rust, and wood density at 20–21 years, estimated by Best Linear Prediction (BLP). The new scores are being used for genetic upgrading of the seed orchard. Four 1988 entries with the lowest scores of the 18 planted were replaced by 1992 progenies to accomplish the first step. Planting of grafts of high–scoring family test trees unrelated to any of the progeny entries, in the expansion area, will complete the upgrade. Most of the required grafts are now on hand. The first cone crop was harvested in 1993, with relatively balanced contributions from the 14 entries planted in 1988.

APPLIED TREE IMPROVEMENT RESEARCH

Gain capture from superior jack pine genotypes by controlled pollination offers the prospect of greater genetic control and flexibility compared with reliance on wind pollination. Early results from trials of crown shaping and nitrogen fertilization suggest that sufficient numbers of seed cone flowers might be produced on trees kept under 2 m in height, while pollen production on these trees is suppressed by high doses of nitrogen. That outcome would improve prospects for relatively efficient controlled mating. A research contract conducted by Morden Research Station, Agriculture and Agri–Food Canada has come close to achieving a system for vegetative multiplication of control- pollinated jack pine seedlots.

Results from all CMPAIF tree improvement experiments, including flowering response to crown shaping and nitrogen fertilization, quantification of realized gain, a retrospective progeny test (all of these with jack pine), and cultural treatments to enhance early growth of black spruce seed orchard seedlings, will be reported in the coming year.

SECOND GENERATION BREEDING OF JACK PINE

Selection of candidate parents has been completed for the second generation of the eastern breeding district jack pine breeding program, using BLP based on family test performance to 21 years from planting. The best 64 unrelated trees had a mean predicted gain of 18% for wood dry weight, and 10% for stem quality and resistance to western gall rust (*Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka) (Klein 1995). A draft breeding plan is under consideration by Manitoba Forestry Branch. Matings to produce second generation progenies will be performed on grafts of 116 candidate parents, most of which are now growing in containers.

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CONE AND SEED PEST MANAGEMENT

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Keywords: Seed orchards, insects, pest management, monitoring and decision tools.

This report outlines my activities in seed orchard pest management from 1993-95.

CONE AND SEED PEST MANAGEMENT

Studies to develop a pheromone-based pest management system for cone beetles, *Conophthorus* spp., centered around the development of a practical and easy-to-use trap and bait system. The effects of trap type, lure type and dosage, trap colour and height, and the use of monoterpenes were investigated. Manuscripts describing these studies are in preparation and should be published within the next reporting period. The chemical identification of the pheromones and the results from earlier work were published (Birgersson et al. 1995; Pierce et al. 1995). A life history study of the white pine cone borer, *Eucosma tocullionana* Heinrich, was completed and the manuscripts are in preparation. A life table study of jack pine cones was published (de Groot and Fleming 1994). Reviews of cone and seed insects, their management and monitoring were published (de Groot 1994; de Groot 1995a; de Groot 1995b, de Groot et al. 1994; Turgeon et al. 1994).

CONE CROP MONITORING AND DECISION SUPPORT SYSTEM

A Cone Crop Monitoring System (CCMS), Information Management System (IMS) and the Decision Support System (DSS) were developed for seed orchard management. The CCMS collects and processes data on the size of the cone crop and expected damage. The IMS provides information on the damage potential of insect pests, pest control products registered in Canada for cone and seed insects, and estimates the efficacy of the products. The DSS uses this data and information and asks the user for additional information about protection costs and seed values to calculate the consequences of various decisions. Although developed specifically for use in jack pine and black spruce seed orchards in Ontario, many of the procedures and much of the information are applicable to conifer seed orchards in most of North America. A user manual that provides step-by-step instructions on the use of the systems on IBM compatible PCs and a field data logger, and a copy of the software (DOS-based), will be available in late 1995 or early 1996.

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POLLEN CONTAMINATION REDUCTION SOFTWARE

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Keywords: pollen dispersal, pollen contamination, seed orchards, *Picea mariana, Pinus banksiana.*

Joint (University of Guelph, Environment Canada and Ontario Ministry of Natural Resources [OMNR]) pollen dispersal studies have been on-going at the Island Lake seed orchard, near Chapleau, Ontario since 1989. These studies were completed in 1994 and have resulted in the production of computer software that can be used to manage pollen contamination at any seed orchard (POLDISP v.1.0).

The software is based on a Lagrangian Markov-chain atmospheric dispersal model which was modified to simulate the dispersal of contaminant pollen from the surrounding natural stand into the Island Lake seed orchard. Pollen diffusion in the atmosphere, impaction on tree branches and receptive seed cones and deposition to the ground are all included in the model.

Model inputs, including physical and vegetation data and meteorological data, were collected at Island Lake during the pollination season for *Picea mariana* and *Pinus banksiana* in 1992 and 1993. These specific input data were used with the model to predict pollen dispersal at that site. Predicted pollen dispersal was compared to the measured dispersal of artificially released and natural pollen. Model predictions and measured dispersal agreed reasonably well providing a validation of the model at the Island Lake site.

The model was incorporated into an interactive program shell which can be used to examine strategies which reduce pollen contamination at other orchards. In order to use the model at other orchards, the model inputs were generalised; thus, the generalised model should be tested at a site other than the original. Discussions are on-going with OMNR to that end.

Where pre-existing contamination problems have been identified at a seed orchard, the software will predict the relative effectiveness of different, physical, anti-contamination scenarios envisioned by the seed orchard manager. These physical anti-contamination scenarios may be the establishment of buffer zones of varying width, or the establishment of wind breaks (to provide pollen filtration) of any particular configuration. The project final report is available, and the software and user manual are available at the discretion of the OMNR from Randy Ford (Phone: 705-567-3372).

Other publications have resulted as spin-off's from this work:

Di-Giovanni, F., Kevan, P.G. and Nasr, M.E. 1995. Settling velocities of some pollen and spores and their variability. Grana 34: 39-44.

Banks, L. and Di-Giovanni, F. 1994. A wind tunnel comparison of the rotorod and samplair pollen samplers. Aerobiologia 10(2-3): 141-145.

Roussy, A-M. 1994. Alleles, cones and pollen: A discreet look into Jack Pine (*Pinus banksiana* Lamb.). M.Sc. Dissertation, University of Guelph. 64pp.

Papers in preparation resulting from this project are:

Di-Giovanni, F., Kevan, P.G. and Caron, G. 1995. Prediction of the timing of maximum pollen release from jack pine (*Pinus banksiana* Lamb.) in northern Ontario.

Di-Giovanni, F., Kevan, P.G. and Arnold, J. 1995. Lower planetary boundary layer profiles of atmospheric conifer pollen above a seed orchard in northern Ontario, Canada.

Publications are also planned on data relating to the pollen production of jack pine trees, and an examination of the pollen dispersal patterns at Island Lake. The author of this report is now an independent, scientific consultant specialising in aerobiology, atmospheric dispersal and atmospheric pollution.

GENETIC DIVERSITY AND REPRODUCTIVE SUCCESS

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Keywords: Conservation biology, genetic diversity, reproductive success and ecology, population viability analysis.

Our conservation research and activities over the past two years has focused on (i) the effects of small population size and population fragmentation on genetic diversity and reproductive success in white pine *(Pinus strobus)*, white spruce *(Picea glauca)*, and pitch pine *(Pinus rigida)*, (ii) the use of PCR-based molecular genetic techniques to quantify genetic variation in genetically uniform species such as red pine *(Pinus resinosa)*, (iii) the national coordination of forest gene conservation activities in Canada, and (iv) the relationships between population parameters such as population size, density and fragmentation on population viability (genetic diversity and reproductive success).

Isozymes and random amplified polymorphic DNA (RAPD) markers were used to quantify genetic variation in the disjunct Newfoundland populations of red pine and white pine for comparison with adjacent mainland populations. These Island populations are declining rapidly in number, due to excessive exploitation without proper forest management controls, and are being threatened with the introduction of diseases such as the white pine blister rust *(Cronartium ribicola)*, and the scleroderris canker *(Greminiella* sp).

Red pine is unique among forest tree species for its genetic uniformity and earlier studies were largely unable to detect any genetic variation at the molecular level. However, some genetic variation in DNA sequence has been detected between Newfoundland populations and those from a range-wide North American mainland sample using RAPD markers in conjunction with restriction enzyme digests. Newfoundland populations of white pine could not be genetically differentiated from mainland seed sources using isozymes. However, analyses of progeny from small, isolated Island populations did show signs of inbreeding. Furthermore, population specific alleles were identified in one of these small, isolated Newfoundland populations. A Newfoundland-wide sample of red pine and white pine germplasm has been established at two locations in central Newfoundland (Wooddale Provincial Tree Nursery and the Thomas Howe Demonstration Forest in Gander).

Cones were collected from a highly fragmented population of white spruce *(Picea glauca)*, adjacent to the Western Arm of Lake Nipissing in 1994 to study the effects of population size and fragmentation on reproductive capacity and genetic diversity. A similar study was also

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[1] With the closure of PNFI, authors will be transferred to the CFS Maritimes Region, P.O. 4000, Fredericton, New Brunswick, E3B 5P7.

initiated in 1994 in collaboration with the Ontario Ministry of Natural Resources and the Eastern Ontario Model Forest Association based on the remnant Canadian population of pitch pine *(Pinus rigida)*, of the St. Lawrence River Valley. The goal of these studies is to develop some guidelines for maintaining minimum viable population sizes in trees. Seedlings from the white spruce study have been established in two short-term nursery tests located at the Petawawa National Forestry Institute and near the seed source origin at the western end of Lake Nipissing. These tests will be used to monitor inbreeding depression related to population size. Similar field tests are planned for pitch pine in 1996. These tests will be established in the St. Lawrence River Valley and at the Acadia Forest Experiment Station in New Brunswick. Although presently rare in Canada, the range of pitch pine may expand considerably with anticipated climatic changes in central Canada.

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PHYSIOLOGICAL GENETICS AND PLASTICITY

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Keywords: climate change, drought tolerance, elevated CO_2, G x E, *Picea mariana*, provenance, water use efficiency, within-stand variation.

DROUGHT TOLERANCE RESEARCH

Intensive physiological assessment of putative drought tolerant and intolerant full-sib families of black spruce was conducted over three growing seasons. The families are part of a 7 x 7 diallele established on three sites at PNFI in 1973 by Dr. E.K. Morgenstern. The sites exhibit a large range in productivity. All physiological evidence supports the contention that the site with the lowest productivity is drier than the other sites.. Two of the families studied have maintained high productivity on all three sites (stable) while the other two (unstable) have had high productivity on the two wetter sites but not the dry site. Gas exchange measurements conducted all years have shown the stable families to have higher net photosynthetic rates on both wet and dry sites and across years ranging from dry and hot, to cool and wet. Family differences in stomatal conductance have been inconsistent and minimal; thus the families have consistently differed in water use efficiency. Analysis of response surfaces indicates that the family differences in P_n is due to differential responses to vapour pressure deficit. The vapour pressure deficit needed to elicit the observed family differences in P_n are common on both wet and dry sites and over many days of a growing season. Work done in cooperation with Dr. Larry Flanagan (Carleton University) using stable isotopes of carbon corroborated family differences in water use efficiency. In addition, over the three years the stable families have displayed higher turgor across all sites with the largest differences occurring on the drier site. Thus, the family differences in growth on the dry site appear due to genetic differences in sink strength (driven by higher turgor) accompanied by higher carbon availability although other traits may also be involved. Dr. Flanagan is currently processing foliar samples for carbon isotope discrimination from the upper diagonal of the entire diallele from all three sites so that a more extensive genetic analysis can be conducted. Marker aided selection will be explored. Grafting was conducted to increase ramet numbers so that original parents can be used to recreate original crosses and make new crosses for further field/greenhouse/growth room research.

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[2] With the closure of PNFI, authors will be transferred to the CFS Maritimes Region, P.O. Box 4000, Fredericton, New Brunswick, E3B 5P7

GENOTYPE X ATMOSPHERIC CO_2 INTERACTIONS

Elevated CO_2 has been shown, at least temporarily, to increase growth rates in forest tree seedlings. Elevated CO_2 can also alter morphological and physiological process components of productivity. For instance, elevated CO_2 has been shown to increase net photosynthesis, increase water use efficiency, decrease photosynthetic capacity, alter biomass partitioning, alter shoot growth phenology, and decrease cold hardiness.

Genetic variation among and within populations of trees is manifested via genetic variation in physiological processes including those listed above. Through its influence on these processes, might elevated CO_2 alter genetic performance in growth and survival? If the answer to this question is yes, then deployment of seed sources and genotypes based on testing under current atmospheric CO_2 conditions might result in decreased potential productivity or even mal-adaptation of future forests. A series of studies have been conducted to examine the potential importance of G x atmospheric CO_2 Environment interactions in black spruce using varying levels of genetic structure.

Wide-range provenances

A greenhouse study using wide-range provenances of black spruce has indicated stable provenance rankings in growth and physiology under both ambient and elevated CO_2. Typical clinal responses were shown with sources ranging from the Yukon to Ontario and growth and physiology responded to varying photoperiod regimes consistent with their origins. Growth and physiological responses of greenhouse seedlings agreed well with results from a concurrent field experiment using 23-year-old trees of the same provenances adding confidence to the greenhouse results. It appears that the profound differences among the wide-range provenances are expressed similarly under ambient and elevated CO_2.

Narrow-range provenances

Seedlots from northern Ontario from four upland and four lowland sites were subjected to a growth room growth analysis under combinations of high and low CO_2 and well-watered and droughted conditions. Seedlings were grown for two simulated growth seasons with chambers programmed using historical weather data to mimic seasonal and diurnal patterns of daylength, temperature and relative humidity. Under ambient CO_2, upland sources grew more than lowland sources with the differences being larger under droughted than under well-watered conditions. These provenance differences were not apparent under elevated CO_2. This experiment is being repeated using the original eight plus eight more seed sources. Following one growth season, results appear similar to the first experiment. The more subtle provenance variation within northern Ontario may be influenced by differential acclimation to elevated CO_2.

Retrospective family test

Seedlings from 20 families of black spruce, were subjected to a greenhouse retrospective test under ambient (409 ppm) and high (686 ppm) atmospheric CO_2 environments. After one season, growth under elevated CO_2 significantly increased total seedling height, root collar diameter, branch number, average branch length, and cumulative branch length. Families were a significant source of variation for all growth parameters. Although family x CO_2 environment was not a statistically significant source of variation, correlations between greenhouse and field performance were generally positive and statistically significant under ambient CO_2 and weaker and non-significant under elevated CO_2. The experiment is continuing for another growth season after which seedlings will be destructively harvested.

Red/Black Spruce Hybrids

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, field plantations were established with the same genetic material. In collaboration with Dr. Judy Loo, CFS - Maritimes Region, we have revisited these plantations to see if negative heterosis is evident in mature trees. Analysis of height growth 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 full black spruce to full red spruce. Thus far, in contrast to seedling results, negative heterosis is not apparent in mature trees.

Gas Exchange Technology

Physiological genetics research is often hampered by the inability to obtain sufficient sample sizes. For instance, due to compounding, very small genetic differences in photosynthetic rate can result in large growth differences at rotation age. These differences can be very difficult to detect using conventional hand-held gas exchange systems which require constant attention and skillful operation. In addition, measurement made in the field, during dynamically changing multivariate environmental conditions can create highly variable data. The result is a series of instantaneous measurement at subtly different specific conditions. In order to detect these differences (if they exist) between genetic entries, gas exchange sampling need to be increased while environmental variation and/or measurement time periods are minimized.

We have invested a substantial effort over the past two years developing a multiple sampling, automated, programmable, steady state, semi-portable, gas exchange system. It can measure up to 32 samples repeatedly over a 24 hour period or more with feedback loops. Together with 2 customized controlled environment chambers which are equipped to control light intensity (up to near full sunlight), light duration, temperature, relative humidity, and carbon dioxide, comparisons between genetic entries can be made under one or a series of static conditions. In addition, some research show that entries can have similar physiological response in one set of environmental conditions but different under another set of environmental conditions. Hence, depending on the experiment, comparisons can be make over a range of one or two environmental conditions while controlling the other environmental variables.

We have just operationally tested the system, and are quite satisfied with the results. The system is relatively flexible and will be adapted to each set of experiments. Also, this is an evolving system which will be modified as component technology improves or as research goals change.

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ONTARIO'S GENETIC RESOURCES MANAGEMENT PROGRAM

Cathy Nielsen, Joan Wild, Randy Ford, Paul Charrette,
Bob White, Dennis Joyce, Frank Schnekenburger, and Peter Nitschke

Overview

Tree improvement, seed management, and gene conservation are carried out through the Ontario Tree Improvement Board. Programs are developed locally in 6 autonomous administrative zones with science, technical, and analytical support being coordinated at the provincial level. A structural evolution continues as the program moves towards financial self-sufficiency. Significant quantities of seed are now being produced by seed orchards and being delivered to growers. Activities which are more conservation-oriented are beginning, especially in southern Ontario where conservation is a major program thrust.

Zonal Highlights

Zone 6: Forest Gene Conservation Association

The Forest Gene Conservation Association (FGCA) held its inaugural meeting on April 14th, 1994. Eighteen organizations have signed the M.O.U. for the Association. The members of the FGCA include: Alfred College, Association of Conservation Authorities of Ontario, Domtar, Canadian Chestnut Council, Canadian Forest Service, City of Toronto Parks and Recreation Dept., Eastern Chapter Society of Ontario Nut Growers, Eastern Ontario Model Forest, Landscape Ontario, M.M. Dillion Ltd., Mohawk Council of Akwesasne, National Capital Commission, Ontario Ministry of Natural Resources, Six Nations of the Grand River, The Arboretum, University of Guelph, The Seed Source, and Townsend Lumber.

During 1994 the main activity of the Forest Gene Conservation Association was the development of a strategy. The objectives of the FGCA as identified in the strategy are:

1) to promote the conservation of the existing genetic base for woody plants species
2) to support ecosystem and species restoration programs
3) to increase environmental, economic and social benefits of planting through genetic management of selected species
4) to support research that contributes to the understanding, maintenance and restoration of the genetic base of woody plant species in Southern Ontario.

Forty–two action items are proposed which address; the conservation of the existing genetic base of species and populations that are relatively abundant and have wide distribution; conservation and restoration of species and populations that are most vulnerable to genetic erosion due to rarity or disease; and the use of existing seed orchards and genetic tests to provide genetic material for a range of commercial uses such as wood products, native and exotic species for landscape stock, nut production and basket weaving.

The strategies proposed by the FGCA recognize the gains in efficiency achieved when activities associated with genetic resource management for commercial gain are integrated with activities associated with gene conservation.

Specific activities undertaken in 1994 and 1995 include:

1) Status reports for vulnerable, threatened and endangered species including Carolinian species, and rare populations including pitch pine in Ontario and white pine in specific areas of southern Ontario.

2) Butternut Canker project to raise awareness of the disease and to develop a conservation strategy for butternut. This is a cooperative project with OFRI with funding support from the Eastern Ontario Model Forest. Over 300 landowners have participated by providing information about butternut growing on their property.

3) Genecology studies for white spruce, white pine and red oak conducted in cooperation with OFRI with funding support from the Eastern Ontario Model Forest.

4) Forest Gene Conservation Workshop held in Ottawa in March, 1995, with funding support from Eastern Ontario Model Forest. This was a two day workshop; one day of genetic theory as it applies to conservation and one day of application of theory in the development of conservation strategies for several forest tree species.

5) Biologically Appropriate Seed Project– A working group of Association members is developing methods to educate the landowners about the importance of planting stock of appropriate seed source and to assist private growers in implementing the use of appropriate seed sources when growing stock for restoration projects.

Zone 5: North Shore Tree Improvement Association

The North Shore Tree Improvement Association was formed ten years ago. The members support programs in gene conservation, collect seed to meet artificial regeneration needs, and cooperate on intensive tree improvement programs to improve jack pine, white pine, black spruce, white spruce, tamarack, and red spruce.

GENE CONSERVATION – Occurrence maps have been completed for red maple, black ash, white elm, and yellow birch. This is part of an ongoing project to identify species of concern in Zone 5. A red spruce status report is planned for 1995.

SEED MANAGEMENT – New seed zones have been developed for Ontario, and Zone 5 has 9 seed zones. These are based on the Ontario Climate Model, and incorporates the Ontario Elevation Model and genecological information. Zone 5 is participating in genecology studies in jack pine, white pine, and white spruce. This information will be used to establish seed transfer rules and define breeding zones.

INTENSIVE TREE IMPROVEMENT – A reduction in artificial regeneration and budgetary constraints have led to a paring down of the intensive tree improvement program.

<u>Jack Pine</u> – Four family tests were measured in the fall of 1993. The first roguing in 3 orchards was completed. Five of the 7 first generation orchards have now been rogued. Only two populations from these original seven orchards will be carried forward into subsequent generations. The remaining program will be archived for gene pool conservation and some seed production. 13.75 hectolitres of cones from rogued orchards have been collected.

<u>Black Spruce</u> – One of the two seedling seed orchards was removed, as this was a site chosen for an oriented strand board mill. A new site was chosen and the site was prepared for planting this orchard in 1995. Three family tests were measured in 1993.

<u>White Pine</u> – There are currently seven clonal seed orchards in place. These orchards have been maintained as needed. This program is on hold, until genecological data is available to define biologically sound breeding zones. Based on the size of the breeding zones and level of interest, the appropriateness of continuing with this species will be decided.

<u>White Spruce</u> – There are three clonal seed orchards and planting was completed in 1994. This program will also be evaluated when genecological information becomes available.

<u>Tamarack</u> – There is one clonal orchard, established in 1985, which received general maintenance.

<u>Red Spruce</u> – The establishment of this clonal orchard was completed in 1992. Since then maintenance, survival assessments, and refilling has occurred. Selections for this orchard are from Algonquin Park, New Brunswick, and Nova Scotia.

Zone 4: Northeast Seed Management Association

In 1993 the Northeast Seed Management Association (NESMA) was formed. The main goal of NESMA is to provide genetically improved seed for forest management, as well as, the wise use and deployment of all seed.

The most notable event in seed management has been the recent revision of the seed zone boundaries based on local long term climatic patterns and a model which incorporates plant growth variables.

Currently the Association's tree improvement work is focussed on black spruce and jack pine; the most recent highlights of this work in the intensive program are summarized below.

<u>Black Spruce</u>

The results of the genecology study lead by Dr. Dennis Joyce has shown that this species is very specialized with respect to its adaptation to climate since a difference in growing season length of more than seven days can result in maladaptation.

The unpublished results from the black spruce flower induction study done in cooperation with Dr. Rong Ho shows that NAA does not beneficially influence the production of male flowers in black spruce.

The farm field tests established in 1991 and 1992 are annually assessed for total height and condition. The 1994 assessment data and information from the assessment of the Aidie Creek seed orchard will be used to rogue the orchard in the fall of 1995.

The establishment phase of the intensive program ended with the planting of a small number of trees in the Ramore, Evelyn and Bonner seed orchards.

It is planned that at least one seed orchard will be rogued each year during the 1996 to 1998 time period. The assessment of the associated family tests will also be a priority during these years.

Jack Pine

A newly developed Cone Crop Monitoring System has been tested and used for two years in three seed orchards. Initial problems with the software and sampling have been overcome and the system is a useful orchard management tool.

Stem form and DBH were recorded for the first time during the assessment of three jack pine family tests and the Aidie Creek seed orchard in preparation for the fall 1994 final roguing of the orchard.

Digested sewage was applied to part of a jack pine seed orchard to study its usefulness as a source of nutrients. There were no positive or negative effects.

Data collection concluded and a user friendly pollen dispersal model was produced for Island Lake Tree Improvement Area. Pollen dispersal and phenology continues to be monitored at the Ramore seed orchards.

A crown management literature review was done and a strategy was written. Initial topping at Aidie Creek required that a substantial portion of some trees be removed; the 10,000 trees topped at Island lake required moderate treatment, while at Ramore training of the crown in younger trees required a very light treatment.

Two farm field tests representing 100 families were planted. The total height and condition of the trees is assessed annually. In addition, six family tests were assessed in preparation for the 1995 first roguing of the Ramore orchard and final roguing of the Island Lake orchard.

By the fall of 1995, all of the jack pine seed orchards will have been rogued at least once. Preliminary plans are being made to advance one population into the second generation.

Zone 3: Lake Nipigon East Seed Management Association

The members made substantial progress in forming a Seed Management Association. The Association works with members of Zone 2 to share in funding and support from a Tree Improvement Specialist. The Association focuses on support for operational seed and stock deployment. Results from Dr. Bill Parker's, 'Focal Point Seed Zone' research at Lakehead University on black spruce and jack pine were provided to members to assist in operational seed and stock deployment decisions.

Two mature black spruce and white spruce clonal seed orchard complexes were maintained and cones were collected. At the Kimberly Clark, Angus McPherson Seed Orchard 3.5 hl. of black spruce cones were collected in 1994. In addition, clone banks for black spruce and white spruce were maintained and a new jack pine clone bank is scheduled to be established this year.

Zone 2: Lake Nipigon West Tree Improvement Association

The industry - government cooperative tree improvement programs, which began in the late 1980's in jack pine and black spruce, have shown excellent progress. Technical and coordinating support is provided to the members by a cooperatively funded Tree Improvement Specialist. The members are now starting to collect improved seed from seed orchards, and are planning for second generation activities. Tending of existing tests and orchards was a major priority during the past two years. The Association also provides support for seed and stock deployment. Results from Dr. Bill Parker's, 'Focal Point Seed Zone' research at Lakehead University on black spruce and jack pine were provided to the members to assist in operational seed and stock deployment decisions. The following are highlights from the tree improvement programs.

Black Spruce

In the breeding zone north of Thunder Bay three family tests and part of the seedling seed orchard were measured. Data analysis will be completed this summer in preparation for first roguing. In the spring of 1994, a new seedling seed orchard (8 ha) was planted by the OMNR at the former Thunder Bay Forest Nursery. Also established at the nursery was a new breeding orchard.

Jack Pine

The first roguing of the Kakabeka Falls seed orchard (14 ha) was conducted by Avenor. Operational cone collections from the orchard have begun with the improved seed being distributed among the members. A cone collection from top ranking families was used in establishing a demonstration plantation in the Abitibi-Price demonstration forest.

Zone 1: Lake of the Woods – English River Seed Management Association

Maintenance and management activities, including the implementation of some advanced techniques in cone crop monitoring and the investigation of flower induction treatments, continued in the Association's 73 tree improvement installations.

Three additional first generation black spruce orchards received initial roguing. In combination with this treatment, two of the orchards were also crown pruned to reduce tree height and to facilitate cone collection and other management activities. A black spruce breeding orchard site was selected and cleared at the Dryden Tree Nursery.

The black spruce seed orchards continue to increase their yield of improved seed. Prior to cone harvest last fall, a cone crop monitoring program developed by the Ontario Forest Research Institute and the Canadian Forest Service was used to make accurate predictions of cone yields. A total of 283 hl. of black spruce cones were collected from seven orchard sites. A phenomenal 138 hl. of cones came from one 12 year–old, 5 hectare black spruce orchard in Dryden alone!

The jack pine orchard and test sites were regularly inspected and management activities, such as pest control, survival assessments and fertilization, were completed as required. White pine weevil and the eastern pine shoot borer were significant concerns in a number of the jack pine test sites. Height measurements and an assessment of damage and condition were completed for three tests in one of the breeding populations. Roguing of the first generation orchard associated with this population is scheduled for the following year.

This year, the Association co–hosted the first Annual Provincial Seed Orchard Manager's workshop with the Ontario Tree Improvement Board. These workshops are designed to provide field managers with an opportunity to improve their knowledge and expertise concerning various technical aspects of orchard management. This year, the workshop was held in Dryden and the participants heard presentations from a number of experts on various aspects of crown management and pest control.

The Association continued its sponsorship of a study of Black spruce genecology being conducted by Dr. Bill Parker of Lakehead University. Population samples were collected in

1993 and field trials were established this year using this material. Results of the study will be used to investigate adaptive variation in black spruce and draft focal point seed zones that will be used to guide stock and seed transfers in artificial regeneration programs.

Science Support

In collaboration with the Canadian Forest Service, several models and decision support tools have been developed. The hierarchical system of homogeneous climatic regions developed from the Ontario Climate Model has provided the base for a new system of seed zones.

In collaboration with the Canadian Forest Service – Ontario Region, an expert system (SeedWhere) was developed to guide long distance transfer of seed and stock. Software to track seed collection and processing costs has also been developed collaboratively with the CFS. The completion of the climatically–based seed zone system has positioned the operational tree improvement program to move into second generation breeding in an efficient manner. A pilot second generation breeding program will be identified and implemented in the summer of 1995.

Genetic Diversity Project

A prospectus outlining the scope and direction of the Genetic Heritage Program has been developed and published. Species status reports and conservation strategies form the core of the program. Status reports for 11 Carolinian tree species have been prepared and/or updated. Regional status reports in Northwestern and Northeastern Ontario are nearing completion. An Ontario Tree Atlas Project was initiated to document the coarse scale distribution and relative abundance of Ontario's trees. The recently developed Ontario Climate Model and the Digital Elevation Model are being used in support of mesoscale spatial analyses of genetic diversity. A conceptual framework for incorporating genetic resource management concepts into forest management planning has also been developed. Practical gene conservation guidelines are the next step to implementing genetic resource management.

Genecology Project

Five studies directed at identifying spatial patterns of adaptive variation are in progress. The data collection for the oldest study (black spruce in northeast Ontario) will be completed in 1995. Data collection in the jack pine, white spruce, red oak, and white pine studies are continuing. Genetic models and seed transfer guidelines will be developed from these studies.

Data Management Project

The Tree Improvement Data Management Project is responsible for the data analysis and systems development needs of the operational program. In the last two years, data from fourteen breeding zones has been analyzed, largely for the purpose of first-generation roguings of black spruce and jack pine seedling seed orchards. Work on the Provincial Tree Improvement Database System (TISYS) is continuing, with the plus tree and grafting modules complete and ready for implementation-testing. In a joint project with Peter de Groot (Canadian Forest Service), funded by NODA, the Cone Crop Monitoring System (CCMS) is complete and now in operational use in seed orchards across the province. Under the same project, a Pest Information Management System and Pest Management Decision Support System have been developed to aid seed orchard managers in dealing with pest problems.

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

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Keywords: DNA fingerprinting, RAPD markers, cold hardiness, isozyme, drought tolerance, antioxidant, Salicaceae, *Picea mariana* (Mill.).

Research at the Forest Genetics Laboratory (FGL) is coordinated by L. Zsuffa, Professor Emeritus of Forest Genetics. The laboratory currently has one Ph.D. and four Master's students. Also contributing to studies are 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: genetic characterization of *Salix* L. and *Populus* L.; pathogen and wound-induced expression of peroxidase in *Populus maximowiczii* Henry; genetic variation in cold hardiness of *Salix* spp.; genetic markers for growth, drought tolerance and response to antioxidant action in black spruce; progeny and clonal testing; biomass quality studies; and assessment of species and clonal variation in resistance to pests and diseases.

GENETIC CHARACTERIZATION OF *SALIX* AND *POPULUS*

This research is coordinated by L. Zsuffa, under the International Energy Agency Bioenergy Agreement (IEA/BA) Genetic Improvement Activity. Several researchers have worked on this project. The study was initiated to determine molecular and biochemical markers for reliable and efficient genetic identification, certification and preservation of poplar and willow clones, and for effective genetic improvement.

RAPD (Random Amplified Polymorphic DNA) fingerprints were used to differentiate species and hybrids of the genera *Populus* and *Salix* as well as to identify individual clones. Fifty-five poplar clones and 81 willow clones were included in this study. Of the 17 random DNA primers tested, only 4 primers were needed to differentiate 55 poplar clones into species and hybrids. Individual clones were further characterized by their DNA polymorphism obtained with two primers tested. Species and hybrids of tree-type willow clones (17) could be differentiated with 3 primers whereas shrubby willow clones (17), originating from North America, could be differentiated with 2 primers. Species of *S. udensis* Trautv. & Mey., *S. dasyclados* Wimm. and *S. viminalis* L. could be differentiated by 2 primers.

The work is progressing well, and has received further support from the IEA/BA.

PATHOGEN AND WOUND-INDUCED EXPRESSION OF PEROXIDASE IN *POPULUS MAXIMOWICZII*

Higher plants have a number of inducible defense mechanisms for protection against abiotic factors, wounding, or pathogens. In trees, one typical response to pathogen attack or wounding involves compartmentalization of affected tissue via lignification. Peroxidase is one of the enzymes involved in lignification. This study investigated the pattern of expression of peroxidase isoenzymes before and after wounding and/or pathogen attack. The results of this study should contribute to the understanding of *Populus* molecular genetics through revealing the isoenzymes that are involved in pathogenesis. Furthermore, it is expected that the remaining constitutively expressed isoenzymes (those present both before and after pathogen attack or wounding) could then be sequentially analyzed with respect to their potential developmental significance in controlling tissue lignin content. This is a collaborative study with Dr. M. Hubbes of the Faculty of Forestry, University of Toronto.

GENETIC VARIATION IN COLD HARDINESS OF *SALIX* SPECIES

The intention of this study is to evaluate interspecific and intraspecific variation in cold hardiness in some *Salix* species. The analyses of variance for frost injury will be conducted at different temperatures during different seasons. The goal of this study is not only to identify species or genotypes which show high vitality after freezing temperatures but also to search for clones whose axillary buds sprout quickly after freezing damage, thereby keeping the loss of biomass to a minimum.

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

The objectives of this research are to: determine fast growing and drought tolerant genotypes of black spruce; investigate the relationship between heterozygosity and growth, and heterozygosity and drought tolerance; determine genotypes with the most significant increase in growth and drought tolerance as a result of antioxidant application; and, using 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. The 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... To date, four pilot plantations have been established, totalling approximately 7 hectares in area.

It is essential that continued breeding be carried out to introduce new varieties into the production population to ensure continued gains in yield, quality, and pest resistance, and to develop clones for specific site conditions.

DISEASE RESISTANCE/SUSCEPTIBILITY

Resistance to disease is an important characteristic to observe in any plant breeding programme. Currently, we are participating in the IEA Rust/Clone Interaction Trial, which is intended to compare clonal resistance/susceptibility to diseases (primarily *Melampsora epitea* Thuem. rust) and pests in several identical trials located in Europe and North America.

TREE-FORM WILLOWS

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 and 1992. These plantations are being studied 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 willow, which permits growth comparisons within and between the genera. Both trials were measured at the end of 1993 and will be assessed again in 1996 - at mid-rotation.

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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 DU PIN GRIS, DU PIN DE MURRAY ET DU PIN SYLVESTRE PAR ROGER BEAUDOIN

Mots-clés : Tests de descendances, tests de provenances, éclaircie génétique, croisements dirigés.

L'éclaircie génétique d'un verger à graines de pin gris (Montreuil) a été réalisée en 1993 et 1994 et celle du verger à graines de Lavergne en 1994. L'éclaircie finale du verger à graines de Harrington a été effectuée en 1994. En résumé, à ce jour un total de 6 vergers, sur 12 établis, ont été éclaircis.

En 1993, des greffons ont été prélevés sur 29 et 46 arbres dans deux tests de descendances de pin gris (Briand et Chasseur) et en 1994 sur 70 arbres dans un autre test (Montreuil) en vue de l'établissement de parcs de croisements.

Onze croisements dirigés ont été réalisés en 1993 et 22 en 1994 sur des clones d'arbres-plus de pin gris d'une provenance recommandée (Briand). Les clones ont été évalués par sélection récurrente.

L'analyse statistique, en 1993, de 11 tests comprenant entre 100 et 134 provenances de pin de Murray de 10 ans et plus et en 1994 de 12 tests de pin sylvestre âgés de 10 ans. Cinquante-six provenances de pin sylvestre sont d'Europe et d'Asie dont certaines de vergers à graines de France, Hongrie, Danemark, Belgique et d'Angleterre. Quinze provenances de pin sylvestre du Québec, utilisées comme témoins, sont issues de plantations. Les résultats obtenus avec ces deux espèces permettront de faire des recommandations pour différentes régions du Québec.

VERGERS À GRAINES SOUS ABRI
PAR STÉPHAN MERCIER

Mots–clés : Pollen, pollinisation, verger à graines sous abri, triage des graines, avortement des cônes, production de plants.

La Division de R-D sur les semences, boutures et plants a initié au cours des deux dernières années un projet portant sur la mise en place de vergers à graines sous abri. Ces vergers sont actuellement axés sur la production de graines améliorées d'épinette noire, d'épinette blanche et de mélèze hybride. 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. Ces vergers sont constitués de six tunnels (4 EPN, 1 EPB et 1 MEH) au Centre de greffage de Duchesnay et de deux tunnels d'épinette blanche à la pépinière forestière de Saint– Modeste.

PRODUCTION ET UTILISATION DE SEMENCES

Les principaux travaux de R-D ont porté sur l'élaboration d'un système de triage des graines de manière à obtenir une semence viable par cavité, sur l'avortement des cônes dans les vergers et l'influence de la dimension des graines sur la production de plants. De plus, nous avons terminé les travaux concernant la contamination pollinique dans les vergers à graines, la levée de dormance des graines d'arbres feuillus et la maturation des graines.

SÉLECTION D'ARBRES FEUILLUS POUR LEUR RÉSISTANCE AUX MALADIES PAR MARIE–JOSÉE MOTTET

Mots–clés : *Populus*, *Betula*, sélection, résistance, *Septoria musiva*, *Hypoxylon mammatum*, *Nectria galligena*.

Les travaux ont porté sur le peuplier et le bouleau. 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 600 semis de peuplier ont été sélectionnés pour leur résistance ou faible sensibilité. La stabilité de la résistance des plants sélectionnés est suivie en plantation. À chaque année des échantillons sont prélevés en plantation et l'évolution de la virulence des isolats de *S. musiva* est étudiée dans un dispositif spécial de 20 clones en pépinière.

Les résultats d'inoculation en serre et en pépinière nous ont permis de noter des différences de sensibilité à *Hypoxylon mammatum* entre certains clones de peuplier. Environ 500 semis de peuplier de la section Leuce ont été sélectionnés à la suite d'inoculations avec *H. mammatum* en pépinière. Quelque 135 de ces semis ont été clonés et seront évalués sur différents sites. La plupart des semis résistants sont des hybrides avec *Populus alba*. Sur une centaine d'autres clones testés en 1993 dans la région de l'Abitibi, aucun clone de peuplier faux–tremble ne montre une forte résistance. Par contre, les 30 clones d'hybrides de baumier sont généralement résistants.

Concernant le bouleau, un volet sur la sélection pour la résistance au chancre nectrien a été entrepris. 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. Deux ans après l'inoculation en pépinière, les observations préliminaires nous permettent de déceler des différences de sensibilité à *N. galligena*, entre huit lots de semences de bouleau jaune issus de différentes régions du Québec. Des tests de provenances–descendances avec une centaine de familles – principalement du bouleau jaune – seront établis en 1996. Des différences de sensibilité ont aussi été observées entre 17 clones de bouleau jaune inoculés en serre. Un bioessai sur des tigelles de bouleau est présentement expérimenté à petite échelle. Une subvention a été attribuée pour étudier la variabilité génétique chez *N. galligena*.

AMÉLIORATION GÉNÉTIQUE DES FEUILLUS À BOIS NOBLE

PAR ANDRÉ RAINVILLE

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

Le programme d'amélioration génétique du chêne rouge (*Quercus rubra* L.) est principalement orienté vers une stratégie à long terme ayant pour base les tests de provenances–descendances. Ainsi, cinq tests de provenances–descendances ont été établis en 1993 à partir de diverses sources de glands de l'Ontario, du Vermont et principalement du Québec; ils sont situés dans la partie sud de la province. Chacun de ces tests sera complété en 1995 et 1996 avec des provenances du nord–est des États–Unis en grande majorité.

Un second volet plus modeste du programme d'amélioration du chêne rouge se déroule simultanément au premier. Une centaine d'arbres–plus ont été sélectionnés en forêt sur des critères de qualité et greffés; ces greffes seront plantées en 1996 pour constituer des parcs à croisements en vue de produire des glands le plus rapidement possible pour le programme de reboisement du MRN. Ces semences seront aussi évaluées dans des tests de descendances.

Dans le programme d'amélioration du bouleau jaune (*Betula alleghaniensis* Brit.), plus de 120 arbres–plus ont été sélectionnés. Plusieurs de ces arbres ont fait l'objet de croisements dirigés intraspécifiques et interspécifques avec *Betula papyrifera*, *B. verrucosa* et *B. pubescens*. Très peu de semences de croisements interspécifiques ont été obtenues jusqu'à présent. Les plants produits à partir de toutes les semences de croisements sont installés en tests; des observations permettront d'évaluer la valeur des croisements. Entre–temps, de nouveaux croisements ont été réalisés aux printemps 1994 et 1995. Ils sont principalement interprovenances et plutôt intraspécifiques qu'interspécifiques; toutes les étapes du processus de pollinisation, allant de la méthode d'extraction du pollen à l'enlèvement des sacs sur les fleurs femelles, ont été étudiées. En 1994, la technique du « pollen mentor » n'a pas permis d'améliorer le taux de réussite des croisements interspécifiques. Le nombre de fleurs étant trop faible en 1995, des essais sont prévus en 1996 avec différentes concentrations de pollen mentor, sans compter l'ajout d'une nouvelle technique appelée « pollen pionnier ».

Chez le frêne d'Amérique (*Fraxinus americana* L.), un verger constitué de 313 ramets représentant 16 arbres sélectionnés (région de Montréal principalement) a été planté en 1994. La constitution de tests de provenances–descendances est retardée depuis deux ans en raison de la production erratique de samares de l'espèce.

Finalement, des efforts d'amélioration plus modestes sont consacrés pour reproduire les noyers noirs ayant démontré une certaine résistance au froid, ainsi que pour identifier des provenances et descendances d'érable à sucre ayant un taux de sucre supérieur et un phénotype intéressant. Pour le noyer cendré, des efforts de conservation *in situ*, hors de l'aire de distribution naturelle, sont consacrés pour protéger l'espèce d'une maladie dévastatrice, le « chancre du noyer cendré ».

AMÉLIORATION 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.

Dans le cadre du projet d'amélioration génétique des mélèzes, durant les deux dernières années, l'accent a été mis surtout sur les éclaircies génétiques dans les plantations expérimentales âgées de plus de 10 ans. Le but de ce travail est de transformer les tests, qui ont fourni des informations sur les performances de différentes provenances ou descendances, en source de graines génétiquement améliorées. Ainsi 17 tests composés de mélèze laricin, de mélèze d'Europe, de mélèze du Japon et de leurs hybrides, d'une superficie totale d'environ 30 ha, ont été traités. Ces éclaircies ont été toujours basées sur les mesures de la hauteur des arbres et sur l'évaluation de la flexuosité du tronc déterminées après 10 ou 15 ans de plantations. Des tests sont prévus pour évaluer les caractéristiques de descendances produites dans ces dispositifs éclaircis dès que la production des graines deviendra régulière.

Notre programme d'amélioration du mélèze vise surtout la production d'hybride interspécifique entre le mélèze d'Europe, le mélèze du Japon et le mélèze laricin. En utilisant les arbres sélectionnés dans nos tests plus âgés, nous essayons de développer des variétés adaptées aux conditions particulières de différentes régions. Dix–neuf croisements dirigés ont été effectués en 1994 et nous avons obtenu plus de 18 000 semences d'hybrides entre le mélèze d'Europe, le mélèze du Japon, le mélèze laricin et aussi le mélèze de Sibérie. En même temps, nous avons récolté 25 lots de pollen sur les arbres sélectionnés. Conservé dans la banque de pollen, ce matériel servira pour les travaux des prochaines années.

Les familles obtenues par croisement dirigé sont évaluées dans des tests de descendances en utilisant des plants multipliés par bouturage. Un test de ce type a été installé en 1993 dans l'arboretum de Verchères. Il est composé de 45 descendances. Un autre, représentant 26 descendances a été mis en marche dans la pépinière de Saint–Modeste en 1994. En plus deux autres tests sont en préparation dans la pépinière de Duchesnay en vue d'obtenir du matériel sélectionné pour les travaux d'hybridation : un test de 60 descendances de mélèze de Sibérie et un autre qui est composé de 20 descendances de mélèze d'Europe var. *sudetica*.

Les travaux se sont poursuivis aussi dans le cadre de notre projet d'amélioration génétique de l'épinette de Norvège. Les éclaircies génétiques ont été effectuées dans 10 dispositifs expérimentaux (7,9 ha) qui pourront servir comme source de graines génétiquement améliorées. Une éclaircie effectuée dans un test de provenances de l'arboretum de Duchesnay avait pour but d'évaluer l'impact du charançon du pin blanc sur la productivité de la plantation.

Les croisements dirigés avec l'épinette de Norvège sont actuellement réalisés par la Division de semences forestières de notre Ministère. Les graines ainsi obtenues sont semées dans la bouturathèque de la pépinière de Saint–Modeste et les plants, multipliés par bouturage servent pour le reboisement et pour les tests de descendances. De cette façon en 1993 un test incluant 49 descendances et un autre composé de 31 familles ont été mis en marche. Des nouveaux croisements dirigés ont été effectués en 1994. La floraison abondante de cette année a permis d'effectuer 93 croisements et de produire plus de 30 000 graines. Les tests avec ces familles seront mis en marche dès que possible. Tous ces croisements ont pour but la production de semences génétiquement améliorées pour le reboisement de la zone C d'amélioration de l'épinette de Norvège (Bas Saint-Laurent – Gaspésie).

Au printemps 1994 trois nouveaux tests composés de 267 descendances ont été transférés sur le terrain dans la zone A (domaine écologique d'érablière à bouleau jaune). Les mesures ont été prises aussi au niveau de la pépinière et la compilation des données est présentement en cours. Les résultats permettront de comparer ces tests avec les dispositifs similaires établis il y a quelques années dans la zone C. Ce projet qui est mené en collaboration avec le Service canadien des forêts se poursuivra l'année prochaine par la mise en marche de la dernière série de trois tests qui seront installés dans la zone d'amélioration B (l'érablière à tilleul et érablière à bouleau jaune).

SÉLECTION DE CLONES ET AMÉLIORATION GÉNÉTIQUE DU PEUPLIER

PAR GILLES VALLÉE

Mots-clés : Populus, croisements, tests clonaux, tests de descendances et de provenances, sélection de clones.

Le manque de bois de peuplier faux-tremble (*Populus tremuloides* Michx.) dans plusieurs régions du Québec augmente l'intérêt de l'industrie forestière et des producteurs de bois (forêt privée) pour les plantations de peuplier. Durant 1993 et 1994 une collaboration plus intense a été obtenue de la part d'industriels et d'organismes de la forêt privée pour la réalisation de travaux d'amélioration génétique des peupliers.

En 1993 et 1994, 1 352 croisements intra et interspécifiques ont été réalisés dont 284 ont produit 21 259 semis. Dans les sections aigieros et tacamahaca, les croisements ont été faits en utilisant les meilleurs génotypes d'espèces et d'hybrides introduits et testés au Québec depuis plus de 20 ans, tels que *P. maximowiczii* Henry, *P. trichocarpa* Torr et Gray *P. nigra* L., *P.* x *interamericana*, *P.* x *euramericana*, (Dode) Guinier croisés avec les espèces *P. deltoides* Marsh, *P. balsamifera* x duroi et les hybrides indigènes *P.* x Jackii Sarg. et *P.* x Rollandii Rouleau. Dans la section leuce les espèces et hybrides *P. alba* L., *P.* x canescens Smith et *P. tremula* L. ont été croisés avec *P. tremuloides* et *P. grandidentata* Michx. Les hybrides de la section leuce sont développés pour les conditions de sols acides.

Durant ces deux dernières années 11 tests de clones de peupliers ont été établis et un test de 31 descendances (demi-fratries) afin d'évaluer et comparer le comportement de descendances de *P. grandidentata*, *P. tremuloides*, *P. tremula*, *P. alba*, *P. maximowiczii*, *P. trichocarpa* et des descendances hybrides de ces espèces sur sol acide.

L'interprétation des données prises dans les tests clonaux ainsi que dans les quartiers de pieds-mères du MRN a permis de préciser les clones à recommander pour les reboisements selon les régions écologiques du Québec. Les données dendrométriques des clones recommandés montrent qu'en pratiquant une bonne culture des plantations, des accroissements annuels en volume marchand de 10 à 15 m^3/ha sont réalisables, selon la fertilité des sols et sur des rotations de 10 à 20 ans, dans les domaines écologiques de 1 à 8 du Québec. Ces résultats confirment que les hybrides de type baumier avec *P. balsamifera* et *P. trichocarpa* sont les plus performants tant sur le plan rusticité que sur le plan d'adaptation au sol de fertilité moyenne et acide (Ph = 5,5 à 6,0). À noter que des nouveaux clones obtenus de croisements avec un peuplier baumier d'Asie, *P. maximowiczii*, donnent des croissances exceptionnelles dans des tests âgés de 5 ans et moins et laissent entrevoir un potentiel supérieur aux clones actuellement recommandés.

AMÉLIORATION GÉNÉTIQUE DE L'ÉPINETTE NOIRE
PAR MICHEL VILLENEUVE

Mots–clés : Épinette noire, tests de descendances, tests clonaux, embryogenèse somatique, croisements dirigés, bouturage.

Le réseau de tests de descendances accompagnant les vergers à graines de 1re génération a été complété en 1993 avec l'ajout de 3 tests. L'implantation des tests de familles issues de croisements dirigés est aussi terminée. Entre 1993 et aujourd'hui, 25 nouvelles plantations expérimentales ont été réalisées. On dénombre maintenant 29 tests de provenances, 67 tests de descendances et 10 plantations clonales. Dans 20 tests de descendances, les plants ont été produits par bouturage. Trois tests clonaux sont en préparation pour 1996. Le premier comparera 200 ortets (clonés par bouturage) des 10 meilleures familles d'un test précoce. Les deux autres compareront des clones produits par embryogenèse somatique, directement (26 clones) et après bouturage (22 clones).

La première prescription d'éclaircie génétique dans un verger de 1re génération a été faite en 1993 pour le verger de Harrington. Cinq autres éclaircies sont prévues pour 1995, d'après les résultats de 10 ans de croissance en tests de descendances. Les gains génétiques calculés pour la croissance en hauteur sont de l'ordre de 5 %.

Deux plantations de démonstration du potentiel de l'embryogenèse somatique ont été établies en 1993. Elles contiennent déjà 2 874 plants de 69 clones d'épinette noire et 1 373 plants de 7 clones d'épinette blanche. Des plants de 6 clones issus d'embryogenèse somatique ont été mis en terre dans 5 arboretums (3 à 5 ramets/clone par endroit). Lorsque la floraison débutera, les mêmes croisements seront faits à chaque endroit, afin de déceler chez les descendances des variations dans l'expression des gènes. Ces variations épigénétiques pourraient être causées par un préconditionnement environnemental.

Les tests précoces en pépinière donnent des résultats intéressants. L'héritabilité familiale pour la hauteur varie de 0,76 à 0,89 dans 3 tests. Après 4 saisons de croissance, on observe des écarts de 32 % à 35 % en hauteur entre les 10 meilleures familles (parmi 70) et les provenances témoins. Ces tests précoces sont utilisés pour éliminer rapidement les moins bonnes familles. Leur validité sera confirmée lorsque les tests à long terme seront mesurés.

Le protocole de suivi démographique des populations familiales de boutures a été complété ce printemps. Le suivi individuel de 7 560 boutures de 21 familles s'est fait depuis le bouturage jusqu'à l'expédition des plants. L'analyse des données est incomplète, mais les étapes critiques semblent être la période d'enracinement et le tri avant expédition. On voulait comparer la diversité génétique des lots de plants livrés pour le reboisement avec la diversité des variétés multifamiliales servant au bouturage.

En 1993, une étude portant sur l'effet du porte–greffe sur la performance du greffon a été entreprise. Dans le cadre de la mise au point d'un parc de croisements sous abri, on veut évaluer la croissance ainsi que la précocité et l'abondance de la floraison de plants greffés en fonction du porte–greffe utilisé. Les résultats du bouturage préliminaire à cette étude sont rapportés dans un article à paraître dans la Revue canadienne de recherche forestière. Il est possible d'obtenir un bon taux d'enracinement avec des ortets âgés de 9 ans, ce qui permet d'envisager le bouturage au lieu du greffage pour reproduire végétativement un arbre élite. Étonnamment, les boutures prélevées sur des arbres florifères se sont mieux enracinées que celles prélevées sur des arbres encore infertiles (48 % *versus* 30 %).

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CURRENT RESEARCH IN FOREST GENETICS AND TREE BIOTECHNOLOGY AT LAVAL UNIVERSITY

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Several positive developments have occurred during the past two years at Laval University in the field of forest genetics and tree biotechnology. Dr. Francine Tremblay from Petawawa National Forestry Institute, who had been from 1990 to 1994 at the Centre de recherche en biologie forestière (CRBF) through the Interchange Canada Programme between the Canadian Forest Service and Laval University, has become professor at the Département des sciences du bois et de la forêt in 1994 and full member of the CRBF. Our molecular biology and in vitro tissue culture labs have moved from the old forestry building to an adjacent newly built research facility regrouping researchers in cell and molecular biology from the Faculties of forestry, agriculture, sciences & engineering, and medicine. This modern research facility offers a higher level of interaction among professors of different departments in these disciplines and is making available several core services such as electron and confocal microscopy as well as DNA synthesis and sequencing. Dr. Jean Bousquet has taken the chair of the CRBF since June 1995. The Center is composed of 13 professors-researchers with their 65 graduate students and a research staff of more than 50 people.

Dr. Louis Parrot has retired in 1995 from his active teaching and research activities, but will remain involved in a number of projects in plant systematics and dendrology. He will be remembered as the pioneer in forest genetics and tree improvement at Laval University and one of the pioneers with Drs. Laurence Roche and Gilles Vallée in this discipline in Québec. While maintaining collaborative links with us, Drs. Armand Séguin and Peng Li have moved with the research teams of Drs. Pierre Charest and Ariane Plourde, respectively, at the Petawawa National Forestry Institute and the Centre de foresterie des Laurentides of the Canadian Forest Service. Dr. Rodolphe Boivin, formerly with Dr. Guy Bellemare in plant molecular biology, has moved in Francine Tremblay's research team by the end of 1993, as senior research associate in the areas of molecular biology of somatic embryogenesis and tree biotechnology. Otherwise, F. Tremblay's team has included, over the last two years, postdoctoral fellows Dr. Aristidis Paterakis (from Aristotelian Univ. of Thessaloniki), Dr. Diane Bergeron (from Laval Univ.), Dr. Roumiana Gulémétova (from Univ. of Sofia), and Dr. Halim Chtourou (from Laval Univ.), Ph.D. students Emanuel Sildor, Abdelmalek El Meskaoui, and Christine Ory, M.Sc. students Slaheddine Khlifi, Fabrice Lantheaume, Claude Bomal, and Ying-Hong Li (co-supervised by A. Séguin and J. Bousquet), and research associates Laurence Tremblay, Caroline Levasseur, Bayardo Beaulieu, Julie Bélanger, Julie Belles-Isles, and Patrice Lavoie.

J. Bousquet's group has been composed over the past two years of postdoctoral fellows Dr. Peng Li (now at Centre de foresterie des Laurentides), Dr. Laurent Maggia (from

Univ. Paris VII, NSERC fellow, now at Univ. Lyon I), and Dr. Sylvain Jeandroz (from Univ. Besançon, Lavoisier fellow), Ph.D. students Nathalie Isabel (co-supervised by F. Tremblay), Jérôme Laroche, Martin Perron, Sylvie Richard (supervised by A. Séguin), and Stéphane Plante, M.Sc. students Éric Forget and Philippe Thériault (co-supervised by Dr. Jean Beaulieu, Nat. Res. Canada), and research associates Alice Roy and François Larochelle.

MOLECULAR PHYLOGENETICS

This area of research is actually taking less importance as funds allocated to molecular phylogenetics are limited to small grants from NSERC and FCAR (Québec research fund). However, owing to collaborative efforts, several projects have again been completed in the past two years. As part of Dr. Louise Savard's (now at IRBV of Univ. of Montréal) Ph.D. thesis now completed, and in collaboration with Drs. Steve Strauss (Dept. Forest Science, Oregon State Univ.) and Mark Chase (Dept. of Biology, Univ. of North Carolina), we have estimated the phylogenetic relationships among the large groups of seed plants including the classes of gymnosperms, using DNA sequences from the chloroplastic gene *rbc*L and the nuclear gene coding for 18S rRNA. We found that gymnosperms are monophyletic and using calibrated and statistically assessed molecular clocks, we have estimated for the first time the divergence time between modern gymnosperms and angiosperms, which was around 300 million years (Savard *et al.* 1994). Using similar approaches and DNA sequences from 12 mitochondrial genes, we have also estimated the divergence between the monocots and dicots of angiosperms at 200 million years (Laroche *et al.* 1995). Other phylogenetic studies conducted in collaboration with Drs. Luc Simon, Maurice Lalonde (from CRBF) and Roger Lévesque (from Faculty of Medicine) have also shown, from the use of 18S sequences, that the origin of endomycorhizal fungi could be traced as far back as 400 million years, pointing at their potentially crucial role at a time where the first land plants emerged from the sea (Simon *et al.* 1993).

Other studies have more focused on the use of DNA sequences from the gene *rbc*L, the gene coding for 18S rRNA, or from the internal transgenic spacer (ITS) of the rDNA repeat unit to achieve the phylogenetic reconstruction of genera, classes, and orders involving dicotyledonous woody species from the families Betulaceae, Fagaceae, and Casuarinaceae (Frascaria *et al.* 1993, Maggia and Bousquet 1994, Savard *et al.* 1993). Our current studies in that area are conducted by J. Laroche on the use of mitochondrial DNA sequences to reconstruct plant phylogenies and by S. Jeandroz on the use of ITS sequences and morphological characters to estimate the phylogeny of the genus *Fraxinus*.

MOLECULAR POPULATION GENETICS AND BREEDING

Our ongoing projects on the use of molecular markers obtained by DNA amplification of arbitrary position have blossomed into quite a number of interesting developments during the past two years. Following the initial use of these markers to assess the genetic stability of black spruce clonal populations derived from somatic embryogenesis (Isabel *et al.* 1993), these markers and microscopic characterization have been used to assess the underlying physiological and genetic causes of rare *variegata* phenotypes recovered after somatic embryogenesis in white spruce (Isabel et *al.* 1995a).

In collaboration with J. Beaulieu (Nat. Res. Canada), the variation at polymorphic DNA loci and at allozyme loci has also been compared within and among black spruce populations in Québec (Isabel *et al.* 1995b). Because haploid megagametophytes were used to obtain maternal genotypes, the dominance problems associated with these markers could be avoided. Heterozygosities and gene diversity estimates were comparable between the two classes of markers. Therefore, the variation at enzyme loci essentially reflected the effects of neutral evolutionary forces. However, when "phenotypic" DNA fingerprints were inferred and used in gene diversity analyses, as would be the case if diploid vegetative tissues were used to genotype mature trees, the gene diversity estimates were strongly biased with most of methods of estimation, owing to the dominance problems. This is a major concern for the studies using "phenotypic" dominant fingerprints instead of genotypic data, especially when typically small population samples are used. Among the solutions, there is a need to increase the sample sizes when working at the nuclear DNA level. There also exist sophisticated methods of numerical analysis that partially correct for the biases induced by the dominance problems.

A similar project aimed at comparing the genetic diversity derived from genotypic data at enzyme and nuclear DNA loci has also been initiated recently by P. Thériault in collaboration with J. Beaulieu. This project is even more important that St-Lawrence Valley populations of white pine have been showed by J. Beaulieu to be genetically less diverse, using enzyme markers. With funding made available by the MRNQ (Ministère des Ressources Naturelles-Québec), specific DNA fingerprints have also been developed in collaboration with Dr. Alan Gordon (Institute of Forestry Research, OMNR) to recognize unambiguously black spruce and red spruce from their hybrids (Perron *et al.* 1995). This collaboration is actually a direct fallout from animated discussions at the last CTIA meeting in New Brunswick! The use of DNA markers also confirmed previous observations derived from isozyme markers that red spruce is genetically depauperated, as compared to black spruce. The markers are currently being used to monitor natural hybridization and for certification of crosses aimed at developing varieties more tolerant to herbaceous competition during the juvenile phase, in collaboration with Mr. Michel Villeneuve (MRNQ). Similar approaches supported by the France-Québec Cooperation Program are also being followed by S. Jeandroz in collaboration with Drs. André Bervillé (INRA-Versailles), Alain Pugin (INRA-Montpellier) and Nathalie Frascaria (Univ. Orsay-Paris) to develop species-specific markers to distinguish closely related species and their natural hybrids in the genus *Fraxinus.*

Several projects in the area of marker-aided selection have been initiated during the past two years. One is concerned with genome mapping and assisting selection for mature wood density in eastern white spruce, and is lead by N. Isabel in collaboration with Drs. J. Beaulieu (Nat. Res. Canada) and John Carlson (from UBC) (Bousquet *et al.* 1994a). A similar project should also be initiated this summer on black spruce with M. Villeneuve (MRNQ). Another project lead by É. Forget in collaboration with André Rainville (MRNQ) and its financial support is aimed at developing markers for increased sap sugar content in sugar maple.

Our projects on the genetics and tree improvement of Acacia and its use in agroforestry systems are now completed (Khasa *et al.* 1993, 1994, 1995). They were lead by Dr. Khasa Phambu, who has completed his Ph.D. in 1993 and who is now part of Drs. Bruce Dancik and Om Rajora's research teams at the University of Alberta. In collaboration with J. Beaulieu (Nat. Res. Canada), Corporation forestière Transcontinental and with the support of the program "Essai, expérimentation et transfert technologique" of the Canadian Forest Service, Dr. Peng Li (now at Nat. Res. Canada) has completed the study on early testing in white spruce and is currently completing the study on the delineation of breeding zones for white spruce in Québec using multivariate approaches. The goals of the project are to select for superior materials adapted to eastern Québec from local sources and 2nd generation materials derived from advanced generation breeding at CFS-Québec. Family and clonal tests have been established on two sites during the past two years.

SOMATIC EMBRYOGENESIS AND BIOTECHNOLOGY

During the past two years, research projects conducted in the laboratory of Dr. F. Tremblay with the financial support of MRNQ have focused on establishing the optimal conditions for somatic embryogenesis of black spruce. Different concentrations of L-glutamine and different nitrogen sources in the medium were compared during maturation of black spruce somatic embryos. This study completed by S. Khlifi showed that organic nitrogen (L-glutamine) can be used as the sole nitrogen source into the maturation medium (Khlifi and Tremblay 1995a) and improved the number of somatic embryos produced by six embryogenic tissue lines. Another study, conducted by L. Tremblay, focused on the physiological and osmotic roles of sucrose during black spruce somatic embryo maturation. The results indicated that the action of sucrose on embryo maturation is mostly achieved through an osmotic control (Tremblay and Tremblay 1995a).

By the end of 1993, F. Tremblay had obtained a Synergie Grant of the Ministère de l'Enseignement Supérieur et de la Science-Québec to work on different aspects of somatic embryogenesis of black and white spruce. Three companies are involved in this five-year cooperative grant with the industry: Bechedor Inc., Centre de Production de Plants Forestiers Inc., and Pampev Inc. One aspect is to develop methods for the dehydration of somatic embryos for long-term conservation as well as for the development of artificial seeds. This project is lead by C. Bomal in collaboration with B. Beaulieu. Under the supervision of Synergie collaborator Dr. Bernard Riedl (Département des sciences du bois et de la forêt, Laval Univ.), Dr. H. Chtourou is pursuing the development of matrices for the encapsulation of artificial seeds. A project conducted by A. El Meskaoui aims at monitoring O_2-CO_2 during the maintenance and maturation stages of somatic embryogenesis. More recently, a study on the effects of salt components of the maintenance and maturation media used in somatic embryogenesis has been initiated by E. Sildor. In addition, C. Levasseur and J. Bélanger are involved in the various steps required for large scale production of somatic embryos-derived spruces for future clonal testing. Synergie grant also supports molecular projects aimed at studying genetic stability and conformity following somatic embryogenesis in black and white spruce. This research, conducted by Dr. R. Boivin, P. Lavoie, and J. Bélanger, utilizes sequences of mobile genetic elements isolated from spruce as genetic markers for embryogenic tissues and somatic embryo-derived plantlets. Other markers of genetic stability, have been shown by C. Ory, to be located in peculiarly variable regions of the spruce chloroplast genome differing among and possibly within embryogenic cell lines.

A project on genetic transformation of white spruce and utilizing the biolistic approach has been sponsored by the MRNQ. In the past two years, Y.-h. Li has evaluated different parameters of the microprojectile bombardment strategy and the culture conditions of pollen and embryogenic tissues to obtain optimum transient expression frequency of the GUS reporter gene (Li *et al.* 1994). More recently, the work of Dr. A. Paterakis and J. Belles-Isles resulted in optimization of the methods for the direct and stable transformation of embryogenic suspensions by the biolistic approach. Presently, the transformation team aims to regenerate transgenic spruces starting from the transformed tissues and to integrate different constructions into the spruce genome.

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

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

Les rapports des membres du groupe de Génétique et amélioration des arbres s'inscrivent dans le cadre des objectifs définis dans la Réflexion stratégique publiée en 1994 par le Service canadien des forêts - Québec (SCF - Québec). Les objectifs du groupe de recherche sont de : (1) définir la structure génétique des populations naturelles d'épinettes blanches (*Picea glauca* [Moench] Voss.) et de pins blancs (*Pinus strobus* L.) et des populations d'épinettes de Norvège (*Picea abies* L. Karst) introduites et adaptées au Québec; (2) déterminer la nature et l'amplitude de la variabilité génétique des trois espèces de conifère en fonction des critères économiques et de vigueur; (3) étudier le comportement des variétés synthétiques dans des environnements multiples; (4) analyser la variabilité génétique de la rouille vésiculeuse du pin blanc (*Cronartium ribicola* J. C. Fisch), évaluer la résistance d'hybrides provenant de croisements inter spécifiques dans le genre Pinus et identifier les gènes responsables de la résistance à la maladie; (5) étudier la réaction du matériel d'origines génétiques diverses par l'observation du comportement du charançon du pin blanc (*Pissodes strobi* Peck.) et intégrer les arbres tolérants ou résistants dans le programme d'amélioration; (6) établir des stratégies d'amélioration efficaces adaptées aux espèces à l'étude; (7) sélectionner des génotypes supérieurs et créer des variétés synthétiques; (8) conserver sous diverses formes le patrimoine génétique des espèces à l'étude, perfectionner les techniques de propagation des espèces, particulièrement celles de culture *in vitro*; et (10) diffuser les innovations technologiques et le matériel génétiquement amélioré. Actuellement, le Service canadien des forêts fait l'objet d'un exercice de réorientation et d'une restructuration de ses activités de recherche. Il est donc à prévoir que les objectifs définis précédemment seront modifiés au cours de la prochaine période.

GÉNÉTIQUE ET AMÉLIORATION DE L'ÉPINETTE BLANCHE PAR JEAN BEAULIEU

Les efforts de recherche sur la génétique de l'épinette blanche et l'utilisation des informations qui en découlent pour le programme d'amélioration se sont poursuivis selon le plan prévu pour la période en revue. Ainsi, huit tests génécologiques, répartis sur le territoire méridional québécois et incluant près de 550 descendances uni-parentales, ont fait l'objet d'un nouveau mesurage. Les arbres étaient alors âgés entre 13 et 17 ans. Les valeurs en croisement des arbres mères ainsi que les indices de sélection permettant d'estimer les valeurs en croisement des descendants ont été calculés en utilisant la méthode BLP («Best Linear Prediction»).

Une sélection en deux étapes a, par la suite, été effectuée à partir des informations recueillies. Ainsi, les familles dont les mères possédaient les valeurs en croisement les plus élevées ont d'abord été retenues. Par la suite, une sélection intra-familiale était effectuée en limitant le nombre de demi-frères à un maximum de quatre et en balançant les arbres sélectionnés sur l'ensemble des tests de descendances. Des greffons ont été récoltés sur quelque 450 individus supérieurs et près de 5 000 greffes ont été réalisées. Ces nouveaux géniteurs viennent compléter les populations d'amélioration de première génération des deux zones d'amélioration qui ont été délimitées. Les greffes sont actuellement conservées à la station forestière de Valcartier (SCF - Québec).

Le programme de croisements dirigés entre les individus du premier groupe de 100 arbres sélectionnés s'est poursuivi. Les croisements de type polycross ont été complétés et les graines ont été ensemencées à l'hiver 1995. Sept tests seront établis dans un proche avenir avec les semis produits et les résultats permettront d'estimer l'aptitude générale à la combinaison des 100 premiers géniteurs. De plus, plusieurs centaines de descendances bi-parentales ont été générées au cours de la période 1993-1995 en croisant entre eux ces mêmes individus. Près d'une centaine de lots de graines générés ont été utilisés pour produire des semis qui seront plantés directement sur un site d'expérimentation pour constituer une plantation de sélection à l'intérieur de laquelle sera sélectionnée une partie des géniteurs de la prochaine génération.

Les tests de descendances initiés en 1992, et visant à démontrer que les descendances des arbres supérieurs sélectionnés pour la première phase des populations d'amélioration étaient bien adaptées aux conditions de croissance de la région du Bas-Saint-Laurent et de la Gaspésie, ont été réalisés **en** 1995 sur trois sites représentatifs des domaines climaciques qu'on y retrouve. En plus des 76 descendances bi-parentales, 24 descendances uni-parentales d'origine locale y sont testées.

Quatre tests génécologiques ont également été réalisés sur la Haute-Côte-Nord en 1995. Ces tests de descendances/provenances comprennent 25 provenances représentées chacune par cinq descendances uni-parentales. Certaines d'entre elles sont d'origine locale alors que d'autres ont démontré des rendements supérieurs dans les régions limitrophes. Ces tests ont été établis pour pallier l'absence de tels tests dans cette région et pour vérifier s'il était possible d'augmenter le rendement des plantations par l'introduction de matériel étranger à la région. Trois des tests établis sont de type «test au champ».

La mise en place d'un réseau de démonstrations des gains résultants de l'amélioration génétique a débuté en 1994. Ce réseau comprendra sept sites de démonstrations et on y retrouvera de l'épinette blanche dans quatre d'entre eux, soit à Mont-Laurier, Saint-Félicien, Dégelis et Causapscal. On pourra y observer des lots témoins d'origine locale, des provenances recommandées, des descendants de la population d'amélioration et des familles performantes et au rendement médiocre tel qu'identifié dans les tests existants. L'établissement des tests de descendances et du réseau de démonstrations au cours de cette période a été rendu possible grâce à un financement obtenu du programme Essais, expérimentations et transfert technologique en foresterie (SCF - Québec).

Une étude visant à trouver des marqueurs RAPD liés génétiquement aux gènes codant pour la densité du bois a été initiée en collaboration avec M^{me} Nathalie Isabel et le D^{r} Jean Bousquet de l'Université Laval et avec le D^{r} John E. Carlson du British Columbia University.

GÉNÉTIQUE DE L'ÉPINETTE DE NORVÈGE

PAR GAÉTAN DAOUST

Le projet entrepris en collaboration avec M. Ante Stipanicic de la Direction de la recherche du ministère des Ressources naturelles du Québec s'est poursuivi au cours de la dernière période. Ainsi, les trois tests de descendances (E390-A) prévus pour la zone d'amélioration des Laurentides ont été établis dans les régions de Mont-Laurier, d'Armstrong et de Valcartier. Le test de descendances implanté à Valcartier a été jumelé avec un test en champ sur le même site. Ce dernier nous permettra d'évaluer à court terme les performances des différentes familles et de les comparer avec les résultats du test régulier. Les sélections familiales précoces qui seront effectuées pourront donc être validées.

En février 1995, les trois tests de descendances (E390-B) pour la zone d'amélioration du Saint-Laurent ont été ensemencés en serre au Centre de foresterie des Laurentides. Ils comprennent 242 descendances provenant de provenances recommandées, de plantations commerciales de belle venue et de provenances polonaises offrant un bon potentiel pour la région du Saint-Laurent. Un test en champ accompagnera également un de ces tests.

Deux tests clonaux, effectués à partir de descendances et de semis sélectionnés en pépinière dans un des tests de descendances (E390-C) qui ont été implantés en 1992 dans la zone d'amélioration des Appalaches, ont été établis à Valcartier et à Biencourt au printemps 1995. Les 360 clones sélectionnés à l'origine ont été réduits par une deuxième sélection à près de 200 clones. En collaboration avec le CEGEP de Baie-Comeau, un essai d'introduction comprenant neuf descendances a été implanté sur leur forêt d'expérimentation.

Des essais d'induction florale en champ et en serre ont démontré, pour un dispositif comprenant les mêmes clones, que le nombre de semences produit par cône en serre est plus faible qu'en champ (7 versus 75) et, qu'en plus, les semences sont 45 % plus légères. Toujours sur une base clonale, les cônes produits en serre sont 70 % plus courts et comportent 70 % moins d'écailles ovulifères que les cônes produits en champ.

Dans le cadre du réseau de démonstration des gains résultants de l'amélioration génétique, décrit dans la section sur l'épinette blanche, deux des sept sites de démonstrations (Mont-Laurier et Causapscal) présenteront de l'épinette de Norvège.

Dans le cadre du projet supporté par le Fonds spécial pour les opportunités de recherche en sciences et technologies du SCF et portant sur la recherche d'outils de sélection d'épinettes de Norvège résistantes au charançon du pin blanc, l'analyse préliminaire des résultats, selon les protocoles utilisés en milieu contrôlé, n'indique aucune préférence des insectes pour le groupe d'arbres dits susceptibles par rapport au groupe d'arbres dits résistants. La D^{re} Ariane Plourde, membre de notre groupe de recherche, assurait la supervision scientifique de ce projet avec le D^{r} Robert Lavallée. Ce dernier a également organisé un colloque de deux jours sur cet insecte en collaboration avec la Direction de la recherche forestière du ministère des Ressources naturelles du Québec.

GÉNÉTIQUE ET AMÉLIORATION DU PIN BLANC
PAR GAÉTAN DAOUST ET ARIANE PLOURDE

Les prises de données concernant la croissance, la survie et les dommages ont été complétées pour les cinq tests de descendances (E600) établis sous couvert partiel de feuillus et les deux tests établis en plantation mixte avec un aulne arborescent (*Alnus incana* Moench). Des analyses préliminaires ont permis de classer, selon la croissance à cinq ans, les 600 familles testées. Au cours des deux dernières années, des semis des 200 meilleures familles ont été produits et seront soumis à des essais d'inoculation pour évaluer la variabilité familiale de la susceptibilité à la rouille vésiculeuse. L'étude de la variabilité génétique de la rouille dans son aire d'introduction s'est poursuivie et est décrite dans la section du D^r Richard Hamelin. L'estimation des paramètres génétiques pour les tests de descendances sera déterminée durant la prochaine période et les prémisses de cette étude sont décrites dans la section du D^r Peng Li.

Dans le cadre du réseau de démonstrations des gains résultants de l'amélioration génétique, décrit dans la section sur l'épinette blanche, deux des sept sites de démonstrations (Mont-Laurier et Saint-Félicien) présenteront du pin blanc.

Le Plan Vert nous a permis de poursuivre l'étude de la diversité génétique du pin blanc au Québec entreprise dans le cadre d'études doctorales du D^r Jean Beaulieu. Les détails de cette étude sont présentés dans la section portant sur la génétique des populations d'épinettes et de pins blancs.

Une production significative de strobiles a été obtenue dans notre parc d'hybridation de pins blancs au cours des dernières années. Cette production nous a permis d'amorcer les plans de croisements requis pour la poursuite des travaux d'amélioration génétique et d'initier des essais de mise en culture de tissus qui nous permettront éventuellement, lorsque la technique sera au point, de multiplier le matériel transformé génétiquement.

POPULATION GENETICS OF WHITE PINE BLISTER RUST
BY RICHARD C. HAMELIN

Our laboratory is developing new approaches to understanding the population biology and epidemiology of tree pathogens using molecular techniques. One particularly relevant question of interest to tree breeders is which source of inoculum should be used to test provenances or potentially resistant material. In order to answer this question, we want to know whether there is genetic differentiation within the rust population, and if so, to learn more about its distribution.

Over 1500 rust samples were collected over a two-year period in natural stands and plantations in Quebec. Our results indicate that there is no genetic differentiation between geographic areas or types of stands (natural stands versus plantations) but that a low level of genetic differentiation exists between populations within regions. This genetic differentiation can be mostly explained by the high level of inbreeding in some rust populations in young plantations (approximately 10 years old). Our interpretation of these results is that the fungus may have been introduced into these plantations on a limited number of symptomless infected seedlings and a micro-epidemic subsequently developed between the local *Ribes* populations and the white pines.

The genetic variability was distributed on a very fine scale, with approximately 90% of the genetic variability being present within single cankers. Our general conclusion is that this pathogen probably travels long distances by means of its aeciospores and/or uredospores thereby homogenizing rust populations across the province, and that extensive genetic recombination takes place in rust populations within single plantations. The lack of any spatial trends within plantations also indicates that the basidiospores originating from surrounding *Ribes* do not tend to clump around the source. Since the genetic composition of rust populations separated by as much as 1000 km is almost identical, the probability of finding rust isolates with different genotypes does not increase by sampling in geographically distant populations in Quebec. Our continuing work will focus on comparing the virulence of rust isolates with different DNA profiles in order to verify whether or not virulence will be almost identical as well.

Seedling Certification In Nurseries Using Molecular Probes

Over 260 million seedlings were inspected in the province of Quebec for certification of health prior to reforestation. Important tree pathogens such as white pine blister rust, scleroderris canker, and root-rots are detected mostly by visual inspection followed by isolations. This process is time-consuming and inefficient, as many tree diseases can remain latent for several years. We are developing DNA oligonucleotides based on sequences of the rDNA genes of these pathogens. They are used in PCR reactions to amplify and detect the presence of these fungi directly from infected symptomless seedlings. Our preliminary results with root-rot organisms indicate that the technique is highly reliable and that the protocol could be automated to process hundreds of samples daily.

ESTIMATING WHITE PINE GENETICS PARAMETERS

BY PENG LI

Eastern white pine is an important timber species but has been overexploited at the beginning of this century. Reforestation of this species encounters two major difficulties: white pine blister rust and white pine weevil. To decrease risks of attacks from blister rust, eastern white pine should be planted in areas with low rust susceptibility. The damage from white pine weevil is controlled mainly by silvicultural methods such as close spacing, planting white pine between hardwood bands, or mixed with hardwood species. Thus, in genetic tests of eastern white pine in Quebec, seedlings were planted either in clearcut strips in hardwood stands or mixed with European speckled alder.

Because these white pine genetic tests were established between hardwood bands in large blocks, spatial autocorrelation phenomena might appear (i.e. genetic entries might perform similarly because they share common microsite environments resulting from patchy patterns of environmental variability within a block and/or from fertility or other environmental gradients). Therefore, we studied the extent of spatial autocorrelation in two eastern white pine provenance/progeny tests, and examined efficiencies of two spatial adjustment methods by analyzing these two tests. At one site spatial autocorrelation was moderate, consequently spatial adjustment procedures improved precision in estimating provenance and family means. However, spatially adjusted and unadjusted provenance and family ranks did not change. At the other site, spatial correlation was weak, and spatial adjustment procedure neither increased the precision of provenance and family means nor changed the ranking of provenance and families. In 1995, we are going to take measurements in genetic tests in which eastern white pine was planted with European speckled alder to examine the effect of competition on the growth of pine trees. Moreover, we also plan to estimate genetic parameters from these white pine provenance/progeny tests with and without spatial adjustment. This project is supported by the Science and Technology Opportunities Fund of the Canadian Forest Service.

GÉNÉTIQUE DES POPULATIONS D'ÉPINETTES ET DE PINS BLANCS
PAR MARIE DESLAURIERS ET JEAN BEAULIEU

L'évaluation globale de la diversité génétique des populations naturelles de pins blancs au Québec sera complétée cette année par l'intégration des données issues des populations localisées à la limite de l'aire de distribution québécoise de l'espèce. Ainsi au cours de la période, les analyses isoenzymatiques ont été effectuées sur le matériel récolté dans les régions de la Gaspésie, de la Rivière-Sainte-Marguerite, de la Mauricie et du Témiscamingue. Les résultats nous permettront de réviser le programme de sélection et de s'assurer que les populations d'amélioration contiennent l'essentiel de la diversité génétique présente dans les populations naturelles de pins blancs du Québec.

L'analyse de la diversité génétique de l'épinette blanche initiée en 1993 sera complétée cette année par l'analyse de cinq populations supplémentaires. En tout, 10 populations naturelles auront été échantillonnées dans des zones de récolte utilisées antérieurement dans le programme d'amélioration. La combinaison des données de diversité génétique et des évaluations plus classiques à partir de mesures prises sur le terrain dans les tests de descendances permettra de tracer une image complète de la diversité génétique de l'épinette blanche au Québec.

Nous terminerons donc cette année l'étude de la diversité génétique de ces deux espèces de conifères qui aura été financée partiellement par le Plan Vert.

Une étude visant à comparer les estimés de diversité génétique obtenus à l'aide de marqueurs isoenzymatiques et RAPD chez l'épinette noire a été réalisée en collaboration avec M^{me} Nathalie Isabel et le D^{r} Jean Bousquet de l'Université Laval. Les indices de diversité génétiques obtenus par les deux techniques sont équivalents chez cette espèce et l'étude démontre le bien-fondé d'utiliser les techniques isoenzymatiques pour les études de génétique des populations chez les espèces allogames arborescentes.

CULTURE *IN VITRO* DES CONIFÈRES
PAR MARIE DESLAURIERS ET ARIANE PLOURDE

Le potentiel embryogène de près de 50 lots d'épinettes blanches a été évalué et du matériel clonal a été produit à partir des meilleures familles. Les clones à haut potentiel ont été cryopréservés.

Par ailleurs, les travaux portant sur l'embryogenèse somatique du pin blanc se sont étendus durant la dernière année. L'aptitude à la culture *in vitro* a été évaluée sur près de 140 familles récoltées dans le parc d'hybridation de Cap-Tourmente. Il nous a été possible d'identifier des clones dont les semences immatures montrent des taux d'induction exceptionnels. Nous avons obtenu un taux moyen de 4 %, avec une variation inter familiale se situant entre 0 et 60 %.

Les cals en maintenance ont été conservés pendant environ six mois, période après laquelle un nombre appréciable de lignées perde leur capacité embryogène. Parallèlement, les cals en maintenance ont été utilisés afin de poursuivre les travaux visant à déterminer les conditions de culture requises pour la maturation de proembryons somatiques. Plus de 40 lignées parmi les plus intéressantes ont été cryopréservées.

Les familles à haut potentiel seront retenues pour les travaux d'optimisation du milieu de culture pour la maturation de proembryons et la récupération d'embryons inter spécifiques.

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FORINTEK'S WOOD QUALITY AND END–USE STUDIES RELATED TO TREE IMPROVEMENT PROGRAMS

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Keywords: *Picea mariana, Pinus banksiana,* hybrid poplar clones, wood density, end–use, growth, dry mass weight, correlation, heritability, selection criterion, genetic gains.

With the increasing proportion of intensively managed plantations, the widespread move to short rotation, wood quality has become one of major concerns in the forest industry. A supply of high quality resource will give the forest products industry the greatest flexibility in manufacturing a variety of high quality and value–added products. To ensure a sustainable supply of quality resource for quality products, we must strive to take end–use and end–use wood characteristics into consideration in tree improvement programs in Canada. In collaboration with a number of research organizations and the industry in the Maritimes (e.g., University of New Brunswick, New Brunswick Tree Improvement Council, CFS, New Brunswick Department of Natural Resources and Energy, Fraser, Irving), we have examined two major species (black spruce and jack pine). The focus in both cases was placed on the alternative selection for maximum genetic gain in fibre production. Since last year we have also maintained a close linkage with major research organizations in Québec which have an active tree improvement program (e.g., Ministère des Ressources naturelles du Québec, Laval University and CFS–Québec Region). A project on hybrid poplar clones was initiated this year by Forintek in collaboration with the Ministère des Ressources naturelles du Québec. This study, as part of a multidiscipline project, is to establish potential end–use of the hybrid poplar clones recommended for the reforestation in Québec.

INCORPORATING WOOD DENSITY INTO BLACK SPRUCE TREE IMPROVEMENT PROGRAM FOR MAXIMUM FIBRE PRODUCTION

In collaboration with Dr. E.K. Morgenstern and Dale Simpson, this project was started in 1992 and completed at the end of 1994. In this study, 40 half–sib families of black spruce (*Picea mariana*) were sampled from Fraser's tests in northern New Brunswick. At the end of 1992 growing season, 8–10 trees (depending on the availability) per family were sampled from 9 blocks. For each tree sampled, DBH and tree height were measured and bole volume was calculated.

Wood density was measured using X–ray densitometry at Forintek's Eastern Laboratory. A radial segment from the pith to the bark was taken from each tree, and was then sawn into 2–mm–thick (longitudinal) X 10– mm–wide (tangential) strip for X–ray densitometric analysis. The analysis provides various data on individual rings scanned: earlywood width, latewood width, ring width, earlywood density, latewood density, average wood density of individual growth rings. Based on the data on individual rings, weighted averages were computed for overall wood density, earlywood density and latewood density and latewood percent of each tree studied. Finally, dry mass weight (bole volume multiplied by overall wood density) was computed for each tree as an index of gross fibre yield.

Analyses of variance and covariance for various traits were performed to estimate various genetic parameters. Implications of genetic parameters for tree improvement were discussed. The results indicate that wood density, earlywood density and latewood density show smaller phenotypic variation than growth traits, are thus under stronger genetic control. Wood density has a strong genetic correlation with earlywood density and latewood density. But earlywood density and latewood density are strongly related to each other. Therefore, these two traits have little value in improving the efficiency of selection for overall wood density. Overall wood density shows a negative genetic correlation with all growth traits. This study reveals that selection for dry mass weight would result in remarkably higher genetic gain in gross fibre yield than selection for bole volume alone. Furthermore, selection for dry mass weight would result in less reduction in wood density, and while holding wood density at zero change, it is still possible to obtain huge genetic gain in gross fibre yield. This study also shows that the relationship of wood density with growth traits varies with both genotype and environment. As a result, it is possible to select families which not only grow fast but maintain high density although a negative correlation generally exists in this species.

A few papers have been written based on this study. Major results of this study were presented to the members of the New Brunswick Tree Improvement Council during its Annual Meeting held in April, 1995, and to the members of the Forintek Technical Advisory Committee.

INCORPORATING WOOD DENSITY INTO JACK PINE TREE IMPROVEMENT PROGRAM FOR MAXIMUM FIBRE PRODUCTION

A project on jack pine (*Pinus banksiana*), the second most important reforestation species in the Maritimes, was initiated in June, 1994. This project funded by the Canada–New Brunswick Agreement on Forest Development and Forintek is a joint effort between UNB and Forintek. In collaboration with Dr. Y.H. Chui and NBTIC, this study was conducted in two phases. Phase I compared measurements from Pilodyn and stress wave methods against actual wood properties (e.g., surface wood density, average wood density of a core sample and modulus of elasticity). It was found that neither of the two methods was considered to be reliable enough for family selection purpose (Note: we intend to test the reliability of a new instrument called RESISTGRAPH for measuring wood density in standing trees). The X–ray densitometry method was therefore adopted in this study as well for measuring wood density and other traits. The Phase II followed a similar approach as adopted in black spruce study.

Wood density in jack pine, as in black spruce, shows the highest heritability among various traits studied. A positive correlation between wood density and all growth traits, however, was found in jack pine. This indicates that selection for bole volume would not necessarily lead to a reduction in wood density. But selection for dry mass weight in this species would achieve the highest gain not only in gross fibre yield (+ 10.8%) but in bole volume (+8.7%) (see Table 1).

A preliminary report was prepared for the New Brunswick Department of Natural Resources and Energy (NBDNRE), and preliminary results were presented to the members of the New Brunswick Tree Improvement Council and NBDNRE's Timber Management Branch in April, 1995. A final report will be completed soon.

Table 1. Expected response in wood density, bole volume and dry mass weight when different selection criteria are used.

	Selection criterion	Gain (%)[1]		
		Wood density	Bole volume	Dry mass weight
1.	Wood density	+ 3.6	+ 4.4	+ 9.3
2.	DBH	+ 0.2	+ 3.6	+ 4.0
3.	Tree height	+ 0.6	+ 7.9	+ 8.4
4.	Bole volume	+ 0.8	+ 7.7	+ 8.7
5.	Dry mass weight	+ 1.6	+ 8.7	+ 10.8

[1] Genetic gain based on family selection at selection intensity i = 1.386.

ESTABLISHING POTENTIAL END–USE OF HYBRID POPLAR CLONES

In collaboration with Dr. Gilles Vallée from the Ministère des Ressources naturelles du Québec, Forintek initiated a multidiscipline project on end–use wood characteristics and utilization of poplars. Part of this project is devoted to fast–growing hybrid poplar clones recommended for the reforestation in Québec. These clones are 15 to 30–year old. The industry has shown interest in utilizing these fast–growing clones as part of their replanting stock. Objectives of this study are to establish potential end–use of the fast–growing hybrid poplar clones and to estimate potential opportunities for value–added products. We first will evaluate key wood quality characteristics which determine end uses and product quality. Trees having reached sufficient size will be selected from the best clones to produce lumber. The quality of the lumber will be evaluated, and the potential of these clones for value–added products (e.g., flooring, MSR stock) will be investigated. This multidiscipline project is scheduled to be completed by 1998.

AN UPDATED RESOURCE ASSESSMENT AND UTILIZATION PROGRAM AT FORINTEK'S EASTERN DIVISION

A Resource Assessment and Utilization Program at Forintek's Eastern Division has been defined recently. This program is to: 1) maximize returns from the present forests, and 2) to determine the impact of silvicultural practices, tree improvement and woodland operations on end–use wood characteristics and product quality to assist in governments and the industry in forest management decision–making. For more information about this program or collaboration, contact us at Forintek's Eastern Division.

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J.D. IRVING, LIMITED - TREE IMPROVEMENT SUMMARY

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Keywords: Spruce species, jack pine, eastern larch, hardwood species, tree breeding, seed orchards, clonal propagation.

SEED ORCHARDS AND SEED PRODUCTION

The company continues to manage clonal seed orchards of black spruce, white spruce, Norway spruce, red spruce, jack pine and eastern larch as well as our two oldest and most heavily rogued black spruce seedling seed orchards. Roguing has started in the clonal orchard of jack pine and will begin in the black spruce and white spruce orchards in 1995. Second generation orchards of black spruce and jack pine are now complete.

In the last two years, record low and record high cone production has been experienced. Amounts of orchard seed processed were 0.4 and 323 kilograms for 1993 and 1994 respectively. Obtaining orchard seed for Norway spruce still remains a problem. Work continues on flower induction however this species may have to be vegetatively multiplied to supply improved reforestation stock. Of significance to our program is the commencement of seed production in second generation orchards of black spruce and jack pine. These orchards have been established since 1989 and early production in black spruce has been improved through the use of flower induction with GA4/7.

TREE BREEDING AND PROGENY TESTING

Tree breeding to determine breeding value is completed for white spruce, black spruce and jack pine in first generation clonal orchards. A complimentary mating scheme is generally followed with the exception of white spruce where first generation clonal orchard selections have been tested using six-tree disconnected half diallels. Breeding is in progress for Norway spruce and red spruce breeding is being conducted by the Nova Scotia Tree Improvement Working Group (NSTIWG). Larch breeding is not a priority at this time. Some pair-matings of black spruce and jack pine remain to be field planted in 1996 for future selection. Preliminary analysis started in 1994 of six-year measurements of white spruce diallels as well as an initial series of black spruce diallels that were established before the complimentary mating scheme was adopted.

Breeding of second generation selections of black spruce and jack pine is being conducted in association with the New Brunswick Tree Improvement Council (NBTIC). Polycrossing is mostly completed for seed orchard parents and field tests have been established.

CLONAL PROPAGATION

A pilot-scale clonal production program for black spruce was initiated in 1993 based on rooted cuttings. Crosses among second generation parents were supplied from the breeding hall and these were hedged. In 1994, 50,000 cuttings were taken from 2,000 one-year-old seedlings with an 89.6% survival rate. In 1995, 300,000 cuttings were rooted and we plan to expand production to 1,000,000 in 1996. Clonal testing was initiated in 1995 based on three clonally replicated five-tree disconnected half diallels. A total of 300 clones will be field tested and the original ortets will be serially propagated to delay maturation during the field testing period.

An extension of this project was initiated in 1995 in collaboration with CFS - Maritimes and PNFI to utilize somatic embryogenesis (SE). A new series of clonal tests will be established using SE derived clones and cryogenic storage of lines during the field testing phase will be utilized. Whether operational production based on clonal test results is by bulking up with rooted cuttings or directly by SE, this method should be superior to traditional methods of serial propagation and hedging to maintain juvenility.

TRIAL PRODUCTION OF HARDWOODS

Experimental plantings of hardwood species have been conducted in the last two years including a number of species of birches and poplars.

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SEED, CONE, AND POLLEN STUDIES AT THE UNIVERSITÉ DE MONCTON

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Keywords: *Picea mariana, Picea glauca, Larix laricina, Pinus strobus, Pinus banksiana,* seed quality, controlled pollination, cone-crop prediction, pollen monitoring, pollen sizing.

This report summarizes on-going and completed research activities at the Université de Moncton over the past two years. These relate to seed quality, seed yield, controlled pollination, cone-crop prediction, pollen monitoring, and pollen sizing.

SEED QUALITY AND YIELD STUDIES

The study of master student Émanuel Sildor relating seed quality to site and family effect is near completion. Half-sib families originating from the Tobique watershed and located in five black spruce (*Picea mariana* (Mill.) B.S.P.) seedling seed orchards in the Maritimes were used. Major cone morphometric measures varied with site and family. However, little variation in seedling growth was noted.

OPTIMAL TIME OF POLLINATION

A study to better define the best time to pollinate black spruce cones was initiated in 1992 and repeated in 1994. Results indicate that controlled pollination should be carried out just before mid-receptivity (Caron 1995a).

CONE-CROP PREDICTION

Yearly seed-cone and pollen-cone production in black spruce seedling seed orchards can be predicted based on the species inherent tendency to bear cones biennially (see Caron 1995b). Furthermore, Dr. R.A. Fleming (C.F.S., Sault Ste. Marie) and I have devised a system to estimate cone production from young individual black spruce trees based on the lowest cone in the crown (Caron and Fleming 1995).

POLLEN MONITORING AND POLLEN SIZING

Various pollen monitoring studies were previously mentioned by Caron (1993). Since then, results have been published: i) 1992 study in a white spruce (*Picea glauca* (Moench) Voss) clonal orchard and a black spruce seedling orchard in St-Elzéar with the Québec Ministry of Natural Resources (Mercier et al. 1994), ii) 1992 and 1993 studies in a white spruce clonal orchard located amidst a large burnover area near LaMalbaie, Québec (Mercier et al. 1994; Caron et al. 1994; Desrosiers 1994a); 1992 and 1993 studies in an eastern white pine (*Pinus strobus* L.) orchard (Mercier et al. 1994; Desrosiers 1994b); 1988 and 1989 study of eastern larch (*Larix laricina* (DuRoi) K. Koch) potential pollen contamination (Caron 1994a); three year variation in pollen production in a black spruce orchard (Caron 1994b).

A study dealing with pollen size variation in jack pine (*Pinus banksiana* Lamb.) clones was initiated in 1992 in collaboration with Dr. G.R. Powell (University of New Brunswick). Pollen was collected in 1992 and 1994 from clones located in J.D. Irving's orchard located 45 km north of Sussex, New Brunswick. An article on this topic has been accepted for publication (Caron and Powell 1995).

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FOREST GENETICS RESEARCH AT CANADIAN FOREST SERVICE - MARITIMES REGION

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Keywords: Breeding strategy, genetic parameters, age-age correlation, stochastic simulation, seed orchards, somatic embryogenesis, genetic engineering, biotechnology, genetic diversity, conservation.

Forest genetics research at Canadian Forest Service Maritimes Region (CFS-M) continued in four major areas: (1) genetic studies of tree species and breeding strategies; (2) cone and seed research; (3) biotechnology involving tissue culture and genetic engineering; and (4) genetic diversity and conservation.

GENETIC STUDIES AND SIMULATION APPROACH TO BREEDING POPULATION MANAGEMENT

Over the past several years, tree breeding strategies using recent advances in vegetative propagation have been given emphasis. A series of clonally replicated tests has been established using rooted cuttings of white spruce obtained from a diallel crossing among parents of a second generation breeding program of the New Brunswick Tree Improvement Council. Also, from the diallel cross, 13 embling clones were mass-produced by somatic embryogenesis, and a field test has been established using mono-clonal blocks and two-clone mixture blocks.

In collaboration with Dr. T.J. Mullin, genetic parameters of height at age 10 years from striking were assessed in a clonally replicated test of black spruce. Field performance at 5 and 10 years was compared with that of the original ortets and families grown in a 25-week greenhouse study, by means of phenotypic and genetic correlation, and rank-change analysis. The strongest age-age correlation was that involving family mean seedling weight in the greenhouse. Family ranking based on greenhouse oven-dry biomass production also showed the most consistent agreement with ranking after 5 and 10 years of field testing, although the analyses suggest that effective early selection is probably limited to culling the worst 25% of the families based on biomass (Mullin and Park 1994).

Again in collaboration with Dr. T.J. Mullin, a computer program, called POPSIM, which simulates changes in genetic structure of managed tree populations under various tree breeding scenarios, has been developed. The purpose of the software is to provide breeders with a generalized decision-support tool to critically examine multi-generation breeding plans using a stochastic simulation approach. The theoretical basis for the simulation is given in an upcoming issue of Silvae Genetica, together with a description of the options currently available to the user. Genetic effects are simulated according to an additive-dominance-epistasis model for a base population of trees that are mated according to a user-prescribed breeding plan. Individual progeny from the breeding plan are generated, simulating the recombination of genetic effects and the random environmental effects during field testing. Selection is carried out among the test progeny to assemble various kinds of production populations for deployment of improved stock, e.g., seed orchard and clonal mixture, and to advance the breeding populations to the next cycle of breeding. Genetic variances are calculated directly from the simulated data and reported for each generation, along with the average level of inbreeding, effective population size, and the cumulative genetic gain realized by each type of production population. Examples are provided that illustrate the statistics generated by the program, and demonstrate the flexibility of the software to handle various approaches to management of genetic gain and diversity. The demonstration version of the POPSIM can be obtained from an FTP site at CFS-M (i.e., FTP Server: **fcmr.forestry.ca** Directory: **psim2016**) by down-loading a self-extracting 'Zipped' file called "**psim2016.exe.**" A user's guide, being published as an Information Report (Mullin and Park 1995), is included in the file.

CONE AND SEED RESEARCH/SEED ORCHARD MANAGEMENT

CFS-Maritimes Region staff from both Forest Genetics and the Forest Insect and Disease Survey continued to provide assistance to orchard managers in the Region through technology transfer activities. Operational, cooperative trials in crown management and cone induction have been completed, and the results are being used routinely in many orchards. Advanced-generation orchards have been established, including three miniaturized and one containerized orchard. CFS-M staff assisted in coordinating a project in which cooperators both established these alternative-type orchards and conducted operational research trials in support of them.

The shift from applied research towards more basic work on reproductive physiology continues. Studies on the effects of gibberellin, cytokinins and adjunct cultural treatments on photosynthesis, stomatal conductance, and bud development have been completed. Ron Smith's PhD program is scheduled to be completed in 1995 and the results will be available shortly thereafter.

SOMATIC EMBRYOGENESIS, MICROPROPAGATION, AND PROPAGULE CULTURE SYSTEMS

The work on somatic embryogenesis (SE) of white spruce, which was initiated in 1991 to determine the degree of genetic control and the effects of cultural treatments on SE with 30 full-sib families derived from six-parent diallel crosses, continued.

After demonstrating strong genetic effects in SE initiation (Park *et al.* 1993), the genetic control in somatic embryos exposed to storage, maturation treatments, germination, and cryopreservation was analysed. These SE processes were under genetic control, but less strongly than the initiation phase. For all the SE characters examined, variance due to clones within families was significant and was often the largest genetic component of variance. This was further partitioned using an additive-dominance-epistasis model. A relatively large proportion of the total genetic variance was due to epistatic variance in the maturation and germination of somatic embryos. Embryogenic lines were cryopreserved easily without a distinct genetic influence being noticed. With declining genetic control during maturation and germination of somatic embryos, it is the initiation phase that can be most effectively manipulated by breeding (Park *et al.* 1994a).

At CFS-M, over 500 embryogenic lines of white spruce resulting from our genetic study of SE have been cryopreserved in liquid nitrogen. To use SE technology in operational tree improvement programs, the long-term effects of cryopreservation on genetic stability must be established. David Barrett, a M.Sc.F. candidate at University of New Brunswick under supervision of Dr. Y. S. Park (Adjunct Professor), is studying genetic stability in morphological traits of cryopreserved embryogenic lines. This research has two main goals. The first is to determine if cryopreserved embryogenic lines are genetically stable after 2 and 3 years of freezing in liquid nitrogen. The second goal is to provide an early screening protocol for future SE ventures by correlating the characteristics of embryogenic tissue growth, form (or category) of mature somatic embryos, germination capacity and *ex-vitro* growth potential with future performance.

Studies on cultural method improvements, primarily during the maturation and germination phases, are continuing. We are focussing on biophysical analysis of embryo and callus membrane properties as diagnostic indicators of physiological status in culture. S. Pond has begun her PhD program at University of Victoria, concentrating on the interaction of temperature and desiccation for embryo conditioning prior to germination.

In a joint study with PNFI, several genetically altered (GUS reporter gene) clones of white spruce were produced, demonstrating the feasibility of a tissue culture laboratory cooperating at long distance with a genetic engineering facility. Another method for transgenic modification, DNA electrophoresis, is being developed for use with conifer embryos as an alternative to biolistic bombardment which, though successful, occurs at low frequency. The goal of this study is the routine insertion of genetic material which will further enhance the value of elite clones.

Attempts to micropropagate mature *Larix* are continuing. Lately we have found that dormant buds, collected in mid-winter, can be kept frozen for at least 9 months and still be suitable for explant excision and culture. Culture of frozen-stored material has resulted in a stimulation of somatic embryogenesis. Mature embryos were produced which germinated and formed shoots, alas without roots.

The Seedling Culture Project has been integrated with Forest Genetics to emphasize research component of the project, particularly development of embling culture and systems for operational production. We have successfully modified existing tissue culture protocols and introduced some new techniques to increase the bulk yield of germinated somatic embryos (emblings) suitable for greenhouse rearing. As a result, the pilot-scale production of 12,000 white spruce emblings for cooperative clonal tests and demonstration plantations, to be established by various government and industrial agencies, was achieved in the past two years, with help of the additional six person-years through Job Creation and Job Development Projects of Human Resources Canada and the Maritime Forest Ranger School (MFRS). Annual workshops were organized for the Container Update series at the MFRS and the Atlantic Forest Nursery Technical Committee.

PHYSIOLOGY AND GENETIC ENGINEERING OF WOOD FORMATION

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 the Swedish University of Agricultural Sciences, Umeå, Sweden, and the University of New Brunswick, Fredericton.

In *Abies balsamea*, nucleolar involvement in the regulation of the activity-rest-quiescence cycle of the vascular cambium was assessed by measuring the seasonal variation in the number, diameter, volume, silver staining and [5-^{3}H]-uridine incorporation of fusiform cell nucleoli isolated from 1- and 19-year-old cambia (Mellerowicz *et al.* 1993). Nucleolar activity exhibited two maxima during the year - a large one during cambial reactivation in April-May and a small one during the rest-quiescence transition in October. The results suggest that repression of ribosomal RNA genes underlies the development of rest, when the cambium will not produce new cells. In additional work (Lloyd *et al.* 1994), it was observed that the amount of ribosomal DNA relative to total genomic DNA (denoted relative rRNA gene content) increased transiently in 1-year-old cambial region cells during the spring prior to the onset of periclinal division, peaking 1 to 2 weeks after nucleolar activity was maximal. These changes are interpreted as reflecting the amplification and loss of genes encoding rRNA to facilitate cambial cell reactivation.

Three investigations were performed to further investigate the role of indole-3-acetic acid (IAA) in the control of cambial growth in *Pinus sylvestris.* First (Sundberg *et al.* 1993), IAA, together with soluble carbohydrates, starch, total nitrogen and amino acids were measured in the cambial region and bark at the top (10-year-old internode) and bottom (1.3 m above ground) of the main stem of trees having different rates and longitudinal gradients of wood production. The results indicated that it is the activity of the cambium rather than the availability of carbohydrates that determines the amount of wood produced along the stem, and that cambial activity, as measured by wood production, was not directly related to the IAA concentration in the cambial region. Second (Sundberg *et al.* 1994), it was demonstrated that the induction of compression wood formation above a ring of N-1-naphthylphthalamic acid (NPA) or morphactin, which inhibit the polar transport of IAA, was not associated with an overall increase in the level or turnover of IAA in the cambial region. Rather, the results suggested that acropetally transported NPA and morphactin induce compression wood formation by interacting with the NPA receptor in differentiating tracheids, thereby locally increasing IAA in these cells. Third (Sitbon *et al.* 1993), changes in protein synthesis in cambial region cells were monitored in 1-year-old shoots during cambial reactivation. Newly synthesized proteins were labelled in vivo with ^{35}S-methionine, and the labelled proteins were resolved with 2-dimensional polyacrylamide gel electrophoresis. The data indicated that quiescent cambial region cells can synthesize proteins as soon as exposed to environmental conditions favoring reactivation, and that only three of the approximately 250 proteins detected were specifically involved in IAA-induced cambial growth.

Work currently in progress is aimed at (1) characterizing an endogenous cambium-specific promoter, and monitoring the expression of two heterologous promotors throughout the annual cycle of activity and dormancy, in a *Populus* hybrid, (2) determining the interaction between IAA and gibberellins in the regulation of shoot growth in *Pinus sylvestris*, and (3) assessing changes in xylem cell anatomy in *Nicotiana tabacum* plants in which the IAA concentration was elevated by transformation with the *Agrobacterium tumefaciens* T-DNA *iaa*M and *iaa*H genes.

GENETIC DIVERSITY AND CONSERVATION

Genetic diversity and conservation projects range from examining impacts of forest management activities on genetic diversity of forest plant populations to studying historical records of forest tree species distribution. Three studies examine genetic diversity of natural populations. The first, nearing completion, addresses the question of how the genetic diversity of selected overstory and understory forest species may be affected by human-caused disturbance of different intensities. A chronosequence approach is employed as a surrogate for time to evaluate temporal changes in diversity of isozymes under different disturbance regimes.

The second study, also nearing completion, surveyed genetic diversity, using isozymes, of mainland and Prince Edward Island populations of several Acadian forest species.

Red oak appears to have less genetic diversity in Prince Edward Island populations than in any of the mainland populations, implying that gene flow is restricted across the Northumberland Strait.

The third genetic diversity study addresses the long term viability of bur oak in New Brunswick. Bur oak presently is restricted to a few small populations mostly located near Grand Lake, in prime cottage habitat. Genetic diversity of these populations will be quantified, again using isozymes, and compared with other isolated populations in New England as well as with populations from within the continuous range in Quebec and Ontario. A gene conservation strategy will be devised for New Brunswick bur oak on the basis of results.

Two other studies are indirectly related to genetic diversity. The first aims to identify areas requiring special management or protection for any of a number of reasons, including gene conservation, within a region of southern New Brunswick, designated as the Fundy Model Forest. Strategies will be developed with landowners to attempt to achieve some measure of protection for the identified areas. The second study addresses the question of how much the forests of New Brunswick have changed since European colonization. Specifically the project aims to provide an understanding of how forest tree species distribution and frequency have changed since the time of the original land grants in Kings County, New Brunswick. Large changes will be highlighted allowing an assessment of conservation needs.

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TREE IMPROVEMENT AND RELATED STUDIES AT THE UNIVERSITY OF NEW BRUNSWICK

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The tree breeding and forest genetics aspects of tree improvement at the University of New Brunswick (UNB) suffered a setback in June 1994 when E. Kristian Morgenstern retired. Kris spent 14 years on the Faculty, at first holding the industry-sponsored Chair in Forest Tree Improvement, and then continuing as the position was taken over by the university. Now, with the university (as others) experiencing financial constraints, all vacated positions are "lost" and any new position is subject to university-wide competition for the limited support-base available. In its staffing submissions, the Faculty of Forestry and Environmental Management has continued to rate highly a position in genetics (but in a broader context than tree improvement). However, in both 1994 and 1995 the position did not receive a high enough position in the university's priorities for approval.

This report is the ninth biennial report from UNB to feature a contribution by Kris. As in the other reports, work related to tree improvement in the programmes conducted by other faculty members is included.

TREE-CROWN AND REPRODUCTIVE DEVELOPMENT, POLLEN, AND SEED

Relationships between patterns of development in tree crowns and patterns of cone production, as described previously (Morgenstern *et al.* 1994), have continued to receive emphasis. In *Larix laricina* (Du Roi) K. Koch, the patterns involve cones produced on both long shoots and short shoots (Powell 1995a). In *Picea* Dietr., distinct phenological patterns exist among comparable shoots (Powell 1995b). Where seed cones occur on the shoots, and precisely when the cones are initiated, are related to these phenological patterns.

The principles used to describe shoot-system development in conifers (e.g., Powell 1995a, 1995b) were extended to evaluating contrasts in development and flowering of seemingly similar woody angiosperms (mistletoes) parasitic on tree crowns (Powell and Norton 1994).

Caron and Powell (1995) evaluated pollen viability and pollen size among clones of *Pinus banksiana* Lamb. Both were variable in two successive years. Number of pollen grains per millilitre of dry pollen averaged 16.4 and 17.7 million in 1992 and 1993, respectively. The highest numbers per clone were 1.8 and 1.7 times the numbers for the lowest values for the two years, respectively.

In the absence of seed from improved *Picea glauca* (Moench) Voss sources in west central Saskatchewan, a large crop of cones was collected in natural stands in 1993 using helicopters and the improved Fandrich Cone Harvester. Experience with the organization of this collection was described (Pinette 1994). For *Picea mariana* (Mill.) B.S.P. in New Brunswick and

Nova Scotia, there was some evidence that seeds and seedlings from separate types of sources (geographical, plus-tree, seed-orchard) could be categorized by specific morphological traits (Scott 1995).

Mahdan (1995) determined for *Acacia mangium* Willd. that pouring two parts of boiling water onto one part of seed by volume, and leaving it for 30 minutes was an effective pretreatment for germination. He also found that direct sowing of pretreated seeds into sand over a suitable growing medium in pots gave better survival and seedling quality than did transplanting of germinants into the same growing medium in pots, which is normal practice for planting-stock production in tropical Asia.

GENETIC VARIATION AND PARAMETERS

The *P. mariana* range-wide provenance experiment at Millertown Junction in Newfoundland was remeasured at age 21 years from seed. Rankings of provenances had changed considerably since the measurement at age 15 years, but the superiority of continental over Newfoundland provenances was maintained (Crocker 1994). A comparison of height growth in the 1978 New Brunswick *Pinus banksiana* provenance experiments with height growth (pooled means) from family tests, based on plus trees selected in the same populations, gave no significant rank correlations, but at the same age the growth of the selected families was generally better than that of the provenance means derived from unselected trees (Nicholas 1994).

A postdoctoral fellow, S.Y. Zhang, completed a study of wood density in New Brunswick *P. mariana* family tests in cooperation with the New Brunswick Tree Improvement Council and Forintek Canada Corp. Earlywood and latewood were found to be under strong genetic control, but showed less phenotypic variation than growth traits. Overall wood density exhibited negative genetic correlations with growth traits, and ranged from -0.34 to -0.41. In a multiple-trait selection strategy, selection should be aimed at improving dry mass while maintaining density at the current level (Zhang and Morgenstern 1995).

TREE PHYSIOLOGY

Regulation of wood formation at the biochemical level continued to be the focus of the physiology research programme. The importance of mature needles of all ages in promoting wood formation in conifers has been stressed (Savidge 1994). Needles evidently export, in addition to known phytohormones such as auxin, one or more factors specifically controlling conversion of cambial derivatives into tracheary elements. The tracheid-differentiation factor from conifer needles has tentatively been identified as D-*myo*inositol 1,4,5-*tris*phosphate (Savidge 1994).

Two enzymes related to lignification in conifers have been purified, characterized, and *N*-terminal sequenced for future molecular genetics research (Leinhos *et al.* 1994; Udagama-Randeniya and Savidge 1994, 1995). Coniferin-specific β-glucosidase from *P. banksiana* was found to be an acidic glycoprotein of the cytosol. The *N*-terminal sequence exhibited negligible homology to known β-glucosidases (Leinhos *et al.* 1994). Purified coniferyl alcohol oxidase (CAO) was found to be a copper-containing glycoprotein distinct from laccase (Udagama-Randeniya and Savidge 1994). Based on immunological and other properties, CAO evidently is a catechol oxidase (Udagama-Randeniya and Savidge 1995).

Using a chip-culture system to produce *L. laricina* tracheids *in vitro* (Savidge 1993), Leitch (1995) found that bordered-pit number, and placement of bordered pits in radial or tangential walls, were both under auxin regulation.

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FRASER INC. – TREE IMPROVEMENT PROGRAMME

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Keywords: *Picea mariana, Picea glauca,* polycross matings, seed orchard, embling, progeny and family tests.

This report summarizes activities at Fraser Inc. since 1993 (Leblanc 1993). It includes information on cone collection, nursery production, tests establishment, and some cooperative projects.

SEEDLING AND CLONAL SEED ORCHARDS

Cone collection from parts of the Second Falls black spruce (*Picea mariana* (Mill.) B.S.P.) seedling seed orchard was carried out in 1994. Cone collection was concentrated on the best five families. The selection criterion was based on results from previously established black spruce tests. A total of 4.25 kg of seed were harvested and kept for future use.

The first generation white spruce (*Picea glauca* (Moench) Voss) clonal seed orchard covering 3.6 ha has contributed 14.49 kg of viable seed in 1994. At the same time, cones were collected for the first time in 1994 from the 1.5 ha second generation black spruce clonal seed orchard that was established in 1991. No cones were kept due to the small amount harvested. These cones were harvested and discarded as a preventive measure against cone–pest proliferation.

SEEDLING PRODUCTION

All black spruce processed by the Second Falls nursery during 1994 and 1995 originated from Fraser Inc. orchards. For the first time in 1995, some seed from the white spruce clonal seed orchard in St–Anne–de– Madawaska were used. Overall, 10.10 kg and 1.83 kg of orchard seed were used for the production of black spruce and white spruce seedlings in 1994 and 1995, respectively. Other species grown at the nursery are red pine (*Pinus resinosa* Ait.), white pine (*Pinus strobus* L.), Norway spruce (*Picea abies* (L.) Karst.) and tamarack (*Larix laricina* (Du Roi) K. Koch). Furthermore, various hardwood species are being seeded on an experimental basis.

TESTING

Fraser Inc. and the New Brunswick Tree Improvement Council (NBTIC) continued to perform polycross matings of specific second generation black spruce selections to obtain third generation material.

In 1994 and 1995, a total of 2.3 ha of tests was planted. This includes one white spruce and two black spruce progeny tests and one white spruce embling test. Over the years, a total of 50.3 ha has been planted by Fraser Inc. for various tree improvement tests.

COOPERATIVE PROJECTS

All major cooperative projects were completed and reports have either been published or are in progress. The master thesis of Mr. Émanuel Sildor relating seed quality to site and family effects is in progress (see Caron 1995a). Results from the study to better define the optimal time to pollinate black spruce cones have been published (Caron 1995b). Another study dealing with seed–cone and pollen–cone production models for young black spruce orchard trees is in press (Caron 1995c).

Furthermore, field assistance and a testing site was provided to the Canadian Forestry Service for the establishment of a white spruce embling test.

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NEW BRUNSWICK TREE IMPROVEMENT COUNCIL UPDATE

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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 18th year of operation in 1994. Technical coordination and direction of NBTIC operations is provided through Canadian Forest Service. A full-time data analyst is funded by members. This important function is being conducted by Victor Steel who replaced David Steeves following his resignation in mid-1992. 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, breeding 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 requirements and production is starting in second generation orchards.

SELECTION

Selection of trees for first generation breeding populations spanned an eleven-year period and was conducted primarily in natural stands. Trees for subsequent breeding cycles will be selected from family or progeny tests and selection plantations. Second generation black spruce and jack pine trees are selected in family tests. Top performing black spruce families are identified based on 10-year height while for jack pine it is 7-year height. 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. Selection is favoured in those tests exhibiting higher individual-tree heritabilities. The goal is to create breeding populations containing 400 trees. Selection work began in 1988 and now a total of 239 black spruce trees have been selected. The jack pine breeding population is complete with 402 selections.

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. All breeding on white spruce and tamarack is carried out in clone banks. Polycrossing of second generation black spruce continues to be carried out primarily *in situ* on the selections located in the family tests. This was employed several years for jack pine but was discontinued due to time and effort expended and ramets beginning cone production in the clone bank. Conducting the breeding directly on the ortets allowed progeny tests to be established 3 to 4 years sooner than would otherwise been possible. Pair-mating of black spruce selections commenced in 1994 to 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	
	No. completed	% completed	No. completed	% completed
White spruce	332	80	314	74
Tamarack	220	82	60	-
Black spruce	174	44	9	-
Jack pine	197	49	-	-

An updated breeding strategy for black spruce was adopted in 1993. This plan incorporates early testing to allow clones to be uniformly deployed to breeding groups and breeding to be conducted in a positive assortative manner. An elite breeding group was established in order to conduct more intensive breeding on the 20 best clones to produce clonal material for testing and production. See Park *et al.* (1993) for further details.

TESTING AND DATA ANALYSIS

Test establishment has been an important component of the NBTIC program. Over the past 18 years, 205 tests were planted on over 250 ha. A number of different tests have been planted including: stand tests, open-pollinated family tests, control-pollinated progeny tests, selection plantations, and realized gain tests. These tests are yielding a wealth of information: on genetic variation of stands and trees, to rogue seed orchards, to estimate genetic parameters, to identify potential second generation selections, and to quantify gains obtained using genetically improved seed.

Polycross progeny tests are being established utilizing an experimental design consisting of two-tree plots randomly planted in each of 15 replications at 4 to 5 sites throughout the province. Progeny resulting from pair-mating among white spruce selections are planted on two sites in unreplicated 48-tree plots. A series of white spruce realized gain tests was planted consisting of several different seed orchard seedlots and unimproved operational collections. These will be measured in 1997.

Data from black spruce and jack pine family tests have been used to develop roguing recommendations for seedling seed orchards. Seedling seed orchards have undergone roguing for the past ten years. All producing orchards have been rogued once and several twice and one jack pine orchard three times. Generally, at least 50% of the families are removed each time. Genetic gain estimates have been high. Table 2 summarizes average gains for different roguings for both species.

During 1994, the Best Linear Prediction (BLP) method for analyzing family and progeny test data (as described by White and Hodge. 1989. Predicting breeding values with applications in forest tree improvement. Forestry Sciences, Vol. 33, Kluwer Academic Publishers) was incorporated into the NBTIC genetic analysis procedure, and is now used exclusively for ranking families/parents, making roguing recommendations, identifying candidate trees for 2nd generation selection and estimating genetic gain. Since this method accounts for tests of varying precision, heritability, imbalance, variance structure, and degree of correlation with the selection/ranking trait, it is more efficient for predicting breeding values that reflect the "true" genetic value of the family/parent. The experience in New Brunswick indicates that BLP improves the efficiency of 2nd generation tree selection by identifying the best trees in the best tests (higher heritability and lower phenotypic variance) and provides for somewhat higher genetic gain estimates due to more robust estimates of genetic value (breeding values).

Table 2. Average gains from roguing black spruce and jack pine seedling seed orchards.

No. times rogued	Black spruce	Jack pine
One	3.7% height (10 years)	2.2 % height (7 years)
Two	5.3% height (15 years) 19.5% volume (15 years)	3.1% height (12 years) 9.4% volume (12 years)
Three	-	3.2% height (14 years) 12.0% volume (14 years)

SEED ORCHARDS AND SEED PRODUCTION

Establishment of first generation seed orchards spanned 10 years with 130 ha of seedling seed orchards and 60 ha of clonal seed orchards. The design of the seedling seed orchards was single-tree plot with a planting spacing of 1 x 2 m. Clonal orchards were laid out at a spacing of 3 x 6 m using a computer generated permutated neighbourhood design (COOL). Second generation seed orchard establishment began in 1989 currently consists of over 11 ha of black spruce and 8 ha of jack pine. The best tree from each of the best 40 families from the three oldest series of family tests is clonally propagated by grafting. Clone placement is determined using COOL. Planting spacing ranges from 2 x 5 m to 3 x 6 m.

Seed production in 1994 was a record with over 350 million seed collected, 240 million of which was black spruce. Good collections from tamarack seed orchards were made possible by instigating effective control measures against the cone maggot. The first seed was collected in 1994 from second generation black spruce seed orchards and collections from the jack pine orchards continue to increase annually.

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TREE IMPROVEMENT PROGRESS IN NEW BRUNSWICK

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Keywords: cone collection, cross-pollinations, progeny tests, seed orchards.

The tree improvement program in N.B. is progressing smoothly. Our efforts continue to be focused on our 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, cross-pollinations and orchard establishment are well underway. The following report highlights our Tree Improvement activities for the past two years.

TREE BREEDING/TESTING

The 1993 and 1994, jack pine polycrosses done on second generation selections provided sufficient seedlots to grow and outplant the first and second series of jack pine progeny tests. The polycross seedlots included those from NBTIC (New Brunswick Tree Improvement Council) members, totalling 119 families. In the past, all crosses on jack pine were carried out in the NBTIC family tests, however, the trees have now reached sufficient size to enable crosses to be done in the breeding garden. Black spruce crosses are still being carried out in family tests, as well as in the breeding garden.

In 1993, white spruce and tamarack progeny tests were outplanted. In addition to the jack pine planted in 1994, tamarack and black spruce progeny tests were established by the Council. Polycross breeding on white spruce is now 86% complete, while tamarack is 89% 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 has begun and breeding work commenced in 1994.

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 60% complete for black spruce selections and 100% complete for jack pine. A total of 4 ha of the total 11 ha second generation black spruce orchard has been established to date. The orchard will be completely planted in 4 years. The jack pine second generation orchard has 3 ha of the total 9 hectares planted. It is anticipated that the orchard will be fully stocked in 4-5 years.

CONE COLLECTION IN SEED ORCHARDS

Both the clonal and seedling orchards have started to produce substantial amounts of seed (Table 1). In 1994 all of the orchards had bumper seed crops and as a result of the great seed production, the black spruce, white spruce, jack pine and tamarack orchards have produced enough seed to meet our annual reforestation requirements with improved seed. A small cone collection in the second generation black spruce and jack pine was made in 1994. It is anticipated that within 5 years the second generation orchards will be producing substantial amounts of seed.

Seed yield from the black spruce seedling seed orchards was phenomenal in 1994 (Table 1.) The tamarack clonal orchard also had a heavy cone crop in 1994 and combined with the control for the larch cone maggot, the seed yield was excellent (Table 1.).

Table 1. Cone collection and seed yield from orchards in 1993 and 1994.

Species	Seed Orchard	Cones (1)		Seed (kg)	
		1993	1994	1993	1994
Black Spruce SSO	Bettsburg	900	23,400	3.5	217.0
	Pokiok	-	4,600	-	36.0
Jack Pine SSO	Otter Brook	4,245	-	38.0	-
White Spruce CSO	Queensbury	-	3,300	-	53.0
Tamarack CSO	Queensbury	-	1,600	-	26.0

MINI ORCHARD PROJECT

In cooperation with the Canadian Forest Service and with funding under the Canada/NB COOPERATION Agreement, a miniaturized seed orchard project was initiated in 1993. The NB DNRE 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 and 25 ramets per clone were grafted and grown in 1993. The grafts were then accelerated the winter of 1994 and outplanted at the Kingsclear Nursery in clonal rows. In conjunction with this project, a research trial was done in the spring of 1994 to investigate the low seed set of jack pine controlled crosses. The three factors that were investigated were the timing of pollen application, the amount of pollen applied per pollination bag and the number of pollen applications. The trial cones will be collected this fall and results analyzed.

COOPERATIVE TREE BREEDING IN NOVA SCOTIA

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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 both Federal and Provincial Governments, Bowater Mersey Paper Company Limited, Scott Worldwide 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.*

ORCHARD ESTABLISHMENT

First generation orchard establishment was completed in 1993. Total first generation orchard area for the five species we work with are as follows:

Species	Type	Approximate area (ha)	1994 Seed Production (kg)
Black Spruce	Seedling	16.6	49.7
White Spruce	Clonal	19.6	129.3
Red Spruce	Clonal	21.5	83.2
White Pine	Clonal	1.8	92.0
Norway Spruce	Clonal	16.9	6.5
	Meadow orchard design	0.6	
	TOTALS	76.4	360.7

Second-generation black spruce material is scheduled to be established into clone banks and two orchards in 1996.

ORCHARD MANAGEMENT

All black spruce reforestation stock in Nova Scotia comes from first generation seedling seed orchards. Further genetic gain is anticipated following a 50% roguing of the 1985 blocks of two black spruce orchards. Roughly half of the families were removed from each orchard on the basis of 10 year heights measured in family tests located throughout the province. Resulting gain is estimated at just over 6%.

Crown management through 'topping' has become a routine exercise in all TIWG orchards. This method of limiting tree height was developed in conjunction with CFS personnel in Fredericton. Following the topping, there is a significant increase in cone production from the orchard.

In 1994, a clonal white pine orchard first established in 1981 was 'topped' for the third time. This third topping was quite severe with often the upper one-third to one-half of the tree being removed, it should prove interesting to monitor the recovery and seed production in this orchard.

Supplemental mass pollination (SMP) was first attempted in 1994 in a clonal red spruce orchard. This operation attempts to increase seed set as well as offset the effects of foreign pollen which originates from a neighbouring forest stand. A research trial attempted to quantify the effectiveness of SMP, with unclear results. In 1995, the trial was repeated with some modifications.

BREEDING

Ninety-seven selections were made from within the 1985 series of family tests for a total of 150 selections to date. This completes 42% of the selection programme.

The current strategy for white and red spruce consists of polycrossing for GCA ranking and paired-matings for deployment into selection plantations. With an abundant flower crop in 1994, significant progress was made in the completion of first generation breeding for both white and red spruce. Breeding of Norway spruce has proven somewhat frustrating due to flowering inconsistency and the range of variation in phenology.

The spring of 1995 was another exceptional breeding year due to abundant flowering. Assuming good seed set, polycross breeding will be virtually complete for white and red spruce. The next species targeted for polycross breeding is second-generation black spruce, this work is expected to begin in the spring of 1996.

PROGENY TESTING

During the spring of 1994, there were two additional white spruce polycross tests series and one red spruce polycross test series established. In the summer of 1995 an additional white spruce polycross test series and two red spruce polycross test series were planted.

Ten year heights and damages in two 1985 series of black spruce family tests were measured and the results analyzed. Individual tree narrow-sense heritability estimates were 0.47 and 0.49 for the two series. Family heritabilities were 0.72 and 0.78 respectively. Families were ranked for both seed orchard roguing and second generation selection.

TREE IMPROVEMENT CONSULTING SERVICES PROVIDED BY GENESIS FOREST SCIENCE CANADA INC.

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Keywords: age-age correlations, China, clonal forestry, computer simulation, decision-support tools, early selection, *Picea mariana*, *Taxus canadensis*.

This writer has offered independent forestry consulting services for the past 5 years, specializing in aspects of forest renewal. Since 1993, these services have been made available to government and private-sector clients through Genesis Forest Science Canada Inc. This report summarizes the major contracts, completed or in-progress over the past 2 years, which are related to tree improvement and forest genetics.

GENETIC PARAMETERS IN A CLONAL TEST OF BLACK SPRUCE AFTER 10 YEARS

Height growth at 10 years from striking was assessed for clonally replicated full-sib black spruce (*Picea mariana* (Mill.) B.S.P.) families tested at three locations by the Nova Scotia Tree Improvement Working Group, under a contract funded by the Canada-NB Cooperation Agreement for Forestry Development (CAFD). Evaluated by means of an additive-dominance-epistasis genetic model, the additive portion of total genetic variance decreased from 66 to 38% between age 5 and 10, while the dominance portion increased from less than 3 to 13%, and the epistatic portion from 31 to almost 49%. As a consequence, narrow-sense heritability estimates were lower at age 10 and gain estimates also decreased, particularly for those strategies that capture gain primarily from additive effects. Age-age correlations were strongest at the half-sib family level and weakest at the clonal level (Mullin 1994*b*; Mullin and Park 1994).

GENETIC PARAMETERS AND CORRELATIONS IN OPEN-POLLINATED BLACK SPRUCE

Open-pollinated black spruce families represented in two field-test series managed by the New Brunswick Tree Improvement Council were also established in retrospective greenhouse and nursery test environments. Under a research contract funded by the Canada-NB CAFD, height growth was assessed after varying numbers of growth cycles and compared to performance in the original field tests after 5, 10, and 15 years. The two test series present conflicting demonstrations of the impact of early selection. Results from one series suggest that family selection can be done with a high degree of reliability after 3 or 4 accelerated cycles in a single nursery environment and that family rankings are reliable after only 5 years in the field tests, whereas those of the other series suggest that longer test periods are required and that short-term nursery tests are only effective in identifying the very worst families (Mullin 1994*b*; Mullin *et al.* 1995).

MANAGEMENT OF GENETIC DIVERSITY IN TREE BREEDING PROGRAMS

A research contract, funded by the Canada-NS CAFD, developed techniques for evaluating the impact of management strategies on genetic gain and diversity in tree breeding programs. A typical breeding plan employs numerous decision steps and a variety of limits, many of them somewhat arbitrary, to maintain diversity in the population while achieving as much gain as possible in each generation. Comparison of breeding strategies based on genetic gain from one round of selection was generally found to be inadequate. This project used stochastic simulation techniques to track changes in genetic structure, accumulated inbreeding and effective population size after several generations of breeding in a closed population. The simulation software has been made available to tree breeders as a generalized decision-support tool that can accommodate a wide variety of mating designs, population structure, and selection methods (Mullin 1995; Mullin and Park 1995*a*, 1995*b*).

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. One 6-week technical mission was performed at the Project site in 1993 (Mullin and Wang 1993) and two in 1994 (Mullin 1994*c*, 1994*d*), during which advice and training were given in the areas of seed procurement, seed biology, seedling physiology, and nursery production technology. Additional services as "Nursery Systems Specialist" have been contracted to perform a 7-month mission on-site in 1995, 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.

PROPAGATION OF SELECTED *TAXUS* FOR PRODUCTION OF TAXOL AND RELATED COMPOUNDS

Scientific advisory services have been provided to Centre sylvicole Forestville inc. (Forestville, Quebec), to assist in the design and implementation of a research project on the Canadian yew (*Taxus canadensis* Marsh.), co-funded by the Canadian Forest Service - Quebec Region (Essais, Expérimentations et Transfert Technologique en Foresterie). Taxol and related taxane compounds are natural products found in tissues of various species of *Taxus* and have demonstrated their potential in the treatment of various cancers. Although the Canadian yew is commonly found throughout eastern Canada, information on nursery propagation and genetic variation in this species is extremely limited. This project is designed to (1) study the mechanisms for regeneration of yew, (2) determine optimum conditions for nursery culture by seedling and clonal propagation, (3) investigate levels of genetic variation for growth and chemical constituents, and (4) evaluate strategies for management of yew in plantations for harvest of foliage and extraction of pharmaceuticals (Mullin 1993; Lalonde and Mullin 1995).

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TREE IMPROVEMENT PROGRESS IN PEI

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Keywords: seed orchard, polycross, flower induction, provenance trials, density trial, gene pool conservation.

The PEI Forestry Division has concentrated its tree improvement efforts primarily on four major reforestation species: black spruce (*Picea mariana* (mill.) BSP), white spruce (*Picea glauca* (Moench) Voss), eastern larch (*Larix laricina* (Du Roi) K. Koch.) and white pine (*Pinus strobus* L.). Secondary species include red spruce (*Picea rubens* Sarg.), balsam fir (*Abies balsamea* (L.) Mill.), yellow birch (*Betula alleghaniensis* Britton.), red oak (*Quercus rubra* L.), white ash (*Fraxinus americana* L.), black ash (*Fraxinus nigra*, Marsh) and hop-hornbeam (*Ostrya virginiana* (Mill). K. Koch).

This report summarizes our tree improvement activities over the past two years.

ORCHARD ESTABLISHMENT

First generation orchards have been completed for our four major reforestation species. The balsam fir orchard, which was established to produce improved seed for Christmas tree growers, is now 85% complete. Our red spruce orchard is approximately 70% complete.

Due to a cut in federal funding, plans for the miniaturized white pine seed orchard, noted in our last progress report, was cancelled. The plus trees that were selected for this project were used instead to expand the conventional seed orchard at Dover. This increased the orchard area for white pine by 0.8ha.

The following table summarizes seed orchard establishment to date.

Table 1 PEI Forestry Division Seed Orchard Summary

Species	Orchard Type	Location	Area (ha)
White Spruce	Clonal	Dover	3.3
Red Spruce	Clonal	Dover	2.5
Black Spruce	Seedling	48 Road	4.0
Black Spruce	Clonal	Upton Road	0.9
Eastern Larch	Clonal	Upton Road	1.5
Eastern Larch	Clonal	Dover	1.1
PEI White Pine	Clonal	Dover	1.5
PEI White Pine (1995)	Clonal	Dover	0.8
NS White Pine	Clonal	Dover	0.5
Balsam Fir	Clonal	Dover	1.0

SECOND GENERATION SELECTIONS

The 1983 black spruce family test was measured in the fall of 1993. From this data, individual selections were made from the top forty families. These selections were grafted during the winter of 1994 and 1995. All available grafts were established in the second generation black spruce seed orchard in the spring of 1995 (90% complete).

TREE BREEDING

The spring of 1994 showed almost no white spruce flower production in our orchards and clone banks, although other parts of the Maritimes had heavy crops. The opposite was true of early 1995 when a high number of clones produced flowers. White spruce polycrosses were completed in 93% of the clones.

CONE COLLECTIONS IN SEED ORCHARDS

All of the orchards have produced seed. The quantities collected have been small with the exception of black spruce and eastern larch. The black spruce seedling orchard has been producing all the required seed since 1988 while the first crop of eastern larch was sown in the spring of 1995. The PEI and NS white pine orchards produced 1.3 and 2.6 kg respectively in 1994.

The remaining orchards are gradually increasing in seed production and should come close to meeting the nursery's requirements within then the next few years.

FLOWER INDUCTION

In June of 1994, all trees of suitable diameter, in the eastern larch orchard were injected with GA4/7. The orchard also received monthly fertilizer applications. These measures, in combination with a dry summer season helped to produce a large cone crop for 1995.

PROVENANCE TRIALS/RESEARCH PLANTINGS

PEI participated in a white spruce embling trial initiated by the Canadian Forestry Service in Fredericton. The trial consisted of two parts: an embling demonstration plantation and a growth and yield plantation.

Several observation tests were also established with the following species: Eastern and Japanese larch, white pine, (native and non-native species), and white, yellow and European birch.

HARDWOOD RESEARCH /GENE POOL CONSERVATION

Tree improvement's hardwood research concentrates primarily on yellow birch, red oak and white ash. The first density trials were established in 1992. These tests contain four planting densities, replicated three times. Plot size was 0.10 ha, for a total of 1.20 ha for each site. The yellow birch and red oak trials were established in 1992 and 1993, while the white ash planting was completed in 1994.

These tests were assessed for survival after the first year and fill planted as required. In addition to survival, height and growth will also be measured at age 1, 5 and 10 years, with diameter and form assessed at age 10.

Tree improvement efforts towards gene pool conservation in black ash and hop hornbeam have made some progress since last report. A small black ash plantation has been established at the Dover seed orchard property. Work is ongoing, to establish a similar plantation of hop hornbeam but successful germination of this species has proven difficult.

THE CURRENT STATUS AND FUTURE DIRECTION OF TREE IMPROVEMENT IN NEWFOUNDLAND AND LABRADOR

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Keywords: Seed orchards, grafting, gene conservation.

The New Directions plan, described in my 1991 report (Harrison, 1991) is nearing completion. The black spruce orchards are complete, the white spruce orchards should be completed by the time this report is published and the eastern larch orchards will be completed in 1996 or 1997. The black spruce progeny tests are in place, but the white spruce and larch progeny tests await acquisition of seed from all plus trees. It is anticipated that flowering, and thus artificial cross pollination, will begin in the orchards and breeding gardens circa 2000 A.D. In the meantime, attention is being turned to a number of other matters.

A major cleaning and remeasurement project was undertaken in the autumn of 1994, in which over 30 tree improvement and silviculture experiments in the field were cleaned and/or remeasured. Competing vegetation was removed with brushsaws, stakes, tags and corner posts were replaced as needed and measurements were taken if due. The data is now being analyzed, and reports are being written for inclusion in Silviculture Notebook, a compendium of published reports on research projects under the direction of the Silviculture and Research Division. For further information on Silviculture Notebook, contact Basil English, Research Silviculturist, at the same address as the author.

A white pine working group has been established and a white pine gene pool reserve/orchard, similar to the one for red pine described in my last report (Harrison, 1993) has been established at Wooddale Nursery. A second reserve is planned for Thomas Howe Forest near Gander, Newfoundland plans to participate in a nationwide series of progeny/provenance trials in cooperation with Ariane Plourde of C.F.S., Quebec Region. Various other measures are underway or planned to preserve and re-establish white pine, red pine and possibly other species with small local populations, such as black ash.

Several new larch experiments are underway. A Japanese larch plantation at Pynn's Brook, 35 km east of Corner Brook, is now 11 years old and beginning to bear cones. A tentative plan is in place to convert the plantation to a seed orchard, pending current experimentation on cone and seed production, germination percentage and quality of seedlings produced. A species/provenance trial of European, Japanese and hybrid larches, in cooperation with Ante Stipanicic of Quebec's Ministere des Forests was established in 1993, and is due for a two year remeasurement this year. A cold-hardiness trial involving Japanese, Siberian, eastern, European and hybrid larches was established in ten locations throughout the province in 1994. A Siberian larch provenance trail, in cooperation with Thomas Nieman of P.N.F.I. is to be established in four locations in Labrador and the Northern Peninsula in 1995.

A Kalmia resistance trial involving progeny of most of our black spruce plus trees will be established in 1995. A demonstration forest is being established at Bottom Brook, in southwestern Newfoundland. Several existing tree improvement and silvicultural experiments, as well as a C.F.S. arboretum established in the late 1960's, are included within its boundaries. A new arboretum, emphasizing hardwoods will be established about 200m from the first one, which emphasized conifers. Additional space is available within the demonstration forest for future genetics and silviculture experiments.

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