

National Tree Seed Centre

Annual Report

2005



Prepared by:

B.I. Daigle & J.D. Simpson
National Tree Seed Centre
Natural Resources Canada
Canadian Forest Service – Atlantic
Fredericton, N.B.
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NATIONAL TREE SEED CENTRE ANNUAL REPORT 2005

EXECUTIVE SUMMARY

It was not a good year for seed production for most species. The exception was black ash (*Fraxinus nigra*) which for the second consecutive year, had a good seed crop in New Brunswick and in the Gaspé region of Québec. As a result 82 single-tree collections of black ash seed were made from 5 sites in New Brunswick and Quebec. Other species of note collected by Seed Centre staff included red maple (*Acer rubrum*: 24 seedlots), pin cherry (*Prunus pensylvanica*: 15 seedlots) and choke cherry (*Prunus virginiana*: 15 seedlots). Donations of 22 seedlots of red maple and 15 pin cherry were also acquired from Newfoundland-Labrador.

A total of 65 requests representing 812 seedlots was processed and provided for research. The majority of the requests were from Canada (55 requests; 743 seedlots) but seed was also sent to China (2 requests; 13 seedlots), England (1 request; 1 seedlot), Finland (1 request; 17 seedlots), France (1 request; 6 seedlots), Sweden (1 request; 13 seedlots), and the United States (4 requests; 19 seedlots).

Seed testing consisted of approximately 1700 germination tests, 1400 moisture content tests, and 1250 thousand seed weight tests.

The total number of seedlots in storage is approximately 12 000 collections with almost half of these stored in the Seed Bank and available to researchers. An additional 3000 are stored for Gene Conservation and the remaining 3000 are old Tree Breeding seedlots or seedlots Reserved by researchers.

Several experiments and trials were conducted:

- Over 500 seedlots of jack pine (*Pinus banksiana*) seed that was tested averaged an annual decline in germination of 0.13% during the 19-year test period.
- Viability testing of black ash (*Fraxinus nigra*), red ash (*F. pennsylvanica*), and white ash (*F. americana*) seed showed variation among provenances, among trees, and among species.
- Sugar maple (*Acer saccharum*) seed from New Brunswick required 12 weeks of moist chilling to alleviate dormancy as opposed to 8 weeks of moist chilling for seed from Québec and Ontario. Storing seed for one year at -20°C did not alleviate dormancy.
- Germination of white spruce (*Picea glauca*) seed stored at -20°C for 25 or 28 years did not change (94.5% before storage vs. 94.1% after storage).

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INTRODUCTION

This report covers the activities of the National Tree Seed Centre (NTSC) for 2005. Similar reports were prepared from 1998 – 2004. The report also captures the results of tests and experiments that were conducted during the year in order to assure that 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 seedlots increased by 404 to 11 855 in 2005. The numbers in brackets in Table 1 represent the numbers reported in the 2004 Annual Report.

Table 1. Seed stored at the NTSC as of December 31, 2005.

Seed Bank		Reserved		Tree Breeding		Gene Conservation	
No. Species	No. Seedlots	No. Species	No. Seedlots	No. Species	No. Seedlots	No. Species	No. Seedlots
175 (189)	5882 (4767)	40 (41)	1945 (1959)	9 (36)	1078 (2804)	18 (8)	2950 (1921)

Seed Bank seedlots are the active collection that are available for distribution. The number of Seed Bank seedlots increased by 1115 to 5882 in 2005. This increase is a result of the large number of collections that were made in 2004 and from the transferral of seedlots from Tree Breeding. 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, 2005, the NTSC Seed Bank had 120 Canadian species (5573 seedlots) in storage (Table 2). An additional 76 exotic species (315 seedlots) are also stored. Exotic species are defined as those that were collected outside Canada which may or may not be present in Canada. With the mandate of the Centre now concentrating on seed from Canadian tree and shrub species, the proportion of seed from exotic species is decreasing although some opportunistic acquisitions may still be made.

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 seedlots west of Ontario. Since collections by NTSC staff are unlikely due to distance and costs, these seedlots will have to be purchased or obtained through donation.

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

Province	No. Species	No. Seedlots	Percent
Alberta	11	50	1.2
British Columbia	31	302	6.7
Manitoba	7	65	1.2
New Brunswick	69	1191	22.6
Newfoundland	13	91	1.7
Nova Scotia	40	405	8.3
Ontario	53	2235	35.9
Prince Edward Island	32	222	2.1
Québec	18	886	17.7
Saskatchewan	8	79	1.8
Yukon Territory	3	47	0.8
Total		5573	100

The Reserved category contains seedlots that have been reserved by researchers. Many of these seed lots were collected for special projects. There were no significant changes in this category in 2005.

The Tree Breeding category is composed of seedlots that originated from the genetics program at PRF and were transferred to the Seed Centre for storage. The number of seedlots in this category decreased by 1726 to 1078 during 2005. Two major activities contributed to this reduction. All of the jack pine seedlots (1133 seedlots) were tested for viability. One hundred and ninety-three seedlots were discarded due to very low quantities of seed or poor germination. Of the remaining 940 seedlots, 160 remained in Tree Breeding while 706 were transferred to the Seed Bank and 74 to Gene Conservation. The other factor that accounts for the reduction in Tree Breeding seedlots is the discarding of white spruce seedlots with low germination. These seedlots were tested in 2001 and kept so that moisture contents could be performed to see if this was a factor contributing to the decline in germination. Once moisture content was determined, most of these seedlots were discarded while a few were transferred to Gene Conservation.

The Gene Conservation category was put in place to assure that genetic material obtained from rare, endangered, and/or unique populations is preserved as well as storing samples from throughout a species' ranges. This collection increased by 1014 to 2935 seedlots in 2005. There are 18 species in this category with the number of seedlots ranging from 1 for *Cephalanthus occidentalis* to 1635 for *Picea glauca*. Other species and number of seedlots contained in this category are: *Acer negundo* (15), *Acer pensylvanicum* (17), *A. rubrum* (28), *A. spicatum* (46), *Fraxinus americana* (186), *F. nigra* (44), *F. pensylvanica* (17), *Picea mariana* (363), *P. rubens* (3), *Pinus banksiana* (80), *P. flexilis* (100), *P. rigida* (4), *P. strobus* (17), *P. sylvestris* (12), *Prunus pensylvanica* (61), and *P. virginiana* (321).

SEED COLLECTIONS

Seed production was poor in 2005 with the exception of black ash (*Fraxinus nigra*) which produced a good seed crop for the second consecutive year. In order to ensure good quality seed, seed is only collected during good seed years. Seed collected in poor seed years may be of lesser quality because of poor pollination. Also, the time required to collect sufficient seed increases when there is a poor seed crop. Therefore, only 142 seedlots were collected by Seed Centre staff in 2005 compared to 549 seedlots in 2004.

Collections of black ash seed were made in Québec (2 sites 30 collections) and in N.B. (3 sites 52 collections). Other collections of note included 20 single-tree red maple (*Acer rubrum*) collections from Upper Smithfield, N.S., 15 pin cherry (*Prunus pensylvanica*) from Island Lake, N.B., and 15 choke cherry (*Prunus virginiana*) from Chicoutimi, Quebec. Table 3 provides a complete list of the collections made in 2005.

Table 3. Seed collections made by Seed Centre staff.

Species	N.B.	N.S.	QC	P.E.I.	Total
<i>Acer saccharinum</i>	1				1
<i>Acer rubrum</i>	3	20		1	24
<i>Fraxinus nigra</i>	52		30		82
<i>Queccus macrocarpa</i>	1				1
<i>Quercus rubra</i>	1				1
<i>Prunus pensylvanica</i>	15				15
<i>Prunus virginiana</i>			15		15
<i>Tilia americana</i>	2				2
<i>Ulmus americana</i>	1				1
Total	76	20	45	1	142

The Seed Centre also acquired 22 pin cherry and 13 red maple seedlots from Newfoundland. Finally, 4 balsam poplar (*Populus balsamefera*) seedlots were purchased from Ontario and 3 red maple seedlots from Minnesota.

A total of 184 seedlots were acquired by the Seed Centre in 2005. Table 1 shows the increase in the number of seedlots in the Seed Bank collection since 1996. The increase in 2005 is a result of the large number of seedlots collected in 2004 as well jack pine seedlots moved from Tree Breeding. Since 1996, the number of seedlots in the Seed Bank collection has increased from 3079 to 5882 which represents an increase of over 90% (Figure 1).

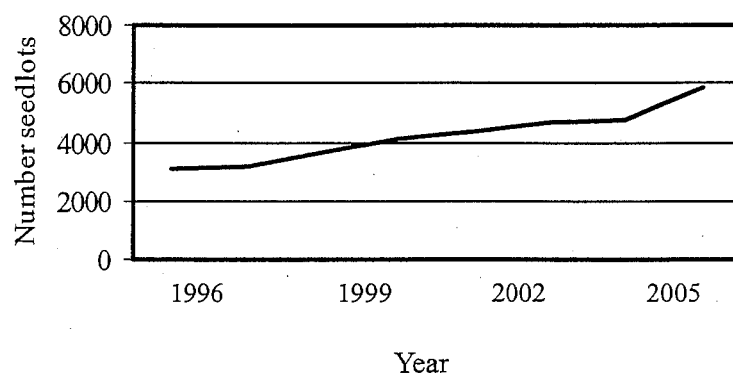


Figure 1. Increase in the number of Seed Bank seedlots at the NTSC since 1996.

The increase shown in Figure 1 represents the net gain in the Seed Bank seedlots. 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 seedlots acquired by the NTSC since 1996.

Table 4. Number of seedlots acquired by the NTSC through collection, donation, and purchase between 1996 and 2005.

Year	Number of Seedlots			Total
	Collection	Donation	Purchase	
1996	239	22		261
1997	75	245		320
1998	284	47	9	340
1999	139	80		219
2000	195	673		868
2001	137	122	45	304
2002	367	36		403
2003	69	142		211
2004	549	381	137	1067
2005	142	29	4	185
Total	2196	1777	195	3994

The reason that the number of seedlots in Table 4 and the number reported in Table 1 do not match is due to the fact that the collection is also being affected by increases due to transfer from Tree Breeding and also through reductions resulting from seedlots being exhausted due to distribution for research or discarded because of low germination.

SEED REQUESTS

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

During 2005, a total of 65 requests representing 812 seedlots was processed. The majority of the requests were from Canada but seed was also sent to China, England, Finland, France, Sweden, and the United States (Table 5). The number of seedlots provided for research by the NTSC since 1967 has ranged from a low of 99 in 1996 to a high of 1603 in 1985 (Figure 2). Canadian researchers received 68% of the seed that was provided by the NTSC while seed sent to researchers outside Canada accounted for the other 32%.

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

Country	No. Requests	No. Seedlots
Canada	55	743
China	2	13
England	1	1
Finland	1	17
France	1	6
Sweden	1	13
United States	4	19
Total	65	812

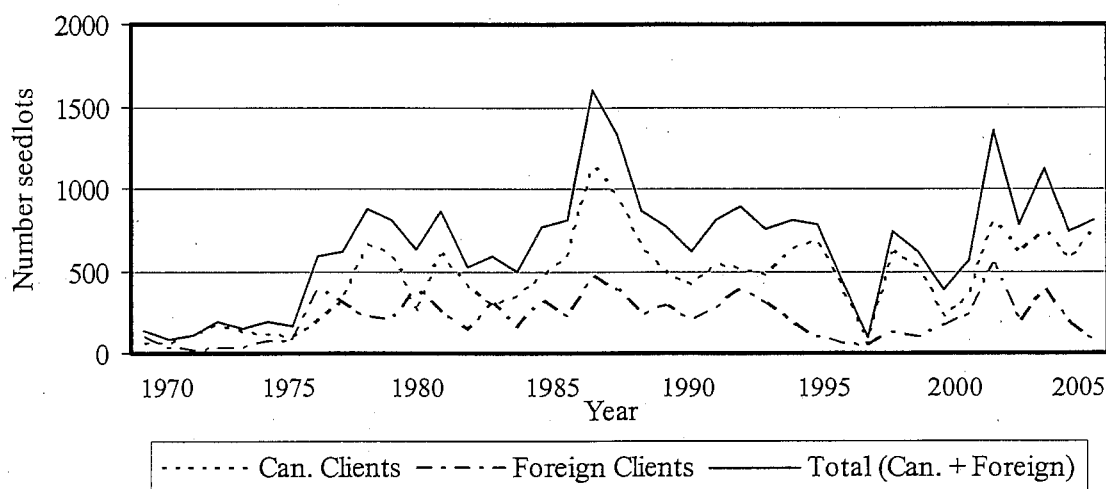


Figure 2. Number of seedlots sent to clients between 1967 and 2005.

SEED TESTING

Germination tests are performed on all freshly collected seedlots as well as seedlots in storage that have not been tested for several years. In most cases, due to small seedlot 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. **One thousand seven hundred and twelve germination tests** were carried out.

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 transferral of the Seed Centre from Petawawa to Fredericton.

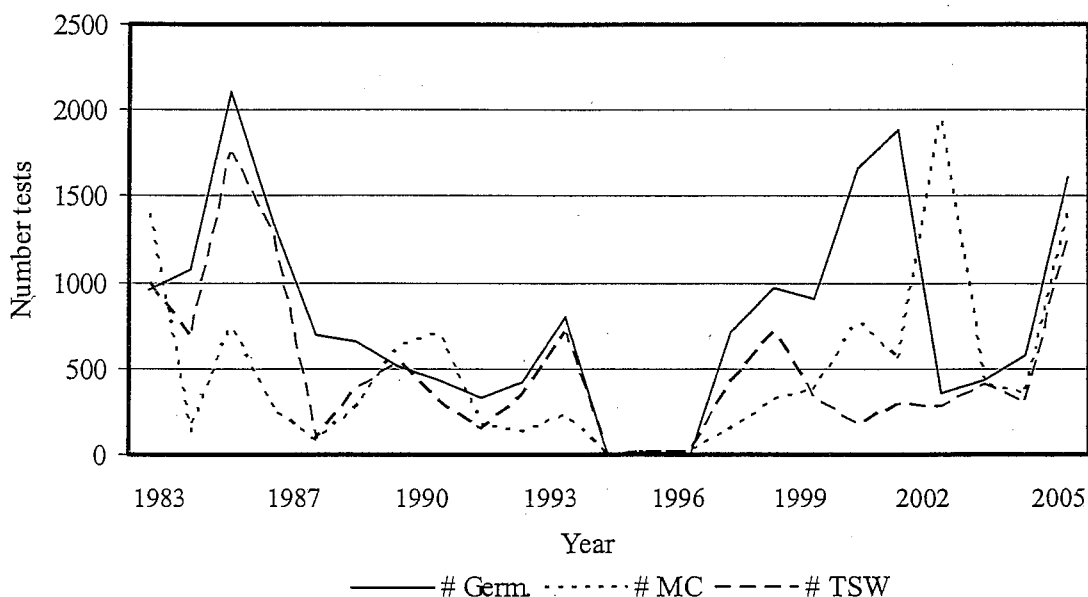


Figure 3. Number of germination tests (# Germ), moisture content tests (# MC), and thousand seed weights (# TSW) carried out by the NTSC since 1983.

The target moisture content for orthodox seed is between 5 and 8 %. Seed that are above this range are further dried before being stored. **One thousand three hundred and ninety-eight 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 1000 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 **one thousand two hundred and sixty-seven 1000-seed weights** was done.

RESEARCH AND DEVELOPMENT

Germination Testing of Jack Pine Tree Breeding Seedlots

In 2005, an assessment was carried out on 1133 jack pine (*Pinus banksiana*) seedlots that were stored in Tree Breeding. These seedlots ranged in size from a few seeds to several hundred grams. Seedlots collected prior to 1961 had been used in a range-wide provenance experiment established in the early 1960s. Most of the seedlots had quantities of less than 10 g. Some of these seedlots had been collected in the 1950s but most were collected in the late 1960s and through the mid-1980s and were presently being stored at -20°C. Seedlots from controlled pollinations (160 seedlots) remained in the Tree Breeding category and were not tested. Seedlots that had only a few seed in storage were not tested and were discarded. A total of 786 seedlots was tested: 706 seedlots were transferred to Seed Bank, 74 to Gene Conservation and 6 were discarded due to poor germination. Germination results were generally very good (Table 6).

Table 6. Germination results in 5-year age increments of jack pine seedlots tested in 2005.

Collection years	Nos.seedlots tested	Germination range	Average germination
1949–1955	14	3–80	61
1956–1960	42	8–97	64
1961–1965	11	0–96	54
1966–1970	180	7–100	81
1971–1975	67	1–100	83
1976–1980	318	0–100	92
1981–1986	154	79–100	94

The only germination testing that was carried out on the seedlots prior to 2005, was done in 1986. Almost 72% (565/786) of the seedlots tested in 2005 were also tested in 1986. This provides a good dataset to examine the storability of jack pine seed. Table 7 shows provenances that contained 5 or more seedlots and were tested in both years.

The loss in viability for all seedlots in Table 7 was 0.13%/year. This is remarkable since all seedlots that met the criteria of having been tested in 1986 and 2005 are included. Some seedlots in this sample were of poorer quality and the difference in germination after 19 years is much greater than the average. There were also some seedlots that increased in germination. In order to obtain a clearer picture of the actual potential for storability of jack pine seed, all seedlots with a germination difference of greater than 5 % or 10 % between the two test periods were removed from the dataset. Results indicate that the change in germination per year went from 0.13 to 0.07% when seedlots with > 10% (+/-) were removed and that this figure was further reduced to 0.03 when the criterion was changed to > 5 % (+/-).

Table 7. Germination results (%) of jack pine seedlots from various provenances tested in 1986 and in 2005.

Provenance	Coll. year	No. Seedlots	Range		Avg. germ (%)		Δ Germ/yr.
			1986	2005	1986	2005	
Deux Rivières	1967	16	82–99	84–99	94.1	93.5	-0.03
Deep River	1967	14	72–99	64–99	94.3	90.4	-0.21
Highview	1967	16	88–99	79–99	94.3	90.4	-0.21
Petawawa River	1967	16	86–99	82–100	95.9	93.8	-0.11
Petawawa Plains	1967	10	94–99	87–98	97.2	94.2	-0.16
Sturgeon Lake	1967	10	28–100	41–95	86.4	80.1	-0.33
Atikokan	1967	9	80–96	75–96	90.4	85.6	-0.25
Grand Calumet River	1969	15	86–99	71–98	91.9	85.9	-0.32
Round Lake	1973	6	72–100	20–100	92.3	76.7	-0.82
Montgomery Plains	1973	5	84–100	47–100	94.0	86.4	-0.40
Sturgeon Lake	1974	16	95–100	80–100	97.9	94.3	-0.19
Petawawa Plains	1975	12	88–100	88–99	95.9	96.0	+0.01
Spoor Lake	1977	142	79–100	61–100	93.7	91.2	-0.13
Spoor Lake	1978	144	89–100	70–100	98.1	96.4	-0.09
Algonquin Park	1985	10	97–100	92–100	98.6	98.0	-0.03
Estaire	1986	5	97–100	88–100	98.6	96.0	-0.14
Russian Lake	1986	24	88–99	83–100	95.1	92.8	-0.12
Ritchie Falls	1986	10	96–100	94–100	98.8	97.9	-0.05
Alces Lake	1986	15	78–100	75–100	94.1	92.7	-0.07
Ritchie Falls	1986	40	56–100	57–100	96.6	95.7	-0.05
All Provenances	NA	535	28–100	20–100	95.5	93.0	-0.13
> 10% difference germination	NA	495	56–100	57–100	95.7	94.4	-0.07
> 5% difference germination	NA	422	56–100	57–100	96.2	95.6	-0.03

Ash Seed Collection Project

In 2004 a project was initiated to collect seed primarily from white ash (*Fraxinus americana*) and black ash (*F. nigra*) and, in addition, red ash (*F. pensylvanica*) from across the species' range. The project was initiated as a response to the threat caused by the emerald ash borer (EAB). This exotic pest has caused tremendous damage to ash stands in Michigan and has established itself in southwestern Ontario. Although considerable research is ongoing, there are no known measures to prevent the EAB from killing ash trees. Seed collected will be set aside for gene conservation and to carry out molecular work to determine genetic variation of the species. Surplus seed will be stored in the Seed Bank collection.

White Ash Collections

In 2004, there was an excellent seed crop on white ash in the Maritimes region as well as in Québec and Ontario. Seed from Maritime locations was collected by Seed Centre staff while seed from Ontario and Québec was purchased from seed collectors. A total of 199 seedlots was collected (Table 8).

Table 8. Results of viability tests performed on white ash seed collected in 2004.

Provenance	Province	No. Seedlots	Viability (%)	
			Range	Average
Darlingside	ON	18	17–88	61
Dempsey's Corner	NS	15	48–87	68
Florence	ON	5	63–95	77
Glenroy	NS	15	52–92	76
Gordon Vale	NB	14	16–87	56
Highgate	ON	14	63–95	77
Judique	NS	15	23–92	65
Lynfield	NB	15	57–93	77
Meductic	NB	13	61–93	81
Richmond	PE	15	77–100	88
Rosevale	NB	14	44–87	63
Shefford	QC	15	72–99	90
Wentworth	NS	15	55–95	76
Smiley Park	NS	16	27–89	74
All provenances		199	17–100	74

White ash seed have wings that occupy a lot of storage space and make the removal of empty and insect damaged seed very difficult. De-winging of the seed was attempted using a Dybvig macerator but this method did not remove all of the wing and also damaged seed. There was too much wing remaining to efficiently blow the seed in the air aspirator. Seed were therefore de-winged manually in denim bags. Work gloves had to be worn as the seed would punch through the denim and pierce the skin. The seed were rubbed twice and sieved and blown between each rub. The seed could then be blown and this removed much of the non-desirable seed and greatly increased viability. Although comparison of viability between winged and de-winged seed was not done, some seedlots had more than half of the seed removed.

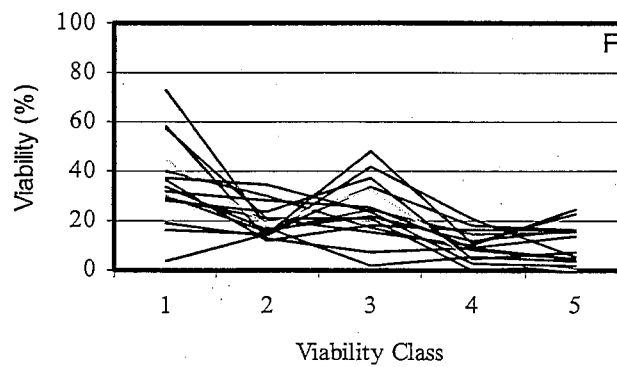
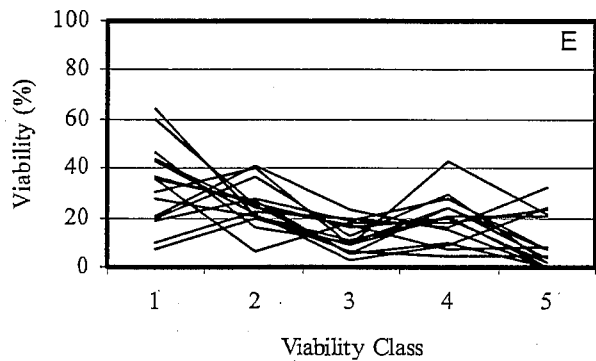
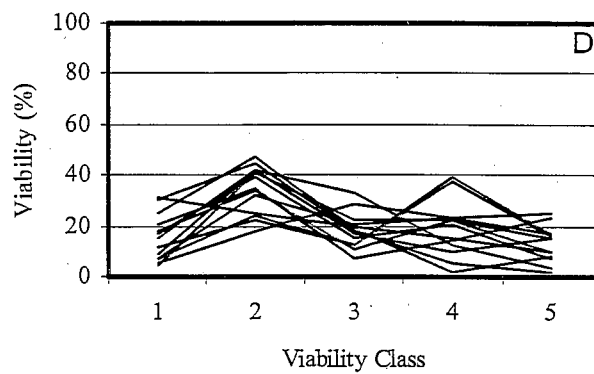
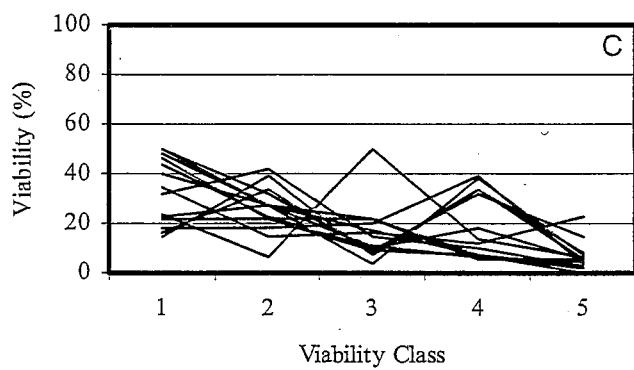
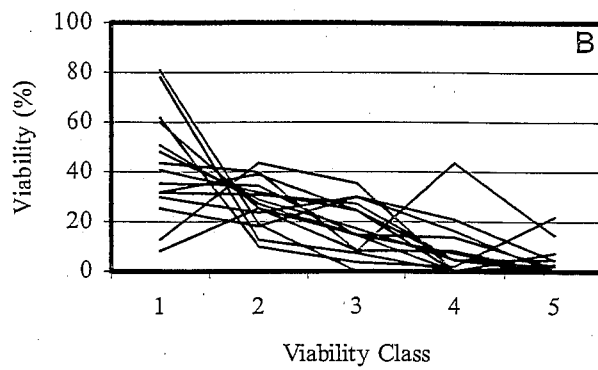
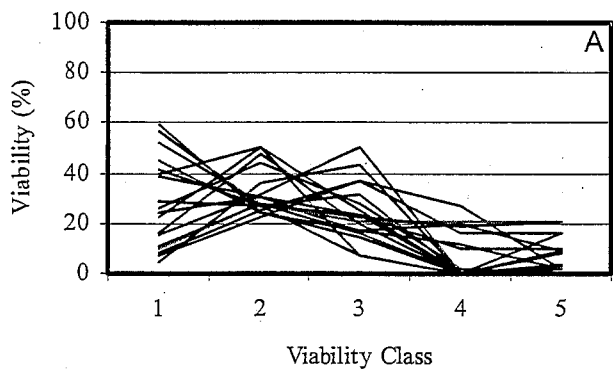
Seed viability was determined by performing excised embryo tests on the seed. Three replicates of 25 seed each were used. The pericarp was removed and the seed were placed in water and soaked at 3°C for 120 hours. ISTA (2006) recommends soaking the seed in tap water for 24 – 96 hours. Although 96 hours is sufficient for good imbibition to occur, many seed were still “starchy” (endosperm inside the seed was sticky which made the embryo difficult to remove). Seed that were empty or damaged by insects or other pathogens were tallied and removed. The remaining embryos were placed on moistened Kimpak in germination boxes and placed in a germination cabinet for 14 days set at 25°C with 8 hours light and 16 hours without light and a relative humidity of 85%.

During an excised embryo test the embryos often exhibit no growth but remain alive and are therefore considered viable. Other embryos show varying degrees of activity ranging from some greening of a single cotyledon to both cotyledons and radicle showing signs of growth. For the purpose of these assessments embryos were classified into 6 categories as follows:

Class 0	Embryo absent, dead, or damaged by insect or pathogen.
Class 1	Embryo alive but exhibiting no activity
Class 2	Embryo alive and exhibiting very little activity (one cotyledon green)
Class 3	Embryo alive and exhibiting moderate activity (both cotyledons green)
Class 4	Embryo alive and exhibiting strong activity (both cotyledons green and some radicle activity)
Class 5	Embryo alive and exhibiting very strong activity (both cotyledons and radicle actively growing)

There was considerable variation in seed viability among the individual collections (Table 8) as well as among trees from the various locations. Figure 4 shows the viability classes of seed from individual trees from 12 populations. In order to provide a more accurate view of the viability classes, only viable seed were used (dead, empty, insect or diseased seed were not included). The populations from Florence and Dempsey’s Corner are not included because seed was collected from only 5 trees at Florence and the seed from Dempsey’s Corner was tested prior to establishing the viability classes.

The difference between populations can be seen in Figure 5 where curves from 12 populations are compared along with a curve representing the average of all trees in all populations. These figures show that there exists a great deal of variability in the viability characteristics of white ash seed between trees in a population as well as between populations. On average, the percentage of viable seed decreases as seed development increases from Class 1 (no activity) to Class 5 (very strong



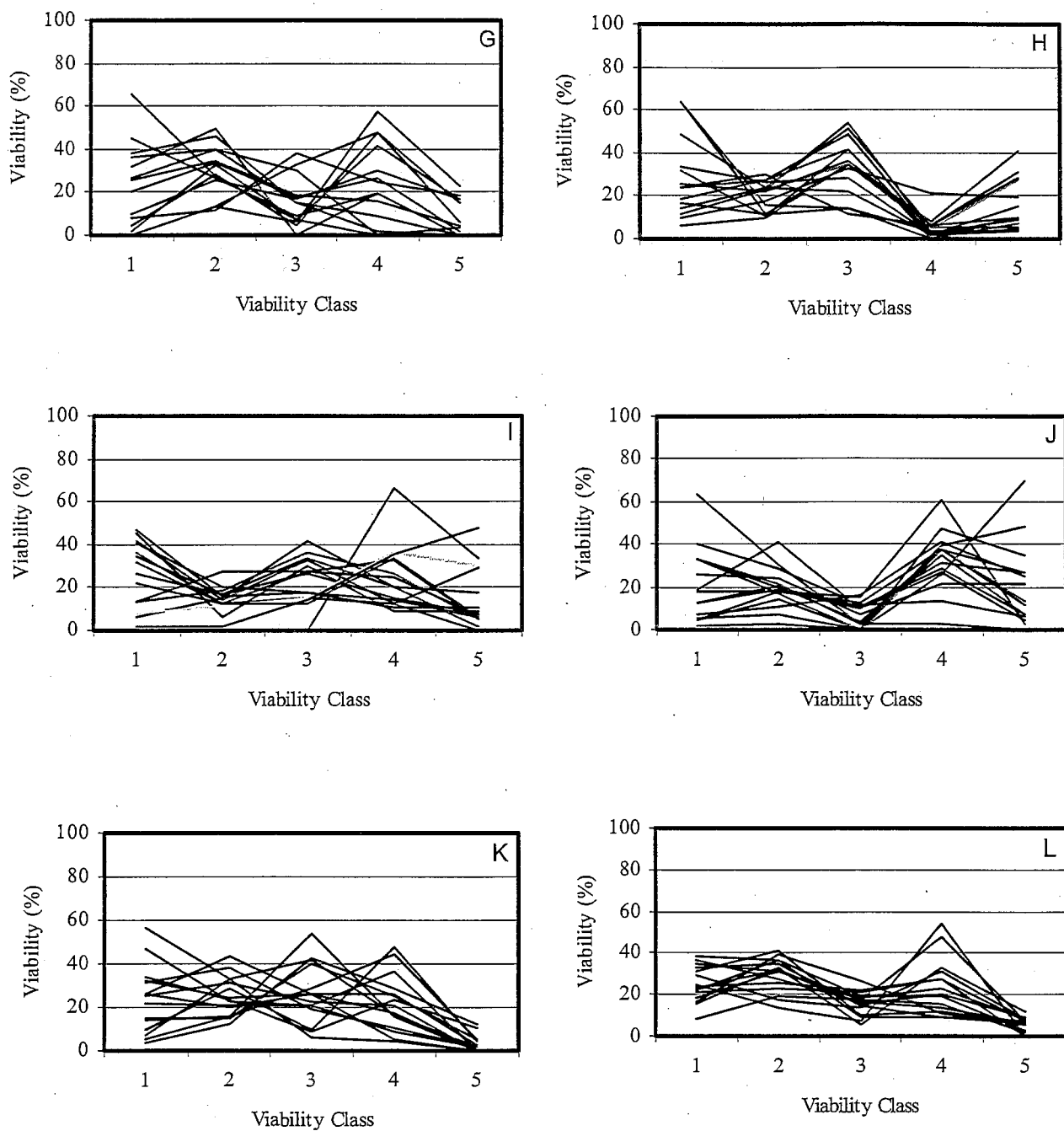


Figure 4. Seed viability curves for individual trees from 12 populations of white ash. (A = Darlingside, B = Glenroy, C = Gordon Vale, D = Highgate, E = Judique, F = Lynfield, G = Meductic, H = Richmond, I = Rosevale, J = Shefford, K = Smiley Park, L = Wentworth).

activity). This indicates that dormancy is probably a contributing factor to the degree of seed activity. The higher proportion of seed in Class 1 is due to their still being dormant, hence no activity.

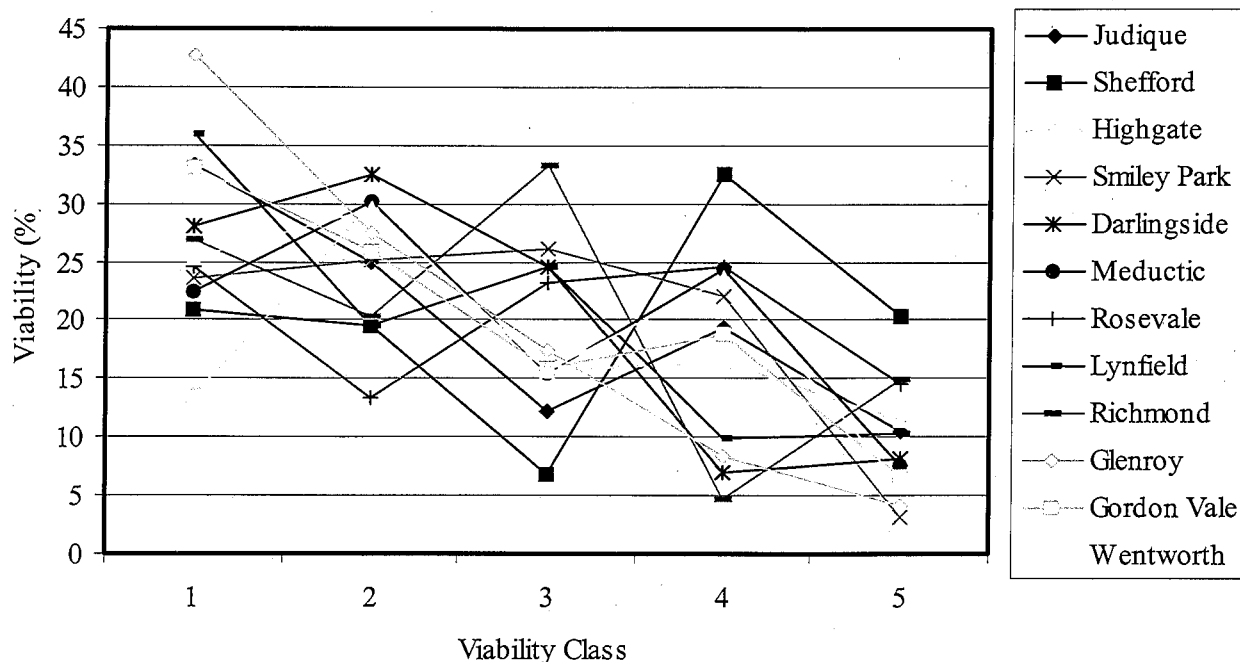


Figure 5. Seed viability curves of 12 populations of white ash.

Black Ash Collections

One hundred and twenty-six seedlots of black ash were collected from 8 locations in New Brunswick, Québec, and Manitoba. The seed from New Brunswick and Québec were collected by Seed Centre staff while the seedlots from Manitoba were acquired from collections made through Agriculture and Agr-Food Canada's PFRA Shelterbelt centre located at Indian Head, Saskatchewan. Table 9 shows the collections of black ash seed that were made in 2004 and 2005.

The range in viability is generally greater than what was found for white ash. The main reason for this is the inability to remove dead, empty and damaged seed. Black ash seed cannot be easily de-winged without damage. The seedlot that was received from Manitoba (Sandilands) did have the wings removed and the average viability of this provenance was higher than the others. However, seeds were broken and even seeds that were apparently intact had damaged embryos.

Table 9. Results of viability tests performed on black ash seed collected in 2004 and 2005.

Provenance	Province	No. Seedlots	Viability (%)	
			Range	Average
Caribou Depot	NB	17	12–89	59
Lynfield	NB	11	9–64	34
Petit Saguenay	QC	6	16–89	65
Riviere Cascapedia	QC	24	12–92	67
Robinsonville	NB	17	49–96	72
Sandilands Provincial Forest	MB	15	34–92	77
Second Falls	NB	18	44–95	74
Watson Settlement	NB	18	12–73	57
All Provenances		126	9–96	63

The difference between populations can be seen in Figure 6 while seed viability graphs for seed of individual trees in 6 of the 8 populations are shown in Figure 7. The results of the tests from the collections made at Petit Saguenay are not included since seed from only 6 trees was collected. Black ash seed are immature when seed are shed and the seed are dormant, requiring several months of warm and cold treatments for the embryo to mature and to alleviate dormancy before the seed is capable of germinating. The graphs clearly show that most of the seed remains in viability class 1 (embryo alive but exhibiting no activity).

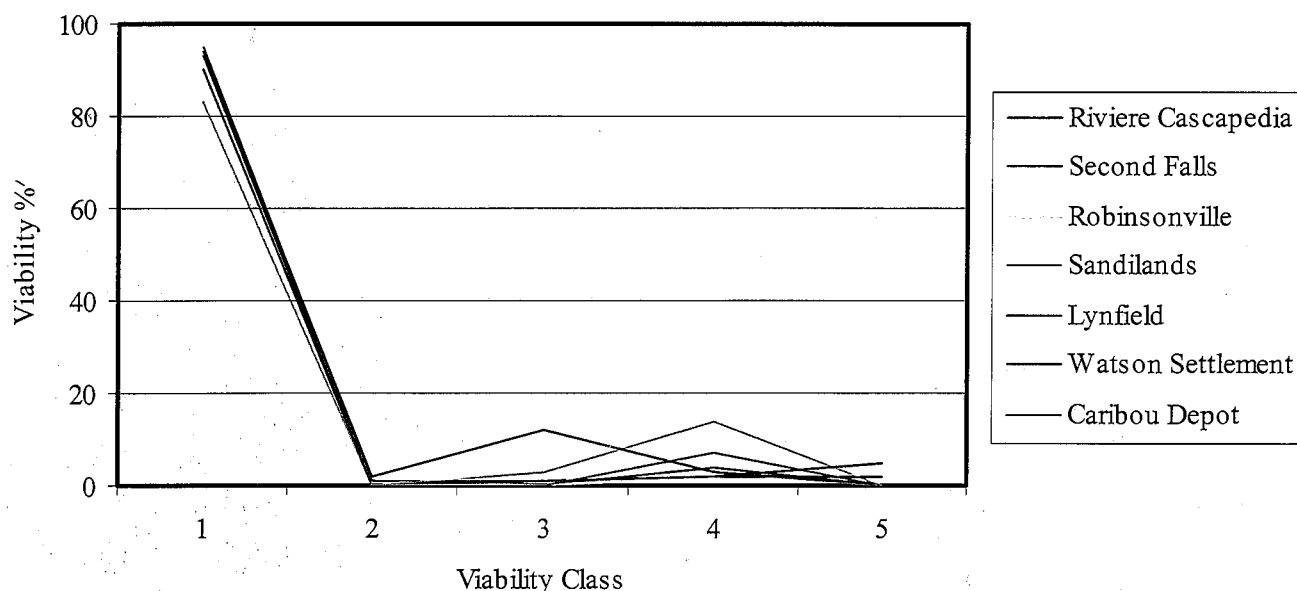


Figure 6. Seed viability curves for 7 populations of black ash.

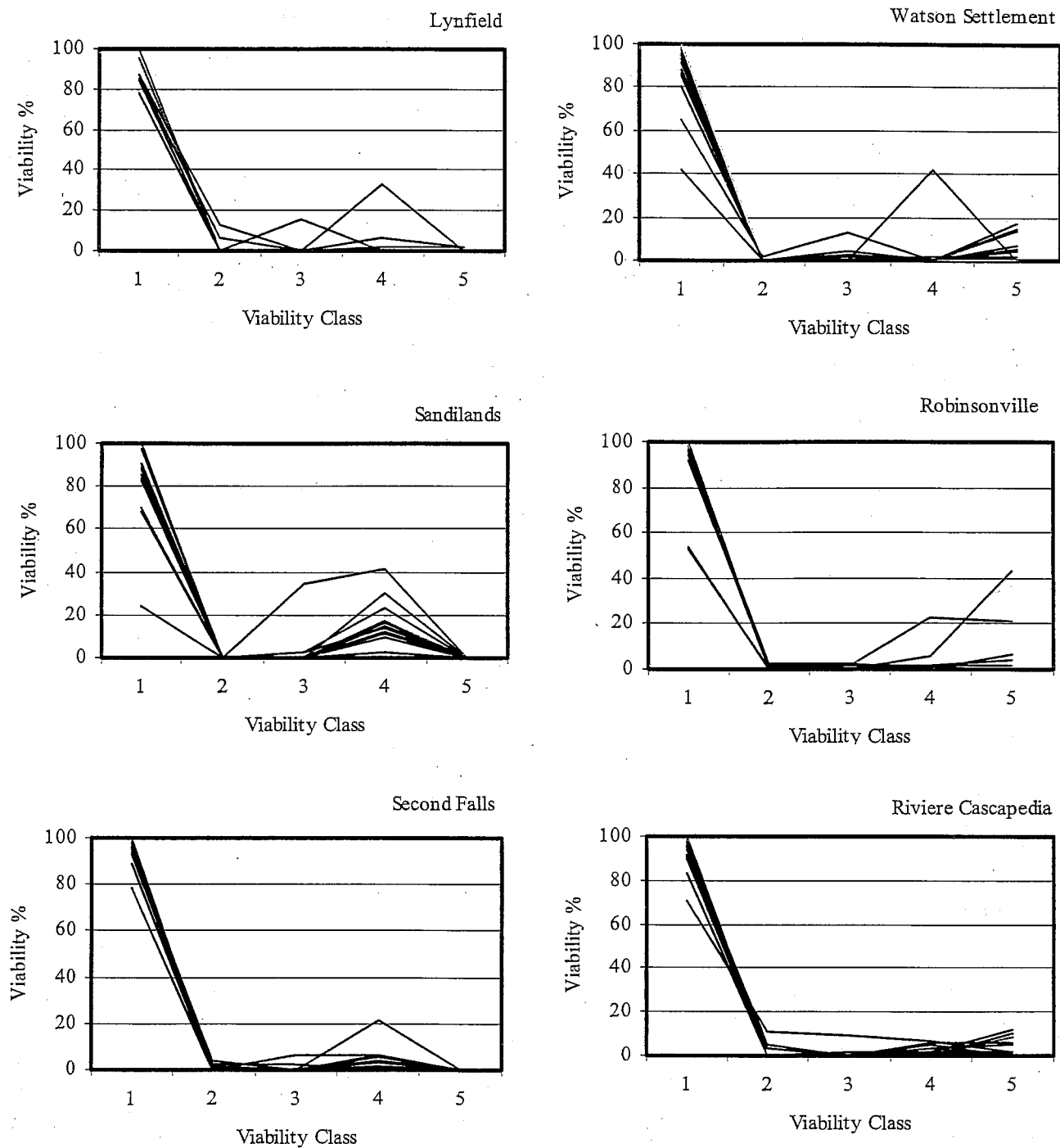


Figure 8. Seed viability curves for individual trees from 6 populations of black ash.

As expected, the black ash embryos were generally more dormant than those of white ash with the majority of the embryos exhibiting no greening of the cotyledons or radicle activity. However, there were some embryos that did show some activity and there were some differences between individual trees. There was less variability among viability classes compared to that found for white ash.

Red Ash Collections

Seed from 5 populations of red ash was collected in 2004. It was possible to remove some of the wing from the samaras. Care was necessary because the seed are more delicate than white ash. Even with most of the wing removed, it was difficult to successfully separate the filled seed from the empty and disease/insect damaged seed. Unfortunately many seedlots were of poor quality and had to be discarded. Thirty-one seedlots were of sufficient quality (16 Manitoba, 4 New Brunswick, and 11 Ontario) (table 10). Seed viability tests were performed using the same criteria as was used for white and black ash seed. Average viability is less than that for white and black ash seed and the range of viability is also less.

Table 10. Results of viability tests performed on red ash seed collected in 2004.

Provenance	Province	Nos. seedlots	Viability (%)	
			Range	Average
Florence	ON	6	41–65	53
Fredericton	NB	4	36–55	46
Birds Hill Park	MB	2	51–64	42
Darlingside	ON	5	40–53	47
Bride Hill Prov. Park	MB	14	27–73	54
All Provenances		31	27–73	52

The viability curves for red ash seed are very different from those of white and black ash. Red ash seed is less dormant and the embryo is mature when the seed is shed. The viability curves support this as only a small percentage of the seed showed no activity (Figure 8). This is in vast contrast to black ash where over 90% of the seed showed no activity.

It is interesting to note that the seed collected from New Brunswick behaved differently than the seed from Ontario and Manitoba (Figure 9). A greater proportion of the seed from New Brunswick was in viability class 5 (embryo alive and exhibiting very strong activity). This may be because the seed collected in New Brunswick are likely northern red ash (*Fraxinus pensylvanica* var. *austini*) and that the characteristics of this sub-species are sufficiently different so as to manifest itself in the viability test. However, further evaluation should be carried out since the New Brunswick population only consisted of 4 trees.

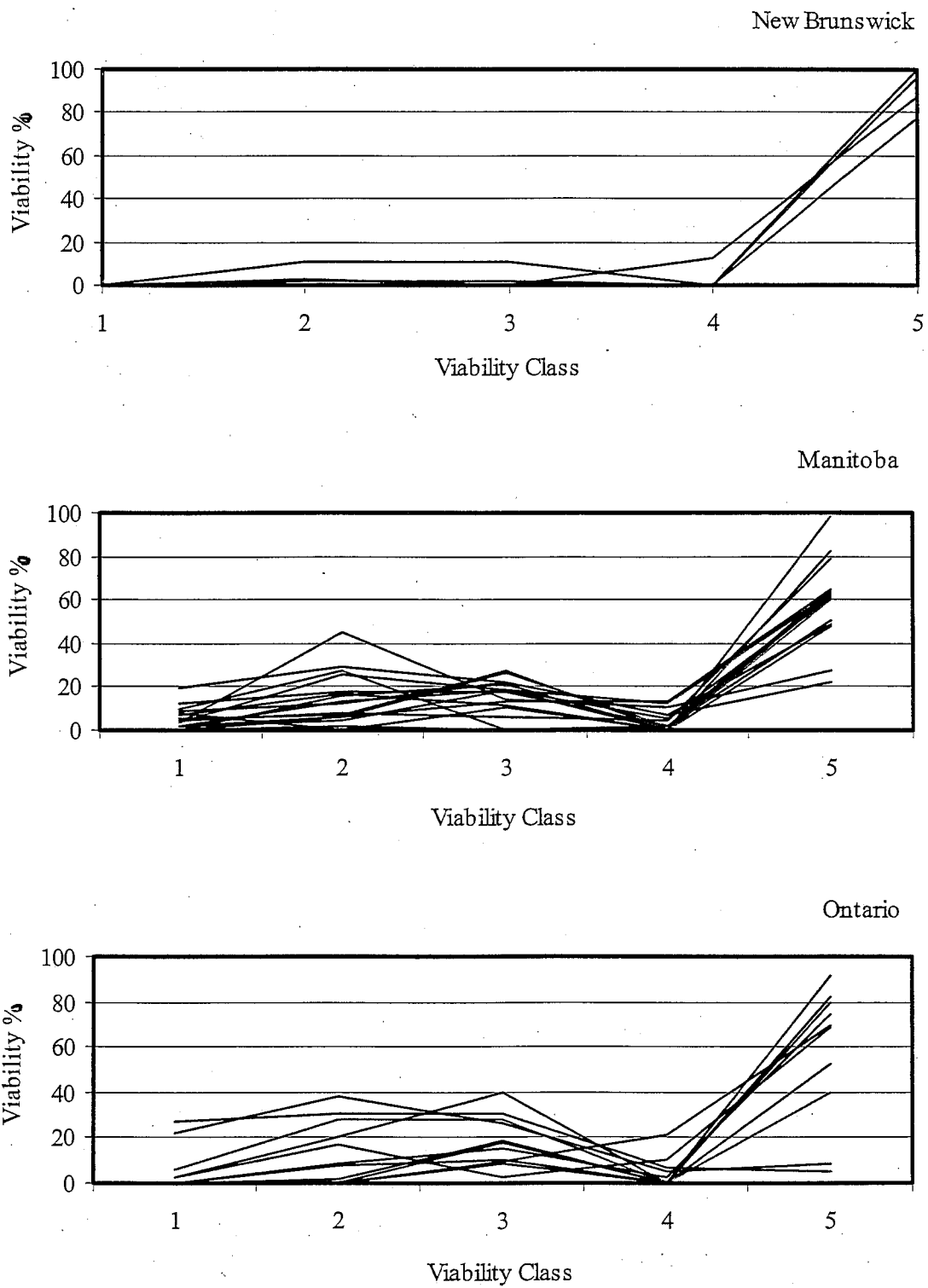


Figure 8. Seed viability curves for individual trees from 3 populations of red ash.

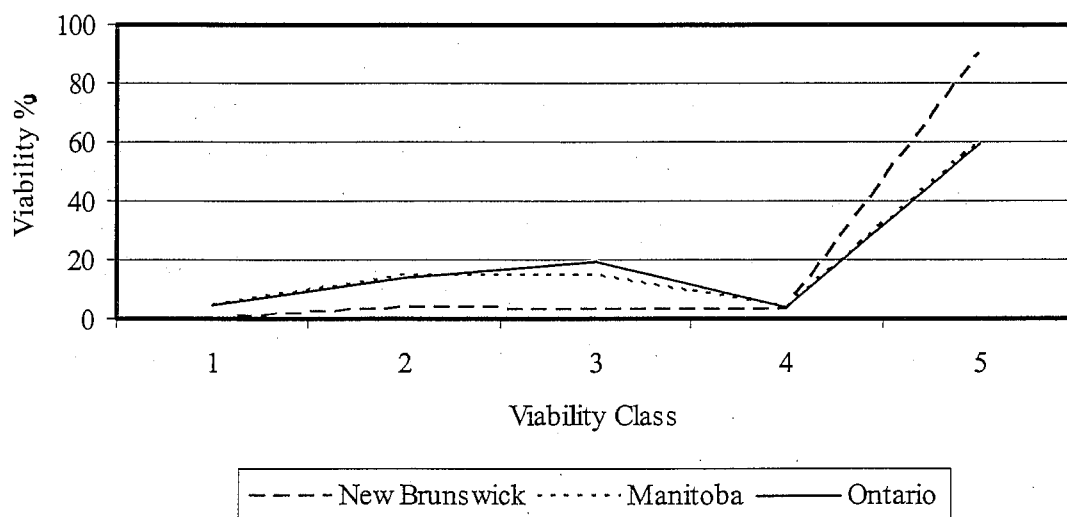


Figure 9. Mean viability curves for red ash seed collected from 3 provinces.

Figure 10 shows the mean viability curves for each of the three ash species. There is a distinct difference between the 3 species with red ash embryos showing very little dormancy, white ash embryos showing intermediate dormancy, and black ash embryos very dormant.

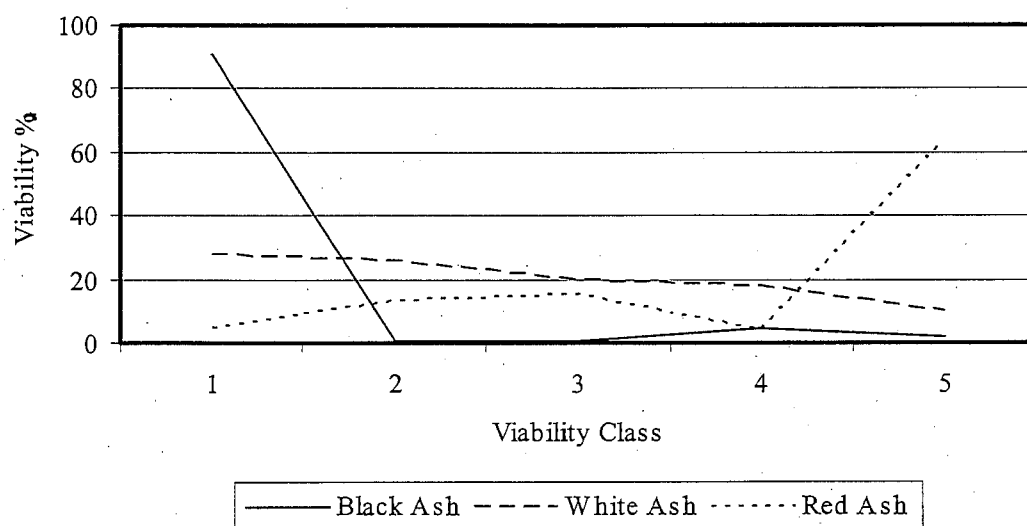


Figure 10. Seed viability curves for 3 ash species.

Sugar Maple Germination Experiment

A preliminary germination trial conducted in 2002, using seed already in storage indicated that seed of sugar maple (*Acer saccharum*) from the Maritimes required a longer period of chilling to alleviate dormancy than what is prescribed by ISTA (2003). The Rules recommend 8 weeks of chilling whereas the trial demonstrated that 12 to 15 weeks were required to maximize germination. This trial also included one seedlot from Ontario which indicated that a chilling time of 9 weeks was sufficient to alleviate dormancy (Daigle and Simpson 2003). A more comprehensive experiment was set up based on the following three hypotheses: 1) seed from Maritime provenances requires a longer period of moist chilling to alleviate dormancy than seed from Québec and Ontario provenances, 2) the chilling time prescribed by ISTA is not adequate to promote germination of Maritime seed sources, and 3) dormancy is diminished when seed is stored at -20°C for at least 12 months.

Methods

Seed collected in 2002 from three single trees at each of two locations in New Brunswick (NB) was used. As well, seed from two bulk collections was obtained from each of the Québec Ministry of Forests and Ontario Ministry of Natural Resources seed centres (QC/ON). Sufficient seed was obtained to allow for storing half the seed at -20°C and the experiment repeated after one year of storage to evaluate the effect of storage on dormancy and germination. This report summarizes the experiment conducted on the stored seed and compares the results to the initial trial conducted in 2003 (Daigle and Simpson, 2004).

The experimental design consisted of: 2 constant germination temperatures of 20°C and 15°C , with 8 hrs light/16 hrs dark and 85% RH; 3 soaking treatments of 0, 72 hrs, and 14 days; and 3 moist chilling durations of 8, 12, and 16 weeks. As well, a control of no soak and no chill was tested. Seed was soaked in beakers using de-ionized water and the beakers placed in a walk-in cooler set at 3°C . Water was changed after 3 days and again after 7 days. The reason for soaking the seed at this temperature was to expose the seed to chilling conditions which may reduce the total chilling time required to overcome dormancy. Three replicates of 25 seed each were placed on moist Kimpak in Petawawa germination boxes. The boxes were placed in a cooler maintained at 3°C for the prescribed chilling times.

To evaluate the rapidity of water imbibition seed was placed on moistened Kimpak in germination boxes and placed in the same walk-in cooler as the seed in beakers of water. A sample from each seedlot was removed from the Kimpak and beakers after 24, 48 and 72 hours and 7 and 14 days and moisture content (MC) determined. Seed was removed from the pericarp after imbibition and surface dried before MC was determined.

Starting at week six, and repeated every second week until week sixteen, seed of the three soak times was scored for radicle emergence which is indicative of dormancy being alleviated. As the duration of chilling increased seed was also scored for radicle growth up to a fully germinated seed.

Seed remained in the germination cabinets for 28 days and germination was assessed every 7 days. A seed was considered to have germinated when the cotyledons were visible and the radicle was well developed. On day 28 all ungerminated seed was assessed. Seed with intact tissue was evaluated by a tetrazolium test to determine whether they were viable or dead. Seed that was empty or insect damaged was eliminated and germination was calculated as a percentage of filled seed.

Results and Discussion

Although all seedlots had about the same initial MC prior to soaking, the NB seed consistently had a higher MC after 24 hrs than the QC/ON seed and maintained this difference over the 14 day duration for seed soaked in water as well as on Kimpak (Figure 11). Seed imbibed water rapidly during the initial 24 hr period and showed a steady increase up to day 14. Seed soaked in water had a greater MC than seed on Kimpak. These imbibition curves for seed soaked in water are practically identical to those from 2003. Within the QC/ON seed sources, the two Ontario sources exhibited a lower MC than the Québec seed throughout the duration of soaking which was consistent with the 2003 trial. Seed MC increased about 10%, on average, for both seed sources between 24 hrs and 14 days for both soaking regimes.

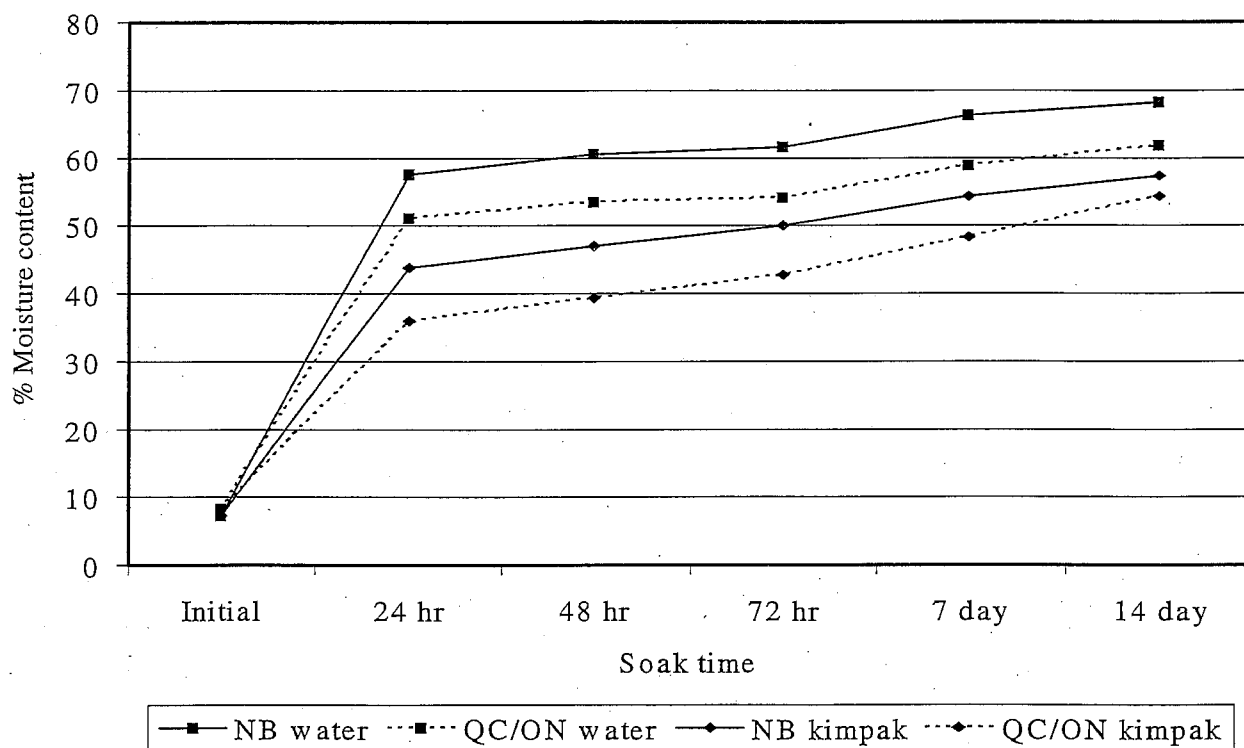


Figure 11. Change in seed moisture content at various intervals during soaking in water and on Kimpak for NB and QC/ON sources.

Seed exposed to the control conditions of no soaking or moist chilling resulted in one germinant in each of the two germination temperatures for one Ontario seedlot. The lack of germination indicates the seed were still dormant and not capable of germinating until dormancy was alleviated. Tetrazolium testing showed that 80–95% of these seed were still viable however, one seedlot had viability of only 42–56%.

Seed germination was significantly different between the NB and the QC/ON seedlots (Table 11). The explanation for this is the higher percentage of dead seed in the QC/ON seedlots across all treatments (Figure 12 vs 13). Soaking seed for 14 days consistently resulted in more dead seed for NB seedlots with twice as many dead seed after 14 days of soaking compared to no soaking. For the QC/ON seedlots, soaking seed for 3 and 14 days produced more dead seed than no soaking but mortality of seed that was not soaked was also high. Two of the four QC/ON seedlots had a consistently higher percentage of dead seed in the 14 day soak treatment. Germination temperature had little impact on seed mortality as the percentage of dead seed varied little between the two germination temperatures and corresponding soaking times within a chilling time (Figures 12 and 13). Seed was likely damaged and even killed as a result of the longer soaking and chilling times. These results are consistent with those found in the 2003 experiment using unstored seed. However, germination was lower and percent dead seed was higher for the stored seed which may indicate that seed quality deteriorated during storage.

Table 11. Results of a Duncan's test for sugar maple seedlots from two regions and treated with three durations of soaking and chilling and germinated at two temperatures.

Region	Mean Germ.	Soak Time	Mean Germ.	Chill Time	Mean Germ.	Germ. Temp.	Mean Germ.
NB	70.0a ¹	0	71.2a	8	58.3a	15°C	67.1a
QC/ON	60.5b	3	73.7a	12	70.8b	20°C	65.3a
		14	53.6b	16	69.5b		

¹ means with different letters are significantly different P = 0.05

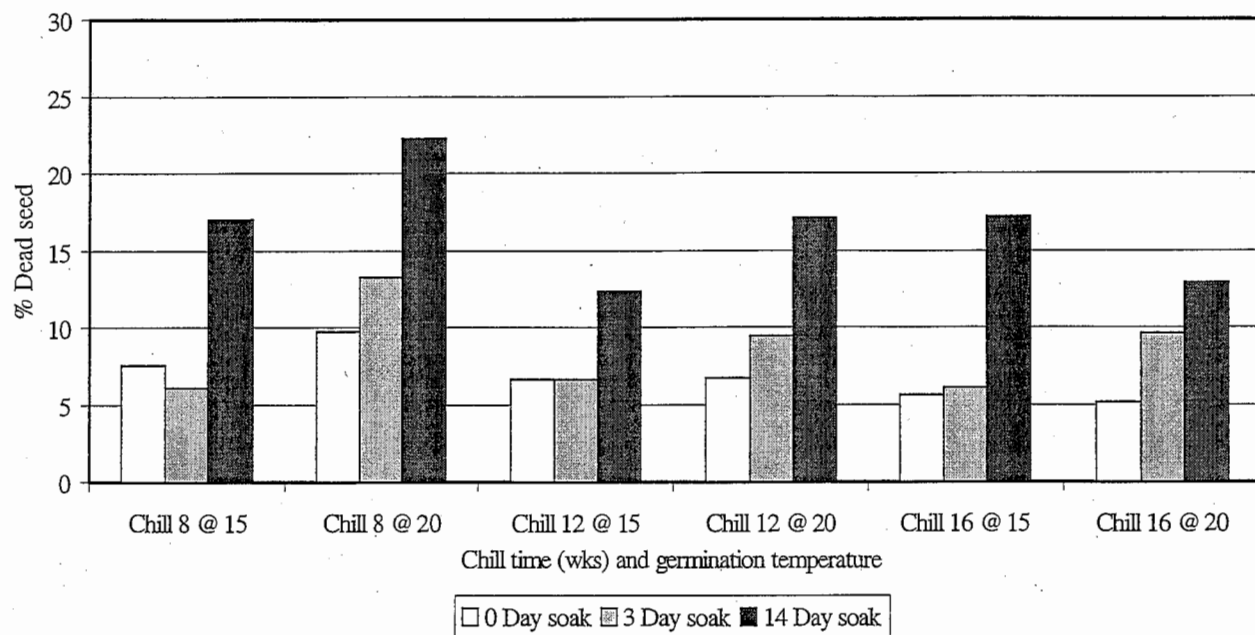


Figure 12. Mean percentage of dead seed for New Brunswick seedlots for three soaking and chilling durations and two germination temperatures.

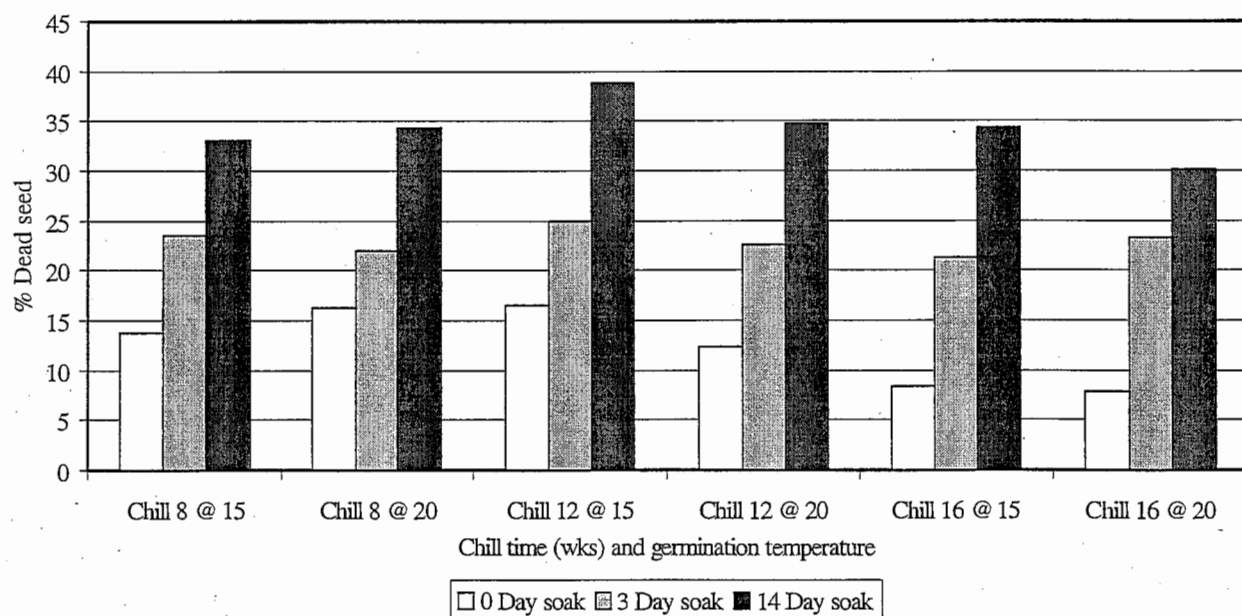


Figure 13. Mean percentage of dead seed for Québec/Ontario seedlots for three soaking and chilling durations and two germination temperatures.

Germination of seed soaked for 14 days was significantly lower than for seed not soaked or soaked for 3 days and chilling seed for 8 weeks resulted in significantly lower germination (Table 11). There was no significant difference in germination between the two germination temperatures, therefore seed germination data from the two germination temperatures were combined to provide an overall indication of the impacts of soaking and chilling durations (Figures 14–19). Soaking seed for 3 days followed by chilling for 8 weeks improved germination of the NB seedlots over the treatment of no soaking and 8 weeks of chilling (Figures 16 vs 14). Soaking seed from QC/ON sources for 3 days resulted in similar total germination for the three chilling durations as opposed to seed not soaked (Figure 17 vs 15). Germination of QC/ON seed soaked for 14 days was substantially reduced from that of seed soaked for 3 days (40 vs 70%) (Figure 19 vs 17) and Figure 13. Germination was also lowest for NB seedlots soaked for 14 days. Extended soaking was clearly detrimental to seed health probably by damaging the embryo.

Janerette (1979) found that soaking sugar maple seed for 14 days at 2° to 3°C reduced the time required for the seed to germinate. She reasoned that because seed was soaked at 2° to 3°C the soaking time could be considered part of the chilling treatment. Seed sources from QC/ON soaked for both 3 and 14 days had a lower percentage of radicle emergence for all three chilling times. Radicle emergence of NB sources soaked for 14 days and chilled for 8 weeks was almost double that of seed soaked for 3 days and chilled for 8 weeks (62 vs. 37%, respectively) (Figure 20). Chilling NB seed for 12 or 16 weeks that had been soaked for 14 days, slightly decreased the percentage of seed with radicle emergence (Figure 20). Figures 12 and 13 show the impact of prolonged soaking and chilling on seed survival. Extended soaking caused seed mortality even if radicle emergence had occurred. Prolonged exposure to the cold, moist conditions during chilling caused radicle tissue to deteriorate and become rotten. The criterion used by Janerette (1979) for a germinated seed was when the radicle had emerged through the pericarp. It is possible that this early assessment of germination may have erroneously lead her to conclude about the beneficial effects of prolonged soaking on germination.

Eight weeks of chilling NB seed that was not soaked or was soaked for 3 days was insufficient to maximize germination (Figures 14 and 16). Twelve weeks of chilling was sufficient for NB sources of all three soak times to germinate well (Figures 14, 16 and 18). Chilling seed longer than 12 weeks did not improve germination on average (Table 11) but did result in an improvement in germination for QC/ON seed that was not soaked (Figure 15). Eight weeks of chilling QC/ON seed soaked for 3 days resulted in seed germinating faster and to the same level as seed chilled for 12 weeks but not soaked (Figure 17 vs 15). Increasing the duration of chilling increased the germination speed for both NB and QC/ON seed sources subjected to no soak and 3 days soak. Chilling seed for 16 weeks resulted in seed germinating during chilling, especially the QC/ON sources.

The 2003 experiment using unstored seed showed that seed from QC/ON sources that was not soaked had maximum germination of 82% with 8 weeks of chilling. Results for the stored seed, illustrated here, showed that germination was maximized at 78% for unsoaked seed chilled for 16 weeks versus 66% for unsoaked seed chilled for 8 weeks. Germination of seed soaked for 3 days was virtually the same for the three periods of chilling (Figure 17). The storage temperature may have

induced the seed to become more dormant. Edwards (2001) stated that eastern white pine (*Pinus strobus*) seed is known to become more deeply dormant when stored at -18°C (or lower) for more than six months.

Starting at week six of chilling, seed in each treatment was assessed every two weeks to quantify when dormancy was broken as indicated by emergence of the radicle. Each seed was evaluated for: radicle length less than the length of the seed coat; radicle length greater than the length of the seed coat; or a germinant with fully developed radicle and hypocotyl and cotyledons emerging or completely emerged from the seed coat. Differences were apparent between the two geographic regions. For NB seedlots, 12 weeks of chilling was required for over 80% of the seed to break dormancy regardless of soaking duration and 16 weeks of chilling resulted in an increase of an additional 2 to 10% (Figure 20). In contrast, 8 weeks of chilling was sufficient for almost 80% of the seed in the QC/ON seedlots that were not soaked to break dormancy with increased chilling times improving it another 10% (Figure 21). As soak time for QC/ON seed increased from no soak to 14 days there was a corresponding gradual decline in the number of seed breaking dormancy in the three chilling times. This observation agrees with what was illustrated in Figures 12 and 13 that seed was likely damaged as a result of longer soaking times.

Germination was lowest for seed from both geographic regions that was soaked for 14 days with the exception of NB sources not soaked and chilled for 8 weeks (Table 12). Figures 12 and 13 demonstrated that extended soaking increased seed mortality. Soaking seed from NB sources for 3 days did not always increase germination for seed chilled 12 and 16 weeks for both germination temperatures. Germination of seed from NB sources was highest after 12 and 16 weeks of chilling but 16 weeks of chilling did not substantially improve germination over that from 12 weeks of chilling. Chilling QC/ON sources for 12 or 16 weeks generally did not result in higher germination than seed chilled for 8 weeks. Soaking QC/ON seed for 14 days was consistently detrimental to germination.

Table 12. Germination (%) of sugar maple seed from two geographic regions subjected to 3 durations of chilling and soaking and 2 germination temperatures.

	Germination temperature 15°C									Germination temperature 20°C								
	Chill duration (weeks)									Chill duration (weeks)								
	8			12			16			8			12			16		
	Soak duration (days)			Soak duration (days)			Soak duration (days)			Soak duration (days)			Soak duration (days)			Soak duration (days)		
	0	3	14	0	3	14	0	3	14	0	3	14	0	3	14	0	3	14
NB	47	73	68	86	80	69	83	84	57	39	58	59	86	84	64	86	83	54
QC/ ON	64	66	43	73	66	42	77	72	37	68	72	47	68	70	41	77	67	40

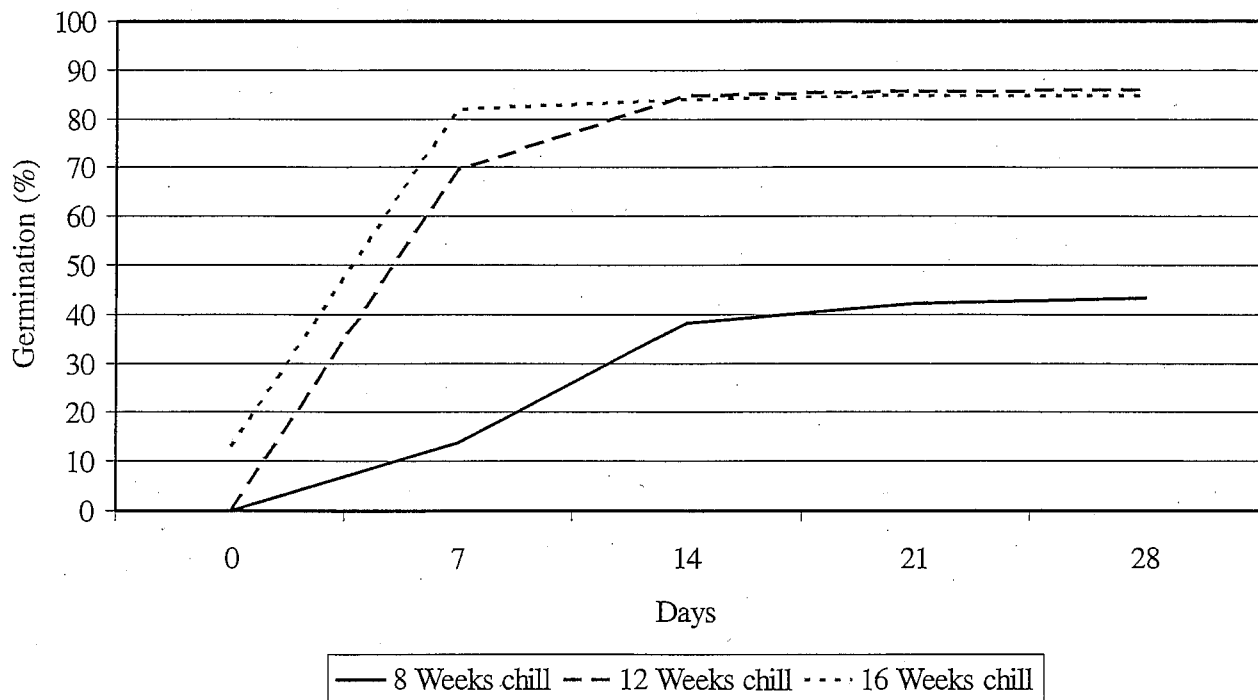


Figure 14. Germination of sugar maple seed from NB sources not soaked and chilled for 3 durations.

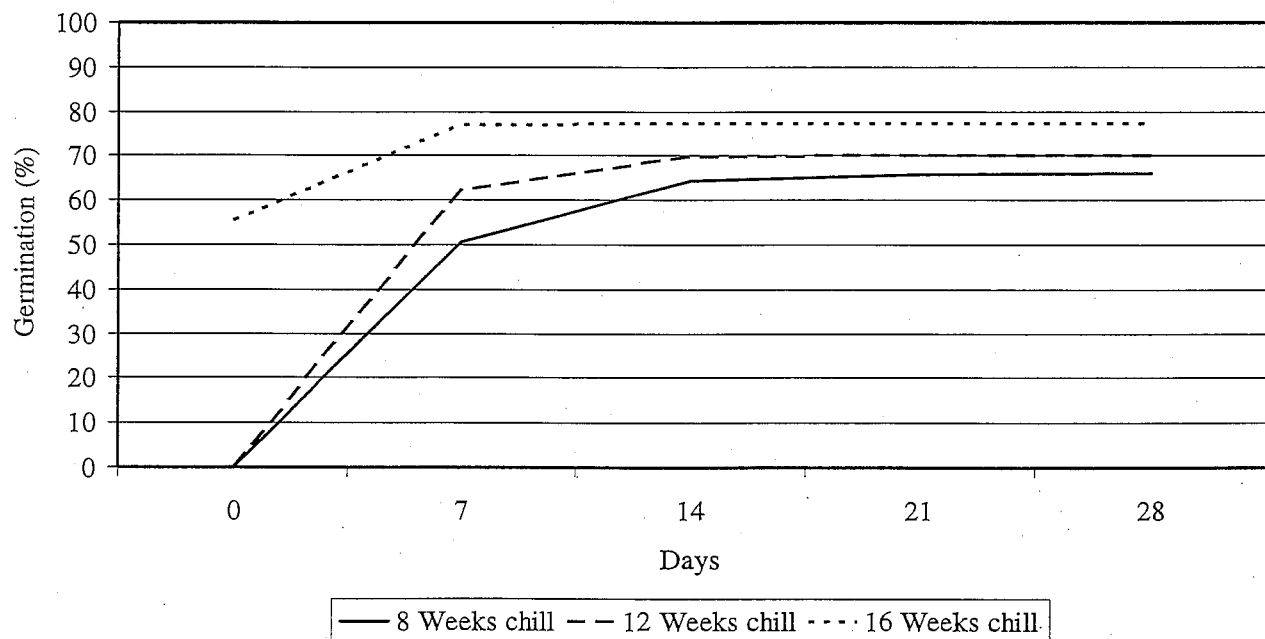


Figure 15. Germination of sugar maple seed from QC/ON sources not soaked and chilled for 3 durations.

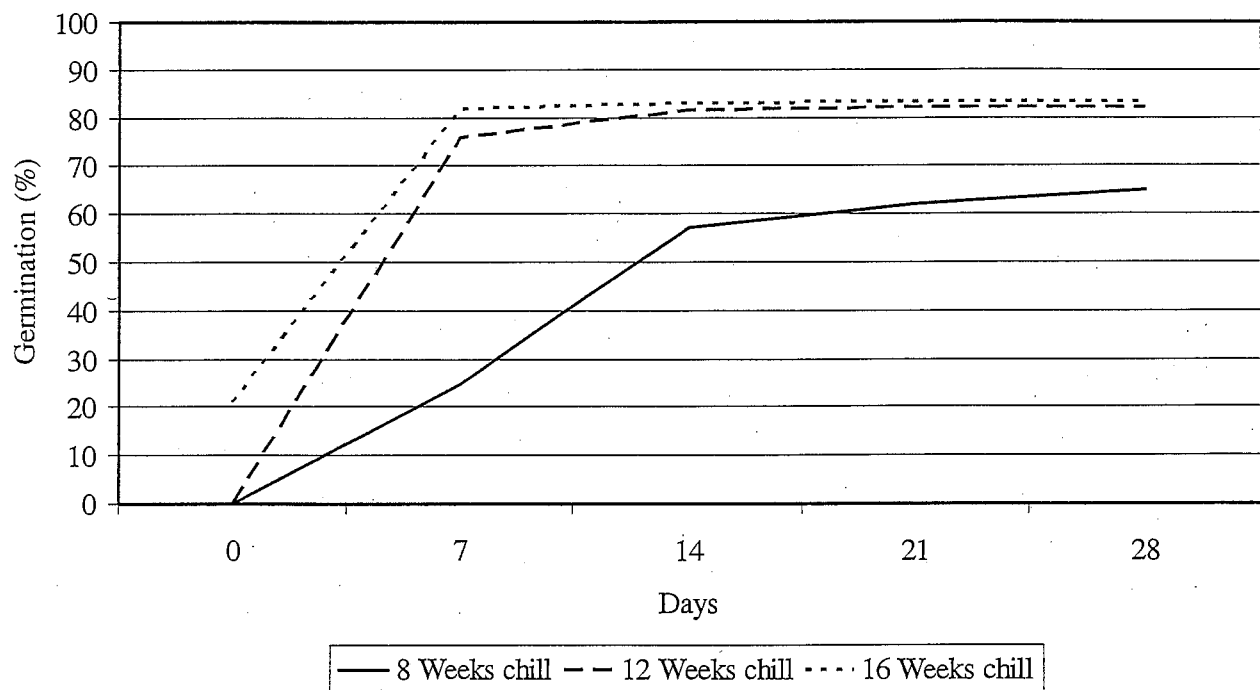


Figure 16. Germination of sugar maple seed from NB sources soaked for 3 days and chilled for 3 durations.

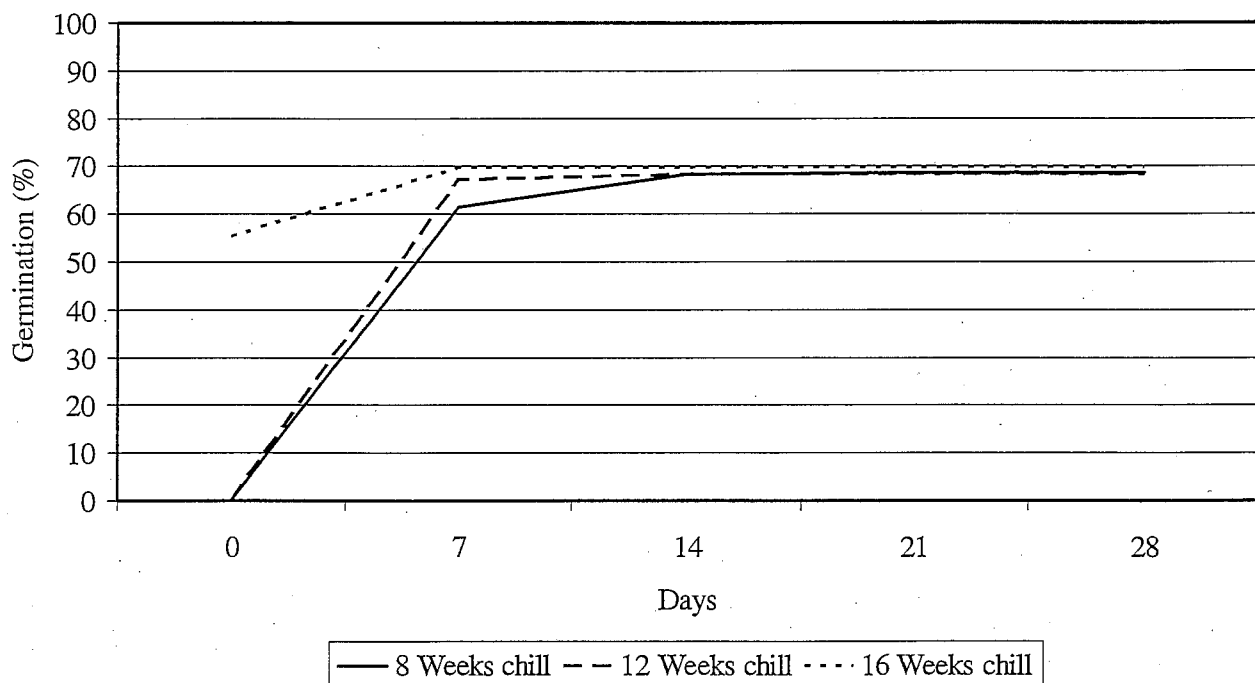


Figure 17. Germination of sugar maple seed from QC/ON sources soaked for 3 days and chilled for 3 durations.

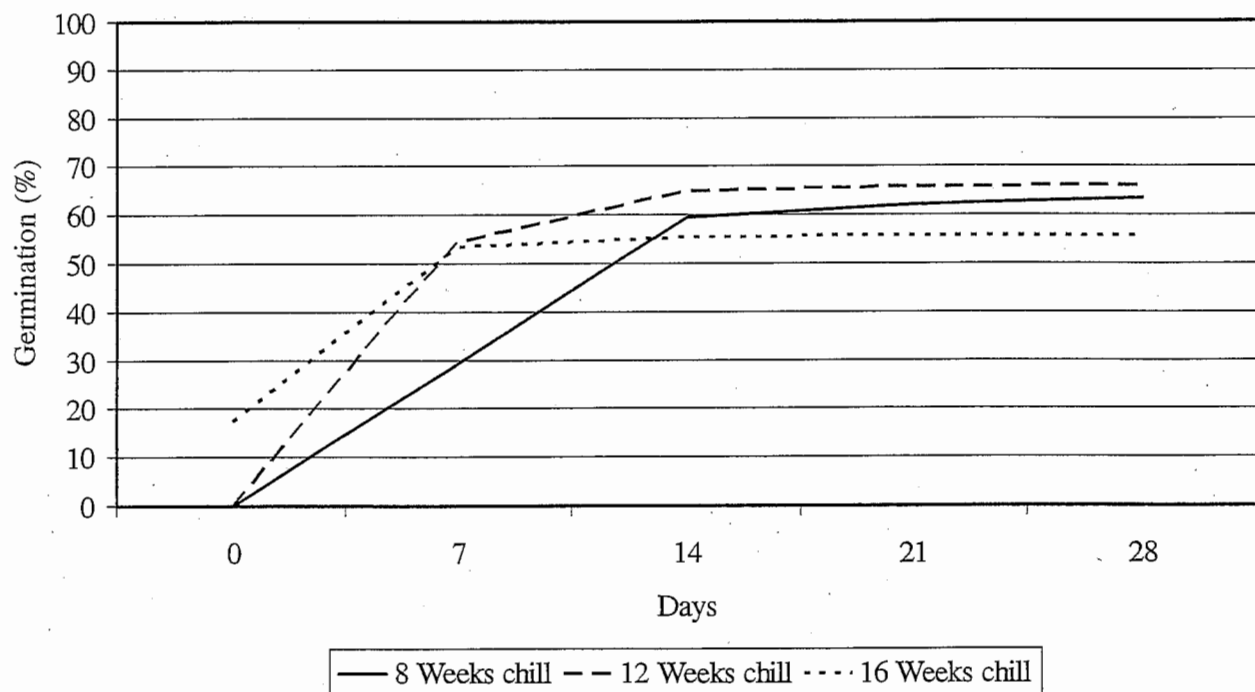


Figure 18. Germination of sugar maple seed from NB sources soaked for 14 days and chilled for 3 durations.

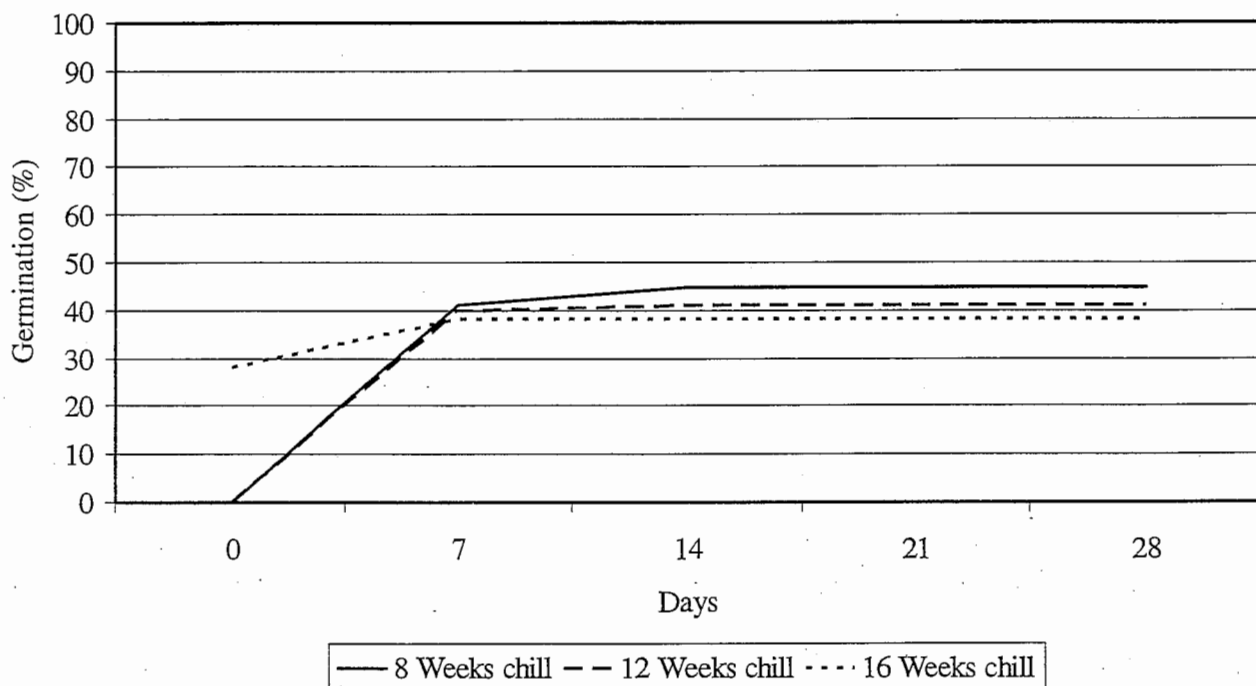


Figure 19. Germination of sugar maple seed from QC/ON sources soaked for 14 days and chilled for 3 durations.

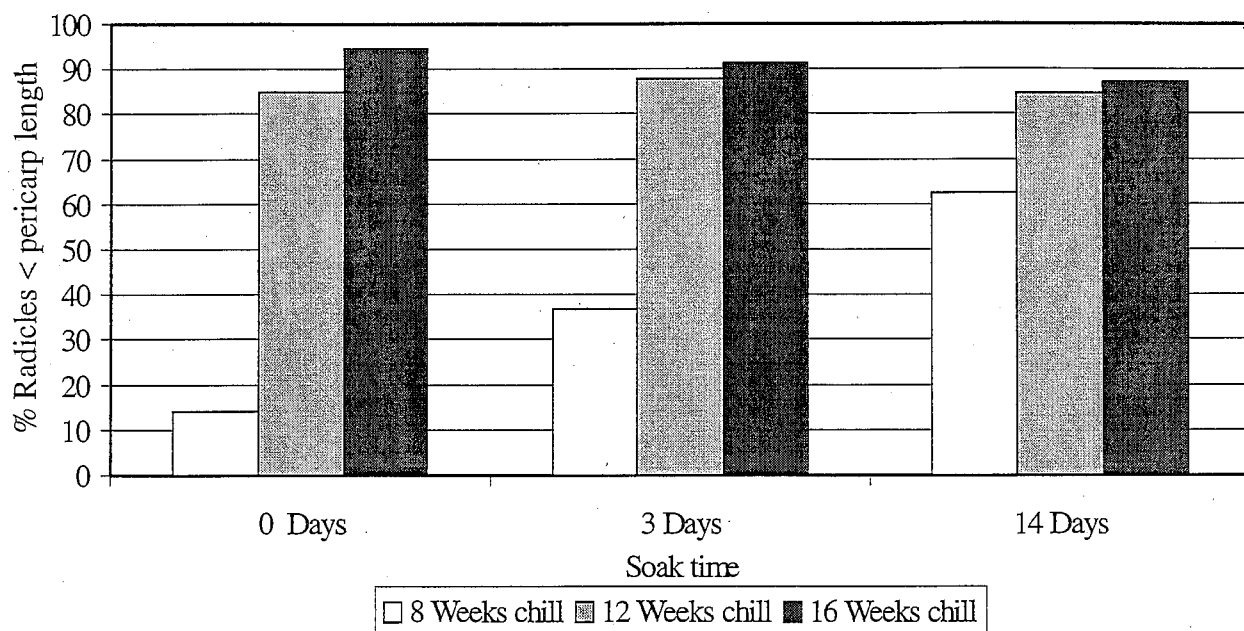


Figure 20. Percentage of seed from NB sources that was soaked and chilled for three durations with radicles less than the length of the seed coat.

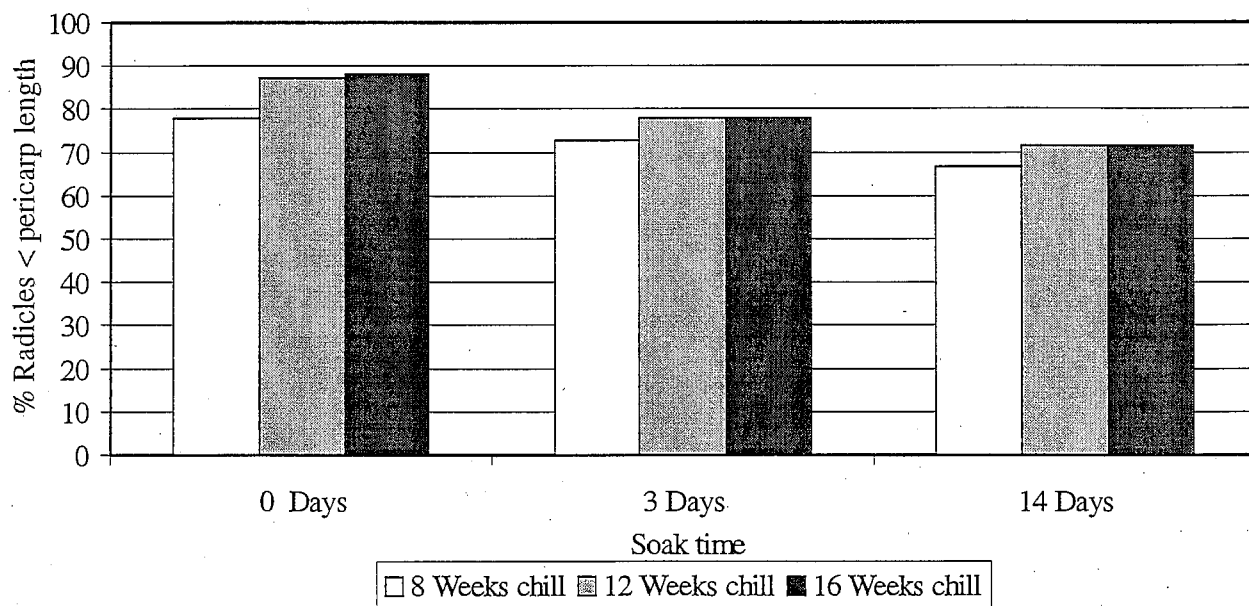


Figure 21. Percentage of seed from QC/ON sources that was soaked and chilled for three durations with radicles less than the length of the seed coat.

Seed imbibed 69 to 85% of its moisture within the first 24 hours depending on seed source and imbibition treatment. Water uptake was more rapid for seed soaked in water than on the surface of Kimpak and NB sources imbibed water faster and to a higher level than did QC/ON sources. The pericarp is not impermeable to water but restricts the amount of water flowing through it (Webb and Dumbroff 1969). The difference in imbibition between seed from the two geographic regions may be related to the permeability of the testa or membrane surrounding the embryo. Webb and Dumbroff (1969) found that the mean flow rate through the testa of sugar maple seed was about 3.75 times less than for the pericarp. It is possible that the testa is thicker or different in some way for the QC/ON sources. Whatever the reason, seed from QC/ON sources imbibed less water. It is accepted that a moisture threshold must be reached before seed become metabolically active. For some conifer species, a MC of 30 to 35% is necessary. It is unknown what the optimum MC is for sugar maple seed but certainly the seed is capable of imbibing a significant amount of water.

The results of this portion of the experiment do not support the hypothesis that dormancy is alleviated or somewhat alleviated in seed stored for one year at -20°C. Only two seed germinated in the controls which were not chilled.

The trend shown in the 2003 trial of seed from NB being more dormant than seed from QC/ON was still evident for the stored seed. Twelve weeks of moist chilling was adequate to maximize germination of NB sources as opposed to 8 weeks of chilling for QC/ON seed sources. Soaking seed from NB sources for 3 days in the 2003 trial increased germination over that of seed not soaked but that trend was not consistent for the stored seed. Soaking seed from QC/ON sources for 3 days in the 2003 trial did not always increase germination much like the results obtained from the stored seed. Extending the soaking time to 14 days had a negative impact on germination for all seedlots but especially for the QC/ON sources. The decline in germination was consistent in both the 2003 trial and the current one using stored seed.

There was no overall difference in seed germination between the two germination temperatures. Therefore, there is no reason to discontinue using ISTA's recommended germination temperature of constant 20°C.

The following conclusions can be made:

1. There is a regional difference in germination of sugar maple seed collected from Maritime Canada vs. central/eastern Canada.
2. Seed from the Maritimes requires 12 weeks of chilling to alleviate dormancy.
3. Eight weeks of chilling is adequate for seed from central/eastern sources to alleviate dormancy.
4. Soaking for 14 days damages seed and causes mortality.
5. Soaking seed for 24 hours is probably sufficient because of the rapid imbibition that takes place during this period of time.
6. Storing seed for one year at -20°C does not alleviate dormancy.
7. A germination temperature of 20°C is appropriate.

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White Spruce Seed Storage Experiment

In 1974 staff at the Petawawa Forest Experiment Station (Petawawa Research Forest) collected white spruce (*Picea glauca*) cones from a number of locations in Ontario for a future range-wide series of provenance trials. At each location cones were collected from individual trees. Seed was extracted and cleaned during the fall of 1974. At some point small quantities of seed were stored at 4°C and -20°C. Seed from one provenance was tested for moisture content and germination in 2002. To determine if those trends were evident in other provenances Darren Hayes evaluated seed from another four provenances and combined this data with the previous data for his undergraduate thesis at the University of New Brunswick (Hayes, 2005).

Methods

Ten grams of seed from each collection was designated for storage at -20°C. Each 10 g sample was subdivided into 4 samples of 2.5 g each and placed into small heat sealed poly bags and the four sub-samples stapled together such that the staple did not penetrate the portion of the packet containing the seed. This operation was conducted in February 1978. The packets of seed were placed in large glass jars with a screw cap and presumably placed into -20°C storage at this time. Seed from each collection that was designated for storage at 4°C was placed in 5 ml vials; 2 vials per seedlot. The 2 vials were placed in a plastic bag which was sealed. All bags from one location were placed into a larger plastic bag which was also sealed. There is no definitive record as to when this was conducted.

Seedlots were chosen for evaluation based on when the seed was initially tested for moisture content (MC) and germination. Only those seedlots tested late 1974 or early 1975 were selected. In 2002 one sample from each of the seedlots from the Whitney provenance was removed from both storage temperatures and tested for moisture content and germination. In 2005 samples were removed from four other provenances to evaluate whether or not the trends found with the Whitney provenance were consistent.

Moisture content of the seed was determined by placing approximately 1 g of seed into each of two aluminum containers and placing them in a force-draft oven at a temperature of $103 \pm 2^\circ\text{C}$, dried for 17 ± 1 hr and MC calculated on a fresh-weight basis. For the germination tests, seed was placed on moistened Versa Pak™ 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 21 days. After 21 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 every 3 to 4 days thereafter until day 21. Seed was considered germinated as evidenced by cotyledons, hypocotyl, and developing radicle.

Data were analysed using SAS. Prior to analysis of variance, arcsine transformation was applied to percentages.

Results

Analysis of the data showed there was a significant difference ($P < 0.01$) in seed germination between test years for seed stored at both temperatures. A subsequent analysis of variance showed that there was a significant difference ($P < 0.01$) in germination among provenances for seed stored at both temperatures. Seed from the single seedlot of Bancroft provenance exhibited 0% germination at 4°C while it had the highest germination of 97% at -20°C. There was no significant difference among provenances for the original seed germination. It was felt that these differences between test years were probably an artifact due to the imbalance of the data because the data set tested in 2002 contained 25 seedlots from one provenance (Whitney) as opposed to 11 seedlots from 4 provenances tested in 2005. Therefore, data from both test years were combined.

Table 13 provides mean MC and germination for each seedlot after seed extraction and after storage at 4°C and -20°C. MC increased significantly during storage (Table 14) however the increase was less for the seed stored at -20°C. Germination of seed stored at -20°C was essentially unchanged from its original value however germination of seed stored at 4°C declined drastically (Table 14) with substantially more variation. It is evident that MC and the 4°C storage temperature had a significant negative impact on seed storability. Seed MC of four seedlots: 7431490, 7431540, 7431620, and 7431700, stored at 4°C was slightly less than that for the same seedlots stored at -20°C but their germination was substantially lower. In contrast, seedlot 7431470 is an exception with a higher MC at 4°C than at -20°C but with the same germination at both storage temperatures.

The impact of MC on germination is illustrated in Table 15. Germination declined sharply when seed MC exceeded 8.5% particularly for seed stored at 4°C. However, germination was substantially lower for seed stored at 4°C with MC ranging between 4.5 and 6.9% than seed stored at -20°C. This again illustrates how MC and storage temperature have interacted.

Storage conditions also had an impact on germination vigour. Seed stored at 4°C started to germinate later and germinated slower than seed stored at -20°C (Figure 22). The MC of seed stored at 4°C was significantly and negatively correlated ($P < 0.01$, $r = -0.5$) with germination. That is as MC increased, germination decreased. There was no correlation between these two variables for seed stored at -20°C.

Discussion

There is no record confirming that the seed was packaged for the purpose of a storage experiment. Two types of packaging were used, small heat-sealed plastic packets for seed stored at -20°C and poly vials for seed stored at 4°C. One would expect that if the intent was for a storage trial the same storage containers would have been used. As well, there is no definitive record stating when the seed was prepared and placed into storage. The date of February 1978 is clearly marked on the ID labels accompanying the seed stored at -20°C so it is assumed that was when this seed was stored. But there is no information indicating when the other seed was put into 4°C storage. This seed had been collected by personnel in the Tree Breeding Project at the Petawawa Forest Experiment Station.

Table 13. Seed moisture content and germination of seedlots following seed extraction and after storage at 4°C and -20°C.

Seedlot	Provenance	Original		4°C storage		-20°C storage	
		MC	Germ.	MC	Germ.	MC	Germ.
7430410	Petawawa	4.5	99.0	9.1	0.0	6.8	95.5
7430420	Petawawa	4.7	97.7	8.6	0.0	6.4	90.5
7430450	Petawawa	4.1	98.5	8.7	0.0	6.5	92.0
7430460	Petawawa	3.8	84.5	5.4	10.0	6.0	94.0
7431910	Petawawa	3.8	82.0	8.5	0.0	6.2	82.5
7430710	Renfrew	3.5	87.5	5.2	38.5	8.5	71.0
7430750	Renfrew	4.2	96.5	9.5	0.5	6.0	98.5
7430830	Antrim	4.0	99.5	9.0	0.0	5.9	94.5
7430850	Antrim	3.9	97.0	8.7	7.5	5.5	93.5
7430870	Antrim	4.4	64.2	8.8	0.0	5.9	83.5
7430970	Bancroft	5.0	99.5	9.4	0.0	6.3	97.0
7431280	Whitney	4.2	97.2	9.6	0.0	6.9	94.0
7431290	Whitney	4.3	99.2	10.0	0.0	5.2	99.5
7431300	Whitney	4.1	93.2	6.2	52.0	5.1	89.5
7431310	Whitney	3.6	98.8	5.6	29.5	4.9	98.0
7431320	Whitney	4.8	98.0	9.7	0.5	6.0	99.5
7431340	Whitney	4.3	99.2	8.6	32.5	5.6	97.5
7431350	Whitney	4.6	94.0	5.7	30.0	5.2	96.5
7431360	Whitney	4.1	99.2	8.4	96.5	6.5	97.5
7431380	Whitney	4.6	95.5	8.7	34.0	5.8	97.5
7431400	Whitney	4.9	97.8	9.6	3.0	5.8	98.5
7431420	Whitney	5.0	97.2	9.1	3.0	5.9	96.5
7431440	Whitney	4.7	97.5	5.7	38.5	5.0	98.5
7431460	Whitney	5.2	87.2	9.2	2.0	6.3	94.5
7431470	Whitney	4.8	97.2	8.2	98.5	6.0	98.0
7431490	Whitney	5.0	98.0	5.9	57.0	6.1	96.5
7431510	Whitney	4.9	95.0	9.8	0.0	6.9	98.0

Table 13. Continued

Seedlot	Provenance	Original		4°C storage		-20°C storage	
		MC	Germ.	MC	Germ.	MC	Germ.
7431520	Whitney	4.4	94.8	6.5	6.0	5.9	97.5
7431540	Whitney	5.3	97.2	5.8	29.0	6.1	96.5
7431580	Whitney	5.0	93.5	6.4	87.5	5.7	96.5
7431600	Whitney	4.4	97.5	9.2	12.0	5.8	95.5
7431620	Whitney	5.1	83.8	6.4	42.5	6.4	84.5
7431660	Whitney	4.4	98.0	6.2	55.5	5.7	97.5
7431680	Whitney	4.0	91.8	5.7	67.5	5.4	89.0
7431700	Whitney	4.6	95.5	5.9	6.5	6.1	92.5
7431720	Whitney	4.4	99.0	6.1	47.5	5.8	96.5

Table 14. Mean, minimum, maximum, and standard error of seed moisture content and germination of 36 white spruce seedlots after collection and after 25 or 28 years storage at 4°C and -20°C.

	Moisture content				Germination			
	Mean	Min.	Max.	SE	Mean	Min.	Max	SE
Original	4.5 a ¹	3.5	5.3	0.08	94.5 a	64.2	99.5	1.17
-20°C	6.0 b	4.9	8.5	0.11	94.1 a	71.0	99.5	0.98
4°C	7.8 c	5.2	10.0	0.27	24.7 b	0.0	98.5	4.93

¹ means with different letter are significantly different at P = 0.05

Table 15. Mean germination and range by seed moisture content class for 36 white spruce seedlots stored at 4°C and -20°C for 25 or 28 years.

MC class (%)	4°C storage			-20°C storage		
	Mean	Range	No. seedlots	Mean	Range	No. seedlots
4.5 – 4.9				98	–	1
5.0 – 5.4	25	10–39	2	95	90–100	5
5.5 – 5.9	37	7–68	7	95	84–99	12
6.0 – 6.4	57	12–88	5	94	83–100	12
6.5 – 6.9	6	–	1	95	92–98	5
7.0 – 7.4	–	–	–	–	–	–
7.5 – 7.9	–	–	–	–	–	–
8.0 – 8.4	98	97–99	2	–	–	–
8.5 – 8.9	11	0–34	7	71	–	1
9.0 – 9.4	3	0–12	6	–	–	–
9.5 – 9.9	1	0–3	5	–	–	–
10.0 – 10.4	0	–	1	–	–	–

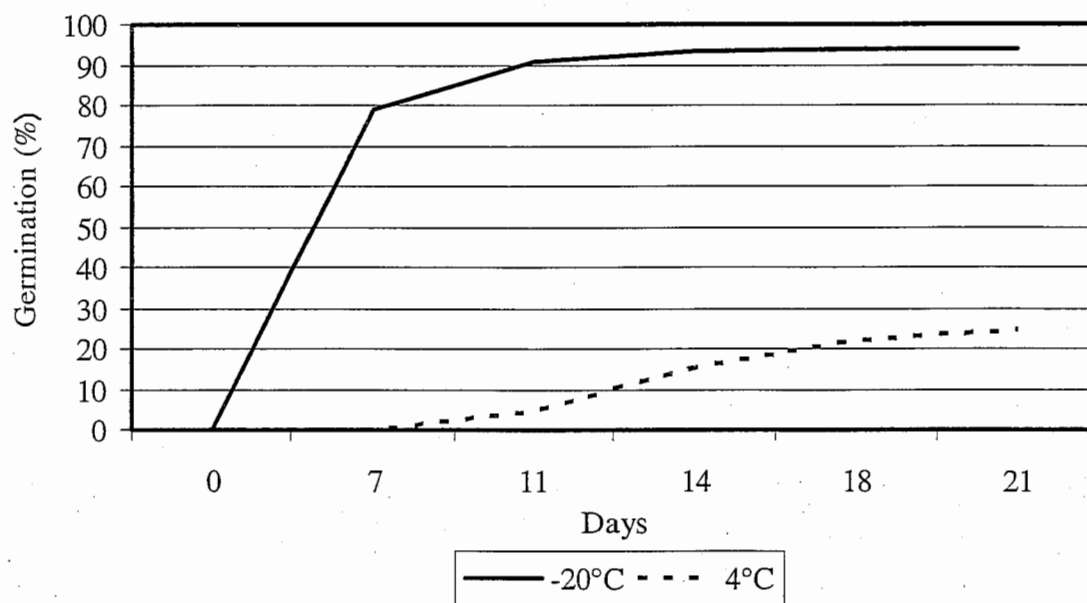


Figure 22. Germination speed of white spruce seed stored at 4°C and -20°C for 25 or 28 years.

At that time, they only had access to 4°C storage and that was probably the temperature the seed had been stored in until this trial was set up.

Another anomaly with this data is the significant increase in seed MC at both storage temperatures compared to the original values determined shortly after seed extraction. With the way the seed stored at 4°C was packaged (2 vials in a small sealed poly bag and all poly bags from one provenance placed into another sealed poly bag) it does not seem likely that the seed would have been able to increase MC by over 3% on average. However, it may be possible because Ellis et al. (2005) found that MC of ultra-dry seed in laminated aluminum foil packets increased after 10 years storage at 20°C. There are a couple of theories about why MC of the spruce seed increased. Seed was provided, over several years, to cooperators who wished to establish provenance trials. It is possible that care was not taken when moving the seed in and out of storage and it acquired moisture. Another possibility is the time of year that the seed was placed in the vials. If this was done during the summer when relative humidity levels are high and seed was exposed to ambient conditions for a period of time it could have gained moisture. Unfortunately, it may never be known when and how these samples were prepared but in any event we are able to evaluate the impact of MC and storage temperature on seed germination.

MC of seed stored at 4°C increased, on average, from 4.5 to 7.8% with a corresponding decline in germination by 70% from 94.5 to 24.7%. The MC of seed stored at -20°C also increased but only by 1.5% with practically no change in germination. It is generally recommended that seed be stored at MCs less than 8 or 10%. Fifteen seedlots stored at 4°C whose MC ranged from 5.0 to 6.9% had an average germination of 31% in contrast to 34 seedlots stored at -20°C with the same range in MC with an average germination of 95%. Clearly a storage temperature above freezing is not sufficient to maintain germinability of seed at low MCs because the seed is continuing to respire therefore depleting its energy reserves resulting in death. Storing seed with MCs above 8.5% is also inferior at both storage temperatures (Table 15). Daigle and Simpson (2003) illustrated that seed MCs above 9% had an increasing negative impact on germination of seed stored at -20°C.

Storage temperature is the other factor. Regardless of seed MC, with the exception of two seedlots, germination of seed stored at 4°C was always inferior. Even in the case of four seedlots with similar MCs at both storage temperatures, the seed stored at -20°C had higher germination. When seed tissues are frozen there is no metabolic activity occurring and therefore the reserves stored in the megagametophyte remain intact.

Vigour is a useful trait to evaluate impact of storage. As seed ages, vigour declines and eventually the seed dies. Total germination is also important but does not take into account vigour. For example, two seedlots could have the same total germination but one seedlot may have required 21 days to achieve this whereas the other seedlot needed only 14 days. The impact of storage temperature on seed vigour was evident in the germination speed. Germination started sooner, was faster, and reached its maximum sooner for the seed stored at -20°C.

Conclusions

1. Germination of white spruce seed stored at -20°C for 25 or 28 years did not change.
2. Seed MC greater than 8.5% negatively impacted germination, particularly for seed stored at 4°C.

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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 23). A total of 329 kg of certified seed was exported in 2005, which was about 90 kg less than in 2004. Of significance was 224 kg of lodgepole pine (*Pinus contorta* var. *latifolia*) from Yukon Territory followed by 66 kg of subalpine fir (*Abies lasiocarpa*) and 37 kg of grand fir (*A. grandis*). 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.

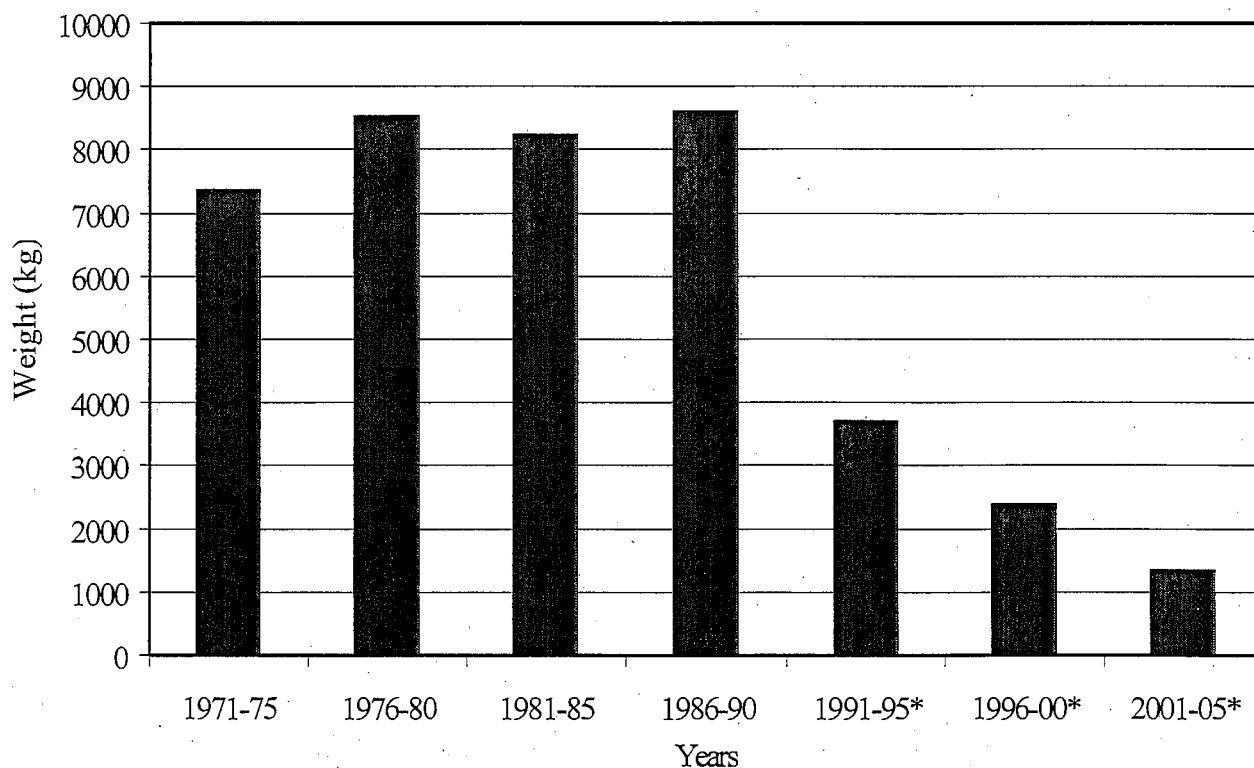


Figure 23. Weight of seed OECD certified or exported* by 5-year periods.

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.

The latest attempt that has been viewed as a positive step forward involved 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 two certification categories with the replaced represent about 99% of the total weight of seed certified annually and therefore it is important to update this text. The EU insisted that text be inserted stating that reproductive material from these two sources does not contain genetically-engineered material at a detectable level. However, the United States rejected this text. Effort is being made to seek a compromise in the wording in order that it be accepted by the EU and US.

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PROMOTION OF THE SEED CENTRE

Throughout the year there are many opportunities to promote the Seed Centre, the Canadian Forest Service, and Natural Resources Canada. This is accomplished through communications with general public, tours of the lab facility, participation in events that promote our organization, and special events when the opportunities present themselves. There are some events that deserve special mention.

- | | |
|------------------|--|
| March 23 | Dr. Yvan Hardy, Chief Scientist with CFS, Dr. John Richards, Ed Hurley, (one other gentleman who was with Dr. Hardy). Tour of lab - talked about gene conservation, freezer space, cryopreservation, partnerships, seed collections, etc. |
| May 19 | NB Minister of Natural Resources Keith Ashfield and other provincial officials. Tour of lab and discussion on mandate of Seed Centre. |
| August 27 | Participated in an event organized by Parc-Nature de la Pointe-aux-Prairies in Montreal. The event was called "Un parc-nature la nuit" and was well attended by the public living in the immediate vicinity.. |
| Sept. 27 - 29 | CFS Days. Display at O'Dell Park as well as in the foyer at the Forestry Centre. Also part of lab tours that were offered to public and special guests attending the event. |
| Oct. 31 - Nov. 4 | Tree Seed Course given to 18 individuals from throughout China. Course duration was one week and presentations were made by Seed Centre staff as well as other CFS researchers. Course participants were also given tours of various provincial and industrial facilities as well as the Seed Centre and the greenhouse. |

SEED CENTRE STAFF

The amount of work carried out by the Seed Centre during 2005 was made possible through "extra" work weeks with funding made acquired through FSWEPA/ASEP and the YMCA Federal Youth Internship Programs. These programs enabled the Seed Centre to acquire employees without having to cover their wages.

Cynthia Caborn was obtained through the YMCA Public Sector Youth Internship Program. Cynthia started the first week of September, 2004 and continued to May 25, 2005. Cynthia proved to be extremely valuable. She was helped with field collections in the fall of 2004 and took on much of the seed processing and testing of the seed in 2005.

Jessica Deluney and Erica Augustine were hired through the FSWEPA/ASEP program for Aboriginal students. They helped with seed collections, seed processing, and testing. They also helped set up a black ash seed germination and embryo maturation experiment. A major project involved testing of Tree Breeding seedlots. All of the jack pine seedlots contained in Tree Breeding were tested. Jessica was able to continue working 20 hours/week until the end of December.

Andrew Vogels was acquired through the YMCA Federal Youth Internship Program. Andrew was hired for a 6 month term and started work in early September. Andrew was involved in seed collections in the Fall and worked on seed processing and testing. He also had the opportunity to work with other people in the lab. He was tasked with setting up, monitoring, and preparing a report of a white pine seed storage experiment established in 2002.

Alex Daigle and Terry Brooks were two students that were made available occasionally during the summer months. They helped with embryo excision of ash species, setting up germination tests, and conducting moisture content and thousand seed weights.

These workers, all of whom were acquired through programs that covered 100% of their wages, accounted for an "extra" 78 weeks of work in 2005. This represents the most help provided to the Seed Centre since it moved to Fredericton in 1996. These programs were beneficial for providing the additional help to complete a number of projects that would not have been possible with current Seed Centre staff. Figure 24 shows the number of "extra" work weeks since 1998.

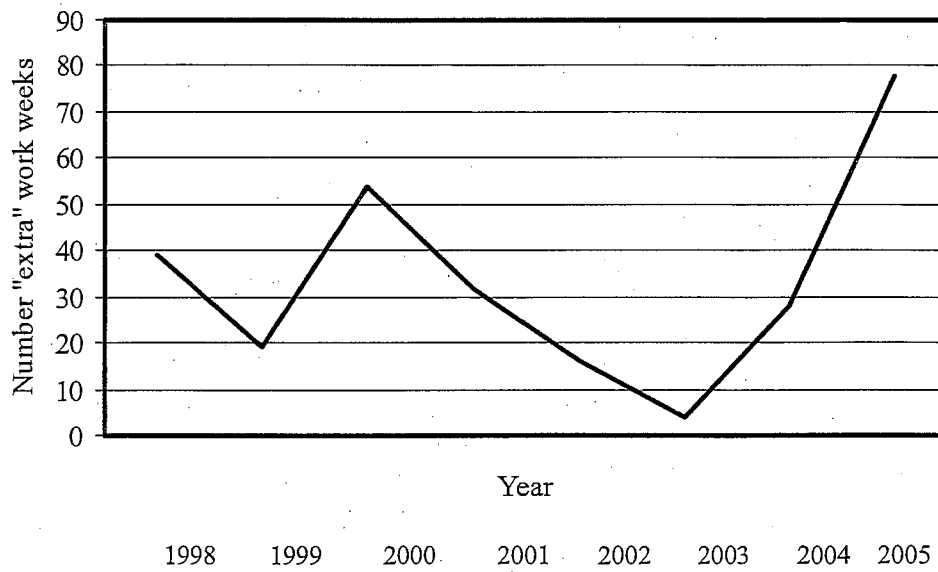


Figure 24. Number of "extra" work weeks provided to the NTSC between 1998 and 2005.