National Tree Seed Centre

Annual Report

2004

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NATIONAL TREE SEED CENTRE ANNUAL REPORT 2004

EXECUTIVE SUMMARY

Seed production was excellent and as a result 549 seed lots from 22 species were collected by Seed Centre staff. Many of the seed collected were as a result of three major projects: Ash Gene Conservation Project, Agroforestry Germplasm Collection Project, and white spruce seed for genetic variation analysis. Seed collected for these projects represented over 65% of the collection. An additional 142 seed lots were purchased, 66 seed lots were acquired through donation, and 305 received from Prairie Farm Rehabilitation Administration (PFRA) Shelterbelt Centre in Indian Head, Saskatchewan as part of the Agriculture and Agri-Food Canada PFRA Agroforestry Germplasm Collection Project. The total number of seed lots in storage is 11 451.

A total of 60 requests representing 740 seed lots was processed and provided for research. The majority of the requests were from Canada (46 requests; 568 seed lots) but seed was also sent to China (1 request; 10 seed lots), France (1 request; 5 seed lots), Greenland (1 request; 3 seed lots), Iceland (1 request; 19 seed lots), and the United States (10 requests; 135 seed lots).

Seed testing consisted of approximately 1 300 germination tests, 350 moisture content tests, and 300 thousand seed weight tests.

With the ever-increasing emphasis and importance on gene conservation, a separate database containing only gene conservation seed lots was set up. This was an opportune time since many "new" collections will be added as a result of collections made and acquired in 2004.

Several experiments were initiated and/or assessed:

• A black ash germination experiment was carried out to determine the conditions necessary for seed germination. Results indicated that seed collected in NB are more dormant and require longer pre-treatments than what is recommended. Germination of up to 90% was achieved with some treatments.

• A five-year assessment of a willow seed storage experiment was carried out. Seed stored at 4°C, -20°C, -80°C, and -145°C were assessed. Results showed that viability remained stable at all sub-zero temperatures while seed stored at 4°C lost all viability by 2 years.

• A white pine seed storage experiment, where seed from two populations stored at two storage temperatures and four moisture contents, was assessed after three years storage. Neither moisture content nor storage temperature had a significant effect on seed viability after three years.

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INTRODUCTION

This report covers the activities of the National Tree Seed Centre (NTSC) for 2004. Similar reports were prepared from 1998 - 2003. The report also captures the results of tests and experiments that were conducted during the year in order to assure 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 gene conservation.

Seed is stored in four different categories: Seed Bank, Reserved, Tree Breeding, and Gene Conservation (Table 1). The total number of seed lots decreased by 235 to 11 451. The main reason for this decrease was the discarding of moldy black spruce seed lots that were part of the Tree Breeding category and stored at 4°C since the 1960's.

Table 1. Seed stored at the NTSC as of December 31, 2004.

Seed Bank		Reserved		Tree Breeding		Gene Conservation	
# Species	# Seed lots	# Species	# Seed lots	# Species	# Seed lots	# Species	# Seed lots
189	4 767	41	1 959	36	2 804	8	1 921

Seed Bank seed lots are the active collection that are available for distribution. 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, 2004, the NTSC Seed Bank had 122 Canadian species (4 452 seed lots) in storage (Table 2). An additional 82 exotic species (315 seed lots) are also stored. Exotic species are defined as those indigenous to other countries 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.

Since the Seed Centre moved to Fredericton, staff have concentrated their efforts in acquiring collections from 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 seed lots west of Ontario. Since collections by NTSC staff are unlikely due to distance and costs, these seed lots will have to be purchased or obtained through donation.

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

Province	# Species	# Seed lots	%
Alberta	11	50	1.1
British Columbia	30	302	6.8
Manitoba	6	60	1.3
New Brunswick	70	1 009	22.7
Newfoundland and Labrador	11	76	1.7
Nova Scotia	40	355	8.0
Ontario	57	1 578	35.4
Prince Edward Island	32	100	2.3
Québec	17	797	17.9
Saskatchewan	8	77	1.7
Yukon Territory	3	47	1.1
Total		4 452	100

The Reserved category contains seed lots that have been reserved by CFS researchers. Many of these seed lots were collected for special projects. No action was taken in 2004 to clean up the Reserved seed lot category. Although clean-up of this category is still necessary, it remains a low priority.

The Tree Breeding category is composed of seed lots that originated from the genetics program at PRF and were transferred to the Seed Centre for storage. As testing progresses, the better quality Tree Breeding seed lots are moved to the Seed Bank category. In 2004, all of the black spruce seed lots (857) that were contained in the Tree Breeding category were assessed. Approximately half of the seed lots (426) were discarded without being tested as the seed were visibly moldy or of insufficient quantity. Of the 431 seed lots that were tested, 174 had to be discarded due to low germination. The remaining 257 were moved to the Gene Conservation collection. There was a significant number of seed lots (301) that had never been entered in the database. Those that were tested and found to have good germination were added to the database.

The Gene Conservation category was put in place to assure that genetic material obtained from rare, endangered, and/or unique populations is preserved. The collection is made up mainly of white spruce (*Picea glauca*) seed lots (1 544 out of 1 565) from range-wide white spruce provenance collections which were made in cooperation with PRF in the mid to late 1970's. Most of these are 5 or 10 gram quantities contained in sealed plastic packets that have been placed in large Mason jars and stored at -20°C. Many of these seed lots are also stored in the Seed Bank (-20°C) and Tree Breeding (4°C or -20°C) categories. In 2004, 100 seed lots of limber pine (*Pinus flexilis*) were added to the Gene Conservation collection.

This collection will grow by several hundred seed lots in 2005 as seed lots collected from populations of white ash (*Fraxinus americana*), red ash (*F. pensylvanica*), and black ash (*F. nigra*) that were collected as part of a gene conservation initiative are added to the collection. The NTSC is also collaborating with the Agriculture and Agri-Food Canada PFRA Shelterbelt Centre in Indian Head Saskatchewan to assist in collecting seeds of a variety of tree and shrub species including: choke cherry (*Prunus virginiana*), pin cherry (*P. pensylvanica*), mountain maple (*Acer spicatum*), striped maple (*A. pensylvanicum*), black ash, red ash, white ash, and green ash (*F. pensylvanica* var. *subintegerrima*). The intent is to make collections across the natural range of these species and evaluate their response and adaptability to climate change. Surplus seed will be stored at the NTSC as part of a gene conservation collection. This is a good example of how the objectives of two separate programs can be mutually beneficial to both organizations. Collections made in 2004 will add several hundred more seed lots to the gene conservation collection.

In view of the increased activity in the gene conservation collection, it was decided to create a separate database that would include only seed lots contained in the Gene Conservation collection. This was relatively easy to accomplish by copying the NTSC database and deleting unwanted records. Some clean-up is still necessary for the white spruce test data records.

SEED COLLECTIONS

Seed production was excellent in 2004. In order to ensure seed of good genetic and physiological quality, 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 549 seed lots from 22 species was collected by Seed Centre staff in 2004 compared to only 69 collections made in 2003.

The majority of the collections were made as part of various projects including the Ash Gene Conservation Project (195 seed lots), Agroforestry Germplasm Collection Project (120 seed lots), and white spruce (*Picea glauca*) collections made for Jean Beaulieu of CFS-Ste-Foy in Québec (60 seed lots). Over 65% of the seed collected by the Seed Centre were as a direct result of these projects.

Other collections of note included 16 single-tree white pine (*Pinus strobus*), 9 single-tree red spruce (*Picea rubens*), and 20 single-tree black spruce (*P. mariana*) collections from Prince Edward Island. Table 3 provides a complete list of the collections made in 2004.

Table 3. Seed collections made by Seed Centre staff in 2004.

Species	N.B.	N.S.	P.E.I.	QC	Total
Acer pensylvanicum	15				15
Acer rubrum	3				3
Acer saccharinum	3				3
Acer spicatum	15		15		30
Betula alleghaniensis	29		12		41
Betula papyrifera	14		10		24
Fraxinus americana	58	76	15		149
Fraxinus nigra	31				31
Fraxinus pensylvanica	15				15
Picea glauca	20	20	26		66
Picea mariana			20		20
Picea rubens			9		9
Pinus strobus			16		16
Prunus pensylvanica	24				24
Prunus serotina	8				8
Prunus virginiana	31	15	15		61
Pseudotsuga menziesii	5				5
Salix bebbiana				3	3
Salix eriocephala	4				4
Salix nigra	6				6
Tsuga canadensis			15		15
Ulmus americana	1				1
Total	282	111	153	3	549

In addition to the seed collected by Seed Centre staff, several seed lots were acquired through donations from various organizations: Québec Ministère des Ressources naturelles de la Faune et des Parcs in Berthierville (9 white spruce, 4 Norway spruce (*Picea abies*), 10 black spruce, 2 red spruce, 7 white pine, 9 jack pine (*Pinus banksiana*), 8 red pine (*P. resinosa*), 4 balsam fir (*Abies balsamea*), and 2 eastern white cedar (*Thuja occidentalis*)), Petawawa Research Forest (6 red maple (*Acer rubrum*)); Ron Smith and Stewart Cameron (5 ground hemlock (*Taxus canadensis*)).

Seed was also acquired through purchase: Silva Enterprises Ltd. (10 lodgepole pine (*Pinus contorta*)); B. Swaile (6 red maple); Jeffries Nurseries Ltd. (3 Manitoba maple (*Acer negundo*), 2 green ash); Allain Fontaine; Québec (15 white ash); Devra Rayvals (18 white ash, 20 green ash, 19 black ash); Mathis Natvik, Orford Ridges Native Plants (15 white ash); Tim Payne, St Clair Region Conservation Authority (15 green ash, 15 white ash); Trinity Tree Company Inc. (3 eastern hemlock (*Tsuga canadensis*)).

Finally, seed was acquired as part of a collaborative effort with the PFRA Agroforestry Germplasm Collection Project. The National Tree Seed Centre agreed to make collections of a variety of tree and shrub species and supply part of the collection to PFRA. In return, PFRA would provide the NTSC with surplus seed collected in other parts of the country. Last year, the NTSC supplied PFRA with 60 seed lots of choke cherry and 15 seed lots of pin cherry. In addition the NTSC also collected 30 seed lots of mountain maple and 15 seed lots of striped maple that will be sent at a later date. The PFRA provided 245 seed lots of choke cherry, and 15 each of pin cherry, green ash, black ash, and Manitoba maple. All seed provided by PFRA will be placed in gene conservation.

A total of 1 070 seed lots were acquired by the Seed Centre in 2004. This is the most seed acquired by the NTSC in a single year since the NTSC moved to Fredericton. Since 1996, the number of seed lots in the Seed Bank collection has increased from 3 079 to 4 767 (net increase of 1689) which represents an increase of over 50 % (Figure 1).

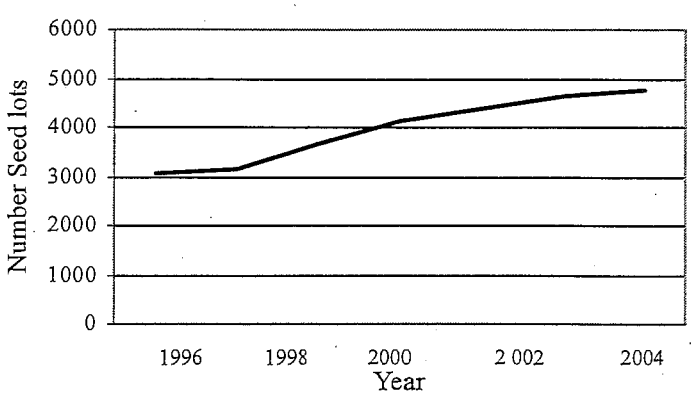

Figure 1. Increase in the number of seed lots at the NTSC Seed Bank since 1996.

The increase shown in Figure 1 represents the net gain in the Seed Bank seed lots. This increase is the result of collections made by Seed Centre staff, donations of seed from various sources, and purchase of seed. Table 4 shows the number of seed lots acquired by the NTSC since 1996.

Table 4. Number of seed lots acquired by the NTSC through collection, donation, and purchase between 1996 and 2004.

Year	Number of Seed lots			
	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	1 067
Total	2 054	1 748	191	3 993

The number of seed lots acquired between 1996 and 2004 is far greater than the increase in seed lots reported earlier. This difference is due to several factors: many seed lots were tested and discarded because of low germination; others were exhausted as they were provided for research; some seed lots were collected and placed in the Gene Conservation collection; and finally, most of the seed lots collected and acquired in 2004 had not been entered into the database by December 31, 2004.

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 of Canada are referred to the Canada Food Inspection Agency to determine if phytosanitary certificates and/or import permits are required.

During 2004 a total of 60 requests representing 740 seed lots was processed. The majority of the requests were from Canada but seed was also sent to China, France, Greenland, Iceland, and the United States (Table 5). The number of seed lots provided for research by the NTSC since 1967 has ranged from a low of 99 in 1996 to a high of 1 603 in 1985 (Figure 2). Canadian researchers received 68 % of the seed that was provided by the NTSC while seed sent to researchers outside of Canada accounted for 32 % of the seed.

Table 5. Number of requests and number of seed lots shipped by country in 2004.

Country	# Requests	# Seed lots
Canada	46	568
China	1	10
France	1	5
Greenland	1	3
Iceland	1	19
United States	10	135
Total	60	740

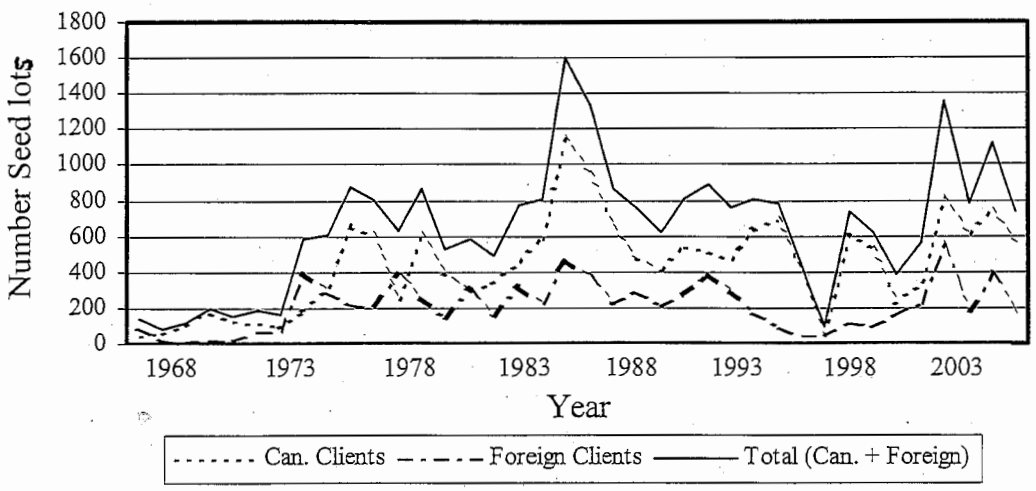

Figure 2. Number of seed lots sent to clients between 1967 and 2004.

SEED TESTING

Germination tests are performed on all freshly collected seed lots as well as seed lots in storage that have not been tested for several years. In most cases, due to small seed lot size, four replicates of 50 seed each are placed on moistened Kimpak in germination boxes. When larger seed is being tested, the number of seed is usually reduced. **Seven hundred and fifty-six germination tests** were carried out. In addition, approximately 600 germination tests were carried out as part of special projects and experiments.

Figure 3 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 transferring of the Seed Centre from Petawawa to Fredericton.

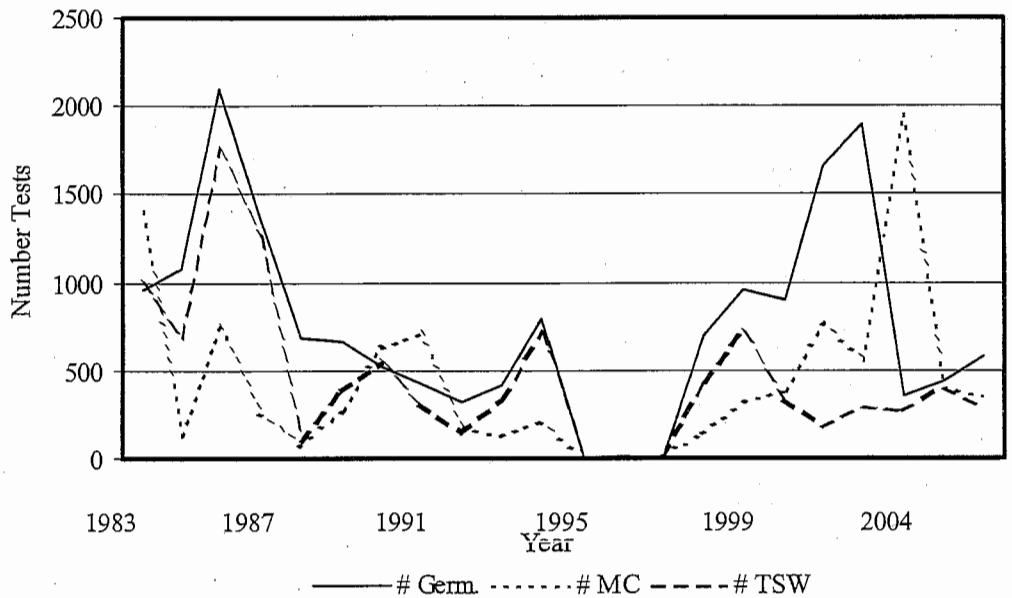

Figure 3. Number of germination tests (# Germ), moisture content tests (# MC), and thousand seed weights (# TSW) carried out by the NTSC since 1983.

The target moisture content for orthodox seed is between 5 and 8 %. Seed that are above this range are further dried before being stored. **Three hundred and fifty-seven moisture content** determinations were carried out.

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

RESEARCH AND DEVELOPMENT

Ironwood Germination Experiment

Ironwood (*Ostrya virginiana*) seed have an internal dormancy that is difficult to overcome. Young and Young, (1992) suggest a 60 day warm stratification period at 20/30°C followed by 140 days pre-chilling. No germination prescriptions are given in ISTA International Rules for Seed Testing (2005) or the AOSA Rules for Testing Seeds (2002).

This experiment was set up as a follow-up to an experiment carried out in 2001 and reported in the 2001 annual report. Fifteen seed lots were tested in 2001. Seed were warm stratified for 9 weeks, moist-chilled for 20 weeks and placed in a germination cabinet set at 20°C without light for 16 hours and 30°C with light for 8 hours and at relative humidity of 85 %. Results varied widely but all seed lots had ungerminated "fresh" seed at the end of the germination period which may indicate failure to break dormancy. In addition, mould had developed on most seed lots by the end of the test period and it is uncertain if this affected results.

This follow-up experiment examined three warm stratification durations (8, 12, and 16 weeks), three moist chilling durations (16, 20, and 24 weeks), and two germination temperatures (20°C and 20/30°C). Four seed lots were selected. Three of these were seed lots previously tested in 2001; the fourth was a seed lot that had been collected in 2002 and had not been previously tested. Prior to treatment, all seed were sanitized using the following procedure: 30 minutes in water plus detergent, 2 minutes in ethanol, and 18 minutes in 5% Javex bleach solution. The seeds were rinsed with water after each soak.

Germination ranged from 0 to 21 % with the better results occurring with treatments involving the longer warm and cold pre-treatments. Best overall results were obtained with the 16 week warm pre-treatment combined with the 24 week cold pre-treatment (Table 6). The results may suggest that the seed pre-treatments are insufficient to mature the embryo and break dormancy.

The sanitation treatment applied to the seed prior to the experiment was not effective in controlling mould. A similar treatment on black ash seed was successful. It is uncertain as to whether the mould had a detrimental effect on germination. The germination temperature of 20/30°C was slightly better than constant 20°C.

Another experiment should be carried out. Based on the results found here and those of the previous experiment, the following is recommended:

- one germination temperature (20/30°C)
- use moist peat to pre-treat seed (warm and cold pre-treatments)
- 3 warm pre-treatments (8, 16, and 24 weeks)
- 3 cold pre-treatments (16, 24, and 32 weeks)

Table 6. Germination results of ironwood seed from 4 seed lots warm stratified at 20°C for 8, 12, and 16 weeks; cold stratified at 3°C for 16, 20 and 24 weeks; and germinated at 20°C and 20/30°C.

Stratification Treatments		Seed lot Number							
		95		1125		1127		1184	
20°C	3°C	20°C	20/30°C	20°C	20/30°C	20°C	20/30°C	20°C	20/30°C
8	16	4	8	0	0	0	0	1	0
8	20	5	17	0	0	1	1*	1	0
8	24	8	21	0	0	3	0*	1	1
12	16	7	1*	0	0*	0	4*	0	0*
12	20	4	5	0	0	1	4	2	4
12	24	6	15	0	0	1	19	3	4
16	16	7	5	0	0	0	0	2	2
16	20	17	14*	0	1	4	9	3	1*
16	24	18	12	1	0	15	13	3	0

* Kimpak dry – added water

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Black Ash Germination Experiment

The range of black ash (*Fraxinus nigra*) in Canada extends from south eastern Manitoba, through Ontario, Quebec, and New Brunswick as well as parts of Nova Scotia, Prince Edward Island and Newfoundland. The range of the species throughout North America lies between latitudes 45°N and 55°N and longitudes 35°W and 95°W. Black ash was more abundant pre-1900 in eastern Canada but the species was heavily sought after for the manufacture of crates used to ship merchandise back to Europe. This affected the quality, quantity, as well as the distribution of the species in eastern Canada. Black ash is often found in wet areas and is commonly found in association with other species that are well adapted to wet conditions such as eastern white cedar (*Thuja occidentalis*), black spruce (*Picea mariana*), balsam fir (*Abies balsamea*), red maple (*Acer rubrum*), and speckled alder (*Alnus rugosa*). Black ash is especially valuable to the native community who use the wood for a variety of products. In addition to its medicinal uses, black ash is used for snowshoe frames, canoe ribs, and especially the ancient art of basket making. Because of its use in basket making it is also called basket ash. Other common names include hoop ash, water ash, swamp ash, and brown ash (Benedict and David, 2000). In recent years there has been increased interest in growing black ash for reforestation and restoration purposes. Overharvesting, habitat intrusion and loss, pollution, lack of sustainable practices, and a naturally low reproductive capacity has jeopardized the supply for current and future black ash for the aboriginal community.

Black ash seed is difficult to germinate and the treatments used to break dormancy require many months. The reason for this has been attributed to various forms of dormancy (embryo and seed coat) as well as an immature embryo at the time the seed is shed in autumn. Although a considerable amount of work has been done on black ash seed, the results do not provide a proven method for germinating black ash from New Brunswick in the lab, the greenhouse, or in the field.

This experiment was designed to explore various treatment conditions for germinating black ash seed. The traditional method involves a warm pre-treatment (90 days) followed by a period (90 days) of moist chilling (ISTA 1996; Wright and Rauscher 2003). The seed are then germinated under 20/30°C conditions in a germination cabinet. Vanstone and LaCroix (1975) reported that germination of black ash seed from Manitoba was best when the seed were subjected to 18 weeks at 21°C followed by an additional 18 weeks at 4°C. Attempts at germinating seed collected in New Brunswick using these recommendations have yielded poor results with many viable seed failing to germinate. The reason for this may be insufficient duration for embryo maturation, cold chilling or both. Horsman (2004) showed that black ash seed collected from a stand in New Brunswick and incubated at 20°C matured at a more rapid rate when the pericarp was removed. The maximum duration used by Horseman was 15 weeks and the data suggested that embryo elongation was still occurring after 15 weeks.

Good seed crops of black ash occur at irregular intervals of up to 7 years (Farrar 1995). The apparent disadvantage of irregular seed crops and challenges posed by an immature embryo and dormancy are mitigated by the fact that black ash seed can remain viable in the litter or soil for up to 8 years (Wright and Rausher 2003).

Black ash is near the northern end of its range in New Brunswick. The literature tells us that many seed only germinate the second year after seed fall (Wright and Rauscher 2003). Since seed fall occurs in October, the embryo has little opportunity to complete its maturation prior to winter but instead undergoes a period of moist chilling. This is then followed by a period of warm stratification the following summer (at which time the embryo probably completes its development). The seed then undergo a second period of moist chilling before germinating the following spring. This experiment attempted to simulate this naturally occurring series of events.

The variables examined included duration of warm pre-treatment, duration of moist chilling, and germination temperature. The traditional warm/cold stratification treatment was compared to a cold/warm/cold treatment which more closely mimics nature. The experiment was carried out simultaneously in the National Tree Seed Centre and at the CFS greenhouse at Fredericton, New Brunswick using different stratification media (moistened Kimpak vs moistened peat). The purpose was to determine if results from laboratory experiments using Kimpak can be readily adapted to germinating seed in a greenhouse which are usually pre-treated and stratified in moist peat.

As the process for germinating black ash seed is lengthy and involves both warm and cold treatments, there is a potential for mould to become a problem. In an attempt to help control this potential problem, all seed were sanitized prior to starting the germination treatments. The method chosen involved a series of steps:

- 30 minute soak in water + dishwashing detergent solution (2 mL soap in 2 L water)
- drain, rinse, and soak in 100 % ethanol for 2 minutes
- drain, rinse, and soak in 5% bleach for 18 minutes (1 L Javex in 500 mL water)
- rinse 3 times in water (first rinse stir for 5 minutes)

The sanitation treatment was used for both the seed treated on moistened Kimpak as well as those stratified in moist peat.

Seed from two single-tree seed lots collected in 2002 at Cross Creek Station, NB, (latitude 46° 16'; longitude 66° 38') and stored at the National Tree Seed Centre for one year at -20°C were used. Two replicates of 25 seed each with pericarp intact were placed on moistened Kimpak in Petawawa germination boxes or in moist peat (75% moisture content) in plastic bags and subjected to the following treatments:

- 2 pre-treatment durations (0 and 90 days at 3°C)
- 3 pre-treatment durations (60, 90, and 120 days at 20°C)
- 3 moist chilling durations (90, 135, and 180 days at 3°C)
- 2 germinations temperatures (20°C constant (16 hours without light, 8 hours with light) and a 20/30°C regime of 16 hours at 20°C without light and 8 hours at 30°C with light).

Germination duration was for 4 weeks and a seed was considered to be germinated once radicle emergence had occurred. Ungerminated seed were cut to determine if the seed was empty, dead, or alive. Germination results are expressed as a percentage of the seed capable of germinating.

Germination temperature had a positive impact on germination. Germination was higher at 20/30°C than at 20°C. This was the case for both the seed germinated with and without the initial pre-treatment of 90 days at 3°C (Tables 7 and 8).

Table 7. Effect of germination temperature on germination of black ash seed pre-treated for 60, 90, and 120 days at 20°C and moist chilled for 90, 135, and 180 days at 3°C.

Pre-Treatment (20°C)	Moist Chilling Duration					
	90 days		135 days		180 days	
	20°C	20/30°C	20°C	20/30°C	20°C	20/30°C
60 days	2 %	6 %	7 %	21 %	0 %	40 %
90 days	2 %	12 %	3 %	44 %	43 %	59 %
120 days	0 %	22 %	23 %	76 %	58 %	64 %

Table 8 also shows that seed treated with a 90 day pre-treatment at 3°C had higher germination, especially at the 20/30°C germination temperature. Another observation is that germination was maximized with up to 90 or 120 days of warm pre-treatment, and 180 days of moist chilling.

Table 8. Effect of germination temperature on germination of black ash seed pre-treated for 90 days at 3°C and 60, 90, and 120 days at 20°C and moist chilled for 90, 135, and 180 days at 3°C.

Pre-Treatment		Moist Chilling Duration					
		90 days		135 days		180 days	
3°C	20°C	20°C	20/30°C	20°C	20/30°C	20°C	20/30°C
90 days	60 days	6 %	40 %	2 %	64 %	46 %	76 %
90 days	90 days	4 %	59 %	16 %	49 %	68 %	92 %
90 days	120 days	3 %	40 %	17 %	81 %	48 %	91 %

There was very little difference in germination results using moist peat or moist Kimpak as pre-germination media.

Another aspect that was looked at was whether or not the extended treatment, sometimes lasting for over one year, damaged the seed. All ungerminated seed were assessed and there was no increase in the number of dead seed among the various treatments (Figure 4).

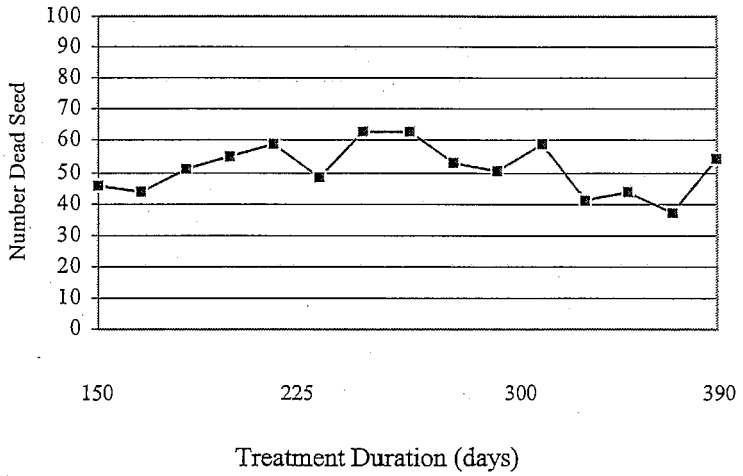

Figure 4. Number of dead black ash seed over time for seed of various durations of cold/warm/cold treatments.

The small sample size, the large number of empty/dead seed, and the placing of both replications in the same germination box may have had an effect and better results may have been obtained if a larger sample size had been used. The number of dead and empty seed per replication ranged from 1 to 13. This problem is difficult to overcome because these seeds cannot be removed during processing. This meant that expressing germination as a percentage of the number of seed used could misrepresent the actual germination results. In order to avoid this, germination was calculated based on the number of viable seed in each replicate. A suggestion for future experiments would be to use a minimum of 50 seed per replication, to increase the number of replications to 3, and to use separate germination boxes for each replication.

The results clearly demonstrated that the recommendation of 90 days warm treatment followed by 90 days of moist chilling is insufficient to germinate black ash seed from New Brunswick as only 6% of the seed germinated using this prescription. The work carried out to support this recommendation was possibly carried out on seed collected in the southern portion of the range of black ash. It appears that black ash seed collected in New Brunswick is more dormant. The fact that black ash seed from the northern limits of the species range is more dormant is not unique to black ash. Other species such as red maple, sugar maple, and eastern hemlock have been shown to exhibit similar characteristics (data on file).

Although the recommendation of Vanstone and Lacroix (1975) of 18 weeks warm treatment followed by an additional 18 weeks cold was not tested, our treatment of 120 days warm and 135 days cold was similar and yielded 76% germination. This was the best result that was obtained using traditional warm/cold treatment combination.

The use of a 90 day cold pre-treatment prior to the warm pre-treatment was effective in increasing germination in all the treatments where seed was germinated at 20/30°C. Furthermore, by subjecting the seed to an initial cold treatment of 90 days it appears that the duration of the warm treatment could be reduced as germinations of 92 and 91% were obtained for seed pre-treated at 20°C for 90 and 120 days, respectively. It is difficult to ascertain whether this is a result of the cold treatment occurring before the warm treatment or if it is due to the cumulative effect of moist chilling on the seed. It may be possible to obtain similar results by increasing the duration of the moist chilling treatment thus eliminating the need for the initial cold treatment.

There appears to be some genetic variation that impacts upon a seed's ability to germinate. The seed used in this experiment were collected from 2 single trees from the same stand. Germination, although low, was observed after only 60 days of warm pre-treatment and 90 days of moist chilling and continued to increase throughout the duration of the experiment. This appears to be part of the reproductive strategy for black ash. This variation makes it possible for some seed to germinate over several years. It would be useful to test seed from different areas throughout the species' range to determine what the requirements for germinating the seed are as you move from north to south and east to west.

The 120 day warm pre-treatment combined with 135 or 180 days of moist chilling succeeded in maturing the embryos and alleviating dormancy of most of the seed. There was, however, about 25 – 35% of the viable seed that failed to germinate. The percentage of ungerminated seed was much lower for the treatment that included the 90 day cold pre-treatment with only 10% of the seed failing to germinate. It is possible that if this warm pre-treatment was increased, the percentage of viable seed that germinate would be higher as the embryos would have more time to complete elongation.

There was very little difference between the seed germinated on moistened Kimpak and those germinated on moistened peat. This is very useful since the information obtained from laboratory experiments can be easily adapted for germinating seed in the greenhouse. The use of a sanitation treatment to control potential mold problems may not be necessary when using peat but this was not evaluated in this experiment.

Finally, based on this preliminary experiment, it appears there may exist some differences in dormancy and possibly embryo maturity of black ash seed in different parts of its range. It is important that the grower know where the seed was collected and what the germination requirements are for those seed. However, thanks to the apparent resilience of the seed (prolonged treatment does not appear to damage the seed), it is possible to extend the treatment to ensure that a high proportion of the seed germinate. Based on this experiment, a combination of 90 days cold / 90 days warm / 180 days cold and germinating the seed at 20/30°C on moistened Kimpak or moistened peat will yield 90% germination of viable seed.

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Willow Seed Storage Experiment

There are 62 species of willow native to Canada where they occur from coast to coast. A country-wide survey of gene conservation needs conducted by the Forest Genetic Resources group at CFS-Atlantic identified 42 of 56 species listed on the survey as requiring some form of gene conservation; *in situ*, *ex situ* or a combination of both. COSEWIC (2004) lists *Salix silicicola*, *S. brachycarpa* var. *psammophila*, and *S. turnorii*, all located in Saskatchewan or Nunuvit, as species of Special Concern defined as possessing characteristics that make them particularly sensitive to human activities or natural events. *Salix jejuna,* which is found in Newfoundland, is listed in the Endangered category which focuses on species facing imminent extirpation or extinction. Therefore, there is a need for long-term preservation of germplasm one means of which is storing seed.

An experiment was initiated using seed from three, common willow species (*S. bebbiana, S. discolor,* and *S. eriocephala*) to evaluate the impact on viability by storing seed at two moisture contents and four temperatures (4°, -20°, -80°, and -145°C). Results from this trial should be applicable to other willow species and will provide guidance for *ex situ* conservation.

Methods

Seed from a single clone of *S. discolor* was collected on May 21, 1999 at Hamtown Corner, located just north of Fredericton, New Brunswick (46° 07' N; 66° 44' W). The catkins were immediately brought into the lab and laid on screen trays at 22°C to allow the capsules to dry and open. On May 25, after 4 days of drying, the partially opened catkins were placed in a rotating screen drum and subjected to warm airflow, which dislodged the seed from the cotton. The seed was sieved to remove larger plant material and lightly blown in an air aspirator to remove lighter particles. Percent moisture content (MC) was determined by drying two samples of seed in a forced draft oven for 16 hours at 103°C (ISTA, 1996) and MC calculated on a wet weight basis. The seed lot was halved and one of the sub-samples dried to a lower moisture content by air drying in a forced draft oven at 30°C and the adjusted MC or target moisture content calculated.

Initial germination testing was carried out on May 26. The high and low moisture content sub-samples were further divided into storage samples weighing 0.35 g each; the seed was 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.

Seed of *S. bebbiana* and *S. eriocephala* was collected on May 28, 1999. The *S. eriocephala* seed was collected at the same location as the *S. discolor* and the *S. bebbiana* seed was collected at Mill Cove, New Brunswick (45° 53' N; 65° 00' W). The *S. bebbiana* seed was a bulk collection consisting of catkins from four clones; the *S. eriocephala* catkins were collected 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, their MC determined, and the lots divided into high and low MCs in the same manner described above. On June 1, a germination test was set up for these species 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 that were to be stored at -80°C and -145°C in the vapor phase of liquid nitrogen were removed from -20°C on June 11.

In addition to the initial germination test, seed was tested after 6, 12, 24 and 60 months storage. Two replicates of 100 seed each were used for the initial tests, while four replicates of 100 seed each were used for the 6-, 12-, 24-, and 60-month tests. Vials of seed stored cryogenically and at -80°C 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. Following this, all vials were left at room temperature for 4 hours before they were opened. The seed was placed on moistened Kimpak™ in Petawawa germination boxes 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 the germinant had chlorophyll and was erect, the seed coat had shed, the cotyledons were open, and the hypocotyl hairs were capable of firmly anchoring the germinant on the substrate as described by Simak (1982).

Results and Discussion

Moisture content of the seed after processing ranged from 8.5 to 9.8% and from 5.1 to 7.2% after drying. The greatest range in MC occurred with *S. discolor,* with high and low MCs of 9.8 and 5.1%, respectively. The difference in MC for *S. bebbiana* and *S. eriocephala* ranged from 8.6 to 7.2% and 8.5 to 7.3%, respectively. Germination test results of the freshly collected seed (tested after processing and before drying) were 89.0% for *S. bebbiana*, 60.5% for *S. discolor*, and 71.5% for *S. eriocephala*.

Seed germination declined with increasing storage time for all species with the greatest decrease at the 4°C storage temperature (Tables 9 – 11). Seed viability was generally maintained for only 12 months at 4°C. Germination of seed in sub-zero storage declined slowly over the 60-month period with the least decline for *S. bebbiana* and the greatest decline for *S. eriocephala*. Differences are evident between the two seed MCs for each species with germination generally less for the lower MC.

Germination after 60 months storage was not significantly different among the three sub-zero temperatures for each of the two MCs for all species except for *S. bebbiana* seed at the higher MC (Table 12). At this MC, seed stored at -20°C germinated slightly higher. An analysis of variance demonstrated that there was no significant difference in germination between MCs within each species after 6 months storage (Table 13). This suggests there is no advantage to further drying the seed following extraction and processing. Germination data were combined for each MC for each species and used to graphically depict changes between each storage interval (Figures 5 – 7). The graphs clearly demonstrate that 4°C is an inferior storage condition for all species. Differences in seed germination among sub-zero storage temperatures are greater for *S. eriocephala* as is the overall decline in germination during the 60 month period. *S. bebbiana* and *S. discolor* seed declined little in germination over 60 months when stored at sub-zero temperatures.

Table 9. Percent germination of *Salix bebbiana* seed stored at two moisture contents and four temperatures.

Storage Duration	4°C		-20°C		-80°C		-145°C	
	8.6%	7.2%	8.6%	7.2%	8.6%	7.2%	8.6%	7.2%
0 months	89.0	89.0	89.0	89.0	89.0	89.0	89.0	89.0
6 months	84.5	84.0	89.3	82.8	89.5	85.5	85.8	82.5
12 months	70.3	78.3	82.8	77.5	86.0	74.8	84.8	83.5
24 months	2.0	2.0	81.5	79.5	84.5	72.3	80.3	79.5
60 months	0.0	0.0	88.8	82.5	84.0	79.3	86.0	79.5

Table 10. Percent germination of *Salix discolor* seed stored at two moisture contents and four temperatures.

Storage Duration	4°C		-20°C		-80°C		-145°C	
	9.8%	5.1%	9.8%	5.1%	9.8%	5.1%	9.8%	5.1%
0 months	60.5	60.5	60.5	60.5	60.5	60.5	60.5	60.5
6 months	55.5	56.0	69.5	48.0	69.0	57.8	68.0	63.0
12 months	34.0	0.0	57.0	51.5	53.0	53.3	62.5	52.5
24 months	8.3	12.5	68.3	55.8	61.0	54.0	56.0	53.3
60 months	0.0	0.0	58.0	51.5	56.3	52.3	50.8	56.8

Table 11. Percent germination of *Salix eriocephala* seed stored at two moisture contents and four temperatures.

Storage Duration	4°C		-20°C		-80°C		-145°C	
	8.5%	7.3%	8.5%	7.3%	8.5%	7.3%	8.5%	7.3%
0 months	71.5	71.5	71.5	71.5	71.5	71.5	71.5	71.5
6 months	53.8	50.8	71.3	46.5	65.8	52.3	59.8	48.3
12 months	5.3	10.3	60.8	50.5	53.3	47.5	59.0	37.0
24 months	0.0	1.5	56.0	43.8	56.0	38.3	62.0	52.0
60 months	0.0	0.5	54.5	44.5	66.0	49.8	63.0	47.8

Table 12. Mean germination of seed at different moisture contents from three willow species stored for 60 months at four temperatures.

Storage Temperature	*S. bebbiana*		*S. discolor*		*S. eriocephala*	
	Moisture Content		Moisture Content		Moisture Content	
	8.6%	7.2%	9.8%	5.1%	8.5%	7.3%
4°C	0.0 a[1]	0.0 a	0.0 a	0.0 a	0.0 a	0.5 a
-20°C	88.8 b	82.5 b	58.0 b	51.5 b	54.5 b	44.5 b
-80°C	84.0 c	79.3 b	56.3 b	52.8 b	66.0 b	49.8 b
-145°C	86.0 c	79.5 b	50.8 b	56.8 b	63.0 b	47.8 b

[1] means significantly different at P = 0.05 determined by a Duncan's Multiple Range Test

Table 13. Analysis of variance summary of effect of seed moisture content on germination of seed stored at four temperatures for four periods for three willow species.

Source	df	MS	P value
S. bebbiana			
Germination at 6 months	1	0.0195	0.017*
Germination at 12 months	1	0.0084	0.301
Germination at 24 months	1	0.0185	0.763
Germination at 60 months	1	0.0280	0.749
S. discolor			
Germination at 6 months	1	0.0767	0.005*
Germination at 12 months	1	0.3016	0.056
Germination at 24 months	1	0.0118	0.671
Germination at 60 months	1	0.0008	0.941
S. eriocephala			
Germination at 6 months	1	0.1419	< 0.001*
Germination at 12 months	1	0.0422	0.411
Germination at 24 months	1	0.0384	0.562
Germination at 60 months	1	0.0742	0.466

* significant difference among moisture content at P=0.05

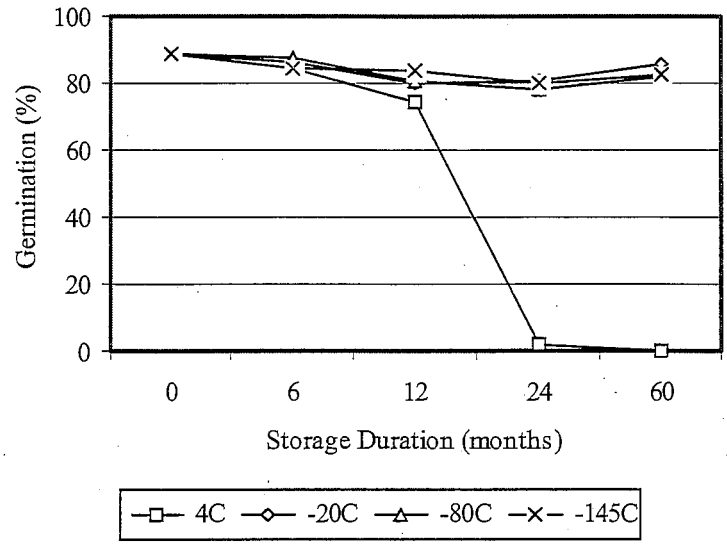

Figure 5. Germination of *Salix bebbiana* seed stored at four temperatures over a 60 month period.

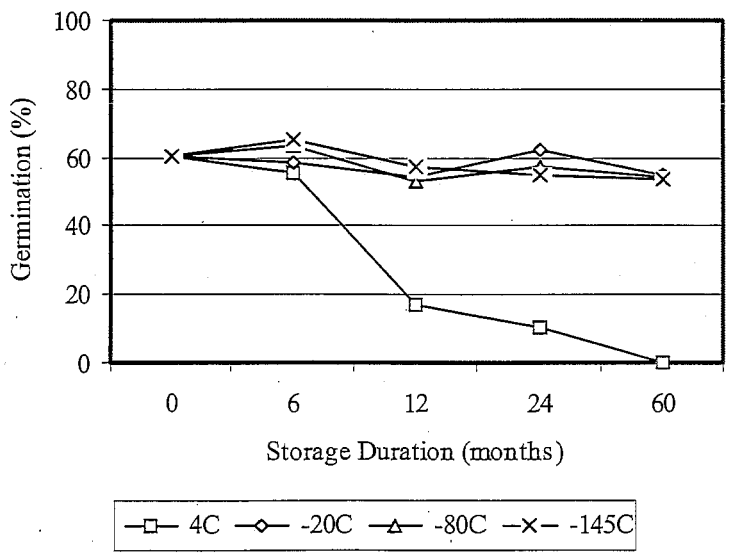

Figure 6. Germination of *Salix discolor* seed stored at four temperatures over a 60 month period.

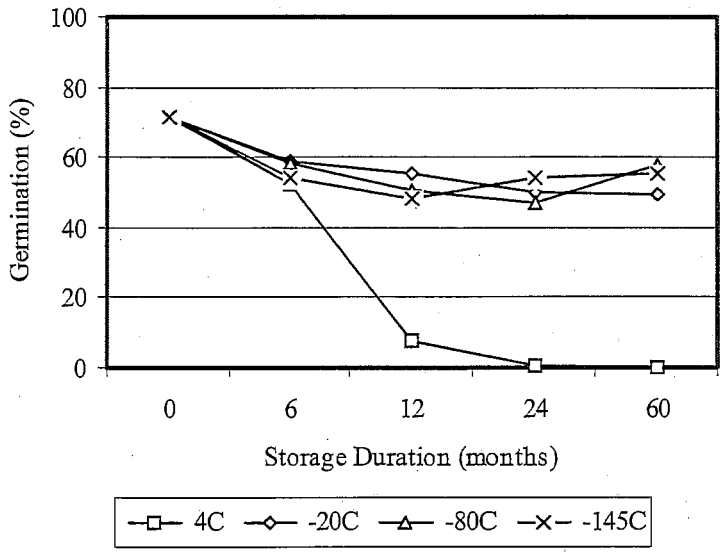

Figure 7. Germination of *Salix eriocephala* seed stored at four temperatures over a 60 month period.

The presence of abnormal germinants can be an indicator that seed is damaged during storage. Abnormal germinants could possess one or more of the following traits: no hypocotyl elongation, bent hypocotyl, no hypocotyl hairs causing germinants to lean, cotyledons do not separate or the seed coat is not shed, and the radicle fails to fully develop resulting in a stump root (Simak 1982). Seed moisture content and time in storage did not impact quality of germinants. *Salix bebbiana* had the lowest percentage of abnormal germinants compared to the other species (Tables 14 – 16). There is no trend of an increase in abnormal germinants with increasing storage time for all species. Seed moisture content generally had no significant impact on the percentage of abnormal germinants for seed in sub-zero storage except for *S. bebbiana* and *S.eriocephala* at 6 months and *S. discolor* at 60 months (Table 17). Data for abnormal germination of seed stored at 4°C was not included in the ANOVA due to missing values for several storage times.

Table 14. Percentage of abnormal germinants of *Salix bebbiana* seed stored at two moisture contents and four temperatures.

Storage Duration	4°C		-20°C		-80°C		-145°C	
	8.6%	7.2%	8.6%	7.2%	8.6%	7.2%	8.6%	7.2%
0 months	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
6 months	5.8	6.3	4.8	6.5	5.0	5.3	4.5	8.3
12 months	8.0	6.5	8.0	8.8	5.5	8.5	9.8	7.0
24 months	3.8	5.0	5.8	6.0	5.8	7.0	5.3	5.3
60 months	-	-	4.5	5.5	6.8	7.3	5.5	8.0

Table 15. Percentage of abnormal germinants of *Salix discolor* seed stored at two moisture contents and four temperatures.

Storage Duration	4°C		-20°C		-80°C		-145°C	
	9.8%	5.1%	9.8%	5.1%	9.8%	5.1%	9.8%	5.1%
0 months	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0
6 months	13.0	14.8	12.5	20.5	11.0	12.8	14.3	9.3
12 months	15.0	-	18.8	22.3	23.5	18.8	19.0	20.3
24 months	4.8	12.0	12.3	15.5	19.5	15.3	16.5	15.8
60 months	-	-	13.8	13.8	22.8	17.5	24.0	12.8

Table 16. Percentage of abnormal germinants of *Salix eriocephala* seed stored at two moisture contents and four temperatures.

Storage Duration	4°C		-20°C		-80°C		-145°C	
	8.5%	7.3%	8.5%	7.3%	8.5%	7.3%	8.5%	7.3%
0 months	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0
6 months	15.0	16.8	7.8	14.8	12.8	14.0	13.5	16.8
12 months	6.8	8.5	18.5	17.8	16.8	18.3	13.8	21.8
24 months	-	0.8	16.8	18.5	19.3	28.8	18.8	15.5
60 months	-	-	12.8	17.0	10.8	9.5	10.5	15.5

Table 17. Analysis of variance summary of effect of seed moisture content on abnormal germination of seed stored at three sub-zero temperatures for four periods for three willow species.

Source	df	MS	P value
S. bebbiana			
Abnormal germination at 6 months	1	0.0091	0.044*
Abnormal germination at 12 months	1	0.0005	0.635
Abnormal germination at 24 months	1	0.0012	0.429
Abnormal germination at 60 months	1	0.0054	0.148
S. discolor			
Abnormal germination at 6 months	1	0.0025	0.544
Abnormal germination at 12 months	1	0.00002	0.937
Abnormal germination at 24 months	1	0.0002	0.804
Abnormal germination at 60 months	1	0.0359	0.040*
S. eriocephala			
Abnormal germination at 6 months	1	0.0208	0.014*
Abnormal germination at 12 months	1	0.0094	0.060
Abnormal germination at 24 months	1	0.0058	0.292
Abnormal germination at 60 months	1	0.0090	0.125

* significant difference among moisture content at P=0.05

Conclusions and Recommendations

1. Storage of willow seed at 4°C is inferior to sub-zero temperatures.
2. Storage at -20°C is sufficient. Although seed can survive at lower temperatures there is no advantage and the costs are greater.
3. Seed moisture contents less than 10% do not have an impact on storability.
4. Willow seed should be quickly processed after collection. Seed deteriorates quickly at room temperature. When removing seed from frozen storage for testing or distribution the remaining seed should be returned to frozen storage as soon as possible.
5. Seed should be stored in several small vials to eliminate damaging seed when a larger quantity would be taken in and out of storage for testing or distribution.

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White Pine Seed Storage Experiment

Historical germination test data at the NTSC have shown that viability of white pine (*Pinus strobus*) seed declines steadily up to 20 years of age. An experiment was initiated to evaluate the impact on viability by storing seed of various moisture contents at -20°C and cryogenically, in the vapor phase, at -145°C. The set-up and initial moisture content (MC) and germination results were reported in the 2001 Annual Report (Daigle and Simpson 2002) and the 12 month results reported in the 2002 Annual Report (Daigle and Simpson 2003). Results to 36 months are summarized here.

Methods

Cones were collected in September 2000 from three trees in the UNB Woodlot and three trees in the UNB Noonan Woodlot (15 km east of Fredericton). Seed was shaken from the cones, debris and dirt removed, seed de-winged by hand rubbing, and full seed separated from empty seed by floatation in 100% ethanol. Following this, MC was determined to establish a control value for each seed lot. Seed was then conditioned to achieve three target MCs of 5, 8, and 11%. A sample from each MC of each seed lot was placed in a 10 ml cyrogenic vial. Six vials were prepared for each MC/tree/storage temperature combination.

After 36 months in storage one set of vials was removed. The vials were immediately placed at room temperature and remained there for 22 hours. Approximately 1.2 – 1.5 g of seed, depending on seed size and target MC, was removed from each vial for MC determination using only one replicate. The remaining seed was used for germination testing. Seed was placed on moistened Kimpak™ in Petawawa germination boxes using a vacuum plate. Four replicates of 50 seed each were placed in each box. The boxes were transferred to a cooler maintained at 3°C for 28 days. After 28 days the boxes were placed in a Conviron™ G30 germinator. Germination conditions were 30°C with 8 hours light followed by 20°C with 16 hours darkness at a constant relative humidity of 85%. Germinants were first monitored at 7 days and at regular intervals thereafter until day 28. Germinants were evaluated according to vigor class (Wang, 1973). Seed was considered germinated when vigor class 3 was reached as evidenced by cotyledons, hypocotyl, and developing radicle.

Results and Discussion

Seed germination was significantly different among the two populations after 36 months storage (Table 18). The difference was greater for seed stored at -145°C (Table 19). However, the differences after 36 months storage were less than before storage. Germination was higher after 36 months than before storage. As well, there were also significant differences in seed germination among the seed lots within each population (Table 18). Seed lots that exhibited lower germination before storage also had lower germination after 36 months storage at both temperatures (Table 20). The consistently poor performance of these seed lots could be related to the seed being slightly immature at the time of collection which is an example of the role genetic variation can play in seed development and maturity. This also highlights the importance of using single-tree collections for experiments in order to account for variation that would otherwise not be detected or as apparent from a bulk seed collection.

Table 18. Analysis of variance of germination of white pine seed stored for 36 months.

Source of variation	d.f.	Mean Square	P value
Population (P)	1	1.0897	< 0.0001
Seed lot (population)	4	2.23	< 0.0001
Moisture content (MC)	3	0.0242	0.472
Storage temperature (ST)	1	0.1014	0.062
Replication (R)	3	0.0784	0.046
P x MC	3	0.0447	0.203
P x ST	1	0.2112	0.008
P x R	3	0.031	0.360
Seed lot x MC	12	0.0604	0.021
Seed lot x ST	4	0.0652	0.065
Seed lot x R	12	0.0203	0.741
MC x ST	3	0.0293	0.386
MC x R	9	0.0072	0.986
ST x R	3	0.0043	0.928
Error	129	0.0286	

Table 19. Mean germination (%) of white pine seed, collected from 2 populations, before storage and after storage for 36 months at 2 temperatures.

		Storage temperature	
Population	Before storage	-20°C	-145°C
Woodlot	75.5	77.4	86.5
Noonan	50.3	70.8	69.1

Table 20. Mean germination (%) of 6 white pine seed lots, collected from 2 populations, before storage and after storage for 36 months at 2 temperatures.

			Storage temperature	
Population	Seed lot no.	Before storage	-20°C	-145°C
Woodlot	130	55.4	50.6	73.8
Woodlot	131	85.4	88.0	90.6
Woodlot	132	85.6	93.5	95.1
Noonan	133	56.8	91.0	93.0
Noonan	134	48.8	73.4	69.5
Noonan	135	45.3	47.5	44.9

There was no significant difference between the two storage temperatures (Table 18). Seed stored cryogenically germinated as well, or slightly better (Tables 19 and 20). It is useful to know that white pine seed can tolerate cryogenic storage conditions. As the remaining samples are tested over time it will remain to be seen whether the extra expense of cryogenic storage can be justified by little or no loss in seed germinability as compared to conventional storage at -20°C. The significant ($P < 0.05$) interaction between population and storage temperature is probably due to the little difference between germination of seed from Noonan stored at the two temperatures versus the much better germination of Woodlot seed stored at -145°C (Table 19).

There was no difference in germination among seed of different MCs although there was a significant interaction between seed lot and MC (Table 18). Figures 8–11 illustrate how germination has changed over time for seed stored at both temperatures and at each MC. Germination tends to be highest after 36 months storage regardless of storage temperature. Edwards (2001) stated that eastern white pine seed is known to become more deeply dormant when stored at -18°C (or lower) for more than six months. This is not demonstrated by this experiment. Germination tended to increase with storage time, however, germination did decrease from 12 to 36 months in several instances (Figs. 8, 10 and 11). The sharp decline from 12 to 36 months for seed stored at -20°C with a MC of 11% is due to much lower than average germination for seed lots 130 and 135 (Table 20). Despite this, over 95% of the ungerminated seed of these two seed lots was fresh (seed had imbibed moisture, the megagametophyte was firm, and the embryo was fully developed) as evidenced from a cut test which was performed at the completion of the germination tests. This indicates that these seed were probably still dormant.

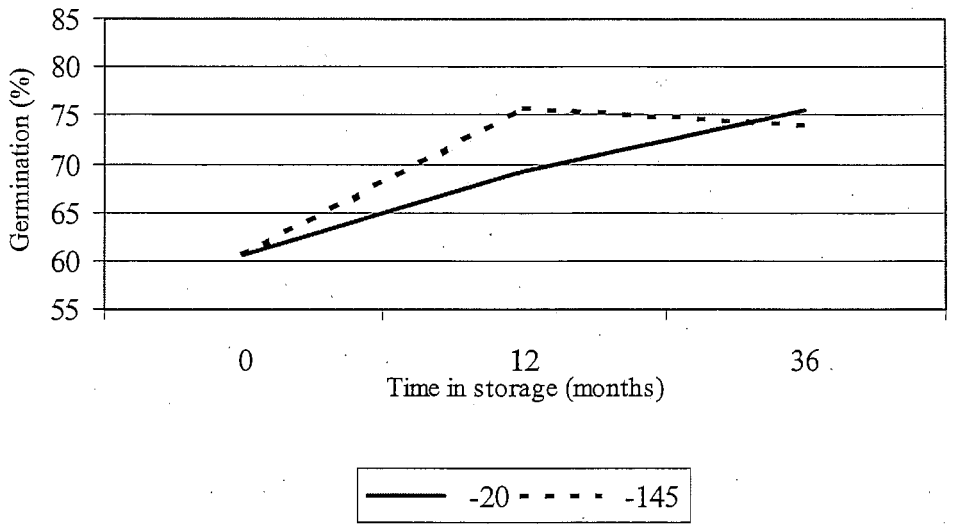

Figure 8. Germination of white pine seed, with 'control' moisture content, after 12 and 36 months storage at -20°C and -145°C.

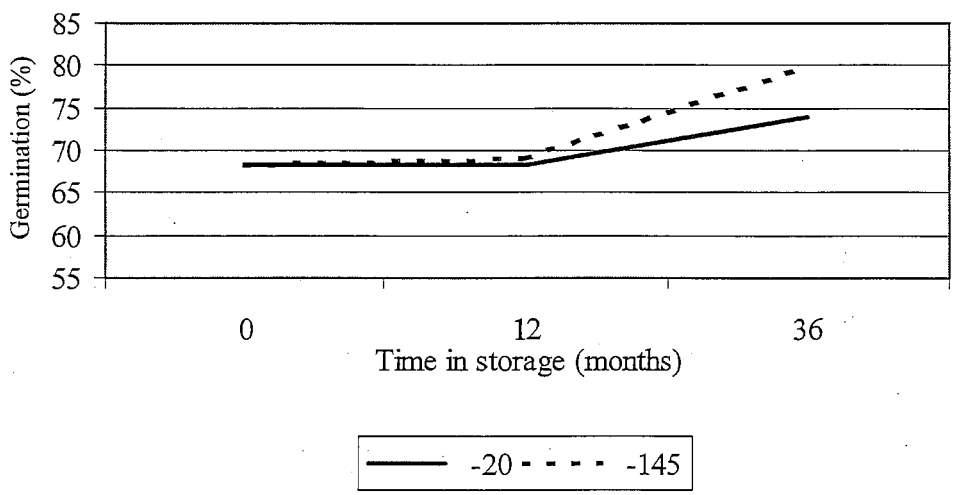

Figure 9. Germination of white pine seed, with 5% moisture content, after 12 and 36 months storage at -20°C and -145°C.

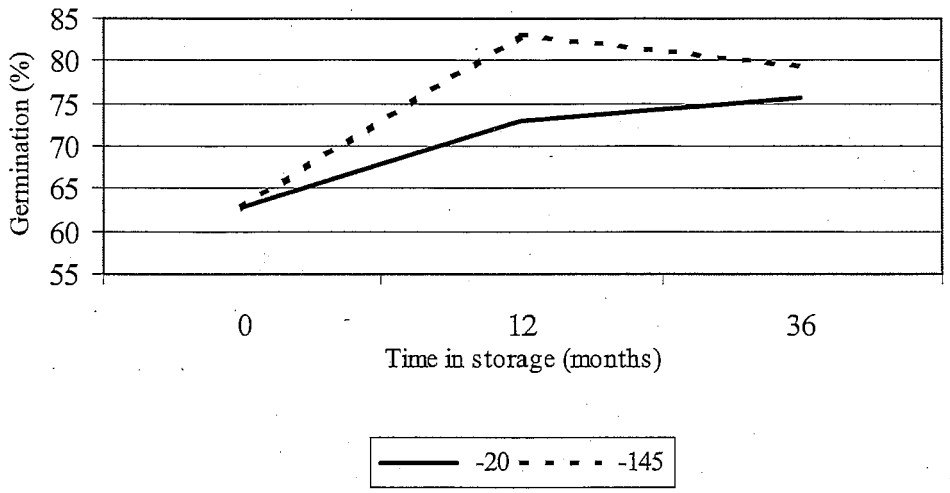

Figure 10. Germination of white pine seed, with 8% moisture content, after 12 and 36 months storage at -20°C and -145°C.

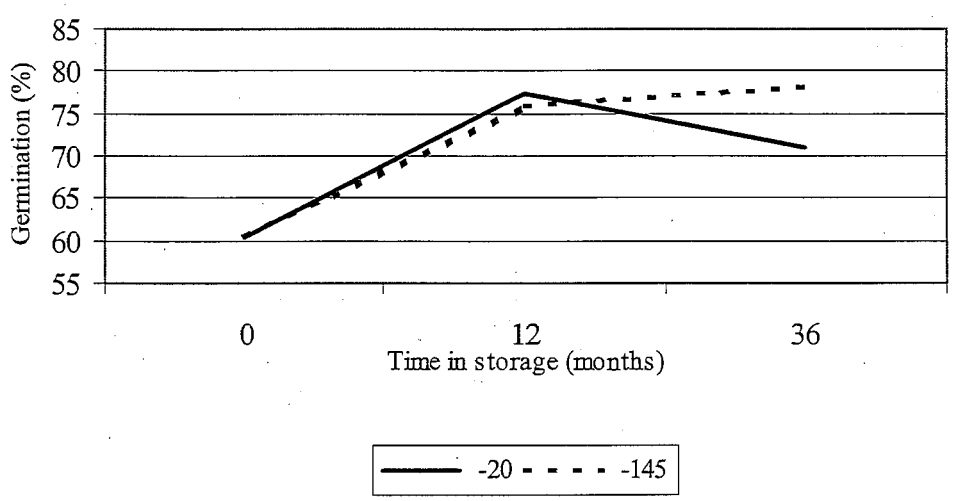

Figure 11. Germination of white pine seed, with 11% moisture content, after 12 and 36 months storage at -20°C and -145°C.

Average MC of the conditioned seed, prior to storage, was close to the target MC (Table 21). The control MC was higher than the lower target MC of 5%. MC of seed stored at -20°C was higher after 12 months in storage and increased further at 36 months. Seed stored at -145°C exhibited a slight increase in MC at 12 months and maintained this level to 36 months although the MC of seed at target MC 11% did increase slightly between 12 and 36 months (Table 21). One could expect MC to be slightly different after storage due to some variation among samples during conditioning. The cryogenic vials are fitted with an o-ring which should create a tight seal so the seed does not gain or lose moisture. At least 20 hours passed from the time the samples were removed from storage and placed at room temperature and the time the vials were opened and the seed removed. Less than 10 seconds passed from the time each vial was opened, seed poured out, and the weight recorded. It is a mystery how seed stored at -20°C is acquiring moisture. MCs were determined on samples of excess seed from each seed lot that was stored in sealed mason jars at -20°C. The average MC of these samples was 5.72% which was only slightly higher than the Control before storage (5.69%) (Table 21). Therefore, all vials in -20°C storage were transferred to sealed mason jars in an effort to halt the increase in seed MC.

Table 21. Change in moisture content (%) of white pine seed stored at 2 temperatures.

		12 months storage		36 months storage	
Target MC	Before storage	-20°C	-145°C	-20°C	-145°C
Control	5.69	6.02	5.78	6.66	5.77
5%	4.89	5.33	4.94	5.73	4.96
8%	8.15	8.25	8.25	8.62	8.26
11%	11.04	11.10	11.18	11.06	11.27

Conclusions

The following conclusions are made from this study:

1. Storing white pine seed at -145°C did not negatively impact germination.
2. There were differences in seed germination among populations and seed lots.
3. Seed MC up to 11% did not have a negative impact on germination of seed stored at -20°C or -145°C.
4. MC of seed stored at -145°C did not change appreciably with time.
5. Generally, seed did not become more dormant with increasing time in storage.

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Growth of Immature Black Ash Embryos

When black ash (*Fraxinus nigra*) seed are shed the embryo is both immature and dormant. In order for the seed to germinate, two processes much occur. First, the embryo must complete elongation and second, the seed must be exposed to appropriate environmental conditions (moisture and cold) to alleviate dormancy allowing germination to occur. The first aspect of this two step process was investigated by Geoffrey Horsman for his BScF thesis project at the University of New Brunswick (Horseman 2004). Two single-tree seed lots, collected in 2002, were used to evaluate the effect of pericarp, incubation temperature, and duration of incubation on embryo growth.

The pericarp was removed from one-half of the seed from each seed lot. Seed with and without pericarp were placed in de-ionized water for 24 hours to imbibe water. Following imbibition, seed were placed on Kimpak™ in Petawawa germination boxes. Each box contained two replicates of 30 seed per seed lot for each pericarp treatment. Only 20 seed from each replication were measured. The ten extra seed were used to replace seeds that were empty, dead, or damaged during embryo excision. The germination boxes were placed in two Conviron™ G30 germinators. One was set to a constant temperature of 15°C, the other was set to 20°C. Every two weeks, germination boxes of each seed lot/pericarp treatment were removed and the total length of the embryo and hypocotyl measured. Embryos were measured from a sample of seed that had only been imbibed to establish a baseline length.

Starting at week 2 and every second week to week 12 and at week 15 germination boxes of each seed lot and pericarp treatment were removed from the germinators. Embryos were excised by making a longitudinal incision along the edge of the seed coat for the complete length. The embryo were carefully removed using tweezers. Total length of the embryo and hypocotyl was measured to the nearest 1/10 mm using a vernier caliper with the aid of a desktop magnifying lens.

There was a significant difference between pericarp treatments throughout the duration of the experiment. Embryos in seed having the pericarp removed grew faster and longer (Figures 12 and 13). Incubation temperature had an impact on embryo growth. Embryos from seed with the pericarp removed were consistently longer after 4 weeks at 15°C. Embryos from seed with an intact pericarp were longer when incubated at 20°C up to week 12. Figure 12 indicates that embryo growth at 15°C was almost complete, at an average length of 16.3 mm, by week 15 for seed with pericarp removed. Seed with intact pericarp would require an additional 3 to 9 weeks for embryo elongation to be completed depending on incubation temperature.

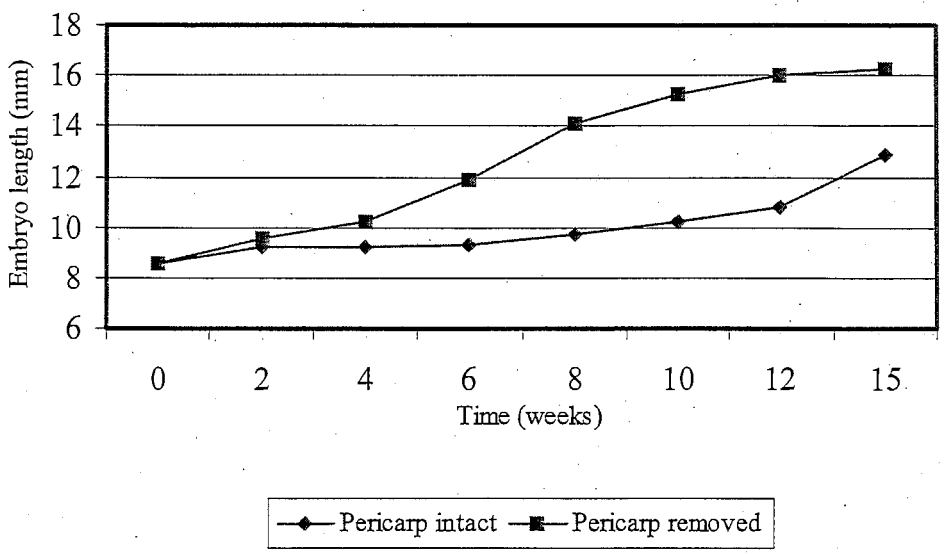

Figure 12. Growth of black ash embryos in seed with pericarp intact and removed incubated at 15°C for 15 weeks.

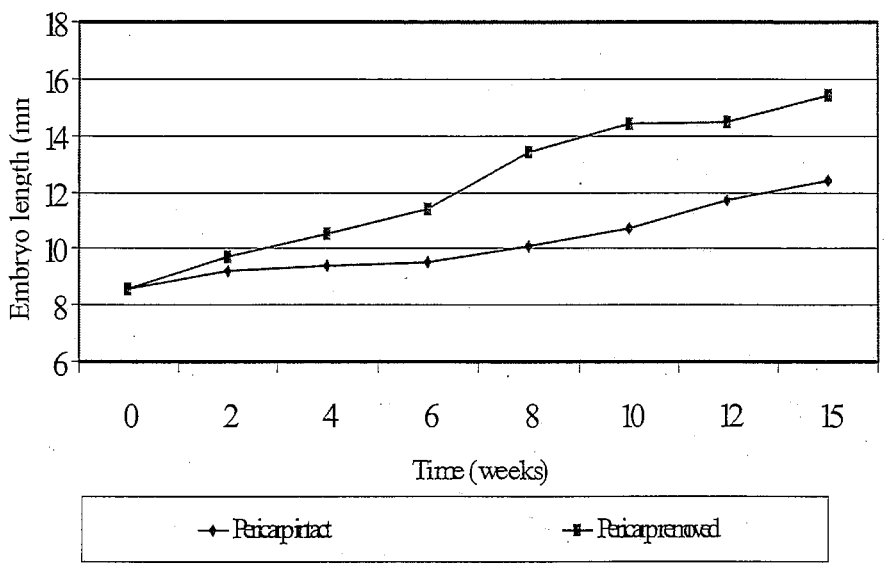

Figure 13. Growth of black ash embryos in seed with pericarp intact and removed incubated at 20°C for 15 weeks.

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SEED CERTIFICATION

Canada has been applying the OECD (Organization for Economic Cooperation and Development) seed certification scheme since 1970. The CFS was nominated by the Government of Canada as the Designated Authority to implement the Scheme. Practically 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.

Demand for certified seed, which was high in the 1970's and 1980's, has declined the past ten or more years (Figure 14). However, a total of 414 kg of certified seed was exported in 2004, which was a substantial increase above previous years. Of significance was 110 kg of Sitka spruce (*Picea sitchensis*), 174 kg of Douglas fir (*Pseudotsuga menziesii* var. *menziesii*), and 50 kg of lodgepole pine (*Pinus contorta* var. *latifolia*). Ten kg of Sitka spruce seed from an untested seed orchard was also exported. The European Union (EU) implemented a revised certification Directive on January 1, 2003. There has 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*. Hopefully this will improve the Canadian tree seed export market.

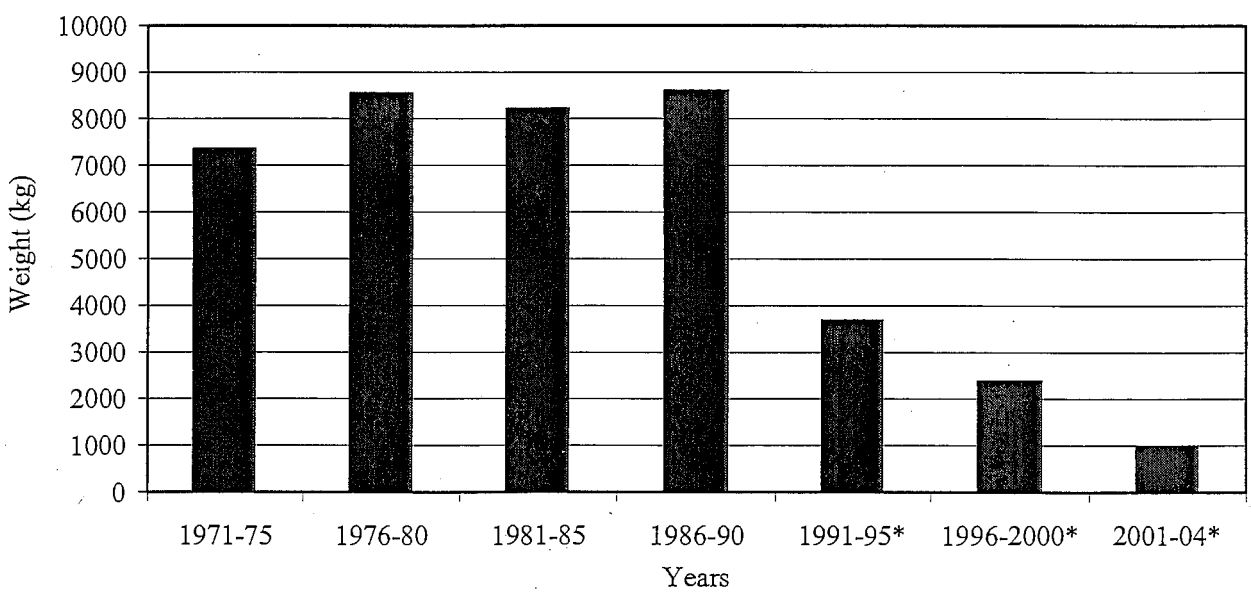

Figure 14. Weight of seed OECD certified or exported* by 5-year periods.

The Scheme which was written in the late 1960's and early 1970's and adopted in 1974, although recognized as being effective, was also viewed in the later 1980's as being somewhat inflexible in some parts. As a result, an Expert Group was tasked with developing a new Scheme which was completed in 1995. This Scheme was accepted as being more flexible to allow for new and different methods and procedures to produce and market reproductive material including GMO (genetically modified organisms). Unfortunately, the inclusion of GMO material has become a contentious issue which has resulted in the new Scheme not being adopted. Several attempts have been made to modify the text of the new Scheme but each revision has not been accepted by all OECD members. An Expert Group met in Montreal in December 2003 to develop another approach.

The proposal was to rescind the 1974 Scheme and replace it with the first two categories of the New Scheme - "Source Identified" and "Selected" which cover about 99% of forest seed used and traded under an OECD label. As well, provision would be provided to have a "blank" OECD label to cover more advanced material which could be applied based on a bilaterally agreed exchange of information. For example, if country X wanted to purchase seed orchard seed from country Y and both agreed that the seed met the "Qualified" category requirements (in the New Scheme), then this special OECD label could be applied. This proposal would allow the implementation of the first 2 categories of the New Scheme and hopefully when the GMO issue becomes resolved, to everyone's satisfaction, at the international level, the remaining 2 categories can be implemented later. This proposal was discussed at the biennial meeting held May 2004. The idea of a "blank" OECD label was rejected but the delegates endorsed the idea of replacing the text of the 1974 Scheme that applied to "Source Identified" and "Selected" with the text of the new Scheme and keeping the text that applies to "Untested Seed Orchard" and "Tested" intact. The EU agreed to this as long as there was text inserted stating that "reproductive material from these two sources does not contain genetically-engineered material at a detectable level". The text of the 1974 Scheme will be revised accordingly and circulated to members for comment. If approved, then members will meet in 2005 to discuss implementation.

PRESENTATIONS / PUBLICATIONS

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COLLABORATION WITH COLLEAGUES

White Spruce Germination Tests

Two seed lots of white spruce were received from Ben Wang at the Petawawa Research Forest. The seed originated from Smoky Lake Forest Nursery Ltd. in Alberta. The seed had been processed using two methods: mechanical and manual. The seed lots were germinated according to ISTA standards. Four replicates of 100 seed each were placed on moistened Kimpak™. Seed were germinated with and without prechill (21 days at 3°C). Chilled and non-chilled seed were placed in germination cabinets with 8 hours with light at 30°C and 16 hours without light at 20°C and at 85% relative humidity. The results were forwarded to Ben.

X-ray of White Spruce Seed

A sample of seed lot 771D collected by Nexfor Fraser Papers Inc. was sent to the NTSC for X-ray analysis by the Atlantic Seed Centre at Kingsclear, New Brunswick. The seed were X-rayed and results indicated that embyos appeared to be fully developed and seed appeared to be normal and showed no sign of physical or insect damage.

Tetrazolium Testing of White Spruce Seed

The NTSC was contacted by Michée Lemieux, nursery supervisor with Nexfor Fraser Papers Inc. in St-Joseph de Madawaska, NB. Seed sown in 2003 had yielded poor germination although tests performed on the seed had shown high germination and Frasers Paper wanted a quick viability test to determine the quality of the seed as there was not enough time to prepare a full germination test.

Two samples of white spruce seed (seed lots 348C and 794C) were sent to NTSC for tetrazolium testing. Two replicates of 25 seed each were cut longitudinally and placed in 1% tetrazolium stain for 16 hours at 20°C All of the embryos (except one in seed lot 348C) reacted positively to the stain indicating that viability of both seed lots was above 95%. These results were provided to Michée Lemieux.

PROMOTION OF SEED CENTRE

January	Expert Committee on Microbial and Plant Genetic Resources in Canada
April	Theresa McGuire. NRCan Departmental Health and Safety Team, with other Health and Safety Officers and Managers from different sectors across the country.
July	Bill Schroeder (Indian Head Nursery)
November	Delegation from Shaanxi province, China

On July 7, a CBC film crew took some film footage at the National Tree Seed Centre. The 6 minute segment was done by CBAT TV and was aired by Canada Now on July 8 and August 30. Following is the text that was aired:

ANITA SHARMA (Host): Welcome back. When your city's trees are wiped out by a nasty beetle, or even a hurricane, the place to go is the National Tree Seed Centre. It's found only in Fredericton.
The federally funded National Tree Seed Centre collects and stores over 11,000 seeds. It's part of the **Canadian Forest Service**. Scientists there gave us a tour of the centre.
DALE SIMPSON (National Tree Seed Centre): These are large white spruce cones that will be collected later this summer and stored at the National Tree Seed Centre, located here at the Hugh John Flemming Forestry Centre in Fredericton New Brunswick.
I'm Dale Simpson, and I will take you on a tour of the National Tree Seed Centre where I'll show you how a seed is processed and stored from tree species from all across Canada.
These are cones that were collected after they dried for a period of time. You can see that when dried the cones are open and the seeds are ready to fall out. We take seeds out of the cones by putting them in this tumbler, and as the tumbler goes around then all the seeds are shaken from the cones.
[Tumbler working]
SIMPSON: As the seed pass through this screen it is collected in this drawer (inaudible), and we can see there are seed with wings. There's also some other dirt which will have to be removed in another process. Once we see that it's come out of the cone tumbler, and at this stage we want to be able to separate the good seed from the empty seed, as well as any other dirt that's in there. This is blowing a column of air, and you can see all the light material is blowing up to the top, as well as the empty seed. At this point relatively all the empty seed is at the top, and all the good filled seed is remaining below in the cup.
BERNIE DAIGLE (Tree Seed Technologist): My name is Bernie Daigle, and I work here at the National Tree Seed Centre with Dale Simpson. My role here is as a tree seed technologist.
The reason for the National Tree Seed Centre, why it's so important, is two-fold. Firstly we provide seed for research and provide our seed to people all around the world. The second reason is for gene conservation. A good example for gene conservation perhaps is the seed we collected a few years ago at Point Pleasant Park in Halifax. That seed is available and could be used for restoration purposes if people in Halifax wish to do that.
After the seed has been cleaned, the next step is to determine moisture content of the seed. Moisture content is very important because if seed is stored at too high a moisture content the seed will not store well. So we check the moisture content of the seed, and we dry it in an oven and once the moisture content is within acceptable limits we'll be able to store that seed and stored seed can last for a long time.
The last bit before we can store seed is to determine the viability or germination of the seed. And that's so we know that the seed will grow after they've been stored. The way we do that is we use these germination boxes, and we place seeds on the germination boxes. We use these vacuum plates that allow for one seed to just to stick on each one of the cavities, and make sure that we have one seed per cavity, and we place them in the germination box. Once that is completed the germination box is ready to go into the germinator

SIMPSON: (Inaudible) from a germination box we just prepared into the germinator.
DAIGLE: These germinators are able to control the temperature, the light, and the relative humidity.
SIMPSON: And these are set according to the international standards, which is quite a full and complete (inaudible) any part of the world. As long as the researcher germinates the seed at the same conditions he or she should attain the same germination results that we have here.
DAIGLE: Once we have completed the germination tests we're able to determine how good the seed is, and if the seed is good we will store it and seed stored can last for many years.
SIMPSON: This is our main seed freezer. Here we have over 11,000 samples collected from thousands of locations across Canada. Some of the seed lots have been in storage here for over fifty years.
DAIGLE: The seed stored here will be used for research, gene conservation, and depending on the type of research being done, we really have no idea what the seeds will be used for in the future.
SHARMA: Well if that story planted a seed in your imagination give us a shout. We'd love to hear your opinion on any story you've seen on Canada Now.

SEED CENTRE STAFF

The Seed Centre benefited from some extra help during 2004. This was fortunate since 2004 was an excellent seed year and a record number of collections were made. The number of "extra" hours that has been provided over the years has varied considerably. It has ranged from a low of 4 weeks in 2003 to a high of 54 weeks in 2000. In 2004, 28 extra weeks were provided (Figure 15).

Aremi Contreras, a Mexican student, worked from mid-February to early May. Her main task was to process and evaluate limber pine seed acquired from CFS–Edmonton. During her stay with the NTSC Aremi had the opportunity to experience may different procedures including: alcohol separation, x-ray tests, moisture contents, seed counts, thousand seed weights, germination testing, cone tumbling, de-winging of seeds, air separation with aspirator, and seed cleaning. She worked an average of 2.5 days /week

Cynthia Caborn was obtained through the Federal Public Sector Youth Internship Program. Cynthia started the first week of September and will end her 9-month term in May, 2005. Cynthia proved to be a valuable asset during the fall collection period. She adapted very quickly to the procedures of cleaning seed and required minimal supervision.

Eugenia Dietrich was hired to help with the processing and testing of the ash seed collections. Over 300 seed lots of white, black, and red (green) ash were collected or purchased by the NTSC in 2004. Eugenia started on December 6 and continued through the end of the fiscal year.

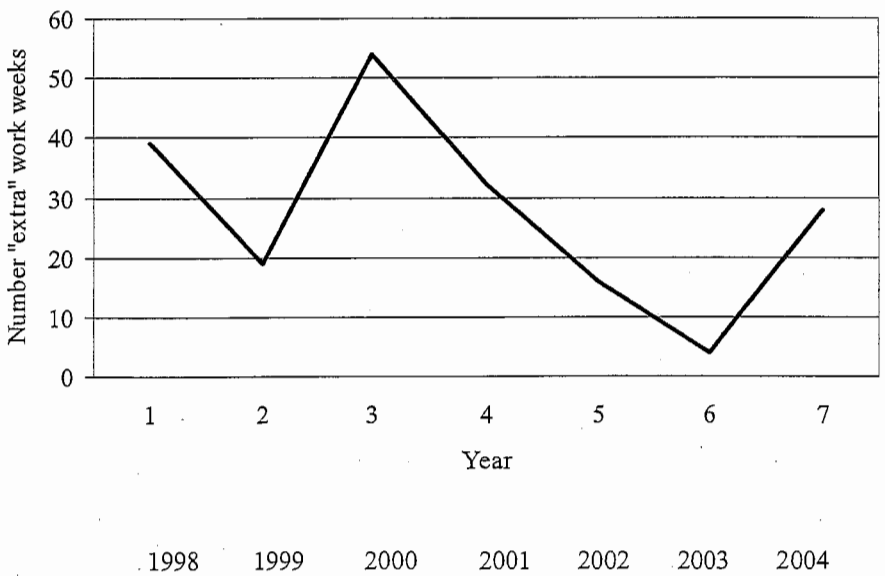

Figure 15. Number of "extra" work weeks provided to the NTSC between 1998 and 2004.