National Tree Seed Centre

Annual Report

2012

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National Tree Seed Centre
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NATIONAL TREE SEED CENTRE ANNUAL REPORT 2012

EXECUTIVE SUMMARY

The number of seedlots in storage increased to 13,593. Of this number, almost 6,700 seedlots are stored under Seed Bank and are available for research, and almost 4,800 seedlots are stored for genetic conservation.

One collection was made by National Tree Seed Centre staff. However, 142 seedlots were donated by the Ontario Ministry of Natural Resources' Tree Seed Plant. As well, the USDA Centre for Genetic Resources Preservation donated 94 ash seedlots for backup conservation storage and Parks Canada contributed 22 *Pinus albicaulis* seedlots for conservation storage.

A total of 37 requests for seed resulted in 310 seedlots provided for research. The majority of the requests were from Canada (25 requests; 134 seedlots) but seed was also sent to China (1 request; 5 seedlots), France (1 request; 9 seedlots), Germany (1 request; 23 seedlots), Great Britain (1 request; 91 seedlots), Slovakia (1 request; 9 seedlots), Spain (1 request; 6 seedlots), Sweden (1 request; 26 seedlots), and United States (4 requests; 6 seedlots).

Seed testing consisted of 435 germination tests, 279 moisture content tests, and 162 thousand-seed weight tests. A significant proportion of the germination testing was re-testing of seedlots tested 10 years ago which provides an up-to-date assessment of seed quality. The data are also used to evaluate long-term storage potential.

Other activities during the year included:

- Re-design of the Seed Centre's web site to make it more efficient and easier to navigate. A significant addition was the functionality to order seed.

- Thirteen-year results from a willow storage experiment demonstrated that *Salix bebbiana*, *S. discolor*, and *S. eriocephala* seed stored best at -145 °C in the vapor phase of liquid nitrogen.

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INTRODUCTION

In the 1960s, tree genetics and breeding research was expanding across Canada. There was increasing demand for small quantities of seed of known origin and quality for provenance testing. In response to this need, the Canadian Forest Service established the National Tree Seed Centre (NTSC) in 1967 at the Petawawa Research Forest (formerly Petawawa Forest Experiment Station), Chalk River, Ontario. At that time, reforestation programs across Canada were being initiated or were expanding and there was a need for large quantities of seed as well as knowledge on how to collect, process, test, and store tree seed. Germination testing protocols existed for the major conifer reforestation species, but in some cases, fine tuning was required. As well, the NTSC played an active role in acquiring and disseminating seed of native and non-native species to researchers to establish provenance trials and other genetic tests. A significant accomplishment of the NTSC was the development of the Petawawa Germination Box. This was in response to the need for a container of appropriate size to permit maximum use of germinator space, allow for full development of germinants, and maintain uniform moisture levels in the germination medium.

Following a review of the research program within the Canadian Forest Service, the NTSC was transferred to the Atlantic Forestry Centre in Fredericton, N.B. in 1996. The mission of the NTSC is to safeguard Canada's forest genetic resources in the face of climate change and other threats by acquiring, evaluating, preserving, and providing a national collection of forest genetic resources to assist in securing the forest biological diversity that underpins the sustainable development of Canada's forests.

This report covers the activities of the NTSC for 2012. Similar reports were prepared from 1998–2011. 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.

INVENTORY STATUS

Seed is stored in four categories: Seed Bank, Genetic Conservation, Reserved, and Tree Breeding (Table 1). The total number of seedlots increased by 207 to 13,593 in 2012. The numbers in brackets in Table 1 represent the numbers reported in the 2011 Annual Report.

Table 1. Seed stored at the NTSC as of December 31, 2012.

Seed Bank		Genetic Conservation		Reserved		Tree Breeding	
No. species	No. seedlots	No. species	No. seedlots	No. species	No. seedlots	No. species	No. seedlots
148 (150)	6,689 (6,661)	50 (49)	4,788 (4,610)	37 (37)	1,726 (1,725)	10 (10)	390 (390)

Seed Bank seedlots are the active collection that are available for distribution for research. Since 1998, the number of seedlots in the Seed Bank collection has increased from 3,079 to 6,689 (Figure 1). This number includes seedlots from native and non-native species. 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 2012, 133 seedlots were discarded and 8 seedlots were depleted.

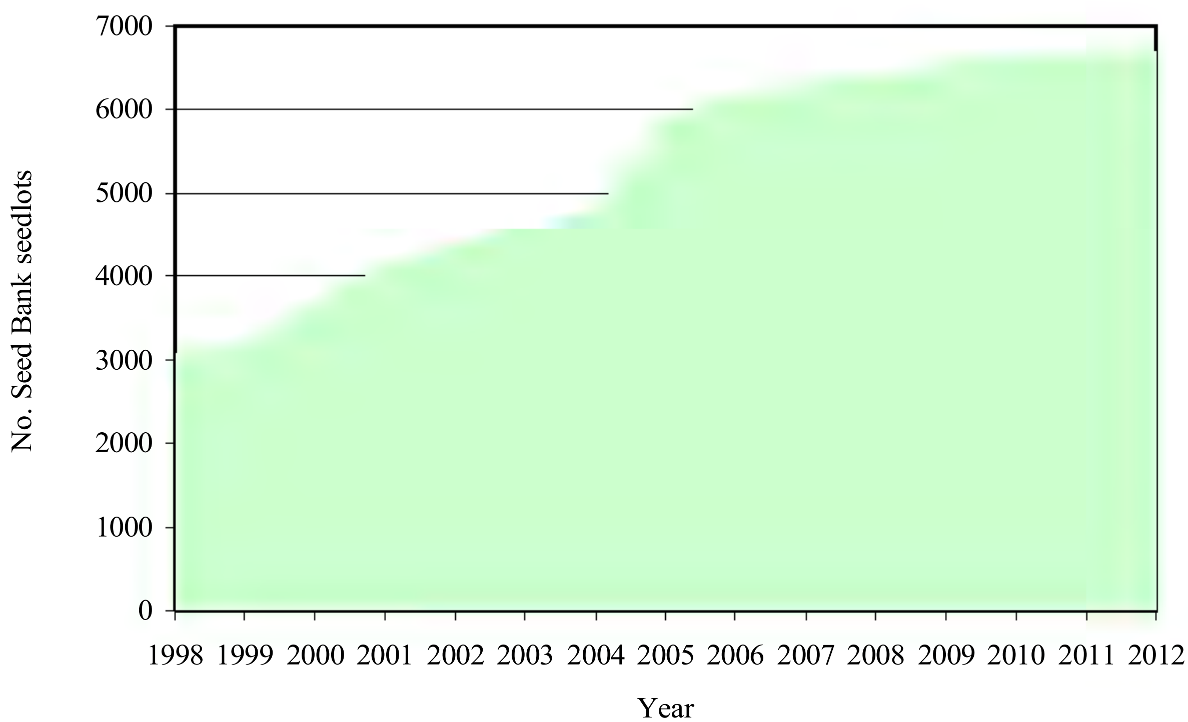

Figure 1. Increase in number of Seed Bank seedlots stored at the NTSC since 1998.

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, 2012, the NTSC Seed Bank had 6,364 seedlots of 105 species in storage from locations in Canada (Table 2). Seed from 43 non-native species as well as native species from the United States (325 seedlots) are also stored. Seed is also stored from non-native species growing in Canada, but most of the seedlots are from species native to other countries. The proportion of seed from non-native species continues to decrease as seedlots become exhausted due to client requests and no effort is made to replace them.

Table 2. Number of native species, number of seedlots, and percentage by province or territory of seedlots stored in the Seed Bank category.

Province	No. species	No. seedlots	Percent
Alberta	12	43	0.7
British Columbia	32	302	4.7
Manitoba	7	65	1.0
New Brunswick	68	1,507	23.7
Newfoundland and Labrador	17	169	2.6
Nova Scotia	39	551	8.7
Ontario	49	2,290	36.0
Prince Edward Island	32	254	4.0
Quebec	26	1,011	15.9
Saskatchewan	10	121	1.9
Yukon Territory	3	51	0.8
Total	105	6,364	100

Since the NTSC moved to Fredericton, staff have concentrated their efforts acquiring collections from New Brunswick, Nova Scotia, and Prince Edward Island. Travel beyond the Maritime provinces is challenging due to limited resources (staff and budget). There is an ongoing effort to acquire seed from other provinces and seed centres when opportunities arise. Since collections by NTSC staff are unlikely due to distance and costs, these seedlots are purchased or obtained through donation.

The Genetic Conservation category was initiated in 2000 using seed already in storage. Its purpose is to conserve the genetic variation occurring in natural populations as well as to ensure that genetic material from rare, endangered, and/or unique populations is preserved. Over the past seven years, seed collecting has focused on expanding the genetic conservation collection. Any surplus seed from these collections is placed in Seed Bank. The collection increased by 178 to 4,788 seedlots primarily due to two important donations. The USDA National Centre for Genetic Resources Preservation, Fort Collins, CO sent 94 ash seedlots for back-up storage as per a storage agreement between the USDA and the NTSC. The NTSC sent 105 ash samples in 2010 for back-up storage at the USDA

National Centre. The second donation was 22 seedlots of *Pinus albicaulis* from three National Parks located in Alberta and British Columbia. The Seed Centre is providing back-up storage of germplasm from this nationally listed endangered species. Figure 2 shows the increase in the number of seedlots in this category since 2000. There is seed from 50 species in Genetic Conservation with the number of seedlots ranging from 1 to 1,641 (Table 3).

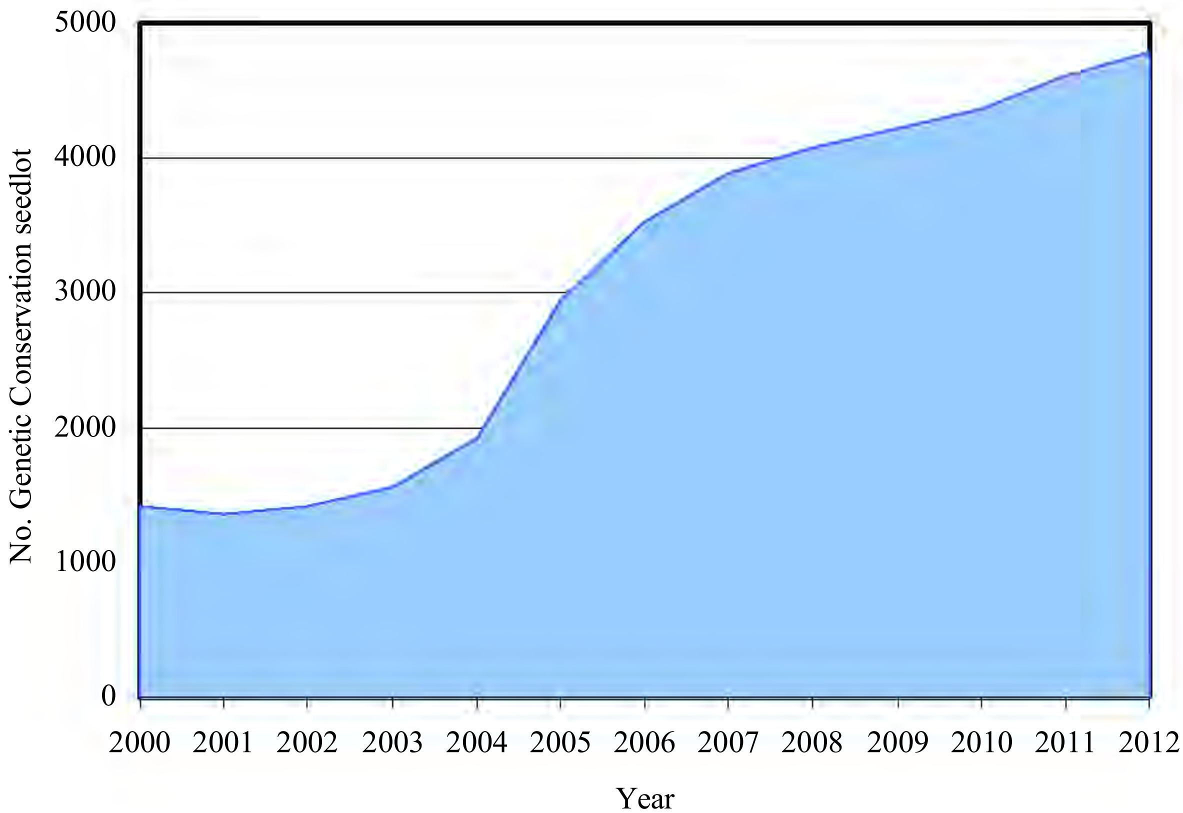

Figure 2. Increase in the number of Genetic Conservation seedlots stored at the NTSC since 2000.

The Reserved category contains seedlots that have been reserved by researchers. Many of these seedlots were collected for special projects.

The Tree Breeding category consists of seedlots that originated from the genetics program at the Petawawa Research Forest and were transferred to the NTSC for storage. There was no change in this category.

Table 3. Species and number of seedlots stored in Genetic Conservation.

Species	No. seedlots	Species	No. seedlots
Abies balsamea	8	*Picea glauca*	1,641
Acer negundo	15	*Picea glauca* var. *albertiana*	9
Acer pensylvanicum	17	*Picea glauca* ssp. *porsildii*	15
Acer rubrum	111	*Picea mariana*	433
Acer saccharum	23	*Picea rubens*	222
Acer spicatum	49	*Pinus albicaulis*	22
Alnus incana spp. *rugosa*	3	*Pinus banksiana*	95
Alnus incana spp.	1	*Pinus contorta* var. *latifolia*	2
Alnus serrulata	2	*Pinus flexilis*	101
Alnus viridis spp. *crispa*	9	*Pinus pinceana*	181
Betula alleghaniensis	57	*Pinus ponderosa*	2
Betula cordifolia	5	*Pinus resinosa*	15
Betula minor	1	*Pinus rigida*	4
Betula papyrifera	10	*Pinus strobus*	52
Betula populifolia	20	*Pinus sylvestris*	12
Betula spp.	1	*Populus balsamifera*	20
Cephalanthus occidentalis	1	*Populus grandidentata*	13
Cornus florida	4	*Populus tremuloides*	16
Fraxinus americana	247	*Prunus pensylvanica*	61
Fraxinus nigra	183	*Prunus virginiana* var. *virginiana*	337
Fraxinus pennsylvanica	222	*Salix lantana* spp. *richardsonii*	1
Fraxinus profunda	1	*Thuja occidentalis*	89
Fraxinus quadrangulata	1	*Thuja plicata*	2
Larix laricina	267	*Tsuga canadensis*	183
Larix occidentalis	1	*Tsuga mertensiana*	1

SEED COLLECTIONS

Seed production was poor to non-existent for most species in the Maritimes. In order to ensure good quality seed, seed is only collected during good seed years. Seed collected in good seed years is of better genetic quality because of ample pollen production and higher physiological quality due to trees allocating significantly more resources to the developing seed crop. Also, less time is required to collect sufficient seed when there is a good seed crop. Only one collection was made that being *Acer saccharinum* (Table 4). These seed were collected primarily for a researcher investigating physiological aspects of storage for species producing recalcitrant seed.

Table 4. Seed collections made by Seed Centre staff in 2012.

Species	Total
Acer saccharinum	1

The NTSC also acquired seed via donation. One hundred and forty-two seedlots from 22 species were provided by the Ontario Tree Seed Plant: 1 *Betula alleghaniensis*, 1 *Cephalanthos occidentalis*, 1 *Cornus sericea* spp. *sericea*, 1 *Fraxinus americana*, 1 *F. pennsylvanica*, 1 *Larix laricina*, 27 *Picea glauca*, 4 *P. mariana*, 2 *P. rubens*, 17 *Pinus banksiana*, 20 *P. resinosa*, 28 *P. strobus*, 3 *Prunus serotina*, 1 *Rhus typhina*, 2 *Sambucus racemosa* var. *racemosa*, 1 *Sorbus americana*, 1 *S. decora*, 12 *Thuja occidentalis*, 6 *Tsuga canadensis*, 3 *Viburnum lentago*, 2 *V. nudum* var. *cassinoides*, and 4 *V. opulus* var. *americanum*. Cooperation from agencies and other seed centres by donating seed to the Seed Centre is greatly appreciated.

Donations received from the USDA Centre of Germplasm Preservation and Parks Canada for genetic conservation were discussed previously.

Table 5 shows the number of seedlots acquired by the NTSC since 1996. About 54% of the seedlots were obtained through collection and a substantial number, 40%, were donated. The remaining 6% were purchased.

Table 5. Number of seedlots acquired by the NTSC through collection, donation, and purchase between 1996 and 2012.

Year	Number of seedlots			
	Collection	Donation	Purchase	Total
1996	239	22	0	261
1997	75	245	0	320
1998	284	47	9	340
1999	139	80	0	219
2000	195	673	0	868
2001	137	122	45	304
2002	367	36	0	403
2003	69	142	0	211
2004	549	381	137	1,067
2005	142	29	3	184
2006	329	42	30	401
2007	190	181	0	371
2008	160	3	0	163
2009	137	75	30	242
2010	37	0	2	39
2011	104	13	57	170
2012	1	258	0	259
Total	3,154	2,349	313	5,816

SEED REQUESTS

The Seed Centre's policy is to provide seed, at no cost, for scientific research. Seed is also provided 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 for screening requests. Seed requests received from international clients are referred to the Canadian Food Inspection Agency to determine if an import permit is required and for the issue of Phytosanitary Certificates.

During 2012, 37 requests representing 310 seedlots were processed. The majority of the requests were from Canada but seed was also sent to China, France, Germany, Great Britain, Slovakia, South Africa, Spain, Sweden, and United States (Table 6). 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 have received about 70% of the seed while international clients accounted for the remaining 30%.

Table 6. Number of requests and number of seedlots shipped by country in 2012.

Country	No. requests	No. seedlots
Canada	25	134
China	1	5
France	1	9
Germany	1	23
Great Britain	1	91
Slovakia	1	9
South Africa	1	1
Spain	1	6
Sweden	1	26
United States	4	6
Total	37	310

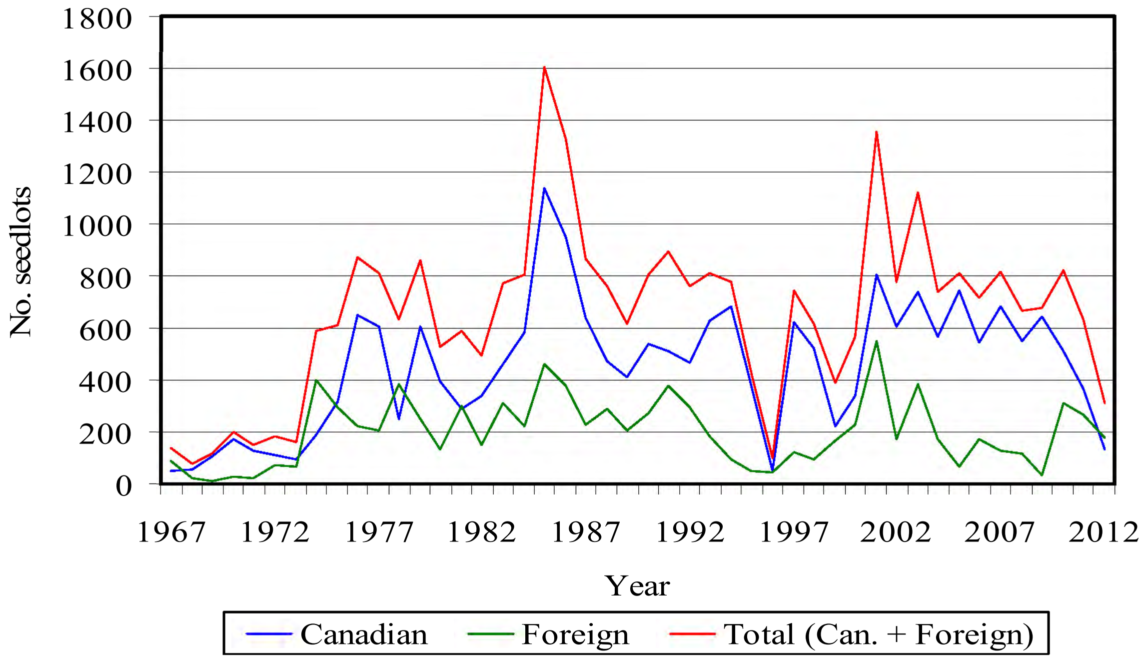

Figure 3. Number of seedlots sent to clients between 1967 and 2012.

SEED TESTING

Germination tests are performed on all seedlots prior to storage as well as seedlots in storage. 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. **Four hundred and thirty-five germination tests** were carried out. Newly acquired seedlots are tested before being placed in storage. Seedlots in storage are tested every 10 years.

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. The reduction in the number of tests between 1994 and 1996 coincided with the transfer of the NTSC from Petawawa to Fredericton.

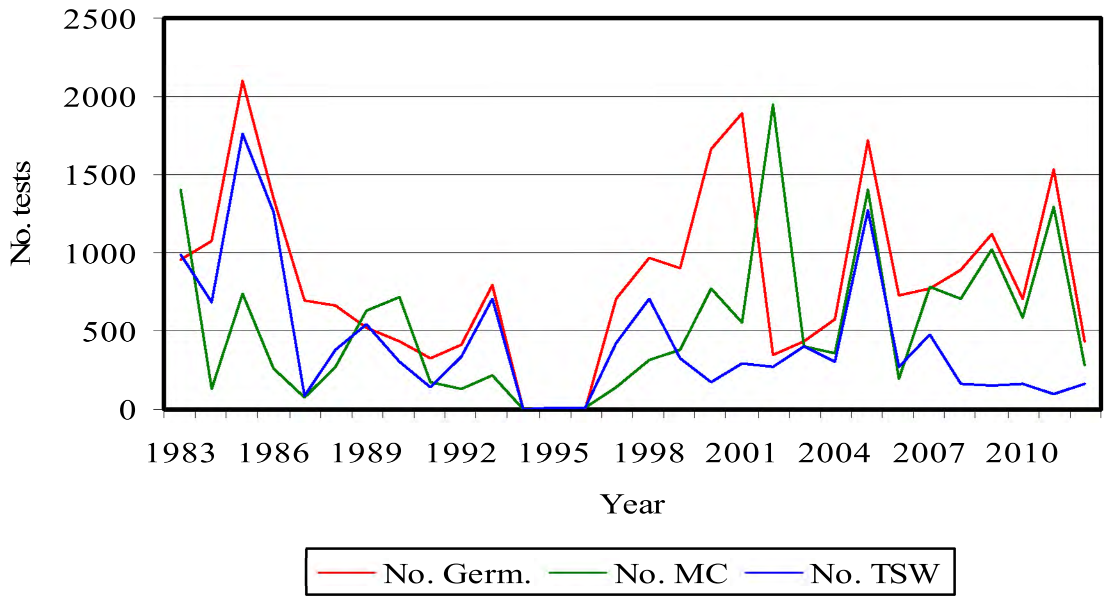

Figure 4. Number of germination tests (No. Germ.), moisture content tests (No. MC), and thousand-seed weight tests (No. TSW) carried out by the NTSC since 1983.

The target moisture content (MC) for orthodox seed is between 5 and 8%. Seed that are above this range are dried before being stored. **Two hundred and seventy-nine moisture content** determinations were carried out. MC is often checked when seed are re-tested particularly those seedlots approaching 8%. If MC exceeds 8% the seed are conditioned to lower their MC. Forty-eight 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 (alders, 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 and sixty-two 1000-seed weights** was done.

SEED STORAGE AGREEMENT WITH PARKS CANADA

Two native pine species, whitebark pine (*Pinus albicaulis*) and limber pine (*Pinus flexilis*), are found at mid to high elevations in the Rocky Mountains. These species are threatened by climate change, Mountain pine beetle (*Dendroctonus ponderosae*), white pine blister rust (*Cronartium ribicola*), and changing land management practices including fire suppression. Loss of these species will impact bears and in particular Clark's nutcracker (*Nucifraga columbiana*) because the seed are a valuable source of nutrition. *Pinus albicaulis* is listed as threatened in Alberta and nationally under the Species at Risk Act.

These species, particularly whitebark pine, are found in most of the National Parks located in the Rocky Mountains. Parks Canada staff are making seed collections in order to provide material for research projects such as genetic screening for blister rust resistance and to preserve the genepool by planting seedlings. However, there are no facilities for storing excess seed. Parks Canada staff were approached in 2011 with the suggestion that the NTSC could store this seed. This resulted in the creation of a seed storage agreement in 2012 involving seven National Parks: Banff, Glacier, Jasper, Kootenay, Mount Revelstoke, Yoho, and Waterton Lakes.

Twenty-two seedlots of *Pinus albicaulis* were sent to the NTSC in 2012 where passport information was entered in the database prior to storage. Basic data such as moisture content and thousand-seed weight were collected and germination tests were set up. Seed are stored and will be returned to Parks Canada upon request.

NTSC WEB SITE RE-DESIGNED

In October 2011 the NTSC's web site was identified as requiring an update in order to comply with the federal government's Web Content Accessibility Guidelines. This resulted in a complete re-design of the site as well as re-writing the content. This resulted in a more streamlined and functional web site that has several important functions added: the ability to order seed online and the capability to view the Seed Centre's database for each species in order to obtain up-to-date information about each seedlot. The new site went online in late February 2012 at this URL http://cfs.nrcan.gc.ca/pages/331?lang=en_CA (Figure 5).

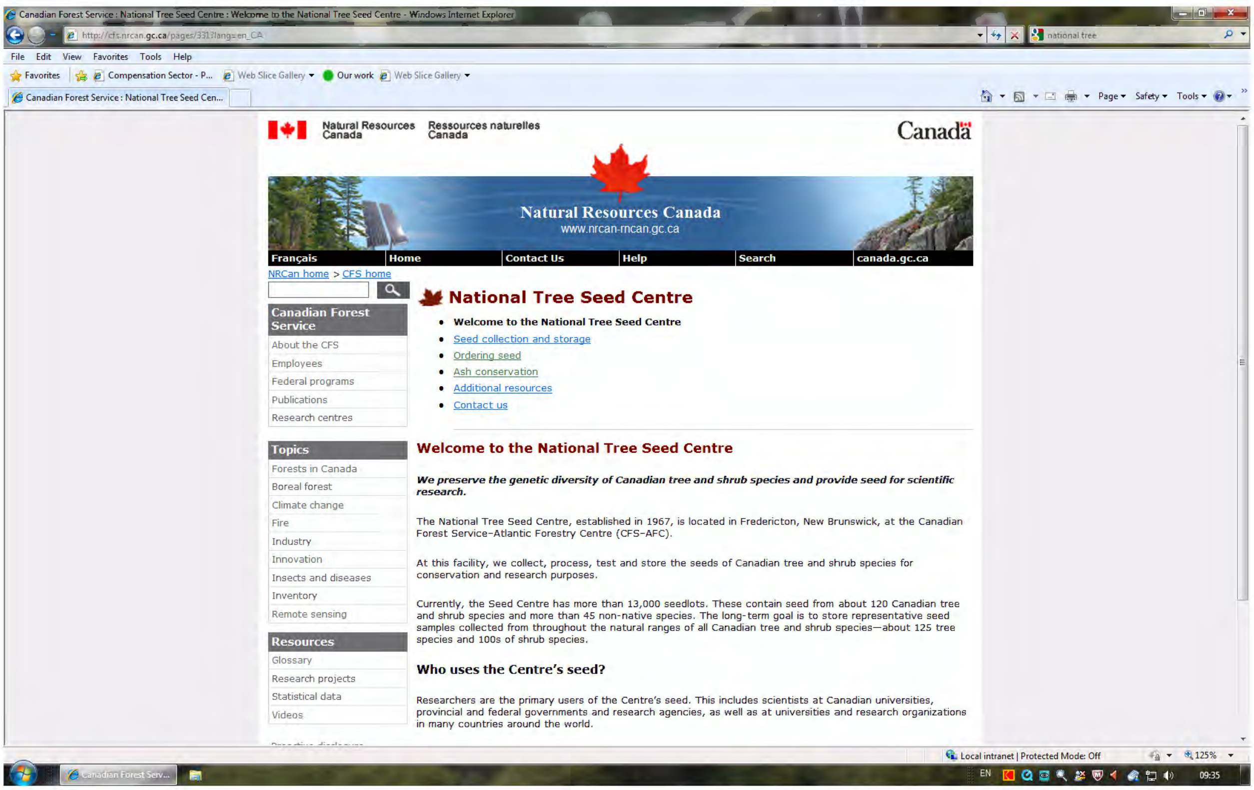

Figure 5. Home page of the National Tree Seed Centre's web site.

RESEARCH AND DEVELOPMENT

Thirteen Years' Storage of Seed From Three *Salix* Species

There are about 400 species of willow worldwide which grow as shrubs or trees. About 100 species occur in North America and 62 species are native to Canada where they occur from coast to coast (Farrar 1995). A country-wide survey of genetic conservation needs conducted by the Canadian Forest Service in 2003 identified 42 of 56 willow species, listed on the survey, as requiring some form of gene conservation; *in situ*, *ex situ* or a combination of both (unpubl. data). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) is a committee of experts that assesses and identifies species that are at risk in Canada. *Salix jejuna*, which is found in Newfoundland, is listed in the Endangered category and this species is facing imminent extirpation or extinction (COSEWIC 2012). *S. chlorolepsis*, growing in the Gaspé region of Québec, is listed as Threatened or likely to become Endangered while *S. brachycarpa* var. *psammophila* and *S. turnorii* located in Saskatchewan and *S. silicicola* located in Saskatchewan and Nunavut are listed as species of Special Concern defined as possessing characteristics that make them particularly sensitive to human activities or natural events (COSEWIC 2012). Therefore, there is a need for long-term preservation of germplasm for these species, one means of which is by storing seed.

The *Salicaceae* family includes *Populus* and *Salix* genera. Poplar and willow seed are small (< 1−3 mm long), short-lived in nature, and must germinate soon after dispersal. Storage temperature and moisture content (MC) of seed are well known factors affecting the storage life of seed. Seed of *Salicaceae*, which consists of an embryo and seed coat with almost no endosperm, does not store well unless frozen. Viability of *S. discolor* seed stored at 20°C decreased from 96 to 31% after 42 days and seed of *S. bebbiana* dropped from 99 to 39% after 28 days (Daigle and Simpson 2001). Douglas (1995) reported that *S. setchelliana* seed stored at room temperature lost all viability after 20 days. Zasada et al. (2008) recommended storing *Salix* seed at temperatures from -5 to -40°C, immediately after cleaning. Simpson and Daigle (2009) reported that germination of *S. bebbiana*, *S. discolor*, and *S. eriocephala* seed stored for five years at -20°C, -80°C, and -145°C ranged from 54 to 86%. Germination of seed from four *Salix* species stored for 36 months at -10°C declined from 96% to 75%, however seed germination of one of the species dropped by only 1% (Zasada and Densmore 1980). Storage of *S. caprea* seed at -20°C gave better results than seed stored at -5 and 0°C (Simak 1982). Seed of *Populus* species also stores well at sub freezing temperatures. Zasada and Densmore (1980) reported that *P. balsamifera* seed stored at -10°C for 36 months declined in germination from 99.5 to 95%. Fechner and Burr (1981) stored *P. tremuloides* seed at -18°C for 24 months and recorded that germination declined from 94.8% to 93.6%. Seed of *P. grandidentata* and *P. tremuloides*, stored at -20°C germinated at 63 and 72% after 32 and 29 years, respectively, in storage (Simpson et al. 2004). Several studies have shown that storing *Salix* seed cryogenically in liquid nitrogen is feasible. Maroder et al. (2000) reported that dry seeds of *S. alba* and *S. matsudana* survived immersion in liquid nitrogen without loss of viability. Seed from two Salix hybrids (*S. rehderinan* x (*S.* x *capreola*) and *S.* x *sericans* x *S. viminalis*) retained viability after immersion in liquid nitrogen for three days (Wood et al. 2003). Optimal seed moisture content for *Populus* and *Salix* seed was reported to be between 4.0 and 7.5% (Buch 1960). Tauer (1979) recommended that *Populus deltoides* seed be dried to a moisture content of 6–10%.

An experiment was initiated at the National Tree Seed Centre using seed from three, common willow species (*S. bebbiana, S. discolor,* and *S. eriocephala*) to evaluate viability following storage at two moisture contents and three temperatures (4°, -20°, and -145°C). Results from these experiments should be applicable to other willow species and will provide guidance for *ex situ* conservation.

Methods

Catkins from a single clone of *S. discolor* were collected on May 21, 1999 at Hamtown Corner, New Brunswick (46° 07' N; 66° 44' W). The catkins were brought into the lab and laid on screen trays at 22°C to allow the capsules to dry and open. After 4 days, the catkins were placed in a rotating screen drum and subjected to warm airflow, which dislodged the seed from the cotton. The seed were sieved to remove larger plant material and lightly blown in an air aspirator to remove lighter particles. Percent MC was determined by drying two samples of seed in a forced draft oven for 16 hours at 103°C (ISTA 2012) and MC calculated on a wet weight basis. The seedlot was halved and one of the sub-samples dried to a lower moisture content in a forced draft oven at 30°C for one hour.

Initial germination testing was carried out on May 26. The high and low MC sub-samples were further divided into storage samples weighing 0.35 g each. The seed were placed in 1.8-mL cryogenic vials and stored at 4°C on May 27 for 24 hours before being transferred to a -20°C freezer.

Catkins from *S. bebbiana* and *S. eriocephala* were collected on May 28, 1999. The *S. eriocephala* catkins were collected at the same location as the *S. discolor* and the *S. bebbiana* catkins were collected at Mill Cove, New Brunswick (45° 53' N; 65° 00' W). The *S. bebbiana* collection was a bulk consisting of catkins from four clones while the *S. eriocephala* collection consisted of a bulk from ten clones. The catkins were brought into the lab and laid on screen trays for 3 days at 22°C to allow the capsules to dry and open. The catkins were processed, seed MC determined, and the lots divided into high and low moisture contents in the same manner described above. On June 1, germination tests were set up and seed was placed in cold storage at 4°C for 24 hours, then moved to frozen storage at -20°C. Samples of all three species were removed from -20°C on June 11 and stored at approximately -145°C in the vapor phase of liquid nitrogen.

In addition to the initial germination test, seed were tested after 6 months and 1, 2, 5, and 13 years storage. Two replicates of 100 seed each were used for the initial germination tests, while four replicates of 100 seed each were used for the other tests. For germination testing, vials of seed stored cryogenically were removed and placed in -20°C for 24 hours, then these vials plus those stored at -20°C were placed at 4°C for 24 hours before being removed and placed at room temperature for 4 hours to allow for gradual warming of the seed. Seed were placed on moistened Kimpak™ or Versa-Pak™ in Petawawa Germination Boxes (Wang and Ackerman 1983) and germinated for 10 days in Conviron™ G30 germination cabinets set at 20°C for 16 hours without light and 30°C for 8 hours with light and at a constant relative humidity of 85%. Germination was considered normal when germinants had chlorophyll and were erect, seed coats had shed, the cotyledons were separated, and the hypocotyl hairs were capable of firmly anchoring the germinant on the substrate as described by Simak (1982). A germinant was scored as abnormal if it possessed one or more of the following traits: no hypocotyl elongation, bent hypocotyl, no hypocotyl hairs causing germinants to lean, fused cotyledons, the seed coat not shed, and the radicle failed to fully develop resulting in a stump root (Simak 1982). Seed MC was determined as above for samples after 2, 5, and 13 years in storage.

Results and Discussion

Salix bebbiana

Moisture content of the seed after processing was 8.6% and it was reduced to 7.2% for one-half of the seedlot after drying. Seed germination at the time of storage was 89%. Germination of seed stored at 4°C declined during the first year with the decline slightly more after 6 months for seed with the higher MC. After two years, germination had decreased to 2% (Table 7). Germination of seed stored at its original MC of 8.6% remained high at both sub-zero storage temperatures with germination of seed stored at -145°C higher after 13 years than seed stored at -20°C. Germination of seed that had been dried was always less. Seed MC of the dried seed was 6.4 − 6.5% after two years but had increased to 7.2 − 7.3% after 13 years storage. Seed stored at its original MC remained below 8.6% for seed stored at -145°C but increased to 9.4% in seed stored at -20°C. The lower germination of the dried seed may have been due to the seed being damaged during oven drying. Seed with an initial MC of 8.6% stored for 13 years at -20°C increased in MC to 9.4% which may explain why the germination was lower than seed stored at -145°C. It is interesting to note that seed MC which had been stable after two and five years storage at both temperatures increased after thirteen years storage. There is no obvious reason for this.

Table 7. Mean germination and moisture content (%) of *Salix bebbiana* seed stored up to 13 years at two moisture contents and three temperatures.

Years in storage	Storage MC 7.2%					Storage MC 8.6%				
	4°C	-20°C		-145°C		4°C	-20°C		-145°C	
	Germ	Germ	MC	Germ	MC	Germ	Germ	MC	Germ	MC
0	89.0	89.0	-	89.0	-	89.0	89.0	-	89.0	-
0.5	84.0	82.8	-	82.5	-	84.5	89.3	-	85.8	-
1	78.3	77.5	-	83.5	-	70.3	82.3	-	84.3	-
2	2.0	79.5	6.5	79.5	6.4	2.0	81.5	7.9	80.3	7.9
5	-	82.5	6.6	79.5	6.4	-	88.8	7.9	86.0	7.8
13	-	77.3	7.2	78.0	7.3	-	81.0	9.4	88.5	8.4

The presence of abnormal germinants can be an indicator that seed is damaged during storage. Generally there was little difference in the proportion of abnormal germinants between storage temperatures within each storage MC and between the two storage MCs over the duration of the trial (Table 8). There is an indication that seed that had been dried tended to have a higher proportion of abnormal germinants than the undried seed but the differences were small.

Table 8. Percentage of abnormal germinants of *Salix bebbiana* seed stored up to 13 years at two moisture contents and three temperatures.

Years in	Storage MC 7.2%			Storage MC 8.6%		
storage	4°C	-20°C	-145°C	4°C	-20°C	-145°C
0.5	6.3	6.5	8.3	5.8	4.8	4.5
1	6.5	8.8	7.0	8.0	8.0	9.8
2	5.0	6.0	5.3	3.8	5.8	5.3
5	-	5.5	8.0	-	4.5	5.5
13	-	8.0	7.5	-	8.0	2.5

Salix discolor

After processing, seed MC was 9.8% and, following drying one-half of the seedlot, MC was reduced to 5.1%. Prior to storage, seed germination was 60.5% which was substantially less than that for *S. bebbiana*. Germination of seed stored at 4°C decreased slightly after 6 months storage but declined rapidly after one year and seed were not viable for both MCs after 5 years storage (Table 9). There is no explanation as to why seed were not viable after 12 months storage at 4°C for the lower MC. Seed stored quite well at both sub freezing temperatures although the decline in germination was greater for seed with the lower MC. It is possible that drying the seed impacted germination especially since the initial pre-storage MC was reduced by almost 50%. However, the MC of these seed increased and was substantially higher after two years in storage. In contrast, MC of seed stored at the higher MC remained below its pre-storage MC of 9.8% up to 5 years in storage and thereafter increased with the most substantial increase for seed stored at -20°C. The MC of 11.5% at 13 years for seed stored at -20°C may have been the reason for the substantial decline in germination compared to seed stored for the same duration at -145°C even though the MC of that seed had increased as well. It is possible that a seed MC of 11% or higher has a detrimental impact on viability.

The proportion of abnormal germinants for all storage durations was higher for *S. discolor* seed than it was for *S. bebbiana* seed. Seed stored at the higher MC tended to have fewer abnormal germinants than seed stored at the lower MC but the differences were sometimes small (Table 10). In combination with the germination results it appears that seed stored at the high MC generally had better germination and fewer abnormal germinants.

Table 9. Mean germination and moisture content (%) of *Salix discolor* seed stored up to 13 years at two moisture contents and three temperatures.

Years in	Storage MC 5.1%					Storage MC 9.8%				
	4°C	-20°C		-145°C		4°C	-20°C		-145°C	
storage	Germ	Germ	MC	Germ	MC	Germ	Germ	MC	Germ	MC
0	60.5	60.5	-	60.5	-	60.5	60.5	-	60.5	-
0.5	56.0	48.0	-	63.0	-	55.5	69.5	-	68.0	-
1	0.0	51.5	-	52.5	-	34.0	57.0	-	62.5	-
2	12.5	55.8	8.8	53.3	8.0	8.3	68.3	9.3	56.0	9.4
5	0.0	51.5	7.3	56.8	7.7	0.0	58.0	9.0	50.8	9.6
13	-	35.5	8.7	48.5	8.3	-	15.5	11.5	56.3	10.3

Table 10. Percentage of abnormal germinants of *Salix discolor* seed stored up to 13 years at two moisture contents and three temperatures.

Years in	Storage MC 5.1%			Storage MC 9.8%		
storage	4°C	-20°C	-145°C	4°C	-20°C	-145°C
0.5	14.8	20.5	9.3	13.0	12.5	14.3
1	-	22.3	20.3	15.0	18.8	19.0
2	12.0	15.5	15.8	4.8	12.3	16.5
5	-	13.8	12.8	-	13.8	24.0
13	-	22.5	21.3	-	12.5	17.3

Salix eriocephala

Seed MC was 8.5% after processing and was reduced to 7.3% following drying in an oven. Before storage, seed germination was 71.5%. For seed stored at 4°C germination declined to around 50% after 6 months in storage and to 5−10% after 12 months storage then to essentially 0% after 2 years in storage (Table 11). Seed at the lower MC stored at -20°C declined in germination after 6 months with small fluctuations until year 13 when it decreased to 28% which may be coincident with the increase in MC to 9.0% at this time. In contrast, germination of lower MC seed stored at -145°C declined as well but was slightly higher than seed stored at -20°C (except after one year storage) but was highest at 63% after 13 years storage. MC of this seed also increased during storage. Seed at the higher MC had higher germination at both storage temperatures except after 13 years in storage. The reduction in germination after 13 years appears to be related to MC especially for seed stored at

-20°C.

The proportion of abnormal germinants was high. There was a tendency for seed at both MCs stored at -20°C to have a higher proportion of abnormal germinants than seed stored at -145°C (Table 12). As well, seed at the lower MC tended to have a higher proportion of abnormal germinants which may have been a result of being dried.

Table 11. Mean germination and moisture content (%) of *Salix eriocephala* seed stored up to 13 years at two moisture contents and three temperatures.

	Storage MC 7.3%					Storage MC 8.5%				
Years in	4°C	-20°C		-145°C		4°C	-20°C		-145°C	
storage	Germ	Germ	MC	Germ	MC	Germ	Germ	MC	Germ	MC
0	71.5	71.5	-	71.5	-	71.5	71.5	-	71.5	-
0.5	50.8	46.5	-	48.3	-	53.8	71.3	-	59.8	-
1	10.3	50.5	-	37.0	-	5.3	60.8	-	59.0	-
2	1.5	43.8	7.4	49.3	8.0	0.0	56.0	8.6	58.5	8.9
5	-	44.5	7.0	47.8	7.5	-	54.5	8.0	63.0	8.0
13	-	28.0	9.0	63.3	7.6	-	14.0	10.0	46.8	8.7

Table 12. Percentage of abnormal germinants of *Salix eriocephala* seed stored up to 13 years at two moisture contents and three temperatures.

Years in	Storage MC 7.3%			Storage MC 8.5%		
storage	4°C	-20°C	-145°C	4°C	-20°C	-145°C
0.5	16.8	14.8	16.8	15.0	7.8	13.5
1	8.5	17.8	21.8	6.8	18.5	13.8
2	0.8	18.5	15.5	-	16.8	18.8
5	-	17.0	15.5	-	12.8	10.5
13	-	22.8	16.3	-	24.0	17.0

General Discussion

Seed germination declined with increased storage time for all species with the greatest decrease at the 4°C storage temperature. Viability was generally maintained for only 6 or 12 months at 4°C. *S. bebbiana* seed stored the best in sub-zero temperatures. Germination of *S. discolor* and *S. eriocephala* seed was lower and the proportion of abnormal germinants was higher than for *S. bebbiana* seed. One possible explanation is that the *S. discolor* and *S. eriocephala* catkins were collected too early and before the seed were fully mature. Overall, seed stored best in the vapor phase of liquid nitrogen demonstrating that the delicate seed of these species tolerates such extreme cold very well.

Germination tended to be less and the percentage of abnormal germinants higher for all species for seed that had been dried. This points to the possibility that seed were damaged during oven drying. *Salix* seed are small and short-lived in nature and are therefore probably sensitive to changes in their environment. In order to reduce the MC, the seed were placed in a forced draft oven set at 30°C for one hour. This process may have damaged the seed. Maroder et al. (2000) found that drying high vigor willow seed from 12% to 4.3% decreased germination whereas germination of low vigor seed decreased when dried to 6.7% MC. Placing seed is a dessicator at room temperature for several hours or overnight may be a better alternative.

Seed MC changed during storage both for seed that had been dried and for seed that was not dried. There is no readily available explanation for this. Seed MC at year 13 was substantially higher than previous years particularly for seed stored at -20°C. When the germination tests were conducted in late spring the relative humidity levels were high (>50%) in the lab. When the jars stored at -20°C were opened (even though they were warmed to room temperature) to remove vials for each testing time point and resealed the moisture in the air trapped in the jars may have somehow passed into the cryogenic vials.

The drastic decrease in germination of seed after 13 years in storage appears to be related to a concomitant increase in seed MC particularly for higher MC *S. discolor* seed stored at -20°C and *S. eriocephala* seed stored at -20°C at both MCs. It would seem that MCs exceeding 9 to 10% have a detrimental impact on seed longevity.

The genetic mix of the seed from each species may have had an influence on the results. The *S. discolo*r seedlot was collected from one clone whereas seedlots for the other species were bulks from four and ten clones. It would have been preferable to have single-clone collections to evaluate if there were clonal differences in seed storage ability.

Conclusions and Recommendations

1. Storage of willow seed at 4°C was inferior to sub-zero temperatures.
2. Storage at -145°C was best for the three willow species.
3. Seed MCs greater than 9 or 10% had a negative impact on storage ability of *S. discolor* and *S. eriocephala* seed.
4. Drying seed for one hour to lower MC using an oven set at 30°C may damage the seed.

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SEED CERTIFICATION

Canada has been applying the OECD (Organization for Economic Cooperation and Development) tree seed certification scheme since 1970 to seed collected for export to Europe. 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 has been certified in the Source-identified category.

Demand for certified seed, which was high in the 1970s and 1980s, has declined the past 20 years (Figure 6) due to less demand from European importers. A total of 58 kg of certified seed was exported in 2012. Grand fir (*Abies grandis)* accounted for 53 kg of the total weight of seed exported. When the European Union (EU) implemented a revised certification Directive on January 1, 2003 there was concern about equivalence between this directive and the OECD Scheme. Fortunately, the EU granted equivalence to Canada for *Abies grandis*, *Picea sitchensis*, *Pinus contorta*, and *Pseudotsuga menziesii* seed to be imported as Source-identified.

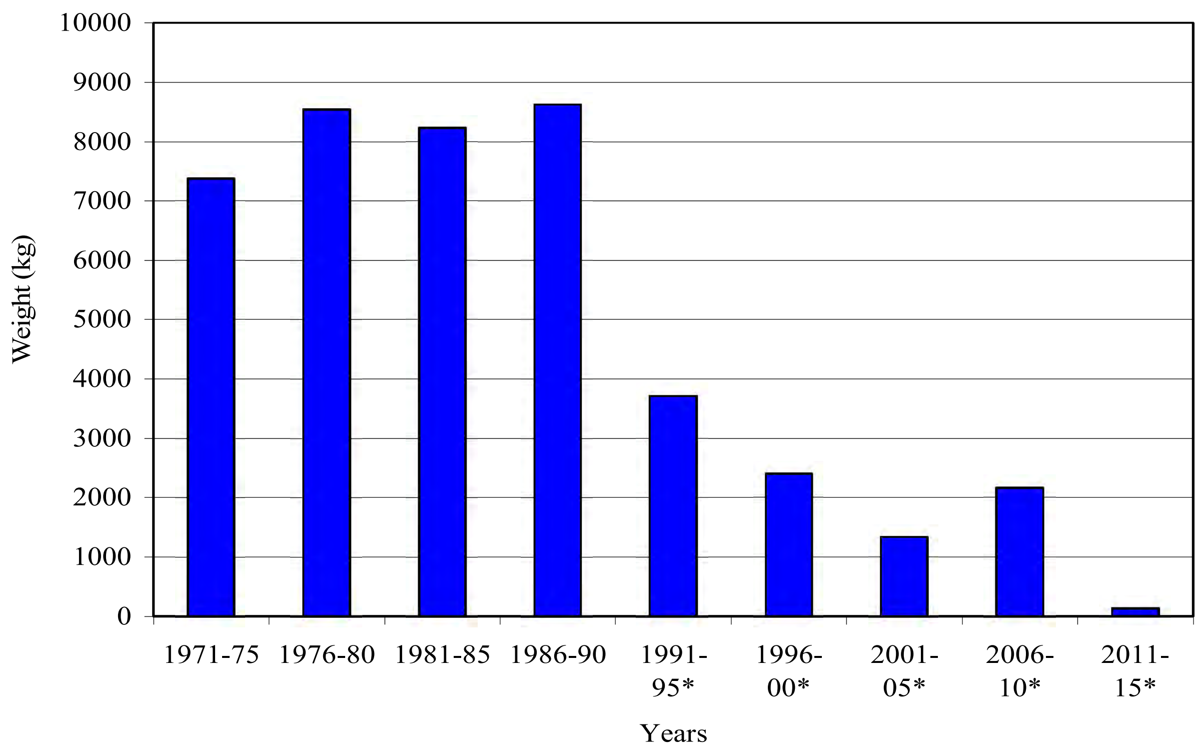

Figure 6. Weight of seed OECD certified or exported* by 5-year periods.

Officially established in 1967, the OECD Scheme for the Control of Forest Reproductive Material Moving in International Trade contained rules and procedures that were adopted in 1974. The Scheme allowed for the certification of seed under four categories: Source-identified (seed collected from a defined geographic area), Selected (seed collected from a stand that was selected for one or more attributes), Untested (seed from untested seed orchards or untested clonal material), and Tested (seed from tested seed orchards or tested clonal material). From its early implementation by a limited number of countries to enable the export of Douglas-fir (*Pseudotsuga menziesii*) seed 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. The Scheme's membership is comprised of 25 countries (primarily European with three African and Canada and United States) working with more than 250 tree species.

During the late 1980s, it became apparent that the 1974 Scheme required revision because of changes in forest management (environmental and social aspects, biodiversity conservation, etc.) in addition to wood production and the growing importance on the market of new types of reproductive material derived from forest tree breeding programs. A revised Scheme was adopted in 2007 and is called the OECD Scheme for the Certification of Forest Reproductive Material Moving in International Trade but only included the Source-identified and Selected categories. These categories benefit all stakeholders, including new applicant countries that are strengthening their domestic control systems for forest reproductive material. Inclusion of the Qualified category was approved in 2010. Having the Qualified category once again provides an opportunity for the marketing of seed orchard seed, the quantity of which is increasing annually. This will afford producers the ability to obtain appropriate monetary value for seed orchard seed.

The Tested category will complete the Scheme. This includes reproductive material from seed orchards as well as parents of families, clones, and clonal mixtures that have been tested thus demonstrating its genetic superiority. A proposal for the inclusion of the Tested category in the Scheme was approved at the annual meeting in October 2012. Now that the Scheme is once again complete it is hoped that it will be an incentive for more countries to join.

Other issues and topics that are being discussed and developed include: guidelines for collecting, processing, selling, sowing and storage of reproductive material; implications of access and benefit sharing to the Scheme; adaptation of the Scheme to tropical countries; and the possibility of using the Scheme's rules for fruit trees.

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SEED CENTRE STAFF

Staff at the NTSC consist of one full-time seed technologist (Bernard Daigle) supervised by Dale Simpson. Mr. Daigle continued full time in the knowledge exchange position that is shared between the Canadian Wood Fibre Centre and Atlantic Forestry Centre. He was able to set up and evaluate a seed pre-treatment trial for *Prunus pennsylvanica*, *P. serotina* and *P. virginiana* var. *virginiana*; completed the testing of seed from three willow species that had been in storage for 13 years and curated/tested seed donated from Ontario Ministry of Natural Resources Tree Seed Plant. Dale Simpson maintained other functions such as filling seed orders and setting up/assessing germination tests.

Matthew Chase was hired for four weeks as a casual to assist with seed processing and setting up germination tests. Figure 7 summarizes the number of "extra" work weeks provided to the Seed Centre. The chart assumes that there was a full-time seed technologist.

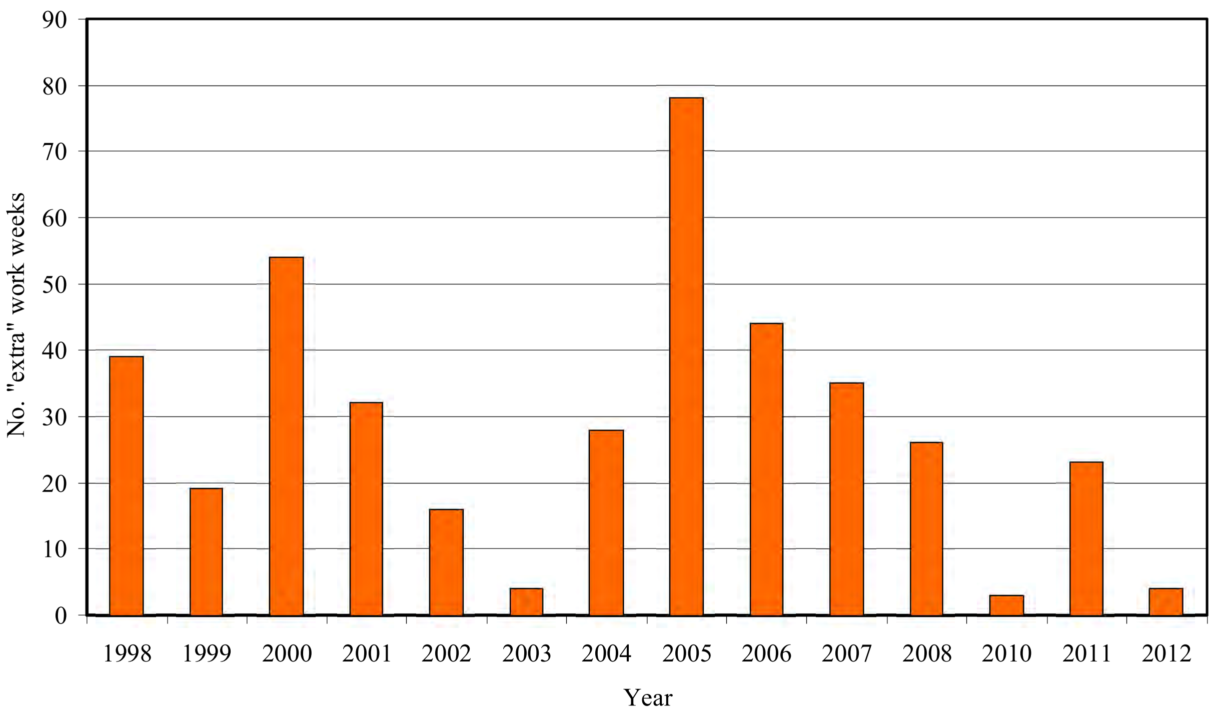

Figure 7. Number of "extra" work weeks provided to the NTSC between 1998 and 2012.