National Tree Seed Centre

Annual Report

2008

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NATIONAL TREE SEED CENTRE ANNUAL REPORT 2008

EXECUTIVE SUMMARY

Seed production was poor in the Maritimes in 2008. This marked the second consecutive year of poor seed production which broke the trend that had gone on since 1996 of good seed crop years every two years. A total of 160 collections from 12 species was made in Nova Scotia, New Brunswick, and Quebec. Significant collections included: 16 red maple (*Acer rubrum*), 18 sugar maple (*A. saccharum*), 17 eastern white cedar (*Thuja occidentalis*), 16 red pine (*Pinus resinosa*), and 20 grey birch (*Betula populifolia*) in New Brunswick; 17 black ash (*Fraxinus nigra*) and 34 red ash (*F. pennsylvanica*) in Quebec; and 15 red maple in Nova Scotia.

A total of 60 requests for seed resulted in 664 seedlots provided for research. The majority of the requests were from Canada (50 requests; 549 seedlots) but seed was also sent to Iceland (1 request; 28 seedlots), Ireland (1 request; 2 seedlots), Slovakia (1 request; 40 seedlots), Sweden (2 requests; 29 seedlots), and United States (5 requests; 16 seedlots).

Seed testing consisted of 894 germination tests, 703 moisture content tests, and 168 thousand-seed weight tests. A significant proportion of the germination testing is re-testing of seedlots tested 10 years ago. Over the next three years, 2500 seedlots that were tested between 1999 and 2001 will be re-tested.

Other activities during the year included:

- Viability of eastern white cedar seed stored for 1 year was higher for seed stored at -20°C than for seed stored at 4°C or in liquid nitrogen.
- Seedlots produced by Dr. Don Fowler for tree breeding work in the 1970s and 1980s, which had been stored in a chest freezer, were re-packaged, weighed, and entered into a database.
- The Seed Centre participated in a test conducted among International Seed Testing Association (ISTA) accredited and non-accredited laboratories comparing different methods using tetrazolium to assess viability of *Abies alba* (European silver fir) seeds.
- After-ripening of white spruce cones collected in 2007 from 15 trees in Newfoundland resulted in improved seed germination.

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INTRODUCTION

This report covers the activities of the National Tree Seed Centre (NTSC) for 2008. Similar reports were prepared from 1998 – 2007. The report also captures the results of tests and experiments that were conducted during the year in order to ensure that this information is synthesized and reported.

The NTSC is a major component of the National Forest Genetic Resources Centre. It was established in 1967 at the Petawawa Research Forest (PRF) in Ontario and was transferred to the Atlantic Forestry Centre in Fredericton, N.B. in 1996. The mandate of the NTSC is to: obtain, store, and provide seed of known origin and quality for forest research; carry out baseline research on seed of Canadian tree and shrub species; and preserve germplasm for genetic conservation.

Seed is stored in four categories: Seed Bank, Reserved, Tree Breeding, and Genetic Conservation (Table 1). The total number of seedlots increased by 229 to 12 775 in 2008. The numbers in brackets in Table 1 represent the numbers reported in the 2007 Annual Report.

Table 1. Seed stored at the NTSC as of December 31, 2008.

Seed Bank		Genetic Conservation		Reserved		Tree Breeding	
No. species	No. seedlots	No. species	No. seedlots	No. species	No. seedlots	No. species	No. seedlots
153 (159)	6,395 (6,302)	41 (30)	4,070 (3,888)	34 (39)	1,920 (1,966)	10 (10)	390 (390)

Seed Bank seedlots are the active collection that are available for distribution. Since 1998, the number of seedlots in the Seed Bank collection has increased from 3,079 to 6,395 (Figure 1). The increase represents the net gain after discarding seedlots due to low germination and the depletion of seedlots as they are provided to clients. In 2008, 20 seedlots were discarded and an additional 24 were depleted.

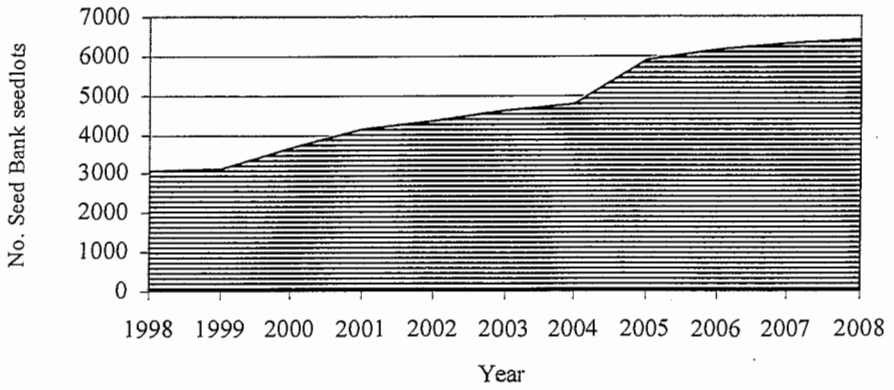

Figure 1. Increase in number of Seed Bank seedlots at the NTSC since 1998.

One of the objectives of the NTSC is to obtain seed samples of Canadian tree and shrub species from across their natural ranges. As of December 31, 2008, the NTSC Seed Bank had 6,167 seedlots from 115 Canadian species in storage (Table 2). An additional 57 exotic species (228 seedlots) are also stored. Exotic species are defined as those from which seed was collected outside Canada which may or may not be present in Canada. With the mandate of the Centre now concentrating on seed from Canadian tree and shrub species, the proportion of seed from exotic species is decreasing although some opportunistic acquisitions may still be made.

Table 2. Number of species, number of seedlots, and percentages by province of seedlots stored in the Seed Bank category.

Province	No. species	No. seedlots	Percent
Alberta	12	45	0.7
British Columbia	32	294	4.8
Manitoba	6	65	1.1
New Brunswick	69	1369	22.2
Newfoundland and Labrador	17	166	2.7
Nova Scotia	39	520	8.4
Ontario	49	2318	37.6
Prince Edward Island	31	234	3.8
Quebec	23	999	16.2
Saskatchewan	8	111	1.8
Yukon Territory	3	46	0.7
Total	115	6167	100

Since the Seed Centre moved to Fredericton, staff have concentrated their efforts acquiring collections from New Brunswick (N.B.), Nova Scotia (N.S.), and Prince Edward Island (P.E.I.). Travel beyond the Maritime provinces is difficult due to limited resources (staff and budget). There is an ongoing effort to acquire seed from other provinces and Seed Centres whenever the opportunity presents itself. The NTSC needs to continue in its effort of acquiring seedlots west of Ontario. Since collections by NTSC staff are unlikely due to distance and costs, these seedlots will have to be purchased or obtained through donation.

The Genetic Conservation category was initiated in 2000 using seed already in storage. Its purpose is to ensure that genetic material obtained from rare, endangered, and/or unique populations, as well as samples from throughout a species' range is preserved. This collection increased by 182 to 4,070 seedlots. Figure 2 shows the increase in the number of seedlots in this category since 2000.

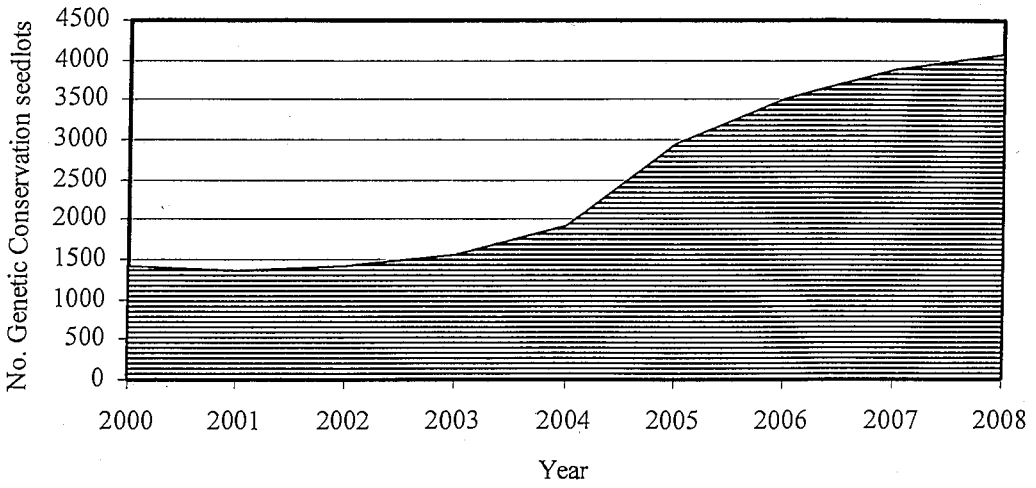

Figure 2. Increase in the number of Genetic Conservation seedlots at the NTSC since 2000.

There is seed from 41 species in Genetic Conservation with the number of seedlots ranging from 1 for mountain alder (*Alnus tenuifolia*), buttonbush (*Cephalanthus occidentalis)*, pumpkin ash *(Fraxinus profunda*), blue ash (*F. quadrangulata*), western larch (*Larix occidentalis*), and mountain hemlock (*Tsuga mertensiana*) to 1,651 for white spruce *(Picea glauca)*. Other species and number of seedlots are: Manitoba maple (*Acer negundo,* 15 seedlots); striped maple (*A. pensylvanicum*, 17 seedlots); red maple (*A. rubrum*, 111 seedlots); mountain maple (*A. spicatum,* 46 seedlots); speckled alder *(Alnus rugosa*, 2 seedlots); green alder (*A. crispa*, 6 seedlots); yellow birch (*Betula alleghaniensis,* 21 seedlots); mountain paper birch (*B. cordifolia*, 5 seedlots); white birch (*B. papyrifera*, 10 seedlots); eastern flowering dogwood (*Cornus florida*, 4 seedlots); white ash (*Fraxinus americana,* 215 seedlots); black ash (*F. nigra,* 120 seedlots); red ash (*F. pennsylvanica,* 17 seedlots); green ash (*F. pennsylvanica* var. *subintegerrima*, 14 seedlots); Northern red ash (*F. pennsylvanica* var. *austini*, 41 seedlots); tamarack (*Larix laricina,* 266 seedlots); black spruce (*Picea mariana,* 404 seedlots); red spruce (*P. rubens,* 16 seedlots); jack pine (*Pinus banksiana,* 95 seedlots); lodgepole pine (*P. contorta* var. *latifolia*, 2 seedlots); limber pine (*P. flexilis,* 101 seedlots); prince pinyon pine (*P. pinceana*, 181 seedlots); ponderosa pine (*P. ponderosa*, 2 seedlots); pitch pine (*P. rigida,* 4 seedlots); eastern white pine (*P. strobus,* 32 seedlots); Scots pine (*P. sylvestris,* 12 seedlots); balsam poplar (*Populus balsamifera*, 20 seedlots); largetooth aspen (*P. grandidenta,* 13 seedlots); trembling aspen (*P. tremuloides,* 16 seedlots); pin cherry (*Prunus pensylvanica,* 61 seedlots); choke cherry (*P. virginiana,* 337 seedlots); eastern white cedar (*Thuja occidentalis*, 34 seedlots); western redcedar (*T. plicata*, 2 seedlots), and eastern hemlock (*Tsuga canadensis*, 168 seedlots).

The Reserved category contains seedlots that have been reserved by researchers. Many of these seedlots were collected for special projects. There was no significant change in this category in 2008.

The Tree Breeding category is composed of seedlots that originated from the genetics program at PRF and were transferred to the Seed Centre for storage. There was no change in this category in 2008.

SEED COLLECTIONS

Seed production was poor for most species in the Maritimes. In order to ensure good quality seed, seed is only collected during good seed years. Seed collected in poor seed years may be of lesser quality because of poor pollination. Also, the time required to collect sufficient seed increases when there is a poor seed crop. A total of 160 seedlots from 12 species was collected by Seed Centre staff.

Most of the collections (93 seedlots) were made in New Brunswick, 52 seedlots were collected in Quebec, and 15 in Nova Scotia. The Quebec collections consisted of red ash *(Fraxinus pennsylvanica*; 34 seedlots), black ash (*F. nigra*; 17 seedlots), and jack pine (*Pinus banksiana*; 1 seedlot). The ash collections are part of an ongoing initiative to collect ash seed from across the ranges of the various ash species in Canada as part of a strategy against the devastating impact of the emerald ash borer (*Agrilus planipennis*). Red maple (*Acer rubrum*) seed was collected in Nova Scotia (15 seedlots) and New Brunswick (16 seedlots). The remaining collections were all from New Brunswick: sugar maple (*A. saccharum*; 18 seedlots), grey birch (*Betula populifolia*; 20 seedlots), red pine (*P. resinosa*; 16 seedlots), eastern white cedar (*Thuja occidentalis*; 17 seedlots), beech (*Fagus grandifolia*; 2 seedlots), and one seedlot each of silver maple (*A. saccharinum*) and red oak (*Quercus rubra*). Seed Centre staff also assisted Dr. Alex Mosseler with collections of pitch pine (*P. rigida)* from two genetic tests in New Brunswick. Table 3 provides a complete list of the collections made.

Table 3. Seed collections made by Seed Centre staff in 2008.

Species	NB	NS	QC	Total
Acer rubrum	16	15		31
Acer saccharum	18			18
Acer saccharinum	1			1
Betula populifolia	20			20
Fagus grandifolia	2			2
Fraxinus nigra			17	17
Fraxinus pennsylvanica			34	34
Pinus banksiana			1	1
Pinus resinosa	16			16
Pinus rigida	2			2
Quercus rubra	1			1
Thuja occidentalis	17			17
Total	93	15	52	160

The NTSC acquires seed through collections made by Seed Centre staff, donations, and purchase. In addition to the 160 seedlots collected by Seed Centre staff, one seedlot each of white birch lodgepole pine, and black spruce, collected in Yukon Territory, was donated by Dr. Rod Savidge of UNB, Fredericton. Table 4 shows the number of seedlots acquired by the NTSC since 1996.

Table 4. Number of seedlots acquired by the NTSC through collection, donation, and purchase between 1996 and 2008.

Year	Number of Seedlots			
	Collection	Donation	Purchase	Total
1996	239	22		261
1997	75	245		320
1998	284	47	9	340
1999	139	80		219
2000	195	673		868
2001	137	122	45	304
2002	367	36		403
2003	69	142		211
2004	549	381	137	1067
2005	142	29	3	184
2006	329	42	30	401
2007	190	181		371
2008	160	3		
Total	2875	2003	224	5102

SEED REQUESTS

It is the Seed Centre's policy to provide seed, at no cost, for scientific research. Seed is also provided, on occasion, to universities and other educational institutions for educational purposes and to arboretums. A seed request form must be completed by the client before a seed order is processed. The purpose of this form is to gather information on the type of research being carried out and to serve as a means of screening requests. All seed requests received from outside Canada are referred to the Canadian Food Inspection Agency (CFIA) to determine if a phytosanitary certificate and/or import permit is required.

During 2008, a total of 60 requests representing 664 seedlots was processed. The majority of the requests were from Canada but seed was also sent to Iceland, Ireland, Slovakia, Sweden, and United States (Table 5). The number of seedlots provided by the NTSC since 1967 has ranged from a low of 99 in 1996 to a high of 1,603 in 1985 (Figure 3). Canadian researchers received 69% of the seed while seed sent to researchers outside Canada accounted for the remaining 31%.

Table 5. Number of requests and number of seedlots shipped by country in 2008.

Country	No. requests	No. seedlots
Canada	50	549
Iceland	1	28
Ireland	1	2
Slovakia	1	40
Sweden	2	29
United States	5	16
Total	60	664

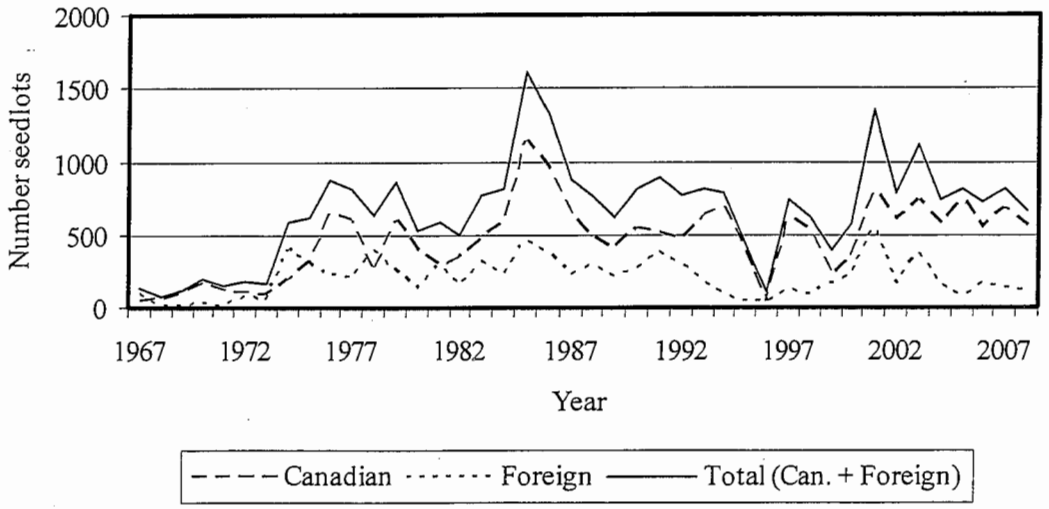

Figure 3. Number of seedlots sent to clients between 1967 and 2008.

SEED TESTING

Germination tests are performed on all freshly collected seedlots as well as seedlots in storage that have not been tested for several years. In most cases four replicates of 50 seeds each are placed on moistened Versa-Pak™ in Petawawa germination boxes. When larger seed are being tested, the number of seed is usually reduced. **Eight hundred and ninety-four germination tests** were carried out. Seedlots in storage are tested every 10 years. Approximately 40% of the Seed Bank seedlots were tested between 1999 and 2001. Once testing of these seedlots is completed, the results will be used to update our seed storage data.

Figure 4 shows the number of tests carried out by the NTSC since 1983. Some testing was carried out prior to 1983 (1970–82), however, the number of tests conducted was low and does not represent a fully operational lab. The reduction in the number of tests between 1994 and 1996 coincided with the transferral of the Seed Centre from Petawawa to Fredericton.

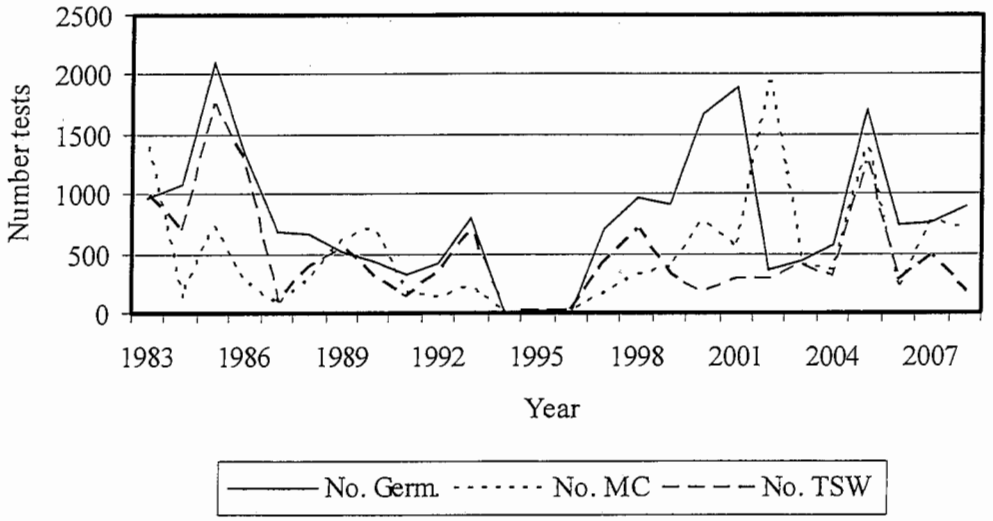

Figure 4. Number of germination tests (No. Germ), moisture content tests (No. MC), and thousand-seed weights (No. TSW) carried out by the NTSC since 1983.

The target moisture content (MC) for orthodox seed is between 5 and 8%. Seed that are above this range are further dried before being stored. **Seven hundred and three moisture content** determinations were carried out. MC is checked when seed are re-tested. If MC exceeds 8% the seed are conditioned to lower their MC. **One hundred and forty-one** seedlots were conditioned.

Once MC is within acceptable limits, the 1000-seed weight is determined. This is carried out by counting and weighing eight replicates of 100 seeds. When dealing with small seed (birches, poplars, willows) fewer replicates are performed. When the collected sample is small (less than 800 seeds), the total number of seed is counted, the total weight of the sample is determined, and the 1000-seed weight calculated. A total of **one hundred sixty-eight 1000-seed weights** was done.

Eastern White Cedar Seed Storage Experiment

There are two species of *Thuja* native to North America.: *Thuja plicata* (western redcedar) and *Thuja occidentalis* (eastern white cedar). In Canada, western redcedar is found almost exclusively in British Columbia while eastern white cedar is common in New Brunswick, Quebec, and Ontario but can also be found in parts of Nova Scotia and Prince Edward Island. The species is listed as vulnerable in Nova Scotia and is not common in Prince Edward Island.

Seeds of both species do not require chilling (AOSA 2002; ISTA 2008). The seed are considered orthodox and can therefore be dried and stored in sub-zero conditions. There is uncertainty as to how long *Thuja* seed can be stored and retain viability. Seed with a moisture content of 5–10% and stored at 0–5 °C should retain viability for up to 5 years (Brand and Schopmeyer 2008). Mean germination of eight eastern white cedar seedlots collected in 1991 and 1992 and stored at -20 °C for 10 and 11 years at the NTSC remained constant at around 61% (Daigle and Simpson 2008).

Thuja seed measure about 3–5 mm in length and have an attached wing, making the seed almost as wide as it is long (Brand and Schopmeyer 2008). Thousand-seed weight varies between 0.75 and 1.50 g. The seed cannot be de-winged and the light seed weight and the attached wing make the seed difficult to clean. Consequently seed germination is often mediocre unless very good seed set occurs.

In 2006, the NTSC received 15 single tree collections of eastern white cedar cones from the Petawawa Research Forest. The seed were processed and moisture content and germination were determined. Seed quality was very good and the quantity of seed received provided an excellent opportunity to set up a seed storage experiment.

Seed from 12 trees were put in 20 ml screw cap vials which were then placed into 250 ml Mason jars for storage at 4 °C and -20 °C and in 10 ml screw cap cryogenic vials for storage in liquid nitrogen (LN (196 °C)). Before setting up the experiment, seed were placed in LN for 7 days to determine the impact of storage under these conditions. Sufficient seed is stored for germination and moisture content assessments at 1, 2, 4, 8, 16, and 32 years.

Four replicates of 50 seed each were placed in Petawawa germination boxes on moistened Versa-Pak™ and put into a Conviron G30 germination cabinet for 21 days. Germination conditions consisted of a daily cycle of 8 hours at 30 °C with light and 16 hours at 20 °C without light with a constant relative humidity of 85%. Seed were assessed every two days starting at day 7. A seed was considered to have germinated when the cotyledons were visible and the hypocotyl and radicle were well developed.

Mean moisture content and germination prior to storage was 6.5% and 86.3%, respectively. After 7 days in LN mean germination was 83.5% which indicates that eastern white cedar seed can tolerate

storage in LN (Daigle and Simpson 2008). Most of the loss in germination was attributable to seedlot 267 whose germination decreased from 59.5 to 39.5%. If this seedlot is removed, mean germination loss is less than 1%.

After one year in storage seed were tested and mean germination decreased for all storage conditions (Table 6). The greatest decrease occurred with the seed stored at 4°C with mean germination decreasing from 86.3–73.1%. The best results were obtained for the seed stored at -20°C which decreased only 3.1%. The seed stored in LN performed slightly better than those stored at 4°C. This is somewhat surprising since there was very little loss in viability after 7 days in LN. Germination of seed in seedlot 267 was higher than the control after one year storage at -20°C but about 50% lower when stored at 4°C. Seed stored in LN for one year germinated slightly better than seed stored for 7 days.

Table 6. Germination (%) of 12 eastern white cedar seedlots stored in liquid nitrogen (LN) for 7 days and at 4°C, -20°C, and LN for 1 year.

	Storage Duration				
	0 days	7 days	1 Year		
Seedlot No.	Control	LN	4°C	-20°C	LN
264	92.5	88.5	88.5	90.5	83.0
265	83.0	87.5	41.0	71.0	57.5
266	98.0	98.5	60.0	96.0	95.5
267	59.5	39.5	31.0	68.5	43.5
268	89.5	82.0	88.5	78.0	87.5
269	89.5	81.0	72.0	85.5	84.0
270	87.0	89.5	84.0	92.0	81.0
271	93.5	94.0	78.5	92.0	79.5
272	85.5	82.0	77.5	81.0	55.0
273	93.5	91.0	86.5	86.0	78.0
274	70.0	72.5	72.0	61.5	48.0
278	94.0	95.5	98.0	96.0	96.0
Mean Germ.	86.3	83.5	73.1	83.2	76.5

Part of the decrease in germination can be attributed to a higher number of low vigour germinants particularly for the seed stored at 4°C and in LN (Table 7). The greatest number of low vigour germinants occurred with seed stored for 1 year in LN (6.3%) and at 4°C (5.8%). Seedlot 265 had the highest percentage of low vigour germinants in each storage tempearture.

Table 7. Low vigour germination (%) for eastern white cedar seed stored for 7 days in liquid nitrogen (LN) and for 1 year at 4°C, -20°C, and LN.

	Storage Duration				
	0 days	7 days	1 Year		
Seedlot No.	Control	LN	4°C	-20°C	LN
264	0.5	1.0	4.0	0.5	2.5
265	1.0	0.5	12.5	14.0	25.0
266	0.5	0.0	11.0	0.0	2.0
267	0.0	0.0	10.0	0.5	5.5
268	1.5	0.0	4.0	7.0	4.0
269	0.0	1.5	3.5	0.5	1.0
270	4.5	2.0	7.0	2.0	10.0
271	0.5	0.5	4.5	1.0	4.0
272	0.0	2.0	8.0	8.0	6.5
273	2.0	0.5	3.5	4.5	4.0
274	3.0	3.0	1.5	4.0	11.0
278	1.5	0.0	0.5	1.0	0.0
Mean LV Germ.	1.3	0.9	5.8	3.6	6.3

Moisture content was determined for all seedlots before storage and after one year in storage (Table 8). Mean MC at time of storage was 6.47% (5.57 – 7.14). After one year at 4°C mean MC increased to 7.52% (5.76 – 10.36). This increase was caused by 4 seedlots that were all contained in the same Mason jar. The jar may not have been properly closed which caused the increase in MC. The mean MC for the seed stored at 4°C if these seedlots are not included is 6.44%. Mean MCs of the seed stored at -20°C and in LN was 6.05% and 6.20% respectively.

The higher MC of the four seedlots stored at 4°C did not negatively affect germination. In fact the decrease in germination for these four seedlots was less than for the remaining eight.

The reason for the high number of low vigour germinants is not clear. The two most likely causes are: natural aging of the seed during storage which would bring about a decrease in vigour or an onset of dormancy caused by storage. It is also possible that a combination of these two factors is in effect as the seed stored at 4°C may be more likely to lose viability during storage than the seed stored at -20°C and in LN.

Six seedlots were selected to try and determine the reason for the high number of low vigour germinants. The seedlots selected were those that exhibited on average the lowest (264, 269, and 278) and highest (265, 270, and 272) percentage of low vigour germinants for the three storage temperatures. The seedlots used were stored at the NTSC Seed Bank collection at -20°C for 1 year and were tested with and without a 21-day moist chilling.

Table 8. Moisture content (%) of 12 eastern white cedar seedlots stored at 4°C, -20°C, and LN for 1 year.

Seedlot No.	Storage Duration			
	0 days	1 Year		
	Control	4°C	-20°C	LN
264	6.13	5.95	5.49	5.09
265	5.57	5.95	5.22	5.49
266	5.97	6.01	5.49	5.69
267	5.62	5.76	5.32	5.42
268	6.23	6.85	5.91	5.91
269	7.14	6.96	6.58	6.71
270	6.82	7.06	6.23	6.48
271	7.14	6.95	6.51	6.87
272	6.97	10.36	6.66	6.69
273	6.63	9.48	6.25	6.31
274	6.72	9.79	6.71	6.62
278	6.68	9.14	6.25	7.13
Mean MC	6.47	7.52	6.05	6.2

Results of this trial showed that chilled seed had mean high vigour and mean low vigour germination of 87.2 and 1.0% compared to 84.3 and 3.0% for the non-chilled seed (Table 9). The results of the seed from the same seedlots in the experiment stored at -20°C for one year were 86.0 and 4.3% respectively.

Table 9. High vigour (HV) and low vigour (LV) germination of 6 eastern white cedar seedlots from storage experiment stored for one year at -20°C, and non-chilled and chilled seed of the same seedlots from Seed Bank stored at -20°C for one year.

		Storage Experiment		Seed from Seed Bank			
				Non-chilled		Chilled	
Seedlot/Vigour		HV	LV	HV	LV	HV	LV
264	LV	90.5	0.5	90.0	3.0	92.0	0.0
269	LV	85.5	0.5	72.0	2.0	82.5	0.5
278	LV	96.0	1.0	95.5	1.5	96.5	1.0
272	HV	81.0	8.0	74.0	5.0	84.0	1.5
265	HV	71.0	14.0	84.0	3.0	82.5	1.0
270	HV	92.0	2.0	90.0	3.5	85.5	2.5
Mean		86.0	4.3	84.3	3.0	87.2	1.0

It appears that the high low vigour value that occurred with seedlot 265 may have been due to sampling error or that something may have happened to the test sample. Based on these results moist chilling improved seed germination in four of the seedlots and reduced the number of low vigour germinant for all of the seedlots. The increase in germination of the chilled seed is slight when compared to seed germination from the storage experiment. According to Brand and Schopmeyer, 2008 the need for moist chilling is not clear. Paired tests will be used for future testing of the seed in this storage experiment.

Literature Cited

AOSA. 2002. Rules for testing seeds. Association of Official Seed Analysts. 166 p.

Daigle, B.I.; Simpson, J.D. 2008. National Tree Seed Centre annual report 2007. Nat. Res. Can., Can. For. Serv.-Atl. 16p.

ISTA. 2008. International rules for seed testing. Edition 2008/1. Int. Seed Test. Assoc., Bassersdorf, Switzerland.

Brand, G.J.; Schopmeyer, C.S. 2008. *Thuja* L. — arbovitae. Pages 1108–1111. *In* F.T. Bonner and R.P. Karrfalt, eds. The woody plant seed manual. USDA For. Serv., Agric. Handbook 727.

Don Fowler Tree Breeding Seedlots

Seedlots used by Dr. Don Fowler, retired tree breeder from Atlantic Forestry Centre, for tree breeding work in the 1970s and 1980s and which had been stored in a chest freezer were re-packaged, weighed, and entered into a database. Seedlots that were determined to have been poorly stored (damaged plastic bags) and that showed signs of mold were discarded. A total of 1262 seedlots are now being stored at -20°C at the NTSC. Seed from controlled crosses and from open-pollinated trees make up the bulk of the collection.

There is a total of 425 seedlots from 8 *Larix* species with 158 of these originating from controlled crosses. (Table 10). Almost half (200 seedlots) involve *Larix kaempferi* as the female parent while an additional 102 have *Larix laricina* and 71 have *Larix decidua* as the female parent.

Table 10. Number of seedlots from open-pollinated and controlled crosses from various *Larix* species.

Species	Pollination/Crosses	Number seedlots
Larix decidua	Open-pollinated	53
Larix decidua	*Larix decidua*	8
Larix decidua	*Larix kaempferi*	9
Larix decidua	*Larix sibirica*	1
Larix decidua x *Larix kaempferi*	Open-pollinated	4
Larix eurolepis	Open-pollinated	24
Larix gmelinii	Open-pollinated	8
Larix gmelinii var. *olgensis*	Open-pollinated	1
Larix kaempferi	Open-pollinated	66
Larix kaempferi	*Larix decidua*	8
Larix kaempferi	*Larix kaempferi*	126
Larix laricina	Open-pollinated	99
Larix laricina	*Larix decidua*	3
Larix occidentalis	Open-pollinated	2
Larix sibirica	Open-pollinated	11
Larix sibirica	*Larix decidua*	1
Larix sibirica	*Larix kaempferi*	2

Picea species comprise the majority of the seedlots. A total of 812 collections consisting of 414 controlled crosses, 347 open-pollinated, 50 self-pollinated, and 1 no pollen seedlots are stored. The majority of the seedlots consist of *Picea glauca* (136 open-pollinated; 278 controlled crosses; 9 self-pollinated) and *Picea mariana* (87 open-pollinated; 95 controlled crosses; 34 self-pollinated). A summary of the *Picea* seedlots from Dr. Fowler's work can be found in Table 11.

Table 11. Number of seedlots from open-pollinated, self-pollinated, and controlled crosses from various *Picea* species.

Species	Pollination/Crosses	Number seedlots
Picea abies	Open-pollinated	70
Picea abies	*Picea abies*	11
Picea abies	*Picea asperata*	1
Picea abies	*Picea mariana*	1
Picea abies	*Picea sitchensis*	1
Picea abies	No pollen	1
Picea bicolor	Open-pollinated	1
Picea breweriana	Open-pollinated	20
Picea engelmannii	Open-pollinated	1
Picea engelmannii	*Picea engelmanii*	2
Picea engelmannii	*Picea sitchensis*	2
Picea glauca	Open-pollinated	136
Picea glauca	*Picea glauca*	146
Picea glauca	*Picea engelmanii*	18
Picea glauca	*Picea jezoensis*	3
Picea glauca	*Picea orientalis*	1
Picea glauca	*Picea pungens* var.	1
Picea glauca	*Picea schrenkiana*	1
Picea glauca	*Picea sitchensis*	108
Picea glauca	Self-pollinated	9
Picea glehnii	Open-pollinated	2
Picea jezoensis	Open-pollinated	2

Table 11. (continued).

Species	Pollination/Crosses	Number seedlots
Picea koraiensis	Open-pollinated	1
Picea koyamai	Open-pollinated	3
Picea mariana	Open-pollinated	87
Picea mariana	*Picea mariana*	88
Picea mariana	*Picea omorika*	1
Picea mariana	*Picea rubens*	6
Picea mariana	Self-pollinated	34
Picea mariana x Picea omorika	*Picea mariana*	9
Picea mariana x Picea omorika	*Picea sitchensis*	1
Picea meyeri	Open-pollinated	1
Picea morrisonicola	Open-pollinated	1
Picea omorika	Open-pollinated	5
Picea omorika	*Picea mariana*	3
Picea pungens var. *glauca*	Open-pollinated	1
Picea purpurea	Open-pollinated	1
Picea rubens	Open-pollinated	6
Picea rubens	Self-pollinated	6
Picea rubens	*Picea rubens*	5
Picea rubens	*Picea abies*	2
Picea rubens x Picea mariana	Open-pollinated	1
Picea rubens x Picea mariana	*Picea mariana*	1
Picea rubens x Picea mariana	*Picea rubens*	1
Picea rubens x Picea mariana	Self-pollinated	1
Picea sitchensis	Open-pollinated	3
Picea smithiana	Open-pollinated	5
Picea wilsoni	Open-pollinated	1

Nineteen seedlots from nine *Pinus* species consisting entirely of open-pollinated seed were also stored (Table 12). Finally there were two seedlots each from *Abies* and *Tsuga* and one *Betula* seedlot. (Table 13).

Table 12. Number of seedlots from open-pollinated *Pinus* species.

Species	Pollination/Crosses	Number seedlots
Pinus banksiana	Open-pollination	1
Pinus densiflora	Open-pollination	1
Pinus nigra	Open-pollination	3
Pinus nigra var. *austriaca*	Open-pollination	1
Pinus nigra var. *banatica*	Open-pollination	1
Pinus peuce	Open-pollination	2
Pinus sibirica	Open-pollination	1
Pinus sylvestris	Open-pollination	8
Pinus tropicalis	Open-pollination	1

Table 13. Number of seedlots from open-pollinated *Abies*, *Betula*, and *Tsuga* species.

Species	Pollination/Crosses	Number seedlots
Abies balsamea	Open-pollination	1
Abies homolepis	Open-pollination	1
Betula x caerulea	Open-pollination	1
Tsuga mertensiana	Open-pollination	2

This is a valuable collection that represents a significant portion of Dr. Fowler's work. However, the quality of the seed is unknown as the seed have never been tested. As time permits these seedlots will be tested, and transferred to the Tree Breeding category of the NTSC collection.

Comparative Test of *Abies alba* seeds

The NTSC participated in a test conducted among International Seed Testing Association (ISTA) accredited and non-accredited laboratories comparing different methods of assessing viability in *Abies alba* (European silver fir) seeds using tetrazolium. The test was organized by the ISTA Forest Tree and Seed Committee and was coordinated by the Chair of the Committee, Dr. Zdenka Prochazkova of the Czech Republic. *Abies alba* seed for the test were provided by Fabio Gorian (ISTA accredited laboratory in Italy).

In the ISTA Rules Table 6A, Part II several methods of seed preparation and evaluation are given for many forest tree and shrub species. The purpose of the test that NTSC participated in was to compare two methods used for determination of *Abies* seed viability using tetrazolium: i) seed soaking before preparation, and ii) no seed soaking before preparation. A germination test was also conducted.

Each participating laboratory selected one of the following methods: a transversal cut along the length of the seed or longitudinal cut at both ends of the seed. Either treatment is performed prior to incubation in tetrazolium solution for 18 hours at 30°C with dry seeds and with seeds that were soaked for 18 hours in water at 20°C (Table 14). The seed coat is removed after incubation in tetrazolium solution to allow for assessment of the megagametophyte and embryo.

Table 14. Treatments used for tetrazolium viability test assessments.

No.	Pretreatment	Preparation before staining	Preparation for evaluation and tissue to be observed
1.	Soak seed 18 hr	Cut transversely at both ends to open embryo cavity	Cut longitudinally through endosperm and expose embryo; remove seed coat
2.	Soak seed 18 hr	Cut longitudinally beside embryo	Expose embryo; remove seed coat
3.	Prepare dry seeds	Cut transversely at both ends to open embryo cavity	Cut longitudinally through endosperm and expose embryo; remove seed coat
4.	Prepare dry seeds	Cut longitudinally beside embryo	Expose embryo; remove seed coat

The treatments chosen by the NTSC were 1 and 3. Dry seeds and seeds that had undergone an 18 hour soak in water at 20°C were cut transversely at both ends before incubation in tetrazolium solution for 18 hours at 30°C. Following incubation, the seed coat was removed and a longitudinal cut made through the endosperm to expose the embryo for assessment. The criteria are very straight

forward for assessment of the embryo and megagametophyte stating that only small superficial necrosis on the outer part of the megagametophyte which is not in connection with the embryo cavity is permitted (ISTA 2008). Based on the illustration in the ISTA Working Sheets on Tetrazolium testing for *Abies* seed (Figure 5) very little non-stained tissue is allowed (ISTA 2003).

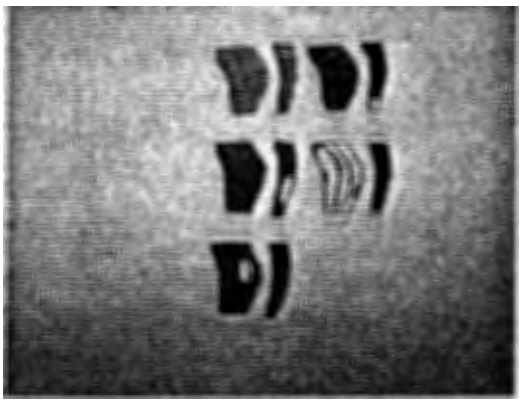

Figure 5. Illustration of non-viable seed taken from ISTA Working Sheets on Tetrazolium.

Staining was usually very good at the cut ends but failed to reach the inner portions of the megagametophyte and embryo (Figure 6). Consequently, parts of the embryo and megagametophyte did not stain. Therefore, based on the criteria, almost all of the seed were assessed as non-viable although we believed that were viable and would have stained if the megagametophyte and embryo had been in contact with the tetrazolium solution.

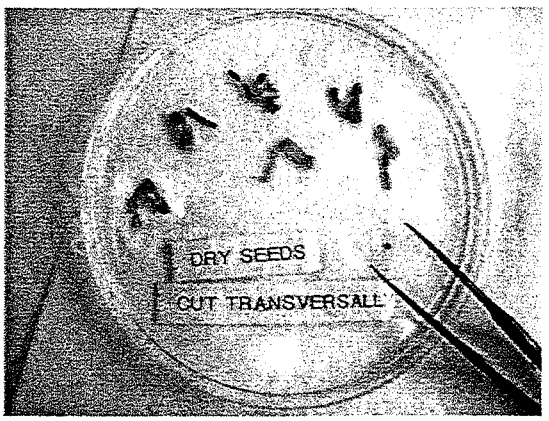

Figure 6. Dry seed cut transversely and incubated in tetrazolium solution for 18 hours at 30°C.

Four replicates of 100 seed each were used for germination testing of the seed. The seed were placed in Petawawa germination boxes on moistened Versa-Pak™ and stratified for 3 weeks at 3°C followed by 4 weeks in a germination cabinet set at 8 hours light at 30°C and 16 hours without light at 20°C. Results of the germination and tetrazolium tests are shown in Table 15.

Table 15. Comparison of germination test results of *Abies alba* seed with viability tests using tetrazolium solution with dry seed and seed soaked in water for 18 hours.

Treatment	Normal (%)	Abnormal (%)	Dead (%)	Empty (%)	Viable (%)	Non-viable (%)
Germination Test	43.50	12.50	17.75	26.25		
TZ 18 hr soak				22.00	0.00	78.00
TZ dry seeds				27.00	0.25	72.75

The results clearly show that the treatment using tetrazolium solution did not accurately assess the quality of the seed. As mentioned earlier, although the staining was not sufficient to assess some of the seeds as being viable it was felt that many were in fact viable and that the inability of the stain to penetrate the tissue was the likely cause of the results that were obtained.

Some seed remained after the completion of the test and a separate test was undertaken. One hundred seeds were soaked for 30 hours (water changed after 12 and 24 hours), the seed coat was removed and a longitudinal cut was made through the megagametophyte to open the embryonic cavity before placing the seed in tetrazolium solution for 18 hours at 30°C. The increased soak time made it easier to remove the seed coat. This modification to the process does not greatly increase the amount of time required because the seed coat has to be removed for assessment regardless of the treatment performed. The advantages are that the megagmetophyte and embryo are in direct contact with the tetrazolium solution and the embryo is not damaged by cutting through the seed. The staining of the megagametophyte and embryo using this method was much better (Figure 7) but some tissue still remained unstained. Although the embryonic cavity was opened to the tetrazolium solution, the radicle section of several embryos and the adjacent megagametophyte tissue did not stain. Upon observation, we noticed that the tissue that had not stained was covered by testa that had not been completely removed. This membrane may have prevented absorption of the tetrazolium.

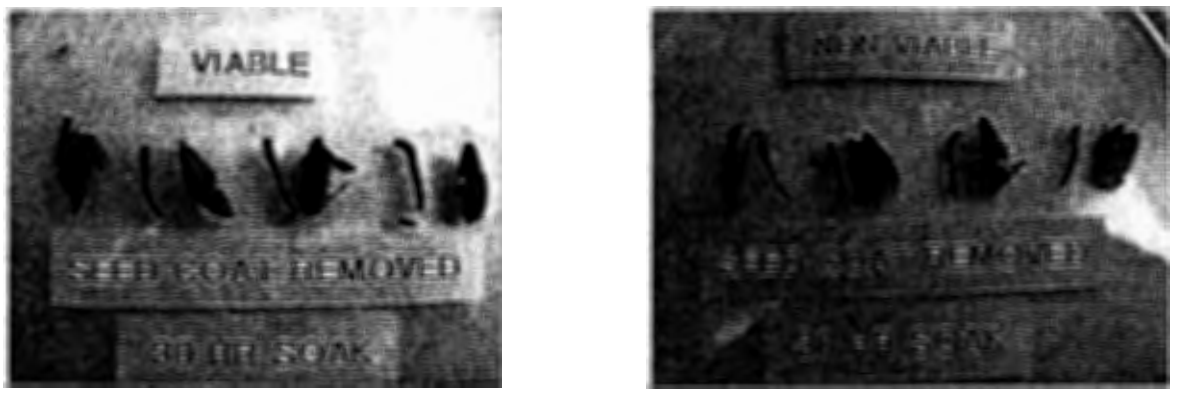

Figure 7. Viable (left) and non-viable (right) *Abies alba* seed soaked in water for 30 hours and with seed coat removed prior to incubation in tetrazolium solution for 18 hours.

Seed that were soaked for 30 hours in water and processed by cutting transversely at both ends did not stain well (Figure 8). The seed coat was not removed prior to incubation in tetrazolium solution for 18 hours at 30°C.

Figure 8. *Abies alba* seed soaked for 30 hours in water at 20°C, cut transversely at both ends, and incubated in tetrazolium solution for 18 hours at 30°C.

A final test was undertaken in which 100 seeds were soaked in water for 30 hours at 20°C and the seed coat removed. The embryo was removed prior to placing the seed in tetrazolium thus ensuring that the tissue adjacent to it would also be in contact with the tetrazolium solution. The excised embryo and megagametophyte for each seed was placed in individual cavities in tetrazolium solution before being incubated at 30°C. Table 16 shows the results for the various tetrazolium tests carried out on the *Abies alba* seed.

Table 16. Results of various treatments using tetrazolium solution to assess viability of *Abies alba* seed.

Treatment	Tetrazolium assessment (%)		
	Viable	Non-viable	Empty
no soak, cut transversely	0.25	72.75	27.00
18 hour soak, cut transversely	0.00	78.00	22.00
30 hour soak, megagametophyte cut open	33.00	45.00	22.00
30 hour soak, embryo excised from megagametophyte	45.00	33.00	22.00

Discussion

We found it very difficult to properly assess the seed using the methods recommended in the ISTA Rules. The main problem is that the tetrazolium was not able to penetrate sufficiently to stain the tissue that needed to be assessed. Furthermore, by cutting the seed transversely at both ends the tip of the radicle may be damaged. Any necrosis occurring at the radicle tip could be removed or be difficult to assess. Consequently, the results of the tests using the transversal cuts yielded only one viable seed. This seed was one of the smallest ones that was assessed and the stain was capable of penetrating sufficiently to fully stain the tissues.

The two other tests that were performed were designed to allow the tetrazolium access to the tissues without damaging the embryo. Removal of the seed coat prior to treatment increased the preparation time but this time is compensated for during assessment. The first treatment, where a longitudinal cut was made along the megagametophyte to open up the embryonic cavity greatly increased the amount of staining (embryo was not excised). However, some tissue did not stain and we believe that the presence of the testa, which was not completely removed, was the likely cause. We obtained 33% viable, 45% non-viable, and 22% empty seed using this method. We then undertook a follow-up treatment similar to the one described above where the testa was completely removed and the embryo excised. The embryo and megagametophyte from each seed were placed in separate wells before incubation in tetrazolium. Results from this treatment were 45% viable, 33% dead and 22% empty compared with the germination test which yielded 43.5% normal, 12.5% abnormal (mostly dead radicles with some low vigour), 17.75% dead, and 26.25% empty. Results from all testing conducted by the NTSC were forwarded to the ISTA Forest Tree and Seed Committee for evaluation.

Completely excising the embryo yielded the best results which were in agreement with the germination test. We feel that the procedure for preparing *Abies* seed for tetrazolium testing as described in the ISTA rules are not adequate and should be changed. Another series of laboratory tests is warranted to evaluate changes to the preparation procedures.

Literature Cited

ISTA 2003. ISTA Working Sheets on Tetrazolium Testing. Vol II. 1st Edition 2003. Int. Seed Test. Assoc., Basserdorf, Switzerland.

ISTA 2008. International Rules for Seed Testing. Edition 2008/1. Int. Seed Test. Assoc., Basserdorf, Switzerland.

After-Ripening of White Spruce Seed

Introduction

The range of white spruce (*Picea glauca)* extends from Newfoundland and Labrador west across Canada and can reach as far north as Hudson Bay, Yukon, and the Northwest Territories (Nienstaedt and Zasada 1990; Farrar 1995). Collection of white spruce cones occurs when it is determined that the seeds are mature, usually just before the cones open, and therefore the collection time is limited. After-ripening of early-collected immature cones could allow for a longer collection period allowing more freedom for the collectors.

Conifers, such as white spruce, have sporadic seed production with good seed years being inconsistent and varied (Edwards 1980). There are no set guidelines as to how to determine when the seeds are mature (as defined as cones beginning to open) and when they can be removed from the tree, as the surrounding environment plays a major factor in seed maturity (Edwards 1980). The seed of most of the forest trees in North America have some form of dormancy, either physiological or physical (Farmer 1997). Farmer (1997) defines dormancy as a mature seed state that "permits germination only under conditions that are favourable for growth" and that it "is released after time spent in a conditioning environment and/or by a special current environment." Seed of most boreal conifers, such as white spruce, will germinate after dispersal (in the fall) only when exposed to light and relatively high temperatures of 25–30°C (Farmer 1997). However, as these temperatures are not reached until the following summer it stands to reason that dormant seed exposed to moisture and temperatures below 5°C will gradually overcome dormancy and develop the capability to germinate (Farmer 1997).

After-ripening, also referred to as artificial ripening, has been defined as the "handling practices for cones collected immaturely" (Kolotelo et al 2001). The definition itself is contentious and hard to place as seen by the definition and warning put forth by Farmer (1997). He states that after-ripening is the release of dormancy after exposing the seed to a period of warm temperatures, but warns that the term has been improperly used in reference to other dormancy-releasing conditions. After-ripening has also been referred to as the exposure of seed to moist, cool conditions for a certain period of time but in this report, this process is referred to as chilling. Edwards (1980) points to three benefits of after-ripening: more flexibility of cone collecting operations, the extension of the collection period, and the use of immature cones from logging operations. There have been a number of experiments, with varying results, that have looked at the collection of white spruce cones over a period of weeks until the beginning of natural cone opening and the use of after-ripening in the hopes of increasing germination.

Edwards and Dobbs (unpublished; cited in Edwards 1980) used various storage conditions to ripen early-collected, immature white spruce cones. The storage conditions ranged from harsh (40°C oven for 2 weeks followed by refrigeration for 5 weeks) to mild (10°C and relative humidity of 60–75% for 7 weeks). Seed were also extracted immediately from the collected cones and tested. It was found that germination increased with collection date of the untreated cones. Cold (mild) storage

increased the germination of seed collected up to August 1 and chilling further increased germination. Collections from late August and early September had increased germination following cold storage, but not to the extent of the earlier collections. Edwards (1980) states that though the conditions for successful after-ripening are ill defined, air temperatures between 5–10°C with relative humidity of 65–75% and good air circulation around the cones have been successfully used.

Haddon and Winston (1981) made five collections of white spruce cones starting on July 11 and ending just before natural seed dispersal. Seeds extracted immediately after collection had no germination. They used two different storage conditions: a well-ventillated but unheated cone shed and cold storage at 5°C with relative humidity of 75–95%. Seed stored in the cone shed had poor germination until the final collection date with chilling increasing germination. They determined that white spruce cones "collected as early as August 1 and subjected to cold storage for twelve weeks produced seed with high germination and that early collection and after-ripening of cones was clearly found to be effective." Further testing of seeds from the cold storage treatment two years later resulted in 80% of the 20 seedlots germinating immediately without chilling, virtually as well as or better than the fresh test. Further analysis showed that chilled samples had less germination than those that were not chilled, but this could not be explained by the authors (Haddon and Winston 1980).

Another study by Caron et al. (1990) indicated that storage of cones in a well-ventilated building for six weeks promoted seed maturation before seed extraction. The average seed dormancy remained close to the same after 2 and 6 weeks of storage, but seeds from some trees became more dormant with storage whereas seeds from others became less dormant (Caron et al. 1990). The authors concluded "that a period of post-harvest ripening of seeds even in mature cones results in higher germination.

The above experiments indicate that after-ripening of early-collected immature seed is beneficial in improving germination and that after-ripening can possibly remove the need for chilling the seed prior to germination. The aim of this experiment was to test the impact of after-ripened white spruce cones on germination.

Methods

Cones from 15 white spruce trees were collected by NTSC staff on September 16, 2007 from St. Andrews, Newfoundland. The cones were put in paper bags and transported via truck to the lab. The cones from each tree were divided into two sub-samples and spread on screened trays. One sub-sample was placed in a cone storage shed (located in an unheated, well-ventilated building) while the other sub-sample was placed in a cooler at 3–5 °C with high relative humidity. After 30 days, the sub-samples which were in the cooler were moved to the cone storage shed.

Both sub-samples were removed from the shed in late March 2008 and brought into the lab. The samples were tumbled to dislodge the seed and the cones were then soaked in lukewarm water for

3–4 hours, the water was drained, and the cones then placed in an oven at 30°C to facilitate further opening of the cones. The cones were then re-tumbled to ensure complete removal of the seed.

The extracted seed were sieved to remove dry pitch, dry-rubbed in a cloth bag, and cleaned using air aspiration to remove the wings and light debris. The seed were wet-rubbed in a cloth bag to ensure complete removal of the wings and were placed on screened trays at room temperature to dry. Once the seeds were dry, alcohol separation was used to remove insect infested and empty seed. The seed were once again dried on screened trays and placed in glass jars for storage. Moisture content, thousand-seed weight, and germination tests of the seed were performed.

Germination of the seedlots (four replications of 50 seeds placed on moistened Versa-Pak™ in Petawawa germination boxes) was done in double replication with one germination box going directly into the germination cabinet (eight hours at 30°C and 16 hours at 20°C) for 21 days, while the other box was placed in the cooler (3–5°C) for 21 days and then placed in the germination cabinet for 21 days. Germination counts started on day seven and continued every second day until day 21.

Results and Discussion

All seedlots germinated, but germination percentage varied among seedlots and among treatments (Table 17). These treatments were: control/no chilling, control/chilling, after-ripening/no chilling, and after-ripening/chilling. Chilling had a beneficial effect on the control sample by increasing germination by almost 10%. Mean germination of after-ripened seed was about the same as for chilled control seed regardless of whether the after-ripened seed was chilled or not.

Seedlot 128 had albino germinants, which ranged from one to seven per box. This seedlot was the only one that had albino germinants and these germinants were present in all treatments. It was observed that germinants were initially pink in color and progressed to pure white when fully germinated (Figure 9).

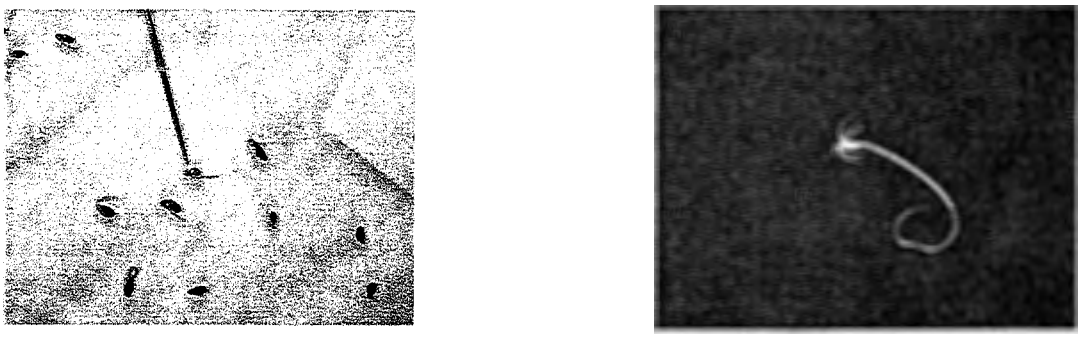

Figure 9. Pink albino germinant at initial germination (left) and fully germinated albino (right).

Table17. Germination (%) of 15 white spruce seedlots with and without after-ripening and with and without chilling.

Seedlot	Control		After-ripened	
	No chilling	Chilling	No chilling	Chilling
122	77.0	95.0	90.0	96.0
123	49.0	59.5	77.0	83.0
124	34.0	65.5	33.0	39.5
125	75.0	80.5	76.5	67.0
126	32.5	50.5	66.5	61.0
127	19.0	25.0	42.5	50.0
128	72.0	71.0	89.5	90.5
129	36.5	24.0	13.5	10.5
130	50.5	67.5	66.5	70.0
132	56.5	59.5	73.5	68.5
133	31.5	65.5	60.0	41.0
134	32.5	31.0	17.5	5.5
135	38.0	45.0	56.5	64.0
136	51.5	50.0	41.0	16.5
137	46.5	57.0	56.0	54.5
Mean	46.8	56.4	57.3	54.5

Mean germination over time of the four treatments was analyzed (Figure 10) and two important outcomes were observed. The first is that treatments with chilling had their peak germination on day 11 while treatments with no chilling reached this peak at day 13. The other outcome was that germination declined more slowly for seeds that were not chilled and more germinants were present on the last day of counts. Unchilled seedlots also had a higher percentage of low vigour germinants.

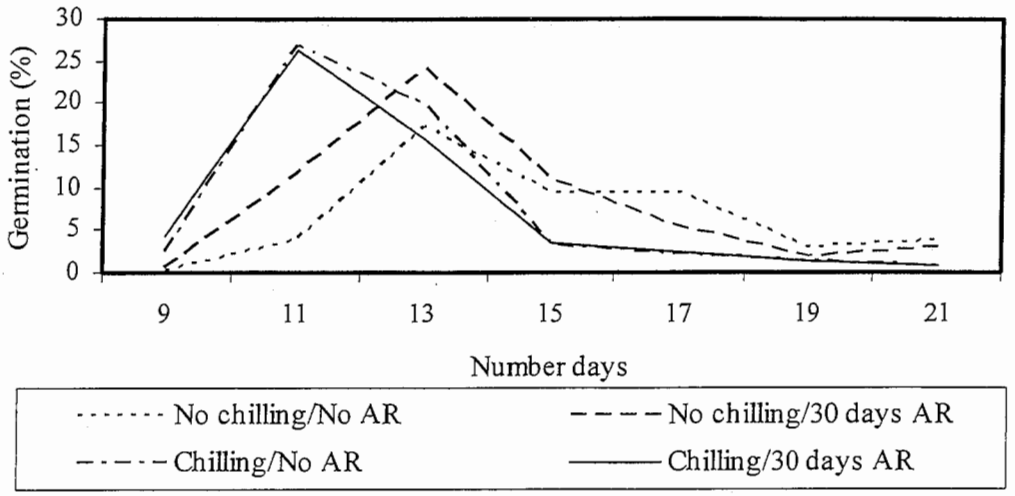

Figure 10. Mean germination over time of white spruce seed with and without chilling and with and without 30 days after-ripening (AR).

Seedlots subjected to after-ripening/chilling treatment had higher germination than their after-ripening/no chilling counterparts, 57.37% to 54.5% respectively (Figure 11). As expected, the control seedlots that were chilled had a higher germination than the control/no chilling treatment, 56.64% to 46.71% respectively. Chilling also increased germination speed.

The after-ripening/no chilling seedlots had a higher overall germination compared to the other three treatments, but seedlots that were not chilled had a later peak in germination and a higher percentage of low vigour germinants compared to chilled seedlots. The after-ripened/chilled seedlots had the highest proportion of abnormal germinants of all four treatments. One possible reason for this is that after-ripening followed by chilling damaged the seeds.

These results agree with those found in earlier research including the findings of Haddon and Winston (1980) where it was also noticed that cold storage treated seed germinated better when not chilled. Dormancy is possibly alleviated during the time the seed are exposed to cool temperatures during after-ripening (Winston and Haddon 1981).

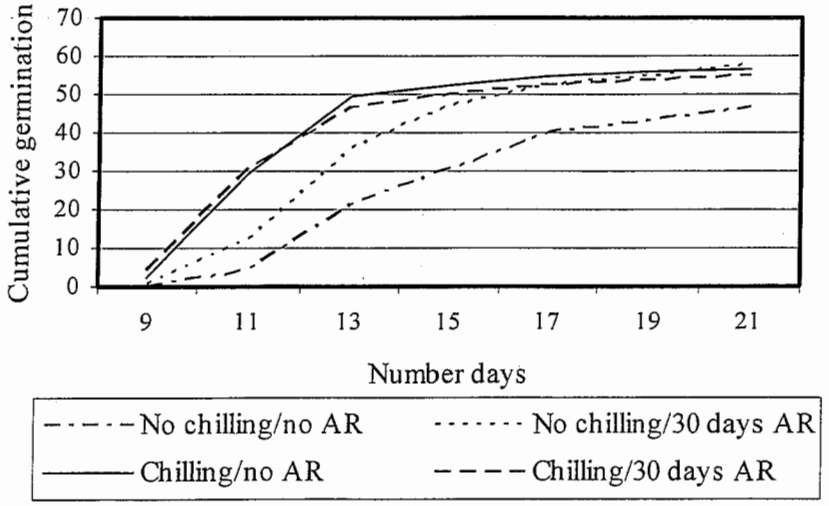

Figure 11. Mean cumulative germination over time of white spruce seed with and without chilling and with and without 30 days after-ripening (AR).

Conclusion

The results agree with previous experiments that examined artificial ripening of white spruce seed in that cones in a cold, moist environment, such as a walk-in cooler at 5°C with high relative humidity, has a beneficial effect on seed germination. This experiment concurs with the results of Haddon and Winston (1980) that chilling of after-ripened seed can be harmful.

There are a number of issues that came forth while analyzing these data. The first issue is that of the collection date. Most of the research done on after-ripening looks at the early collection of immature cones and the maturation of the seed after collection. The cones and subsequent seed used in this experiment were collected in mid-September around natural cone opening indicating the possibility that the seed had already reached maturity and were not in need of further treatment. This could explain the small difference in germination between the control/chilled treatment and the after-ripened treatments.

Another issue is whether the design of the experiment was rigid enough. There was no initial extraction of seeds when the cones were collected and before treatments were done. Though it was thought that placing the cones in a cone shed would have no major effect on the seed, this should not be assumed as these seeds were subjected to freezing temperatures during the winter. Placing cones that were after-ripened in a cone shed following their initial chilling could have also affected the seeds.

It is suggested that further research on after-ripening white spruce seeds should be undertaken. There should be a number of trees where cones are harvested over a period of time starting mid-July until natural cone opening. The cones collected should be divided into 3 sub-samples, those whose seeds

are extracted immediately and germinated, those that are placed in the cone shed, and those that are placed in cold storage for a set period of time. Instead of placing the cones subjected to cold storage into the cone shed, the seed should be extracted and analyzed immediately. This will remove any questions of whether the cone shed has an effect on the cold treated seed.

This experiment found similar results to those previously reported showing that after-ripening seed increases germination and that chilling of after-ripened seed has a detrimental effect on germination.

Literature Cited

Caron, G.E.; Wang, B.S.P.; Schooley, H.O. 1990. Effect of tree spacing, cone storage and prechilling on germination of *Picea glauca* seed. For. Chron. 66(4):388–392.

Edwards, D.G.W. 1980. Maturity and quality of tree seeds — a state-of-the-art review. Seed Sci. Technol. 8:625–657.

Farmer, R.E. 1997. Seed Ecophysiology of Temperate and Boral Forest Trees. St. Lucie Press, Delray Beach, Florida.

Farrar, J.L. 1995. Trees in Canada. Fitzheny & Whiteside Ltd., Markham, ON. 102p

Haddon, B.D.; Winston, D.A. 1980. Germination after two years storage of artificially ripened white spruce seed. *In* B.S.P Wang and J.A. Pitel, eds. Proc. Int. Symp. on Forest Tree Seed Storage. Environ. Can., Can. For. Serv., Petawawa Nat. For. Inst.

Kolotelo, D.; Van Steenis, E.; Peterson, M.; Bennett, R.; Trotter, D.; Dennis, J. 2001. Seed handling guidebook. BC Min. For., Tree Imp. Br. 36p

Nienstaedt, H.; Zasada, J.C. 1990. *Picea glauca. In* R.M. Burns and B.H. Honkala, eds. Silvics of North America: Volume 1, Conifers. . USDA For. Serv., Agric. Hdbk 658.

Winston, D.A.; Haddon, B.D. 1981. Effects of early cone collection and artificial ripening on white spruce and red pine germination. Can. J. For. Res. 11:817–826.

SEED CERTIFICATION

Since 1970, Canada has been applying the OECD (Organization for Economic Cooperation and Development) tree seed certification scheme. The CFS was nominated by the Government of Canada as the Designated Authority to implement the Scheme. All seed certification has been conducted by the Pacific Forestry Centre in response to demand, primarily by European seed dealers, for seed from west coast tree species. Practically all seed is certified in the Source-identified category.

Demand for certified seed, which was high in the 1970's and 1980's, has declined the past 15 years (Figure 12). However, a total of 571 kg of certified seed was exported in 2008, which was 90 kg less than that of 2007. There continues to be a demand from Sweden for lodgepole pine (*Pinus contorta* var. *latifolia*) seed from Yukon Territory resulting in 377 kg exported. Other significant species include 88 kg of grand fir (*Abies grandis*) and 104 kg of Douglas-fir (*Pseudotsuga menziesii*). The European Union (EU) implemented a revised certification Directive on January 1, 2003. There had been concern about equivalence between this directive and the OECD Scheme. Fortunately, the EU has granted equivalence to Canada for *Abies grandis*, *Picea sitchensis*, *Pinus contorta*, and *Pseudotsuga menziesii* seed to be imported as source-identified.

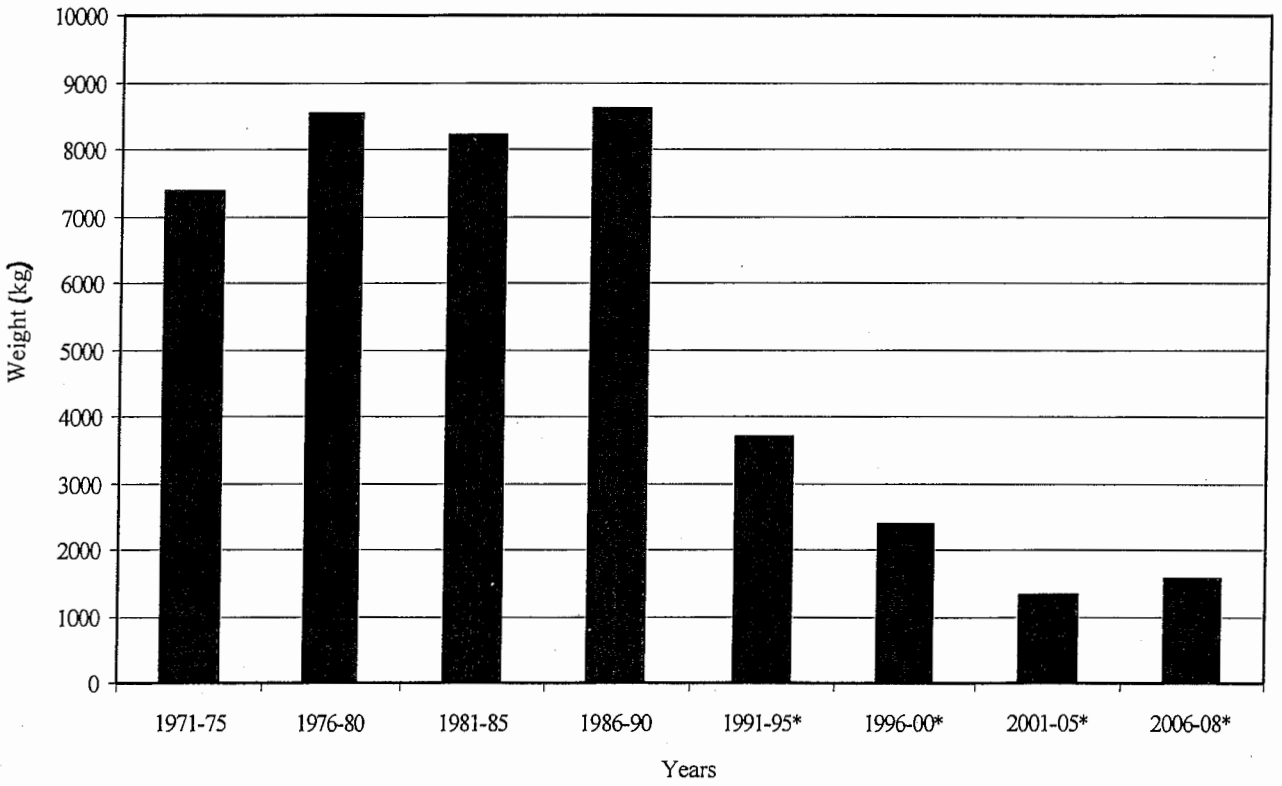

Figure 12. Weight of seed OECD certified or exported* by 5-year periods.

Officially established in 1967, the OECD Scheme for the Control of Forest Reproductive Material Moving in International Trade developed rules and procedures that were adopted in 1974. From its early implementation by a limited number of countries for Douglas-fir seed exported from North-America to Europe, the scope of the Scheme was progressively enlarged over time to attract new participants and to deal with many forest tree species. Three countries were granted approval in 2008 to join the Scheme: Burkina Faso, Croatia, and Serbia. The Scheme includes 25 participating countries working with more than 250 different species.

In the early 1990s, it became apparent that the 1974 Scheme required revision because of changes in forest management in addition to wood production (environmental and social aspects, biodiversity conservation, etc.) and the growing importance on the market of new types of reproductive material derived from forest tree breeding programs. This resulted in the formation of a Group of Experts that worked between 1992 and 1996 to prepare a comprehensive proposal.

A revised Scheme was adopted on 20 June 2007. The Scheme includes only the "*Source-identified*" and "*Selected*" categories as these immediately benefit all stakeholders, including new applicant countries that are strengthening their domestic control systems for forest reproductive material. As a result of these updated categories it is hoped that the Scheme will be an incentive for other countries to join. The two "advanced" categories of forest reproductive material, "*Qualified*" and "*Tested*", will be incorporated at a later time. Work has begun on developing text for the "*Qualified*" category which includes seed produced in seed orchards.

PRESENTATIONS / PUBLICATIONS

Loo, J.; Beardmore, T.; Simpson, D. 2008. Coordinating gene conservation for climate change action plans: a role for CONFORGEN. *In* S.J. Colombo, B. Boysen, K. Brosemer, A. Foley and A. Obenchain. Managing tree seed in an uncertain climate: conference summary. Ontario Min. Nat. Res., Climate Change Res. Inform. Note No. 8, 8 p.

Simpson, Dale. 2008. Conservation of genetic diversity by tree seed centres. Presented at CTIA Tree Seed Working Group Workshop, IUFRO-CTIA 2008 Joint Conference, Quebec, Quebec, 25-28 Aug 2008.

Simpson, Dale; Daigle, Bernard. 2008. Ash seed collections for genetic conservation. Pages 15–17. *in* Can. For. Gen. Assoc. Tree Seed Work. Group, News Bull. No 48.

SEED CENTRE STAFF

The amount of work carried out by the Seed Centre during 2008 was increased through "extra" work weeks that were funded through the Federal Public Sector Youth Internship Program. This program covers the cost of wages.

Hendrikje (Erica) Zwaneveld was hired through the Federal Public Sector Youth Internship Program. Her term started on February 4 and ended August 1, 2008. In addition to assisting with seed collection, processing, and testing Erica also conducted an experiment and produced a report on after-ripening of white spruce seed which is included in the Research and Development section of this report.

Erica provided an "extra" 26 weeks of work to the Seed Centre. The number of "extra" weeks worked in 2008 is below the 10-year average of 34 extra weeks (Figure 13).

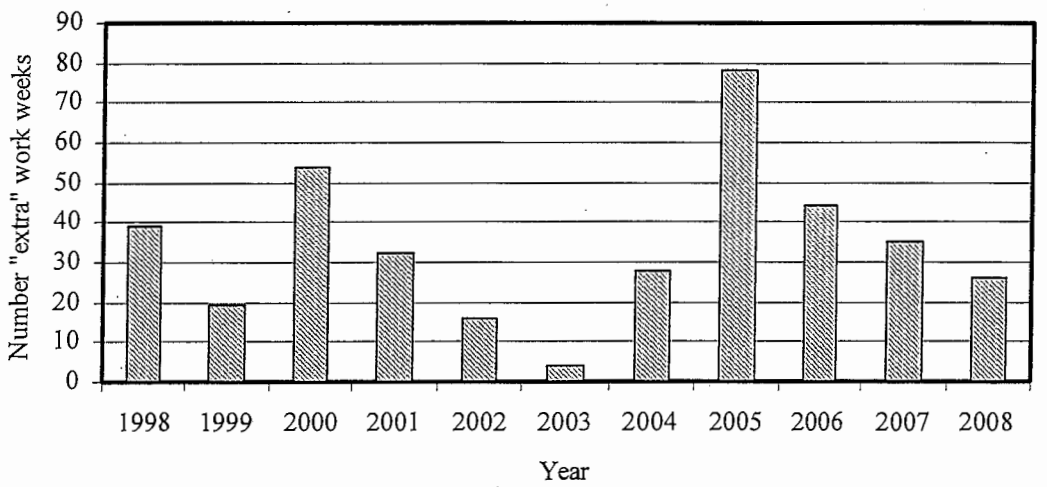

Figure 13. Number of "extra" work weeks provided to the NTSC between 1998 and 2008.