

***Ex situ* Conservation of Forest Biodiversity in British Columbia**

D. George W. Edwards

Forestry Canada, Pacific Forestry Centre
Victoria, B.C.

Yousry A. El-Kassaby

Canadian Pacific Forest Products Ltd., Saanich Forestry Centre
Saanichton, B.C.

Introduction

Biological diversity of our forests, which has become a preeminent concern of forest practitioners, users, and the public, has three major components: species diversity, ecosystem diversity, and genetic diversity. This project focuses on the conservation of genetic diversity because no similar studies were being conducted; work on species and ecosystem diversity is under way elsewhere. Since little information is available on the impact of *ex situ* conservation on the genetic diversity of tree seeds, this project was initiated.

Ex situ conservation in crop plants relies on frequent rejuvenation (i.e., production of new plants, conducting of controlled crosses, and harvesting of fresh seeds for further storage). *Ex situ* conservation in forestry, specifically conifers, cannot accommodate this rejuvenation component because of the well-known long duration required by seedlings to reach sexual maturity. Thus, the genetic integrity of stored seedlots has to be assumed to remain constant, unchanging over time. This project is designed to test the efficacy of this assumption through simulated (accelerated), long-term ageing.

Before any testing is begun, two fundamental questions must be addressed: 1) what genetic differences exist in germination parameters; and 2) what genetic differences exist in dormancy levels. These questions are essential for establishing benchmarks against which deterioration during simulated ageing can be evaluated, and for testing the hypothesis that genotype-specific differences in dormancy are related to rate of deterioration.

Germination/dormancy-breaking is a by-product of enzymatic activity. Most of the proteins involved in this are stored in the nutritive (megagametophyte) tissue. It is expected, therefore, that rate of deterioration in stored seeds will be associated with changes in the protein matrix. The protein matrix will be monitored through the simulated ageing process to determine the presence or absence of associated changes.

Objectives

The objectives of the project are:

- to determine if commonly used forest tree seed storage practices result in a reduction of genetic diversity;
- to relate any reductions to specific genotypes;
- to identify specific changes (gene markers) in the protein matrix with deterioration during storage; and
- to propose alternative approaches to current seed storage procedures to circumvent any changes in the genetic composition of stored seedlots.

Materials and Methods

The species to be studied will include all coniferous species under domestication programs (coastal: Douglas-fir, Sitka spruce, western hemlock, western redcedar; and interior: lodgepole pine, "interior" spruce), minor species (mountain hemlock), and a representative broadleaved species (red alder) (Table 1).

TABLE 1. *Ex situ* Conservation Work Plan 1992–1997

Species	Genetic differences	Accelerated aging	Biochemical analysis
Douglas-fir	Inheritance of germination - published 1992 Germination speed - publication 1992 Manipulation of dormancy - data analysis - publication 1993	Completed - data analysis ongoing - reporting in progress	In progress
Sitka spruce	Inheritance and germination differences - completed - publication 1992	Completed - publication 1993 (in press)	1994/95
western hemlock	Inheritance, germination differences and dormancy - in progress	1993/94	1994/95
western redcedar	Germination differences - completed Preliminary data presented	1994/95	1994/95
mountain hemlock	Germination differences - completed	1993/94	1994/95
lodgepole pine	Inheritance, germination differences and dormancy manipulation - in progress	1993/94	In progress
interior spruce	1993/94	1994/95	1994/95
red alder	1993/94	1994/95	1994/95

The methods to be used include: 1) traditional seed pretreatments; 2) modified seed pretreatments; 3) simulated (accelerated) ageing techniques; and 4) biochemical analyses (2-D electrophoresis).

The standard method developed for simulating ageing in crop plants will be modified by means of pilot tests, to suit individual tree species. (Work already carried out on Douglas-fir, Sitka spruce, and mountain hemlock has indicated that custom-made ageing treatments are required for every species.) The modified method will then be used to determine whether differences in resilience to simulated long-term storage exist, and whether these differences are genotype specific.

Standard germination tests will be employed to establish benchmarks for germinability/dormancy and to establish the presence of genetic differences among genotypes.

Biochemical analyses (2-D electrophoresis) are being used to determine changes in the protein matrix and whether such changes (losses/additions) are genotype specific.

Results

1. Genetic differences in germination parameters and dormancy have been found in Douglas-fir (El-Kassaby *et al.* 1992), Sitka spruce (Chaisurisri *et al.* 1993b), and western redcedar (El-Kassaby *et al.* 1993). Evidence of strong genetic (maternal) control ($h_b^2 > 0.5$) has been found for Douglas-fir, Sitka spruce, western redcedar (El-Kassaby *et al.* 1993a) (Table 2), and mountain hemlock. The unique structure of coniferous seeds dictates this strong maternal effect (4:1 maternal:paternal) (El-Kassaby *et al.* 1992). A novel interpretation of germination parameters has also been reported (Thompson and El-Kassaby 1993).

2. Since strong genetic control over germinability/dormancy has been established, similar differences in rate of seed deterioration can be expected. Genotype-specific differences in rate of deterioration under simulated ageing have been documented for Sitka spruce (Chaisurisri *et al.* 1993a) and in Douglas-fir (in preparation); work has begun on western hemlock, mountain hemlock, and lodgepole pine. These results indicate that the genetic makeup of a seedlot will be different before and after simulated ageing.
3. Biochemical analyses are under way to establish the protein matrix for fresh, untreated seeds of Douglas-fir and lodgepole pine. Investigation of changes in the matrix caused by simulated ageing are under way.

TABLE 2. Estimates of variance components, significance level, and broad-sense heritabilities (h_b^2) for germination parameters of Douglas-fir, Sitka spruce, and western redcedar (Source: El-Kassaby *et al.*, 1993)

Species	Source of variation	Degrees of freedom ^a	Germination parameters ^b		
			GC	PV	GV
Douglas-fir (Df)	Among trees	t-1	0.058**	3.602**	1.681**
	Residual	t(r-1)	0.005	0.348	0.134
	h_b^2		0.92	0.91	0.93
Sitka spruce (Ss)	Among clones	c-1	0.003**	0.710**	19.537**
	Residual	c(r-1)	0.004	0.204	6.744
	h_b^2		0.42	0.78	0.74
Western redcedar (Cwr)	Among clones	c-1	0.020**	0.521**	23.055**
	Residual	c(r-1)	0.005	0.151	5.733
	h_b^2		0.79	0.78	
0.80					

* = $P < 0.05$, ** = $P < 0.01$

^a t = no. of trees (Df, t = 19, t = 12),

c = no. of clones (Ss, c = 18, Cwr, c = 22),

r = no. of replications = 4.

^b GC = germination capacity; the percentage of seeds that had germinated at the end of the test (transformation = arcsin).

PV = peak value (Czabator 1962); a mathematical expression of the break of a sigmoid curve representing a typical course of germination (no transformation).

GV = germination value (Czabator 1962); a mathematical expression combining speed and completeness of germination into a single value (transformation = $\sqrt{X} + 0.5$ for Df).

References

- Chaisurisri, K., D.G.W. Edwards, and Y.A. El-Kassaby. [1993a]. Accelerated ageing of Sitka spruce seeds. *Silv. Genet.* In press.
- _____. 1993b. Genetic control of seed size and germination in Sitka spruce seeds. *Silv. Genet.* 41: 348-354.
- Czabator, F.J. 1962. Germination value: an index combining speed and completeness of pine seed germination. *For. Sci.* 8:386-396.
- El-Kassaby, Y.A., D.G.W. Edwards, and D.W. Taylor. 1992. Genetic control of germination parameters in Douglas fir and its importance for domestication. *Silv. Genet.* 41: 48-54.
- El-Kassaby, Y.A., K. Chaisurisri, D.G.W. Edwards, and D.W. Taylor. 1993. Genetic control of germination parameters of Douglas-fir, Sitka spruce, western redcedar, and yellow-cedar and its impact on container nursery production. *In Proc. IUFRO Symp. Dormancy and Barriers to Germination*, Victoria, B.C., April 1991. D.G.W. Edwards (compiler and editor). *For. Canada, Pac. For. Cent. For. Tech. Rep.*, pp 37-42.
- Thompson, A.J. and Y.A. El-Kassaby. 1993. Interpretation of seed germination parameters. *New Forests* 7:123-132