

National Tree Seed Centre

Annual Report

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NATIONAL TREE SEED CENTRE ANNUAL REPORT 2005

EXECUTIVE SUMMARY

Seed production was good for most species in 2006 and 329 seedlots were collected by Seed Centre staff. Seed from black ash (*Fraxinus nigra*) in Nova Scotia was collected from a population along River Philip near Oxford. Black ash is rare in Nova Scotia and a viable seed source has been sought by the Nova Scotia Mi'kmaq for several years. Eastern hemlock (*Tsuga canadensis*) seed was collected from three populations in Québec. This seed is important to allow researchers to continue genetic diversity work on the species. Collections of eastern white cedar (*Thuja occidentalis*) were made in Nova Scotia and Prince Edward Island. The species is listed as vulnerable in Nova Scotia and is not common in Prince Edward Island. Seed was also collected from several species in Kejimikujik National Park. Some of the seed is to be used as part of a restoration project in the park.

A total of 63 requests representing 716 seedlots was processed and provided for research. The majority of the requests were from Canada (53 requests; 544 seedlots) but seed was also sent to Finland (1 request; 1 seedlot), France (2 requests; 16 seedlots), Japan (1 request; 82 seedlots), Slovakia (2 requests; 61 seedlots), and United States (6 requests; 12 seedlots).

Seed testing consisted of 731 germination tests, 198 moisture content tests, and 270 thousand-seed weight tests.

The total number of seedlots in storage is over 12 000 with over 6,000 of these stored in the Seed Bank and available to researchers. An additional 3,500 are stored for Gene Conservation and the remaining seedlots are old Tree Breeding seedlots or seedlots Reserved by researchers.

Several experiments and trials were conducted:

- Water of different pHs did not have an effect on germination or formation of mold on seed of two white pine seedlots.
- Chilling duration of 8 weeks affected germination speed of white pine seed but did not increase overall germination when compared to seed chilled for 4 weeks.
- Germination of black ash seed was higher when seed were subjected to an initial cold treatment. Best germination was obtained using the treatment combination of 60 days cold, 120 days warm, and 180 days cold.
- Alcohol separation was effective in removing hemlock seed infested with hemlock seed adelgid (*Megastigmus hoffmeyer*). The adelgid was present in eastern hemlock seed from four collection sites. Many countries insist that seed must be free of *Megastigmus* species before seed can be imported.
- Testing of 211 tamarack seedlots showed an annual rate of decline in germination of 0.64%/year over a 13-year period.
- De-winged and alcohol separation consistently improved germination of white birch seedlots.

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INTRODUCTION

This report covers the activities of the National Tree Seed Centre (NTSC) for 2006. Similar reports were prepared from 1998 – 2005. 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 seedlots increased by 160 to 12 015 in 2006. The numbers in brackets in Table 1 represent the numbers reported in the 2005 Annual Report.

Table 1. Seed stored at the NTSC as of December 31, 2006.

Seed Bank		Reserved		Tree Breeding		Gene Conservation	
No. Species	No. Seedlots	No. Species	No. Seedlots	No. Species	No. Seedlots	No. Species	No. Seedlots
171 (175)	6,157 (5,882)	38 (40)	1,947 (1,945)	10 (9)	390 (1,078)	22 (18)	3,521 (2,950)

Seed Bank seedlots are the active collection that are available for distribution. The number of Seed Bank seedlots increased by 275 to 6157 in 2006. This increase is a result of collections that were made in 2005 and from the transferring of seedlots from Tree Breeding as these were tested. 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, 2006, the NTSC Seed Bank had 5,857 seedlots from 121 Canadian species in storage (Table 2). An additional 69 exotic species (300 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	49	0.8
British Columbia	34	307	5.2
Manitoba	7	67	1.1
New Brunswick	70	1281	21.9
Newfoundland and Labrador	14	99	1.7
Nova Scotia	41	424	7.2
Ontario	52	2298	39.4
Prince Edward Island	31	221	3.8
Québec	20	951	16.2
Saskatchewan	8	111	1.9
Yukon Territory	3	49	0.8
Total	121	5857	100

The Reserved category contains seedlots that have been reserved by researchers. Many of these seedlots were collected for special projects. There was no significant change in this category in 2006.

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 688 to 390 during 2006. This is mainly due to the testing of 558 tamarack (*Larix laricina*) and 223 Scots pine (*Pinus sylvestris*) seedlots. Seedlots that had sufficient quantities of seed and adequate germination were kept while the rest were discarded. After testing, 246 tamarack seedlots were moved to Gene Conservation and 69 to Seed Bank and 115 Scots pine seedlots remained in Tree Breeding.

The Gene Conservation category was put in place to assure that genetic material obtained from rare, endangered, and/or unique populations, as well as samples from throughout a species' range is preserved. This collection increased by 571 to 3521 seedlots in 2006. There are 22 species with the number of seedlots ranging from 1 for buttonbush (*Cephalanthus occidentalis*) to 1,635 for white spruce (*Picea glauca*). Other species and number of seedlots contained are: black spruce (*P. mariana*, 363 seedlots); red spruce (*P. rubens*, 2 seedlots); Manitoba maple (*Acer negundo*, 15 seedlots); striped maple (*A. pensylvanicum*, 17 seedlots); red maple (*A. rubrum*, 65 seedlots); mountain maple (*A. spicatum*, 46 seedlots); white ash (*Fraxinus americana*, 200 seedlots); black ash (*F. nigra*, 120 seedlots); red/green ash (*F. pensylvanica*, 17 seedlots); tamarack (*Larix laricina*, 246 seedlots); jack pine (*Pinus banksiana*, 80 seedlots); limber pine (*P. flexilis*, 100 seedlots); pitch pine (*P. rigida*, 4 seedlots); eastern white pine (*P. strobus*, 17 seedlots); Scots pine (*P. sylvestris*, 12 seedlots); largetooth aspen (*Populus grandidentata*, 13 seedlots); trembling aspen (*P. tremuloides*, 16 seedlots); pin cherry (*Prunus pensylvanica*, 61 seedlots); and choke cherry (*P. virginiana*, 321 seedlots).

SEED COLLECTIONS

Seed production was good for most species in 2006. In order to ensure good quality seed, seed is only collected during good seed years. Seed collected in poor seed years may be of lesser quality because of poor pollination. Also, the time required to collect sufficient seed increases when there is a poor seed crop. A total of 329 seedlots was collected by Seed Centre staff.

Seed from black ash in Nova Scotia was collected from a population along River Philip near Oxford. Black ash is rare in Nova Scotia and a viable seed source has been sought by the Nova Scotia Mi'kmaq for several years. Most of the black ash stands in Nova Scotia (few scattered trees in most cases) consist of low quality trees with poor crown development. The Oxford population may provide a useful seed source for the native community in Nova Scotia. Eastern hemlock (*Tsuga canadensis*) seed was collected from three populations in Québec. This seed is important to allow researchers to continue genetic diversity work on the species. Collections of eastern white cedar (*Thuja occidentalis*) were made in Nova Scotia and Prince Edward Island. The species is listed as vulnerable in Nova Scotia and is not common in Prince Edward Island. Seed Centre staff collected seed from several species in Kejimikujik National Park. Some of the seed is to be used as part of a restoration project in the park. Table 3 provides a complete list of the collections made.

Table 3. Seed collections made by Seed Centre staff in 2006.

Species	N.B.	N.S.	QC	P.E.I.	Total
<i>Acer rubrum</i>	20	21			41
<i>Acer saccharinum</i>	1				1
<i>Acer saccharum</i>	15	1			16
<i>Betula alleghaniensis</i>		16	1		17
<i>Betula papyrifera</i>	17				17
<i>Fagus grandifolia</i>	16	2			18
<i>Fraxinus nigra</i>		15			15
<i>Fraxinus pensylvanica</i> var. <i>austini</i>	41				41
<i>Picea abies</i>	1				1
<i>Picea glauca</i>	1				1
<i>Picea mariana</i>	1				1
<i>Picea rubens</i>		15			15
<i>Pinus strobus</i>		15			15
<i>Populus grandidentata</i>	13				13
<i>Populus tremuloides</i>	15				15
<i>Prunus virginiana</i>		16			16
<i>Quercus macrocarpa</i>	8				8
<i>Thuja occidentalis</i>		19		13	32
<i>Tsuga canadensis</i>			45		45
<i>Ulmus americana</i>	1				1
Total	150	120	46	13	329

The Seed Centre also acquired 15 eastern white cedar and 15 eastern hemlock seed collections from Petawawa Research Forest. Fifteen green ash and 15 white ash seedlots were purchased from a seed collector in Ontario. We also acquired 4 seedlots of eastern flowering dogwood (*Cornus florida*), 2 seedlots of common hoptree (*Ptelea trifoliata*), 1 seedlot of pumpkin ash (*Fraxinus profunda*), 2 seedlots of green ash, and 3 seedlots of red maple from Minnesota.

A total of 401 seedlots was acquired by the Seed Centre in 2006. Figure 1 shows the increase in the number of seedlots in the Seed Bank collection since 1996. The increase in 2006 is a result of the seedlots collected in 2006 and from tamarack seedlots moved from Tree Breeding. Since 1996, the number of seedlots in the Seed Bank collection has increased from 3,079 to 6,157.

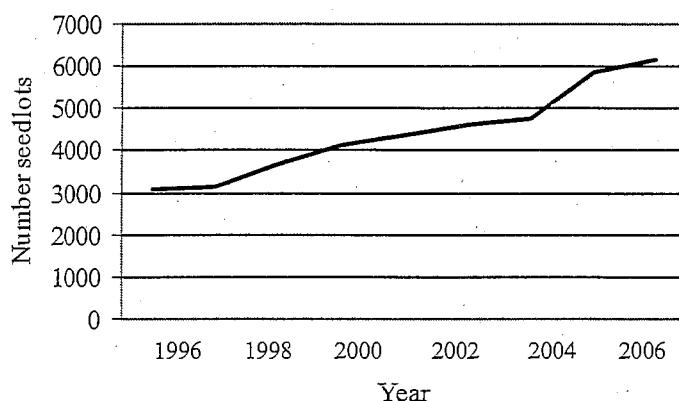


Figure 1. Increase in the number of seedlots at the NTSC Seed Bank since 1996.

The increase shown in Figure 1 represents the net gain in the Seed Bank seedlots after losses of seedlots due to low germination and seed quantities exhausted have been taken into account. 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 2006.

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	1,067
2005	142	29	3	184
2006	329	42	30	401
Total	2,525	1,819	224	4,568

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 arboreturns. A seed request form must be completed by the client before a seed order is processed. The purpose of this form is to gather information on the type of research being carried out and to serve as a means of screening requests. All seed requests received from outside Canada are referred to the Canadian Food Inspection Agency (CFIA) to determine if a phytosanitary certificate and/or import permit is required.

During 2006, a total of 63 requests representing 716 seedlots was processed. The majority of the requests were from Canada but seed was also sent to Finland, France, Japan, Slovakia, and 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 1,603 in 1985 (Figure 2). Canadian researchers received 69% of the seed while seed sent to researchers outside Canada accounted for the other 31%.

Table 5. Number of requests and number of seedlots shipped by country in 2006.

Country	No. Requests	No. Seedlots
Canada	53	544
Finland	1	1
France	2	16
Japan	1	82
Slovakia	2	61
United States	6	12
Total	63	716

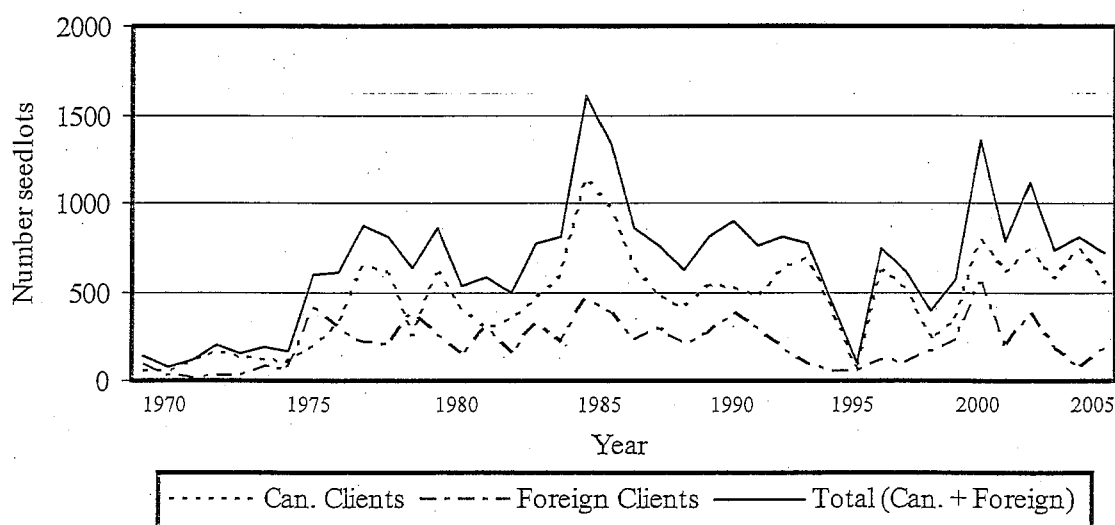


Figure 2. Number of seedlots sent to clients between 1967 and 2006.

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 seeds each are placed on moistened VersaPak™ in germination boxes. When larger seed is being tested, the number of seed is usually reduced. **Seven hundred and thirty-one 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 transferring of the Seed Centre from Petawawa to Fredericton.

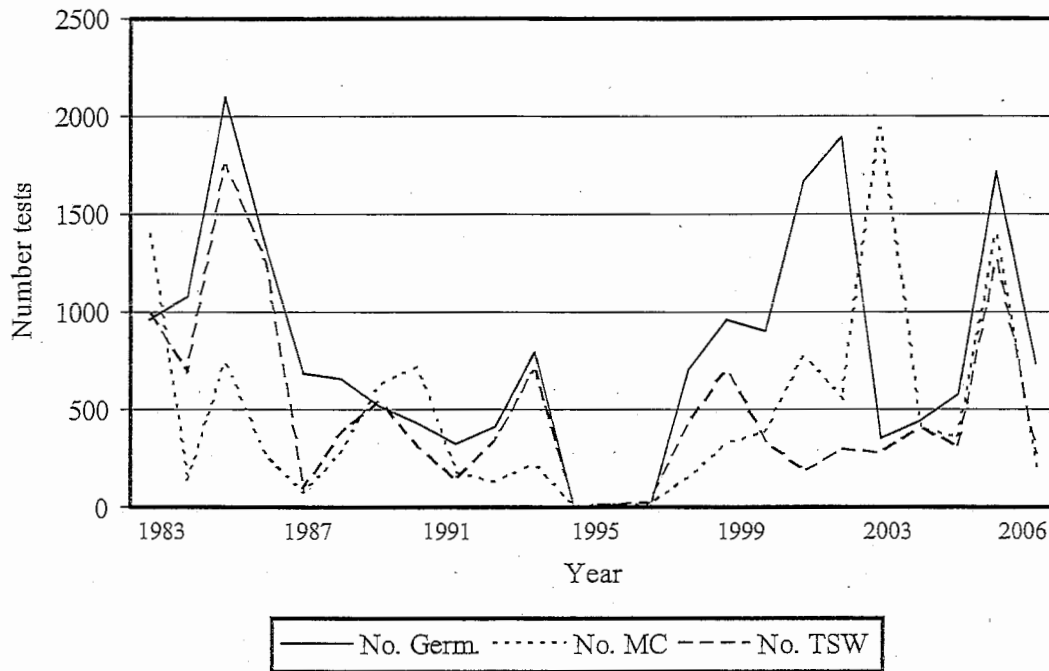


Figure 3. Number of germination tests (No. Germ), moisture content tests (No. MC), and thousand seed weights (No. TSW) carried out by the NTSC since 1983.

The target moisture content for orthodox seed is between 5 and 8%. Seed that are above this range are further dried before being stored. **One 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 800 seeds), the total number of seed is counted, the total weight of the sample is determined, and the 1000-seed weight calculated. A total of **two hundred and seventy 1000-seed weights** was done.

RESEARCH AND DEVELOPMENT

Impact of Acid Water on Formation of Mold on White Pine Seed

Introduction

In the fall of 2005 the Seed Centre was contacted by Sterling Greene, The Greene Light Company Ltd., Fredericton, NB regarding an instrument that he had developed that can acidify water. He felt that acid water may have a beneficial effect on seed germination. After discussing the possible benefits of treating seed with acid water it was decided to set up an experiment to determine if acid water could control the growth of mold on the surface of seed while not adversely impacting germination. Two graduate students attending University of New Brunswick, Marianela Ramirez and Jakub Olesiński conducted the experiment for a statistics course they were enrolled in.

Material and Methods

Two white pine (*Pinus strobus*) seedlots were chosen based on previous history of mold during germination. One seedlot (A) consistently had mold on the surface of seed while the other seedlot (B) tended not to have mold. Two water samples of different acidity (pH 5.9 and 6.3), that had been produced from Mr. Greene's equipment, were used. Each seedlot was treated by soaking for 20, 40, or 80 minutes in each water sample. A no soak treatment was used as a control. Seed was placed on VersaPak™ in Petawawa Germination Boxes such that 50 seeds from one seedlot from each soak/non-soak treatment was randomly placed in each of four germination boxes for each seedlot resulting in eight germination boxes for each of the two water acidity samples. The boxes were placed in a walk-in cooler at 3 °C for four weeks of moist chilling. Following chilling the boxes were moved to a germinator maintained at 30 °C with 8 hours light and 20 °C with 16 hours darkness at a constant relative humidity of 85%. Starting at the fifth week, from the beginning of establishing the experiment, seed was assessed for mold development and germination. Monitoring was completed at the end of the seventh week.

Results and Discussion

Soaking seed in acidified water did not control the development and growth of mold (Figure 4). Seed that was not soaked had the least frequency of mold. There was little difference in the proportion of seed with mold between the two water pH levels (Figure 5) however seed soaked in pH 6.3 water did have a lower incidence of mold. Surprisingly, seedlot B had a higher percentage of seed with mold (Figure 6). If acidified water has the potential to control development and growth of mold, as was hypothesized by Mr. Greene, that was not demonstrated by this trial. It is possible that the pH of the water was not low enough. At the time the water samples were prepared it was unknown what the potential impact of water pH would be on seed viability and it was decided to err on the cautious side. As well, there is little difference in the pH of the two samples.

Germination was not impacted by soaking seed in acidified water (Figure 7) although germination of seed soaked for 40 minutes was about 8% less than that of seed from the other soak treatments

at week 7. There was little difference in seed germination between the two levels of water pH although there was a tendency for slightly lower germination of seed soaked in water of pH 6.3 (Figure 8). Seed from seedlot B had faster and slightly better germination than seed from seedlot A (Figure 9). The results show that mold did not have a negative impact on seed germination by the end of the trial.

Conclusions

1. Soaking white pine seed in water at pHs of 5.9 and 6.3 did not reduce or control the growth of mold on the surface of the seed or negatively impact seed germination.
2. There was little difference between the two pH levels in the percentage of seed with mold and percentage of seed that germinated.
3. The trial could be repeated with a lower range of water pHs.

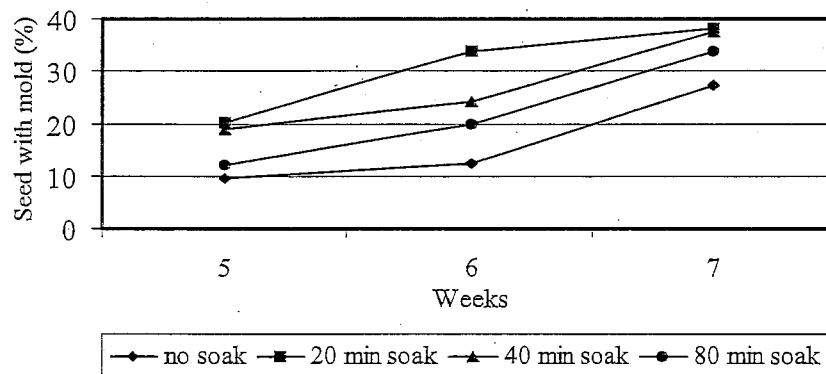


Figure 4. Percentage of white pine seed with mold after various soaking durations in water of two levels of pH.

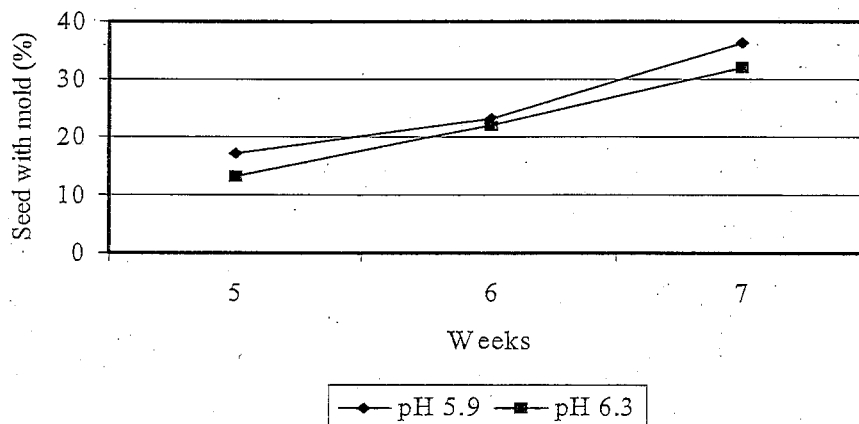


Figure 5. Percentage of white pine seed with mold following soaking in water of two pHs.

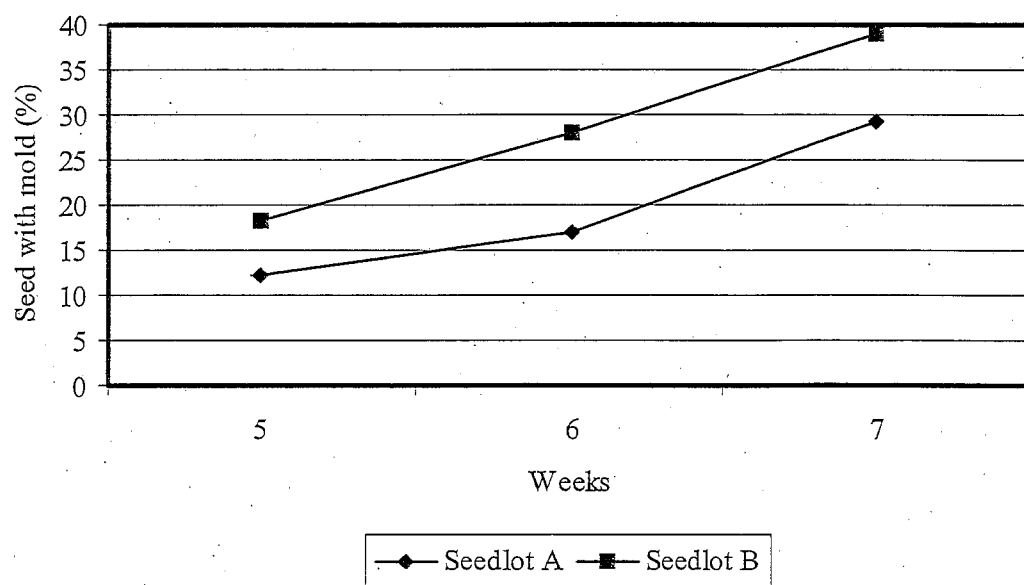


Figure 6. Percentage of seed in two white pine seedlots with mold after various soaking treatments in water at two pHs..

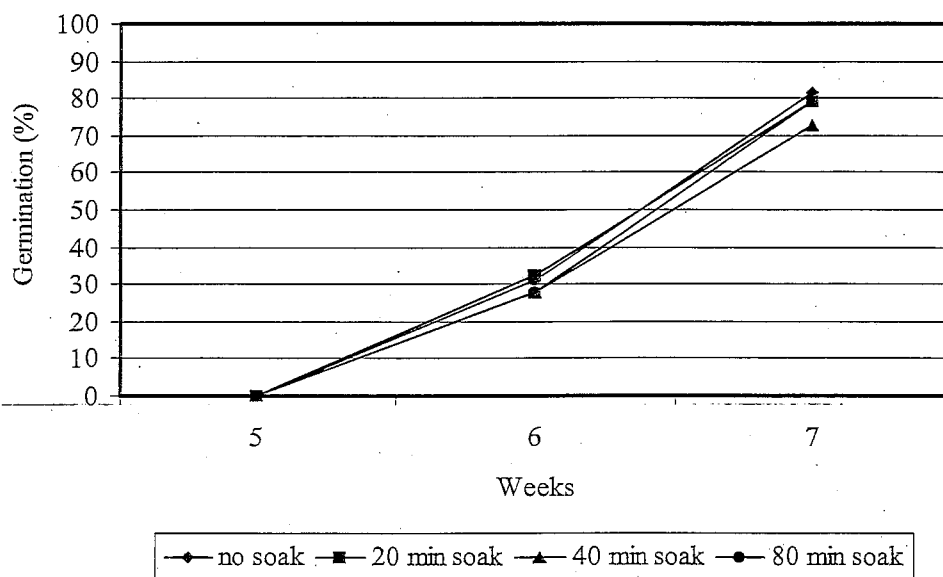


Figure 7. Germination of white pine seed after various soaking durations in water of two levels of pH.

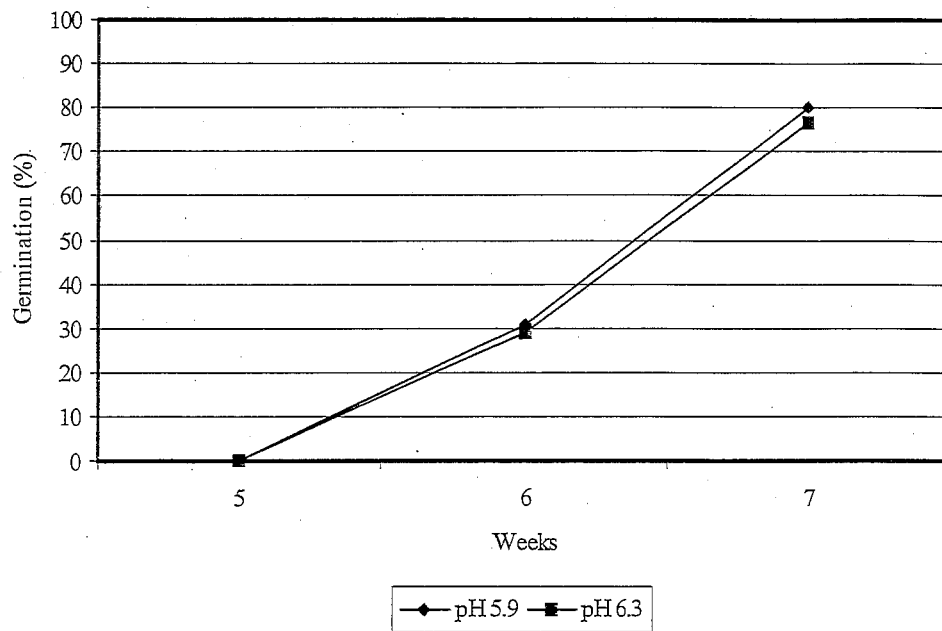


Figure 8. Germination of white pine seed following soaking in water of two pHs.

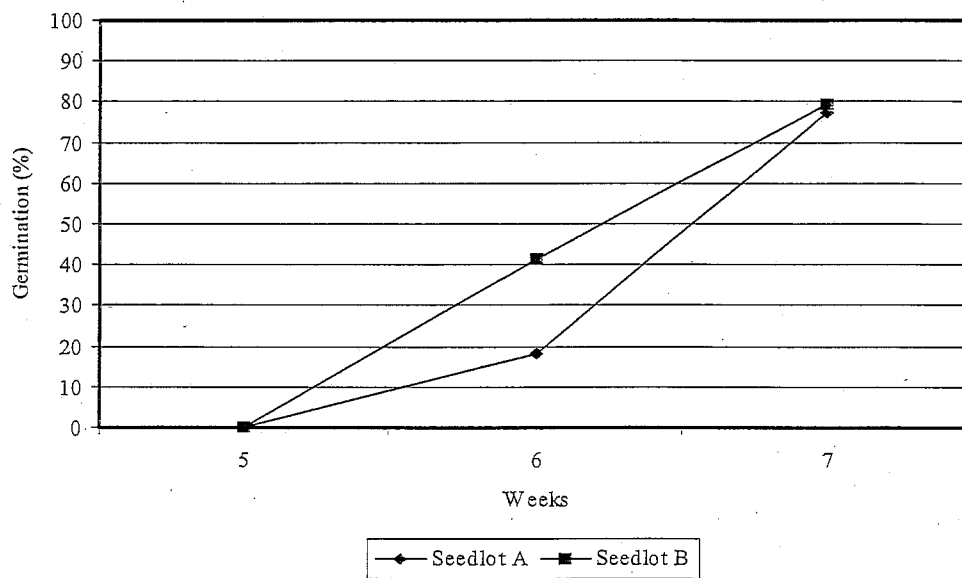


Figure 9. Germination of seed in two white pine seedlots after various soaking treatments in water at two pHs.

Impact of Extended Chilling on Germination of White Pine Seed

This study was conducted by Andrew Vogels who worked in the Seed Centre under the Federal Public Sector Youth Internship Program. In previous work conducted at the National Tree Seed Centre on germination testing of white pine (*Pinus strobus*) seed for a storage experiment, it was determined by a cut test that seed that failed to germinate appeared fresh (firm, white megagametophyte and embryo) and the embryo was fully developed (Daigle and Simpson 2005). These seed were considered to be dormant which raised the question of whether extending the period of moist chilling from four to eight weeks would alleviate dormancy on a higher proportion of ungerminated seed.

Methods

White pine cones were collected in September, 2000, from two sites in New Brunswick; three trees in the UNB Woodlot and three trees in the UNB Noonan Woodlot. After processing, moisture contents, thousand seed weights, and germination tests were performed on the seed. Seed was packaged into vials which were stored at -20°C and in liquid nitrogen vapour at about -145°C. Seed was removed after 12 and 36 months storage and tested for moisture content and germination (Daigle and Simpson 2005). Excess seed was stored in mason jars at -20°C. Samples of this excess seed were used for the present study after being in storage for almost 48 months.

On November 21, 2005 six Petawawa Germination Boxes were set up using moistened VersaPak™. Each box contained four replicates of 50 seeds from each of the six seedlots. The boxes were then transferred to a 3°C cooler and moist chilled for eight weeks. On December 19, 2005 the previously mentioned procedure was repeated and the boxes were placed in the 3°C cooler for four weeks.

On January 16, 2006 the twelve boxes were removed from the cooler and placed in a Conviron™ G30 germinator at conditions of 8 hours light at 30°C and 16 hours dark at 20°C for four weeks. Seed was assessed every two days beginning on day seven. Germinants were classified based on laboratory germination vigour classes (Wang, 1973); germinants reaching Class 3 were considered successfully germinated and were removed. At the completion of the four weeks and final germination counts, the number of abnormal and low vigour germinants were tallied. Tetrazolium tests were performed on seed that failed to germinate to determine the quality of the seed. The tip of the hypocotyl end of the seed was cut off and the seed placed in multi-well plates containing a 5% tetrazolium solution for 18 hours at room temperature. Seed was assessed as healthy or dead according to ISTA (2003).

Results and Discussion

Overall germination was significantly different among the two populations and among seedlots within a population but not significantly different between the two chilling durations (Table 6). Seed undergoing the 4 week pre-chill had an average germination of 71.2% compared to the 8 week

pre-chill that had an average germination of 68.3% (Table 7). These results are similar to those previously found for seed germination before and after 36 months storage which was 62.9 and 74.1%, respectively. Poor germination in some replicates within seedlots 133, 134, and 135, particularly for the 4 week chilling would account for the slight difference between the two chilling treatments. Seedlots collected from the Noonan Woodlot (133, 134, 135) showed more variation in germination than the seedlots collected from the UNB Woodlot (130, 131, 132) for the 4 week chill. Eight weeks of chilling reduced germination and increased the standard deviation of seed from UNB Woodlot. For seed from Noonan Woodlot germination either increased, decreased or remained the same while the standard deviation decreased except for seedlot 135 which had one poor replicate (Table 7).

Table 6. Analysis of variance of high vigour germination of white pine seed chilled 4 and 8 weeks.

Source of variation	d.f.	Mean Square	P value
Population (P)	1	1.5620	<0.0001
Seedlot (population)	4	0.2084	0.0004
Chilling (C)	1	0.0242	0.3750
Replication (R)	3	0.0277	0.4383
P x C	1	0.0391	0.2614
P x R	3	0.0155	0.6715
C x R	3	0.0554	0.1577
Error	31	0.0299	

Figure 10 illustrates that there was little difference in germination between the chilling treatments. Seed that was chilled for 8 weeks started to germinate sooner and germinated more rapidly, but at the end of the 4 week test period final germination was similar for both chilling treatments.

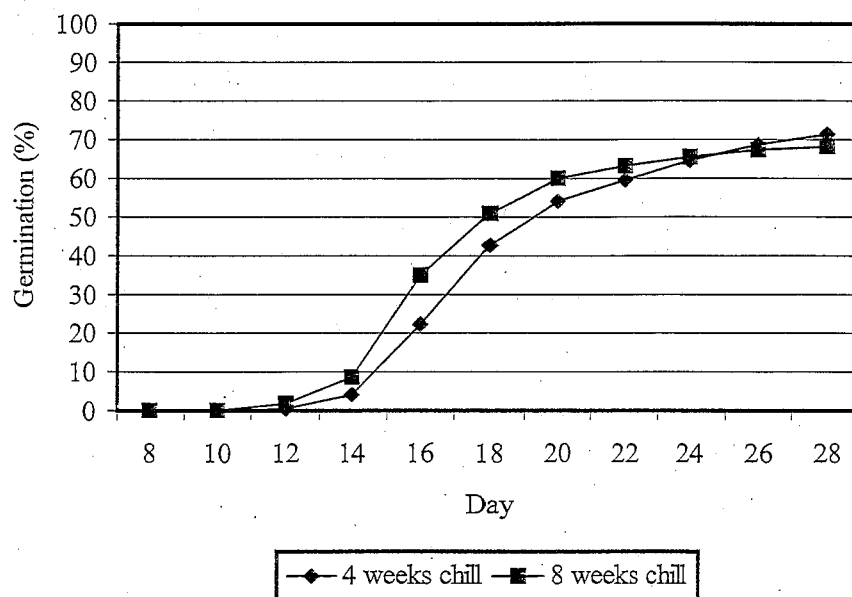


Figure 10. Average germination of six white pine seedlots subjected to 4 and 8 weeks of moist chilling.

Table 7. Percent germination by replicate, the mean, and standard deviation (SD) for six white pine seedlots from two sites chilled for two time durations.

Seedlot	Chill time	Rep 1	Rep 2	Rep 3	Rep 4	% Germ	SD
130	4 weeks	76	78	74	80	77.0	2.24
131	4 weeks	94	98	84	94	92.5	5.18
132	4 weeks	88	92	100	98	94.5	4.76
133	4 weeks	84	34	84	60	65.5	20.66
134	4 weeks	36	34	64	78	53.0	18.68
135	4 weeks	60	26	68	24	44.5	19.72
Average						71.2	
130	8 weeks	50	76	68	80	68.5	11.52
131	8 weeks	96	68	90	88	85.5	10.52
132	8 weeks	96	90	78	96	90.0	7.34
133	8 weeks	72	92	62	88	78.5	12.12
134	8 weeks	50	38	50	36	43.5	6.54
135	8 weeks	66	38	12	60	44.0	21.22
Average						68.3	

Tables 8 and 9 summarize the germination and tetrazolium test results on the ungerminated seed. Based on previous studies using these same seedlots it was thought that the ungerminated seeds were still dormant because a cut test showed the embryos to be fully developed and the megagametophyte to be firm and white. This appeared not to be the case as the tetrazolium test revealed that most of the ungerminated seed in this study were dead. The number of dormant, living, ungerminated seed found through the tetrazolium test was low. There was a slightly higher number of low vigour germinants found within the Noonan Woodlot seedlots (Table 9) particularly in those seedlots chilled for four weeks. Along with the large number of dead seed this accounted for the large number of seed from Noonan Woodlot that did not germinate. Abnormal germination was not common.

Table 8. Percent of white pine seed of six seedlots, subjected to two chilling durations, exhibiting healthy, low vigour, and abnormal germination plus percentage of ungerminated seed that were alive or dead as determined by a tetrazolium test.

Seedlot	Chill time	Germination			Tetrazolium test on ungerminated seed	
		Healthy	Low Vigour	Abnormal	Alive	Dead
130	4 weeks	77.0	6.0	0.0	1.5	15.5
131	4 weeks	92.5	4.0	0.0	0.0	3.5
132	4 weeks	94.5	3.5	0.0	0.0	2.0
133	4 weeks	65.5	7.5	0.0	1.5	25.5
134	4 weeks	53.0	10.5	0.0	0.5	36.0
135	4 weeks	44.5	9.0	0.0	0.5	46.0
130	8 weeks	68.5	5.0	0.0	1.0	25.5
131	8 weeks	85.5	4.0	0.0	0.0	10.5
132	8 weeks	90.0	2.5	3.0	0.0	4.5
133	8 weeks	78.5	3.0	0.0	1.5	17.0
134	8 weeks	43.5	5.0	0.0	0.5	51.0
135	8 weeks	44.0	7.0	0.0	0.5	48.5

Table 9. Percent of white pine seed subjected to two chilling durations, exhibiting healthy, low vigour and abnormal germination plus percentage of ungerminated seed that were alive or dead as determined by a tetrazolium test.

Location	Chill time	Germination			Tetrazolium test on ungerminated seed	
		Healthy	Low Vigour	Abnormal	Alive	Dead
Overall	4 Weeks	71.17	6.75	0.00	0.67	21.42
	8 Weeks	68.33	4.42	0.50	0.58	26.17
UNB	4 Weeks	88.00	4.50	0.00	0.50	7.00
	8 Weeks	81.33	3.83	1.00	0.33	13.50
Average		84.67	4.17	0.50	0.42	10.25
Noonan	4 Weeks	54.33	9.00	0.00	0.83	35.83
	8 Weeks	55.33	5.00	0.00	0.83	38.83
Average		54.83	7.00	0.00	0.83	37.33

Conclusions

The following conclusions are made from this study:

1. Subjecting seed to 8 weeks of chilling rather than 4 weeks did not increase total germination.
2. Subjecting seed to 8 weeks of chilling showed an increase in the rate at which seed germinated.
3. There were significant differences in seed germination between seedlots.
4. There were significant differences in seed germination between populations.
5. Seed that failed to germinate was dead rather than dormant.

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Black Ash Germination Experiment

Introduction

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. Germination prescriptions in the literature vary somewhat but all recommend a combination of warm and cold treatments. The most common prescription involves 60–90 days at room temperature followed by 90 days at 2–5°C (AOSA 2002; Wright and Raucher 2003). 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 while ISTA (2006) recommends 60 days at 20°C followed by 7 months at 3–5°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 because of an insufficient duration for embryo maturation, cold chilling or both. Horseman (2004) showed that embryos of black ash 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 at 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 Raucher 2003).

Black ash is near the northern limit of its range in New Brunswick. Many seed only germinate the second year after seed fall (Wright and Raucher 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 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.

In 2003, a germination trial was set up to look at several factors that may be affecting germination (Daigle and Simpson 2004). The factors examined were: 2 treatment media (moist peat and moist Kimpak™), 2 germination temperatures (20°C constant and 20/30°C), 3 warm (20°C) stratification durations (60, 90, and 120 days), and 3 cold (3°C) stratification durations (90, 135, and 180 days). Finally, pre-treatment durations of 0 and 90 days cold were applied to determine if an initial cold treatment prior to the warm would increase germination. This treatment would more closely mimic the events occurring in nature. The results of this preliminary experiment helped to identify the parameters that were examined in this experiment.

Methods and Materials

Seed used for this experiment was collected by National Tree Seed Centre staff from individual trees from 2 sites in New Brunswick: 3 trees at Cross Creek (lat 46° 16'; long 66° 38') collected in 2000 and 3 trees at Watson Settlement (lat 46° 05'; long 67° 27') collected in 2002. The experiment examined three elements of seed germination: imbibition rate of water by the seed, embryo maturation, and germination following varying treatments.

Imbibition of water

Samaras (seed with pericarp intact) were placed in moistened peat (moisture content ~ 75%) at 22 °C (room temperature) and at 3 °C (cooler) and tested for moisture content after 1, 2, 3, 7, 14, 21, and 28 days. After the prescribed duration, samaras were removed from the peat and the pericarp was removed. Moisture content of the seed was determined by placing approximately 1–2 grams of seed into each of two aluminum containers, that were then placed in a force-draft oven at a temperature of 103 ± 2 °C and dried for 17 ± 1 hours; moisture content was calculated on a fresh-weight basis (ISTA, 2006). The same procedure was used to determine the moisture content of the peat at each time interval.

Seed germination

Samples of 100 black ash samaras were placed in plastic bags with moist peat (moisture content ~ 75%) and subjected to the following treatment combinations: 4 durations of cold pre-treatment (0, 60, 90, and 120 days); 2 durations of warm pre-treatment (90 and 120 days); and 3 durations of moist chilling (150, 180, and 210 days). The cold treatments were carried out in a walk-in cooler (3 °C) and the warm treatment was at ambient laboratory temperature (22 °C). Bags were moved from cold to warm and warm to cold according to a schedule. The bags were opened every 30 days to allow for air exchange. Upon completion of a treatment, four replicates of 25 seed were placed on moistened VersaPak™ in Petawawa Germination Boxes and transferred to a Conviron G30 germination cabinet for 28 days using germination conditions of 8 h light at 30 °C followed by 16 h darkness at 20 °C with a constant relative humidity of 85%. Germination was assessed every 7 days and successful germination was achieved once radicle emergence had occurred. On day 28, seed that failed to germinate were cut and examined to determine if they were still viable.

Embryo maturation

A baseline set of measurements for each seedlot was established by placing seed with pericarp removed in tap water for 120 hours, measuring the length of the seed, excising the embryos and measuring the embryo length. All measurements were made using a digital caliper and measurements recorded to the nearest 0.01 mm.

Sufficient samaras to provide 25 viable embryos were placed in moist peat (moisture content ~ 75%) in plastic bags and subjected to the same treatment conditions as described above. Measurements were also taken at all intermediate steps in the process (every time seed was moved from one condition to the next). This provided data on embryo development at various stages of the process and not just the condition of the embryo at the end of the treatments.

Results

Moisture content

The increase in moisture content of the seed was more rapid for the seed that were imbibed at 22 °C than for those at 3 °C (Figure 11). Moisture content of the seed imbibed at 22 °C increased steadily for 14 days and stabilized. Water uptake for the seed imbibed at 3 °C was slow for the first 3 days then increased to day 14 after which it slowed.

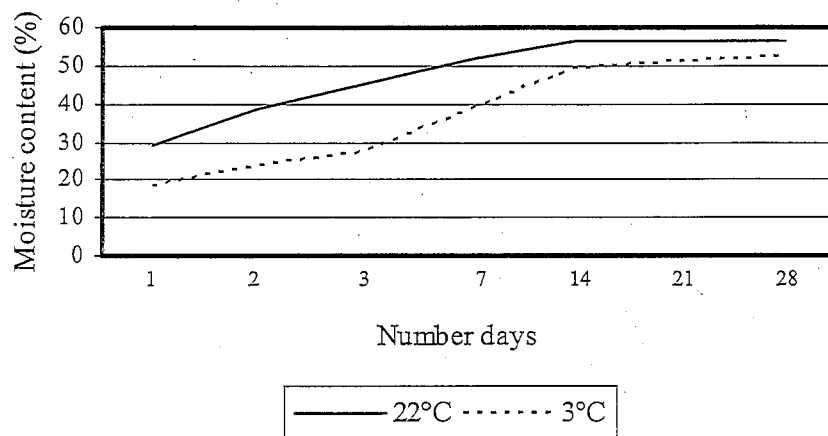


Figure 11. Increase in moisture content of black ash seed placed in moist peat (MC ~ 75%) and incubated at 3 and 22 °C for 1 – 28 days.

Embryo maturation

The baseline measurements for embryo maturation (elongation) are presented here as the ratio of the length of the embryo to the length of the seed (expressed as %). The reason for this is to mitigate the effects of varying seed lengths. Average seed length, as determined from a sample of 25 seeds, ranged from 15.03 to 17.68 mm (mean of 16.81) while the length of the embryos ranged from 8.28 to 10.27 mm (mean of 9.21 mm). There was also a difference in the seed and embryo lengths between the two populations with seed from Watson Settlement having longer seed and embryos than those from Cross Creek.

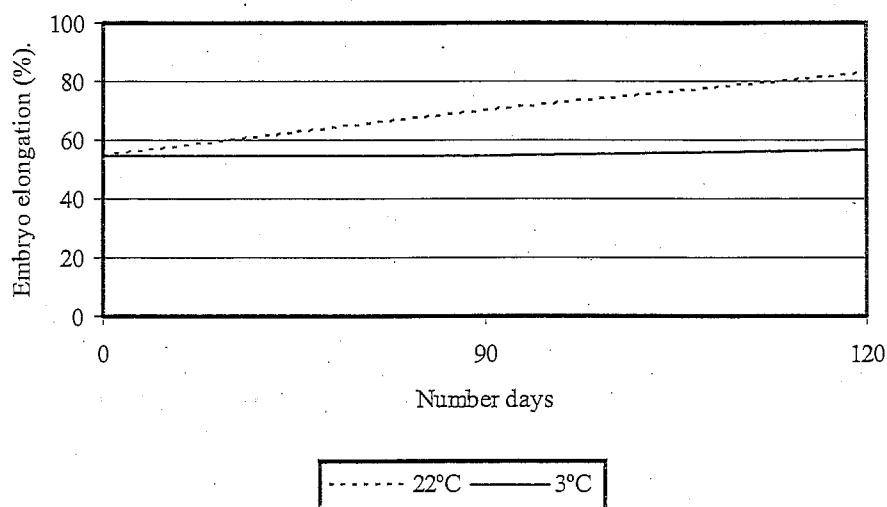


Figure 12. Mean change in embryo elongation for black ash samaras treated in moist peat at 3 and 22°C after 0, 90 and 120 days.

Embryo elongation was very slow at 3°C with very little elongation occurring after 120 days but was much faster at 22°C (Figure 12). The curves represent the average of the 6 seedlots. There was very little variation among seedlots treated at 3°C. However, the seedlots treated at 22°C showed more variation (Figure 13).

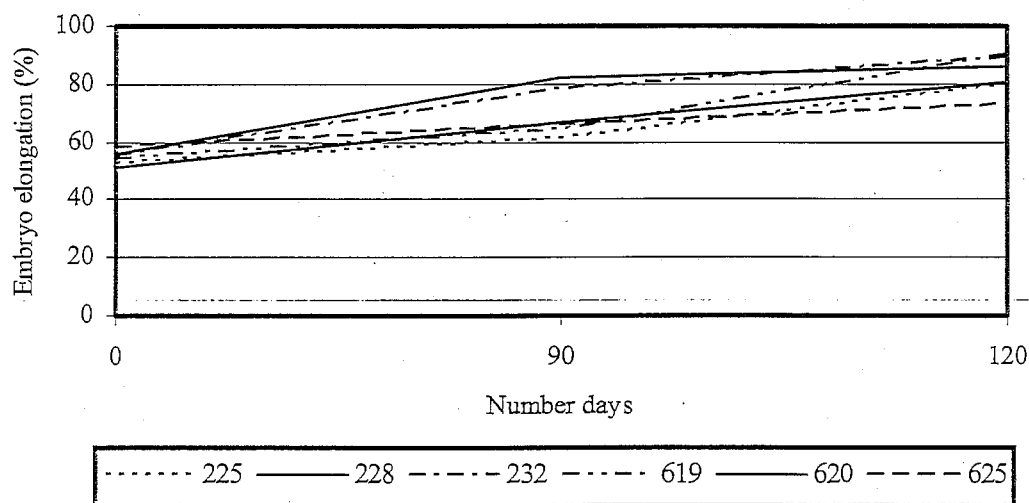


Figure 13. Change in embryo elongation of individual black ash seedlots for samaras treated in moist peat at 22°C after 0, 90 and 120 days.

Figure 13 indicates that embryo elongation was likely to continue beyond 120 days for all seedlots. The scheduled treatments did not include a warm duration of more than 120 days. However, a set of seedlots was not moved according to schedule and was left at 22°C for an additional 30 days. This set had a pre-treatment of 60 days cold (3°C). Embryos were measured on some of these to determine if the additional 30 days at 22°C had an impact on embryo elongation. Figure 14 shows that embryo elongation after a warm treatment of 120 days is the same for seed with and without the 60 day pre-treatment at 3°C. This indicates that the pre-treatment has little effect on embryo elongation. However, the embryos that were left for 150 days continued to elongate.

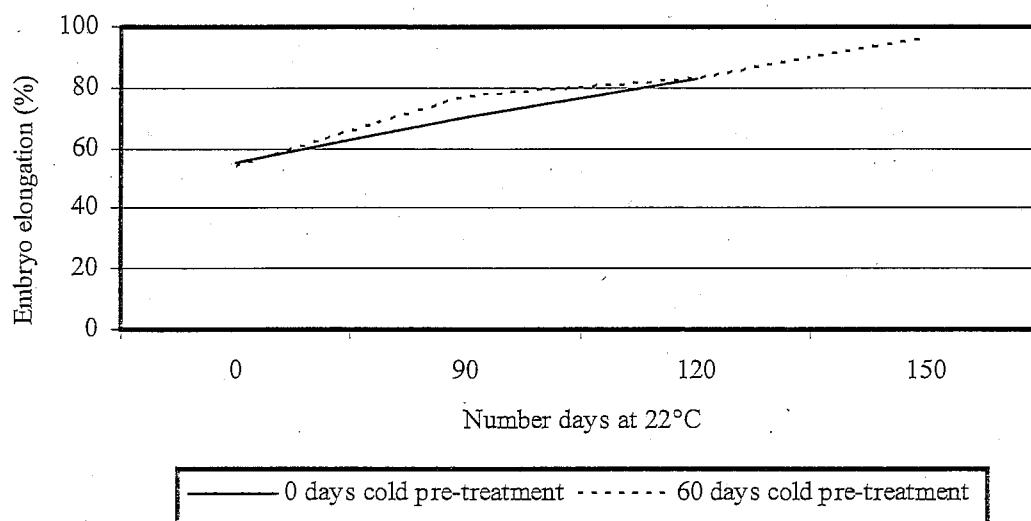


Figure 14 Mean change in embryo elongation for black ash seed treated at 22°C in moist peat for 0, 90, 120, and 150 days with and without an initial treatment of 60 days cold at 3°C.

Germination Results

Percent germination was calculated by excluding dead seed. Germination varied among seedlots and among treatments (Table 10). Three factors were examined: duration of initial cold pre-treatment (0, 60, 90, and 120 days); duration of warm pre-treatment (90 and 120 days); and duration of moist chilling (150, 180, and 210 days). Germination generally increased as the total duration of the treatments increased. Each of these factors had an impact on germination. The impact of the initial cold pre-treatment had a positive effect on germination for all treatment combinations for each of the cold pre-treatment conditions (Figure 15). Germination was lowest for the treatments that did not include a cold pre-treatment. The best results were obtained when a pre-treatment of 90 days was used. Germination declined slightly when the pre-treatment duration was extended to 120 days in all cases except for seedlot 620.

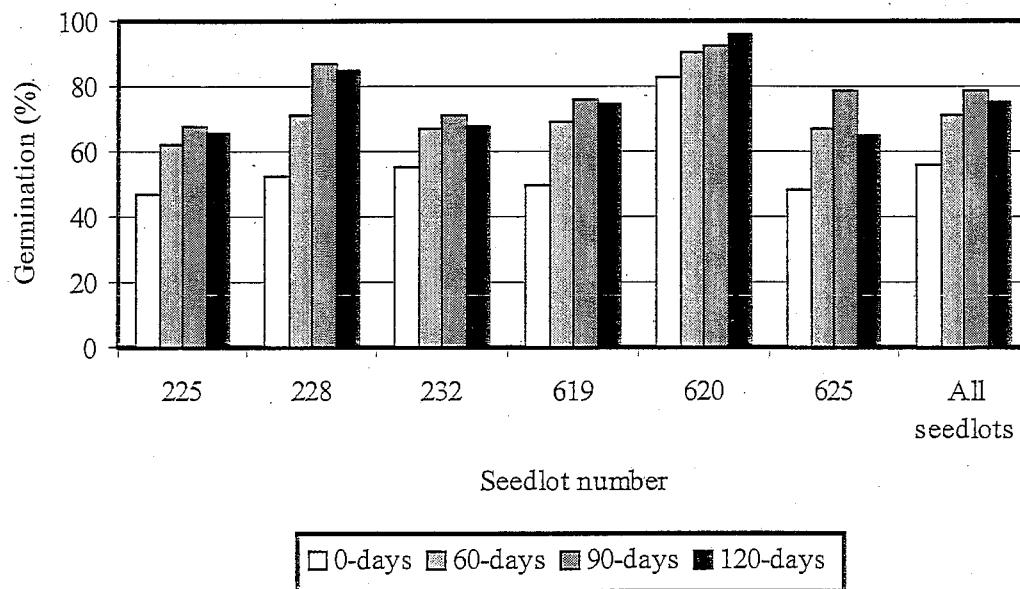


Figure 15. Effect of initial cold pre-treatment on germination of black ash seed.

The purpose of the warm treatment was to mature the embryo. Two warm treatment durations were used (90 and 120 days). All seedlots showed higher germination after 120 days warm than after 90 days (Figure 16). Mean germination for all seedlots increased from 63.0 to 78.5% with increases of individual seedlots ranging from 10.3–20.0%.

Table 10. Effect of varying durations of cold pre-treatment, warm pre-treatment, and moist chilling on germination (%) of 6 single-tree black ash seedlots.

Cold	Warm	Cold	Seedlot					
			225	228	232	619	620	625
0	90	150	42	31	38	38	76	33
0	90	180	47	56	56	39	76	37
0	90	210	39	55	42	38	71	31
0	120	150	48	53	59	49	88	59
0	120	180	55 46	85	69	82	91	79 76
0	120	210	49	87	68	54	98	52
60	90	150	39	42	41	39	82	42
60	90	180	51	58	60	68	84	53
60	90	210	67	77	67	63	91	71
60	120	150	46	67	51	65	91	54
60	120	180	83	87	90	92	93	93
60	120	210	85 86	96	93	88	100	87
90	90	150	47	70	52	63	80	69
90	90	180	59 58	91	75	75	92	77
90	90	210	90 90	92	84	90	93	80
90	120	150	69	87	55	63 62	93	94
90	120	180	88	92	80	83	100	87
90	120	210	54	90	79	82	98	66
120	90	150	59 57	81	44 41	84	89	58 75
120	90	180	73	87	76	70	95	59
120	90	210	44	69 82	51	33	95	51 63
120	120	150	69	90	84	91	98	92
120	120	180	75	86	76	85	99	63
120	120	210	71	95	76	84	99	---

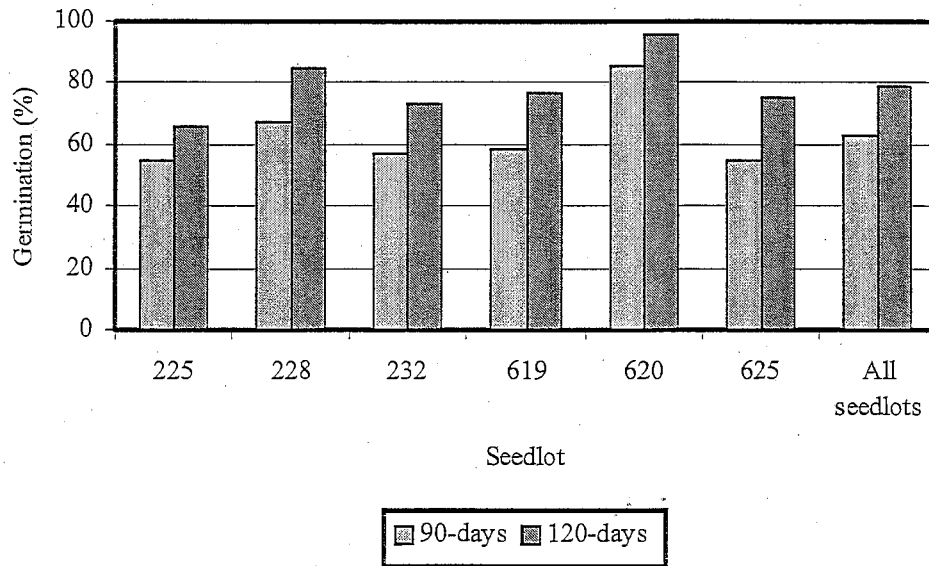


Figure 16. Effect of warm pre-treatment on germination of black ash seed.

The overall effects of the moist chilling treatments can be seen in Figure 17. When germination for all seedlots is considered, 180 days moist chilling provides the highest value with mean germination increasing from 63.4 to 75.6% as the duration of moist chilling increased from 150–180 days and then dropping to 72.9% as the duration of the moist chilling treatment increased to 210 days. However, two seedlots (228 and 620), showed greater germination after 210 days. This may be due to a greater degree of dormancy in these seedlots.

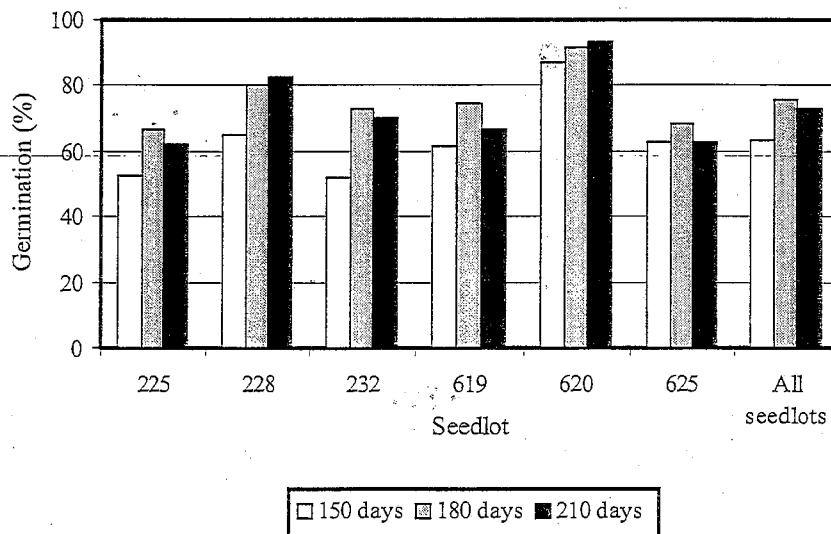


Figure 17. Effect of moist chilling duration on germination of black ash seed.

Figures 15–17 show general germination trends for the three treatments. The variation that exists among seedlots can be more clearly seen when germination is plotted for a given treatment.

Figures 18 and 19 provide a more detailed account of the variation that exists among seedlots under specific conditions. The curves in Figure 18, where samaras did not receive a cold pre-treatment, clearly indicate variation among seedlots. The differences between Figure 18A and 18B are the result of an additional 30 days warm treatment. It is interesting to note that the germination requirements of seedlot 620 have mostly been satisfied whereas germination of other seedlots has not yet reached full potential.

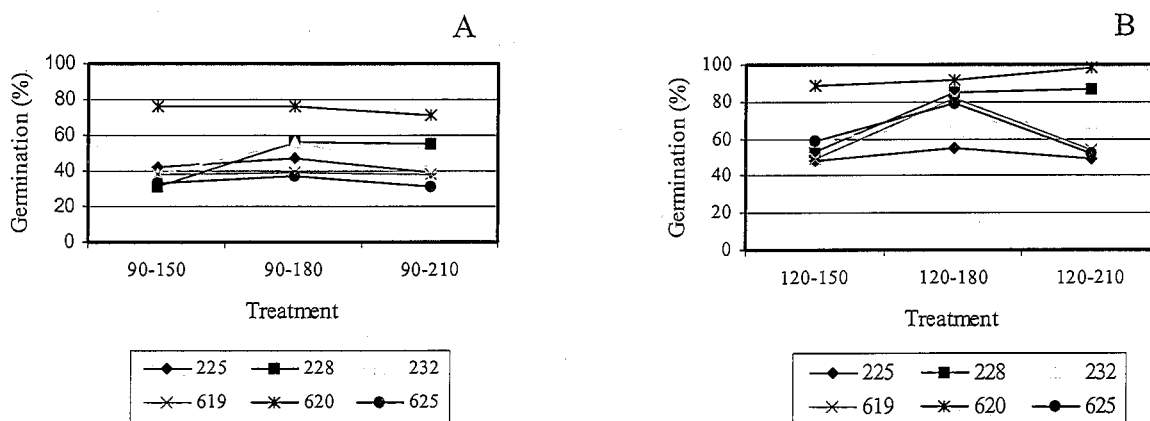


Figure 18. Germination (%) of six single-tree black ash seedlots subjected to 0 days initial cold pre-treatment at 3°C, 90 (A) and 120 (B) days warm treatment at 22°C, and 150, 180, and 210 days moist chilling at 3°C.

The differences that can be observed in Figure 19 are the result of applying an initial 60 days of cold pre-treatment to the seed. Germination has increased for all seedlots which supports the idea that an initial cold pre-treatment improves germination. Figure 19B shows that germination requirements for all seedlots have been essentially satisfied after 60 days cold pre-treatment followed by 120 days warm and 180 days moist chilling.

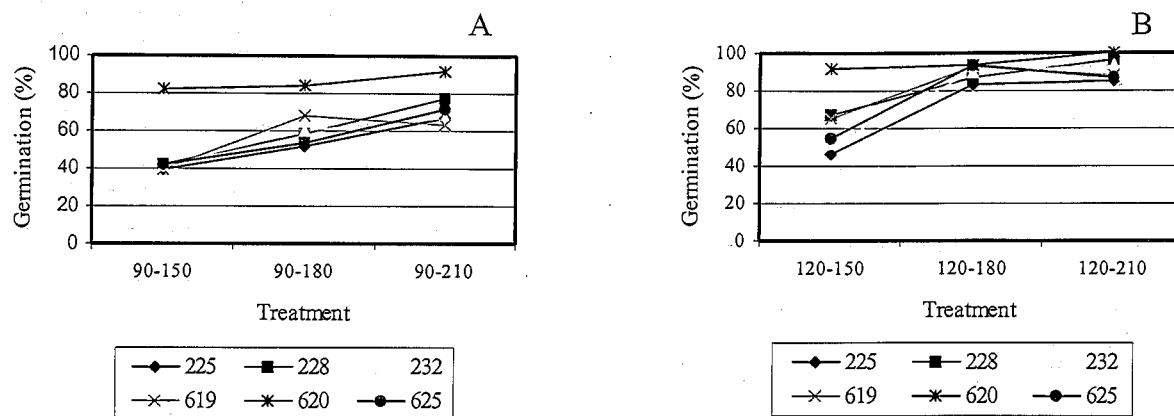


Figure 19. Germination (%) of six single-tree black ash seedlots subjected to 60 days initial cold pre-treatment at 3°C, 90 (A) and 120 (B) days warm treatment at 22°C, and 150, 180, and 210 days moist chilling at 3°C.

The total treatment durations used in this experiment ranged from 240 to 450 days. A concern when seed are subjected to prolonged treatments is potential for damage of the seed. Seed that failed to germinate were dissected and visually assessed for viability. Seed quality did not deteriorate during the treatments. Figure 20 shows the mortality of the 6 seedlots over the course of the treatment durations. There is quite a bit of variation among the individual seedlots with mortality fluctuating up to 10% within a seedlot over the duration of the experiment.

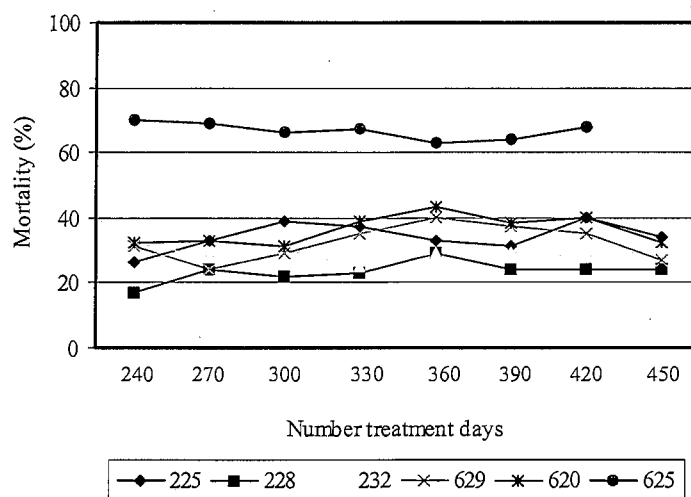


Figure 20. Mortality of black ash seed during treatments.

As with germination, the effects of initial cold pre-treatment, warm treatment, and moist chilling

As with germination, the effects of initial cold pre-treatment, warm treatment, and moist chilling were examined to determine if seed mortality over time could be attributed to these factors. The duration of the cold pre-treatment did not seem to have an effect on seed mortality (Figure 21). Some variation occurred among seedlots but the average of all seedlots showed very little difference between treatments.

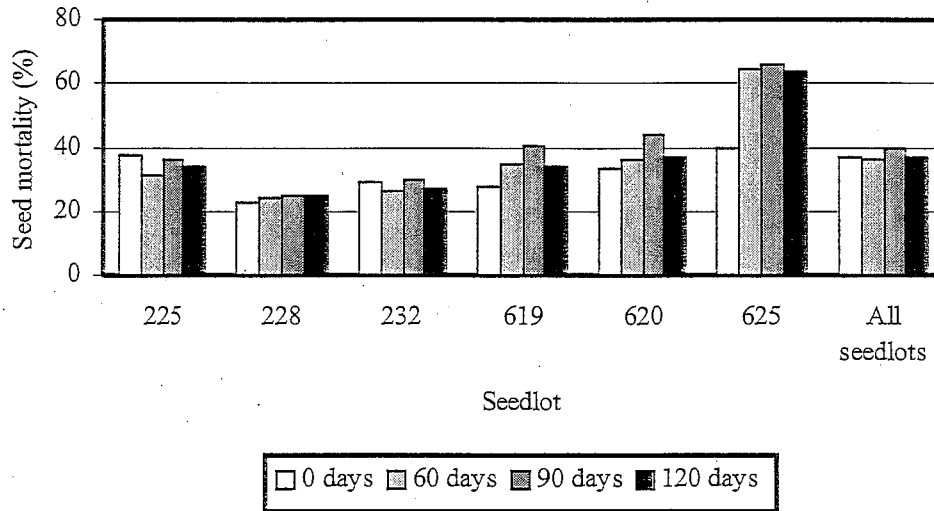


Figure 21. Effect of cold pre-treatment on mortality of black ash seed.

The effects of the warm treatment and the moist chilling were also examined to determine if they impacted seed mortality (Figures 22 and 23). Results were similar to those found with the cold pre-treatment.

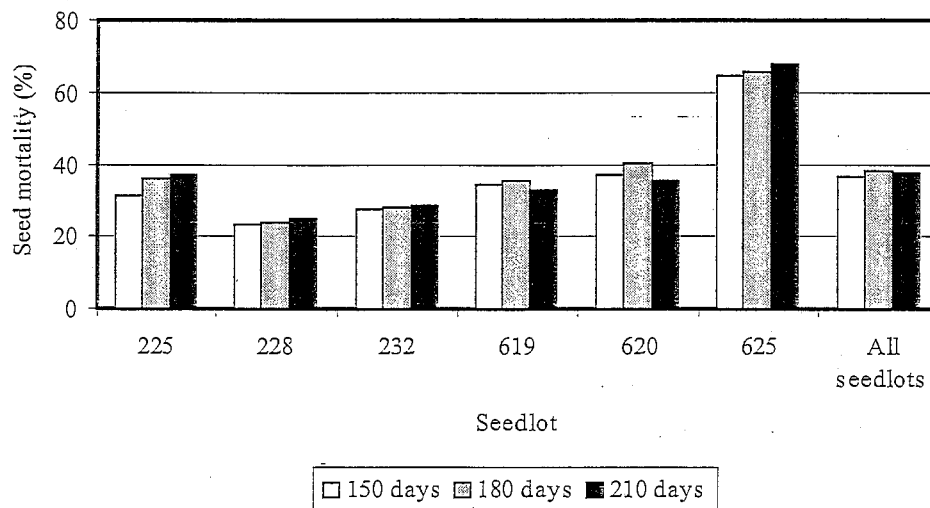


Figure 22. Effect of warm treatment on mortality of black ash seed.

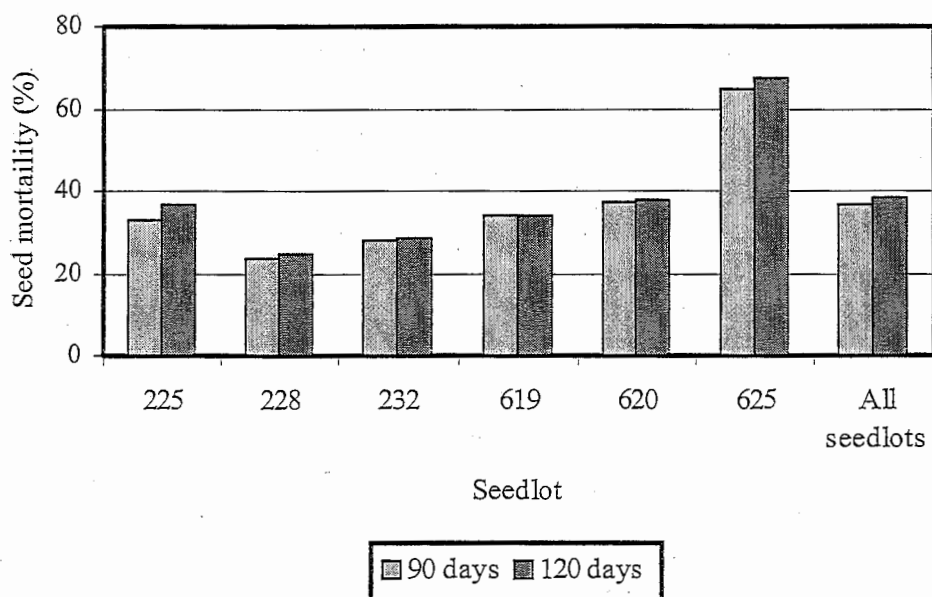


Figure 23. Effect of moist chilling treatment on mortality of black ash seed.

The viability of the six seedlots was also determined by excised embryo tests performed in 2007. The seed were removed from the pericarp and placed in tap water for 120 hours at 3 °C. The embryos were excised by carefully cutting through the seed coat and removing the embryo and placing the embryo on moistened VersaPak™. The embryos were placed in Petawawa Germination Boxes and incubated for 14 days at 25 °C in a Conviron G30 germination cabinet. Three replicates of 25 seed each were used.

Seed were assessed by scoring the level of activity of the embryos as follows:

- 0 non viable seed (no embryo or embryo dead or damaged)
- 1 intact embryo with no activity
- 2 intact embryo with very little activity (some greening of cotyledons)
- 3 intact embryo with moderate activity (cotyledons green)
- 4 intact embryo with good activity (cotyledons green and some radicle elongation)
- 5 intact embryo with very good activity (cotyledons green and radicle growing)

Results of the excised embryo tests showed very little embryo activity in any of the seedlots.

The results of the viability test were compared with the average germination from the 6 best treatments (Table 11). These results represent actual germination figures and are not adjusted to account for dead seed. Viability was higher in all cases and averaged 10% more than germination. The difference in viability and germination is largely attributable to viable seed that failed to germinate.

Table 11. Comparison of viability and germination (%) of black ash seed.

Test Method	Seedlot						All Seedlots
	225	228	232	619	620	625	
Viability (excised embryo)	69	84	61	73	63	40	65
Mean germination of 6 best treatments	53	71	60	57	59	30	55
Mean viable seeds of 6 best treatments	13	6	11	8	2	4	7

Discussion

Black ash seed requires extensive durations of treatments in order to satisfy its germination requirements. Traditional treatments have included varying durations of warm and cold treatments. These serve to mature the embryo and alleviate dormancy.

Embryo maturation (elongation) occurred mainly during the warm stratification phase (Figure 12). After 120 days at 3 °C the increase in the length of the embryos was less than 2% compared to a 27% increase after 90 days and a 51% increase after 120 days at 22 °C. These data suggest that most of the elongation occurred after 90 days and support the findings of Horseman (2004) who suggested that embryo elongation was still occurring after 15 weeks. These results were compared to seed that had undergone a 60-day cold pre-treatment (Figure 14). After 120 days there was no difference between the seed that had a 60-day cold pre-treatment to those that had no pre-treatment. This suggests that the initial cold pre-treatment does not have an effect on embryo elongation. Because of a failure to move some seed from warm to cold, data were available for 150 days of warm stratification and these embryos showed a 78% increase in length. Unfortunately, there is no corresponding germination data available to determine if this would impact germination. This may be something to consider in future experiments.

Germination test results showed some interesting relationships between the various parameters that were tested. The use of a cold pre-treatment consistently yielded better results. This is consistent with earlier tests using a 90-day cold pre-treatment (Daigle and Simpson 2005). Data support the fact that this pre-treatment does not help elongate the embryo. The reason for the increase in germination may be due to the seed starting to lose dormancy before embryo elongation occurs. In nature, black ash seed usually do not germinate the year after seed fall but the following season. The cold pre-treatment therefore simulates more closely the manner by which black ash seed germinate in nature.

The variation that exists among seedlots is a factor that cannot be overlooked. Seedlot 620 was much less dormant than the others. Since most seedlots are a product of bulk collections (consisting of seed from many trees), it is important to satisfy the requirements of the seed with the most demanding dormancy. This is especially important to nursery growers who want all of their seed germinating at the same time. In the past, growers have had to give multiple treatments to black ash seed because many of the seed failed to germinate after the first series of treatments. The findings of this experiment should allow growers to accurately plan their seeding schedule.

The embryo excision test is a reliable method of estimating the quality of black ash seedlots. When compared to seed germination, viability was 10% greater. This was not unexpected as the viability test indicates the potential of seed to germinate whereas the germination test gives the actual results. The advantage of the excised embryo test is that an estimate of the quality of a seedlot can be achieved in less than 3 weeks compared to one year for a germination test. It is interesting to note that most of the 10% difference can be attributed to viable seed that failed to germinate. It is possible that our treatments did not satisfy the dormancy requirements of all the seeds.

Moist peat worked very well as a pre treatment medium. This is important because this is the method commonly used by growers to stratify black ash seed. The peat helps control mold and does not appear to damage the seed.

Recommendations

Based on this experiment, black ash seed should be germinated in the following manner:

- use moist peat (MC 70 – 75% to stratify seed
- 60 days at 3°C
- 120 days at 22°C
- 180 days at 3°C
- open bag for 24 hours every 30 days to allow for air exchange
- use germination conditions of 20°C for 16 hours and 30°C for 8 hours

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Presence of Hemlock Seed Adelgid in Four Eastern Hemlock Populations

Cones from eastern hemlock (*Tsuga canadensis*) trees were collected between October 10–12, 2006 from three locations in Québec and between September 27–October 5 from one location in Ontario. Fifteen trees were sampled at each site: Charny, Sherbrooke, and Lytton Lake in Québec and Petawawa Research Forest in Ontario. Cones were placed in a ventilated greenhouse to allow for drying and opening. They were then brought into the lab and processed by tumbling. Cones that failed to open were soaked and dried to encourage opening. The extracted seed were de-winged by gently rubbing in a cloth bag and blown in an air aspirator to remove empty seed and debris.

Moisture contents were determined for the seed. Seedlots with a high moisture content were conditioned to lower moisture content to acceptable levels. However, the moisture content of some seedlots could not be lowered and cut tests were performed on seed to determine the cause. The cut tests revealed insect larvae inside the seed. These larvae were of a much higher moisture content than the seed and were causing the high readings. The larvae were identified as the hemlock seed adelgid (*Megastigmus hoffmeyer*) which is a seed insect of eastern hemlock (Turgeon et al. 2005). One of the characteristics of this insect is that there is no visible evidence of damage to the seed.

Alcohol separation was used to separate the seed with insect larvae from healthy, normal, filled seed. Alcohol separation of eastern hemlock seed did not affect germination when seed was immersed in alcohol for up to 15 minutes (Daigle and Simpson 2003). This is important because hemlock seed have resin vesicles that are damaged by alcohol and the long-term effects of alcohol on the storage ability of hemlock seed is not known. However, it is also critical that insects be eliminated from the collections whenever possible because of the risk of transfer to other areas or countries when seed is requested.

Alcohol separation was accomplished by placing the seed in a funnel containing absolute ethanol. A rubber tube at the bottom of the funnel was clamped shut. The heavier filled seed sank while the lighter insect infested or dead seed floated. Once the seed had flowed down the funnel, the clamp was released and the “sinkers” were collected in a sieve and immediately rinsed in tap water. The sample was then laid on paper towel on a screen tray and allowed to dry for 24 hours. The “floaters” were collected by releasing the clamp and flushing the seed into the sieve. Any remaining seed were rinsed out of the funnel with ethanol and collected. These seed were also rinsed and laid to dry for 24 hours in the same manner as the “sinkers”.

A sample of 50 seeds from each of the “sinkers” and “floaters” was assessed by performing a cut test and determining whether the seed was “fresh” (normal seed with firm megagametophyte), dead (decayed or empty seed), or contained insect larvae. Because of small quantities of seed for some of the “sinkers” collections, 7 of the 60 seedlots were not assessed. However, none of the sampled seed from the “sinkers” samples contained any *Megastigmus hoffmeyer* larvae. In fact, only 1 seed out of the 2,650 seeds that were assessed was not classified as “fresh”.

The results from the "floaters" samples were much more variable and results varied between populations and among trees within a population. Table 12 shows the results of from the Lytton Lake population.

Table 12. Percentage of insect, dead, and fresh eastern hemlock seed from Lytton Lake after alcohol separation.

Seedlot	MC (%)	Seed Quantity		Floaters Classification (%)		
		Sinkers	Floaters	Insects	Dead	Fresh
20062167	5.63	41.00	2.04	64	26	10
20062168	5.61	27.04	1.11	72	24	4
20062169	6.20	7.33	0.96	34	64	2
20062170	9.33	22.28	12.11	38	56	6
20062171	4.37	34.35	0.38	0	70	30
20062172	6.31	18.12	1.98	100	0	0
20062173	5.68	50.11	3.24	72	14	14
20062174	5.45	22.16	1.13	36	44	20
20062175	4.37	14.20	0.48	2	12	86
20062176	7.10	19.81	3.25	86	8	6
20062177	5.86	12.12	1.07	84	0	16
20062178	7.29	24.85	4.33	88	6	6
20062179	5.66	26.98	6.03	16	78	6
20062180	5.71	31.14	2.62	32	64	4
20062181	5.35	51.38	5.83	22	8	70

Alcohol separation removed on average 10% of the seed (floaters). Some seedlots had very little damage with only 1% of the seed being removed while others had as much as 35% of the seed removed. The incidence of insect infested seed ranged from 0–100%. The alcohol also removed dead seed and also some filled (fresh) seed.

Tables 13 – 15 give the results of the alcohol separation treatment for the other three populations.

Table 13. Percentage of insect, dead, and fresh eastern hemlock seed from Charny after alcohol separation.

Seedlot	MC (%)	Seed Quantity		Floaters Classification (%)		
		Sinkers	Floaters	Insects	Dead	Fresh
20062182	5.27	6.29	0.65	4	80	16
20062183	4.93	20.74	4.01	0	76	24
20062184	5.55	10.56	1.27	2	92	6
20062185	5.13	7.00	1.00	0	94	6
20062186	4.34	11.77	0.31	0	86	14
20062187	5.50	9.08	1.88	0	98	2
20062188	4.53	16.19	2.76	0	98	2
20062189	4.41	2.49	0.19	0	18	82
20062190	5.40	6.44	1.10	0	94	6
20062191	6.03	6.37	2.07	2	98	0
20062192	4.75	10.76	0.29	8	54	38
20062193	5.12	12.48	1.16	0	96	4
20062194	5.96	1.33	0.42	0	90	10
20062195	5.43	17.34	2.03	4	64	32
20062196	5.92	5.39	4.45	0	100	0

Based on our sample, only one third of the trees in the Charny population had *Megastigmus hoffmeyer* larvae in the seed. Although the incidence of the insect was lower, the alcohol separation still removed about 14% of the seed. Most of the seed removed were dead but some filled seed were also among the "floaters".

Table 14. Percentage of insect, dead, and fresh eastern hemlock seed from Sherbrooke after alcohol separation.

Seedlot	MC (%)	Seed Quantity		Floaters Classification (%)		
		Sinkers	Floaters	Insects	Dead	Fresh
20062197	5.53	12.93	0.36	0	52	48
20062198	6.18	19.41	0.48	22	16	62
20062199	5.58	8.60	0.73	0	82	18
20062200	5.64	28.13	5.03	6	66	28
20062201	5.66	13.32	6.43	0	98	2
20062202	4.90	15.82	2.60	0	2	98
20062203	5.00	29.79	1.48	2	38	60
20062204	6.10	15.53	2.18	0	90	10
20062205	5.73	47.06	0.81	0	16	84
20062206	6.14	28.62	5.48	0	72	28
20062207	5.53	25.25	1.04	8	60	32
20062208	5.72	27.69	3.27	0	92	8
20062209	5.67	18.45	4.93	2	22	79
20062210	5.26	29.84	0.36	2	40	58
20062211	4.92	9.84	5.89	0	12	88

*Megastigmus hoffmeyer*i larvae was found in 40% of the Sherbrooke seedlots. The amount of seed removed by the alcohol was similar to Lytton Lake and Charny.

Table 15. Percentage of insect, dead, and fresh eastern hemlock seed from Petawawa Research Forest after alcohol separation.

Seedlot	MC (%)	Seed Quantity		Floaters Classification (%)		
		Sinkers	Floaters	Insects	Dead	Fresh
20063212	4.72	41.56	0.80	0	4	96
20063213	5.00	194.55	1.98	0	50	50
20063214	5.07	139.24	9.50	4	52	44
20063215	4.98	102.15	3.49	0	84	16
20063216	6.39	75.08	9.54	48	28	24
20063217	5.10	73.38	8.44	2	88	10
20063218	5.42	111.07	10.44	2	98	0
20063219	5.25	114.16	6.21	10	48	22
20063220	4.55	104.77	15.83	2	82	16
20063221	4.88	41.95	0.48	62	8	30
20063222	5.87	60.83	6.73	10	90	0
20063223	6.19	33.42	6.32	16	84	0
20063224	5.10	65.58	2.92	0	92	8
20063225	5.03	95.30	3.76	2	94	4
20063226	4.63	71.20	0.60	2	0	98

Megastigmus hoffmeyer was present in most of the trees in this population. The quantity of seed removed by the alcohol separation process was less than for the other populations (6%). Seed that were classified as "fresh" were also removed by the alcohol which was the case for all of the populations.

The results clearly show the effectiveness of using alcohol separation to remove insect infested and dead seed from a seedlot. Unfortunately, some "fresh" seed are also removed by this process. It may be possible that the "fresh" seed that are removed by the alcohol have not completed their development and are immature. It would be possible to test this by taking seed from the "floaters" that show a high percentage of "fresh" seed and germination testing them and comparing the results to the "sinkers" from the same seedlot.

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Germination Testing of Tamarack Tree Breeding Seedlots

During 2006, a total of 348 tamarack (*Larix laricina*) seedlots were tested. These seedlots were the result of collections made between 1970 and 1984 to establish provenance trials. It is believed that the earlier collections were stored at 4°C. In 1986, seedlots stored at 4°C were conditioned (dried) and stored at -20°C. Germination tests were not carried out on freshly collected seed. The only testing carried out prior to 2006 was in 1976 when seedlots collected in 1970 were tested and again in 1993 when 211 seedlots were tested.

Eleven seedlots collected in 1970 were tested in 1976, 1993, and 2006 (Table 16).

Table 16. Germination(%) of 11 tamarack seedlots collected in 1970 and tested in 1976, 1993, and 2006.

Seedlot	Germination year		
	1976	1993	2006
7021900	72.5	14.0	0.0
7021970	56.5	2.0	3.0
7021980	72.5	26.5	17.0
7031510	98.5	35.0	14.0
7031540	96.5	37.5	36.0
7031550	98.5	78.0	62.0
7031560	92.5	10.5	7.0
7031570	90.5	9.3	11.0
7031580	87.5	50.0	18.0
7031590	85.0	31.0	20.0
7031620	92.5	1.3	0.0
Average	85.7	26.8	17.1

Although the viability of the fresh seed is not known, the germination results from the tests carried out in 1976 indicated that these were good quality seedlots (mean germination of 85.7%). Germination tests carried out in 1993 showed a marked decrease in mean germination (85.7 to 26.8%). This represents an annual average loss in germination of 3.46%/yr. Average germination in 2006 was 17.1%. Although this is low, it represents an annual average loss in viability between

1993 and 2006 of 0.75%/yr which is considerably lower than the loss which occurred between 1976 and 1993. It appears that the conditioning treatment applied to the seed in 1986 along with moving the seed to a more favorable storage condition (-20°C) helped to stabilize the seed and reduced the rate of deterioration.

In 1993, 211 of the 348 seedlots were tested (Table 17). The germination data do not show any definite trends with regard to the loss of germination and the age of the seed. The mean germination loss/year was 0.64% and ranged from a low of 0.46% for seed collected in 1979 to a high of 0.85% for seed collected in 1978. However, the figure obtained for 1978 is only based on 3 seedlots.

Table 17. Tamarack seedlots collected between 1970 and 1984 and germination tested in 1993 and 2006.

Collection year	No. seedlots	Mean germination		Δ Germ(%) / Yr
		1993	2006	
1970	13	23.5	14.5	- 0.69
1976	66	50.8	41.6	- 0.71
1978	3	47.6	35.7	- 0.85
1979	46	24.9	18.9	- 0.46
1980	2	23.0	14.0	- 0.69
1982	46	42.3	32.6	- 0.74
1983	3	74.8	69.0	- 0.45
1984	33	51.8	44.4	- 0.57
1970 – 1984	211	42.0	33.7	- 0.64

Earlier germination curves developed for 41 seedlots and 63 tests indicated an annual loss in germination of - 0.81%/year for seed stored for 22 years. Germination testing of 535 jack pine (*Pinus banksiana*) seedlots in 2005 showed an annual loss of - 0.13%/year. The results suggest that conditioning of seed is effective in slowing the rate of loss of viability.

White Birch Processing

White birch (*Betula papyrifera*) seed, in its natural form, is a winged nutlet that is borne on catkins. The quality of the seed varies greatly from tree to tree and from site to site (Daigle and Simpson 2003). Seed quality is greatly improved through de-winging and alcohol separation. The advantages are higher germination and more efficient use of storage space.

Approximately 3 L of catkins were collected from 17 white birch trees at Semiwagan Ridge, New Brunswick on August 16, 2006. The seed were brought into the lab to dry and the catkins were lightly rubbed to separate the nutlets and bracts from the catkin axis. The winged nutlets were separated from the bracts by sieving and air aspiration and a sample of winged seed removed. The remaining winged seed were lightly rubbed in a cloth bag to remove the wing and this sample was lightly blown in an attempt to remove any empty or damaged seed. A second sample was taken after the blowing was complete. Alcohol separation using absolute ethanol was carried out as a final treatment to separate the filled seed. Emersion of white birch seed in alcohol for a short duration does not affect the viability of the seed (Daigle and Simpson 2001).

Germination tests were conducted on the seed. Four replicates of 50 seed each for the winged, de-winged, and de-winged + alcohol treatments were placed on moistened VersaPak™ and placed in germination cabinets for 21 days at 20°C for 16 hours without light and 30°C for 8 hours with light and at a constant relative humidity of 85%. Results of the germination tests are shown in Table 18.

Germination of winged seed ranged from 0.5 to 23.0 % (mean of 5.8%) and from 39.0 to 92.5% (mean 74.5%) when the seed was de-winged and separated using ethanol. De-winging alone did not improve germination of the seed. Air aspiration which is commonly used to improve seedlot quality, is an ineffective technique for white birch. These results are consistent with results obtained in 2003. The yield of seed after the alcohol separation treatment ranged from 0.6 to 24.4 grams. Much greater quantities would be expected from 3 L of catkins.

Although the production of white birch catkins was abundant in 2006, the proportion of viable seed was very low. The quality of the catkins at collection time did not suggest insect or disease damage so it is likely that the low germination is due to inadequate pollination. GPS coordinates were obtained for all of the trees sampled. It is therefore possible to return to the site and sample the same trees to see if the poor germination occurs another year.

Table 18. Germination (%) of winged, de-winged, and de-winged + alcohol separated white birch seed.

Seedlot	Winged	De-winged	De-winged + Alcohol
20061076	1.0	1.0	53.5
20061077	5.0	3.0	82.5
20061078	11.0	9.5	88.5
20061079	2.5	4.0	90.5
20061080	2.5	3.5	73.5
20061081	2.5	2.5	85.5
20061082	3.0	4.0	68.0
20061083	5.0	3.5	85.0
20061084	3.5	2.0	86.5
20061085	0.5	0.0	39.0
20061086	4.5	2.5	75.0
20061087	6.5	5.5	52.0
20061088	23.0	19.0	87.0
20061090	6.0	5.5	88.5
20061091	10.5	5.0	92.5

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- Daigle, B.I.; and Simpson, J.D. 2001. National Tree Seed Centre annual report 2000. Nat. Res. Can., Can. For. Serv.-Atl., 46p.
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De-winged Yellow Birch Seed

Past experiments measuring the effects of alcohol separation using absolute ethanol on de-winged white birch (*Betula papyrifera*) have produced favorable results by increasing seed germination. Due to ethanol's low specific gravity and low surface tension, filled seed can be separated from empty, partially filled, and insect infested seed. Non-viable seed float whereas viable seed sink where they can be collected (Daigle and Simpson 2004). The purpose of this experiment was to determine if results involving white birch would be similar when applied to yellow birch (*Betula alleghaniensis*) seed. This study was conducted by James Bird while working under the Federal Public Sector Youth Internship Program.

Methods

Fifteen single-tree yellow birch seedlots were collected from Williamsdale, Nova Scotia on September 8, 2006. All collections were made from trees with good catkin production. Following collection, the catkins were allowed to air dry indoors. Processing involved breaking apart the catkins by hand and separating winged seed and bracts by sieving, air aspiration, and finally by manually extracting any unwanted debris with tweezers. After cleaning, seed from each tree was separated into three testing samples and labeled as follows: winged, de-winged, and de-winged + alcohol.

Germination tests were performed on all three categories of seed. Winged seed were not available for two seedlots. The winged seed were placed into 13 Petawawa Germination Boxes on top of moistened VersaPak™. Four replicates of 50 seeds were positioned in each box and germinated at 20/30°C for 21 days. Seed were assessed every 2 days after the 7th day. Seed that satisfied germination criteria (clearly developed radicle, hypocotyl, and cotyledons) were tallied, removed, and discarded.

The remaining seed from each seedlot were de-winged by gently rubbing the seed in a thin cloth bag. Seed were separated from the crushed wings by sieving and air aspiration. The de-winged seed were tested using the same method as the winged seed. A sub-sample of remaining seed was floated in ethanol to separate filled from empty seed. After this process the seed were rinsed in tap water and dried at ambient room conditions of 21°C and 20% RH for 24 hours. Germination tests were performed on the de-winged + alcohol separated seed using the same method used for the prior two seed categories.

Results and Discussion

Mean germination for all winged seedlots was 17.1% with seed germination ranging from 2.0 to 41.5% while mean germination of the de-winged seed was 15.8% and ranged from 2.0 to 49.0%. Mean germination for de-winged + alcohol separated seed was much better at 39.4% and a range of 4.5 to 92.0% (Table 19). This follows results found for white birch where germination of

winged seed was 32.2% compared to 81.8% for de-winged + alcohol treated seed (Daigle and Simpson 2004).

Table 19. Germination (%) of 15 seedlots of winged, de-winged, and de-winged + alcohol separated yellow birch seed and quantity and 1000-seed weight of de-winged + alcohol separated seed.

Seedlot	Winged	De-winged	De-winged + alcohol	Quantity (g)	1000-seed weight
20061107	6.5	6.5	12.0	46.2	1.12
20061108	8.0	9.5	12.0	29.6	1.26
20061109	14.0	14.0	58.0	13.1	1.39
20061110	34.0	23.0	76.5	58.5	1.23
20061111		10.0	11.5	32.7	1.24
20061112	16.5	11.5	35.5	23.9	1.05
20061113	8.5	7.0	42.5	5.9	0.98
20061114	17.5	15.5	26.5	42.4	1.07
20061115	8.0	8.5	7.0	119.7	1.10
20061116		19.5	28.5	10.8	1.34
20061117	9.0	8.5	61.5	7.0	1.05
20061118	37.0	49.0	92.0	12.6	0.93
20061119	2.0	2.0	4.5	46.5	1.34
20061120	20.0	19.5	84.0	14.4	1.07
20061121	41.5	34.0	39.5	31.6	0.96
Mean	17.1	15.8	39.4		

Results indicated that there is little difference in mean germination between winged and de-winged seed (Figure 24). In some cases, de-winged actually showed signs of hindering germination in some seedlots while there was no apparent difference between other seedlots. These differences could be explained by the fact that air aspiration is sometimes ineffective in separating all dead and partially empty seed from good viable seed. The equipment is not sensitive enough to easily separate light seed such as yellow birch.

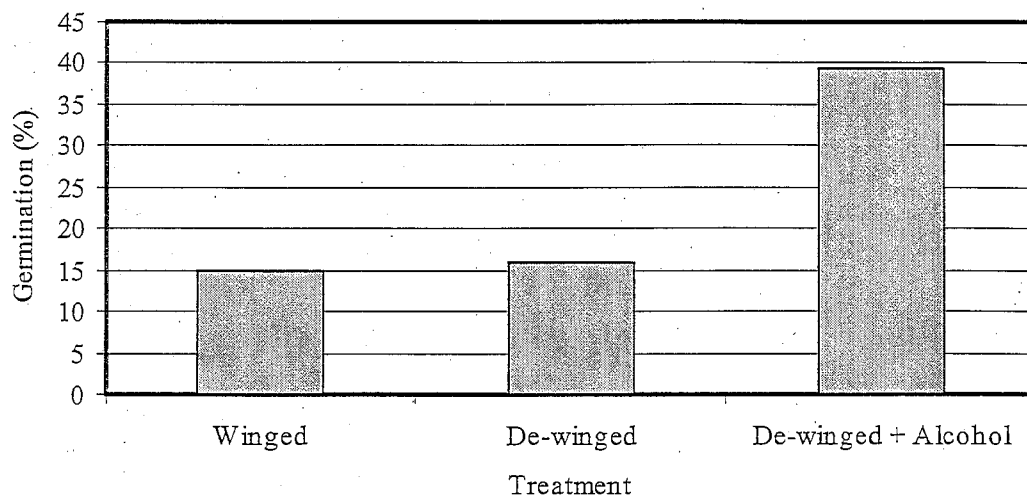


Figure 24. Mean germination of 15 winged, de-winged, and de-winged + alcohol separated yellow birch seedlots.

Although the quantity of catkins collected from each tree was similar, some trees produced more viable seed. This production of viable seed, as evidenced by the germination results of all three test conditions, varied greatly among trees and is apparent in Table 19 and Figure 25. Germination of seed following the de-winging + alcohol treatment was generally improved for each seedlot.

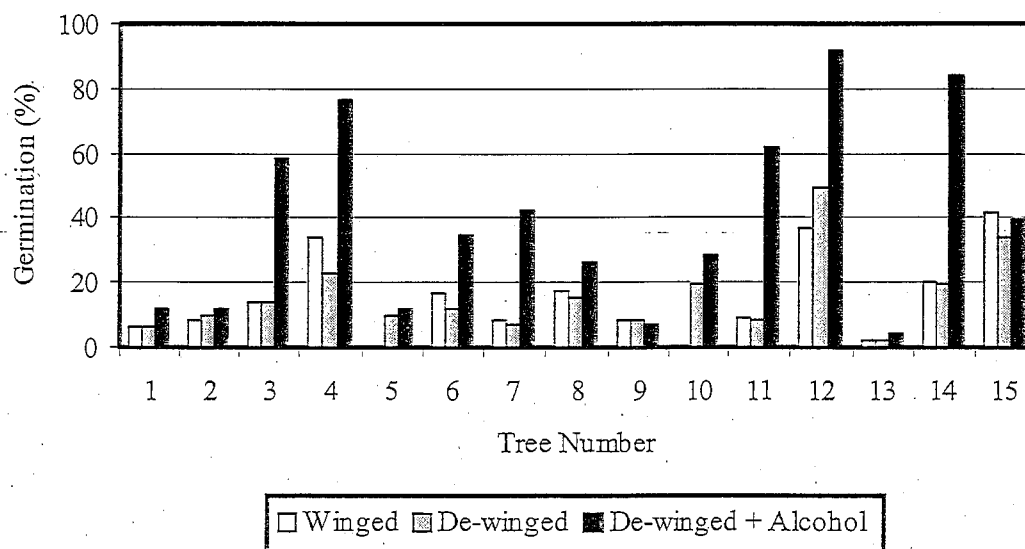


Figure 25. Comparison of germination of winged, de-winged, and de-winged + alcohol treated yellow birch seed collected from 15 single trees.

There are a few observations that should be noted based on the results that were obtained. The first is that good catkin production does not necessarily guarantee good production of viable seed. There is no known reason for these poor reproductive yields but poor pollination or insect and disease damage on the seed could be possible reasons (Daigle and Simpson 2004). Damage may also be occurring while seed is being de-winged. Too much pressure could damage the seed and result in poorer germination as observed in some seedlots with de-winged seed exhibiting germination lower than that of winged seed.

Conclusions

As with prior experiments, there are obvious advantages gained by treating the seed with ethanol. This process not only increases overall germination but also lowers the volume of seed stored by 10-20 times its original volume. Finally, as discussed in an earlier report by Daigle and Simpson (2004), de-winging and immersion in alcohol does not appear to damage the seed.

It should also be noted that in the future catkins should be collected at a later date to ensure that they are fully developed. Fully developed catkins reduce processing time substantially because the catkins are easily broken apart thus preventing breaking of bracts allowing for easier separation of the seed from bracts and other debris. Closed and not fully developed catkins however, increase processing time and make it difficult to separate seed from unwanted debris.

Based on these results and with results found in prior research involving white birch, it is recommended to continue to treat freshly collected yellow birch seed using the de-winging plus alcohol separation method.

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SEED CERTIFICATION

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

Demand for certified seed, which was high in the 1970's and 1980's, has declined during the past 15 years (Figure 26). A total of 339 kg of certified seed was exported in 2006, which was about the same as in 2005. Of significance was 174 kg of lodgepole pine (*Pinus contorta* var. *latifolia*) from Yukon Territory followed by 61 kg of subalpine fir (*Abies lasiocarpa*), 73 kg of grand fir (*A. grandis*), and 25 kg of Douglas-fir (*Pseudotsuga menziesii*). Five kilograms of Sitka spruce (*Picea sitchensis*) was exported certified as Untested Seed Orchard. 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.

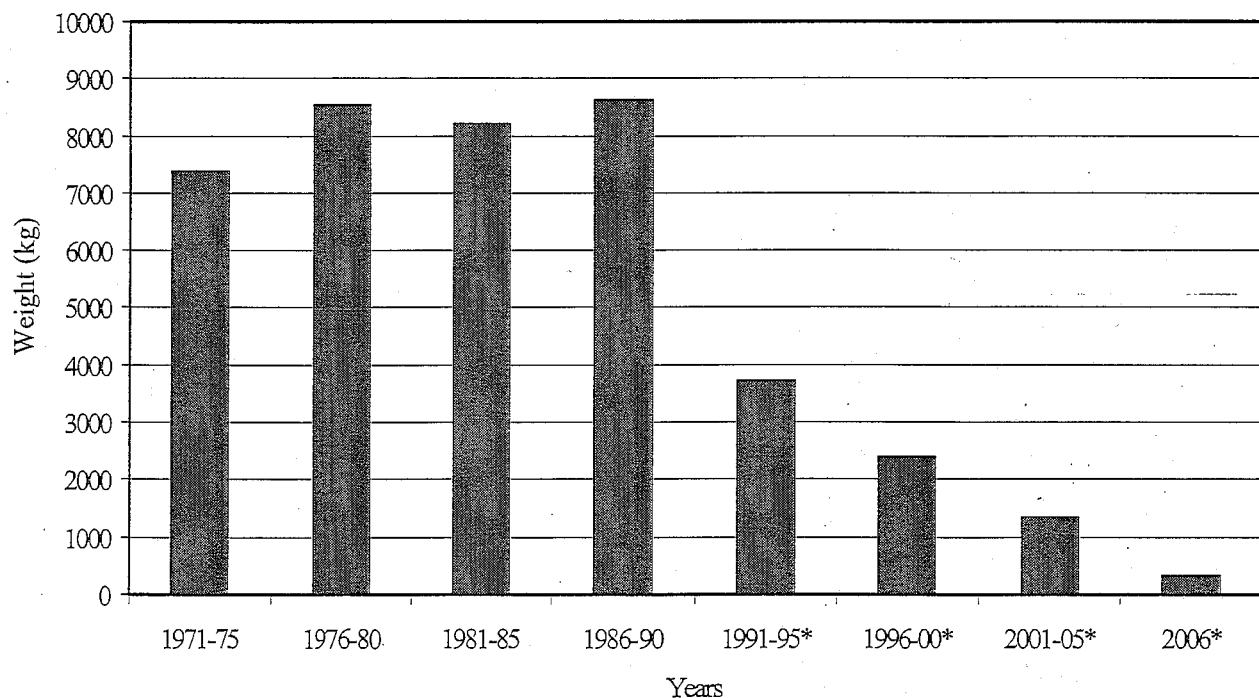


Figure 26. Weight of seed OECD certified or exported* by 5-year periods.

Officially established in 1967, the OECD Scheme for the Control of Forest Reproductive Material Moving in International Trade developed rules and procedures that were adopted in 1974. From its early implementation by a limited number of countries for Douglas-fir seed exported from North-America to Europe, the scope of the Scheme was progressively enlarged over time to attract new participants and to deal with many forest tree species. To date, the Scheme includes 22 participating countries and three official applicants, working with more than 250 tree species.

During the late 1980s, it became apparent that the 1974 Scheme required revision because of changes in forest management in addition to wood production (environmental and social aspects, biodiversity conservation, etc.) and the growing importance on the market of new types of reproductive material derived from forest tree breeding programs. This resulted in the formation of a Group of Experts that worked between 1992 and 1996 to prepare a comprehensive proposal.

A revised Scheme was presented to the OECD Committee for Agriculture in November 1996 but could not be accepted at that time, mainly due to divergences among member countries on the matter of identifying new types of trees derived from modern biotechnology. During the following years, several solutions were explored to try finding a compromise, in particular for the more advanced categories of forest reproductive material (Qualified and Tested).

At the Biennial Meeting of Representatives of the National Designated Authorities held in Hungary on 5-6 October 2006, delegates discussed the matter and agreed to amend the Scheme as follows:

1. It was urgent to revise the Scheme after many years in a stand-by situation. An improved text for "Source-identified" and "Selected" categories would immediately benefit all stakeholders, including new applicant countries that are strengthening their domestic control systems for forest reproductive material.
2. The two "advanced" categories of forest reproductive material [*"from Untested Seed Orchards"* and "Tested" categories of the current Scheme, "*Qualified*" and "*Tested*" categories of the previous proposal] would be suspended, given the lack of an unanimous agreement for the time being.
3. Discussion on more "advanced" forest reproductive material would continue within the framework of the Scheme. These categories relate to small quantities that could, at present, be traded under bilateral contracts. However future development is expected to show an increased trade of forest seed and plants from advanced breeding, and to cover this an extended OECD Scheme will be needed at the international level.

A pre-draft proposal was examined by the participants at the Biennial Meeting and it was agreed that the final text be prepared for approval by all delegates in early 2007. If approved, it will be submitted to the OECD Committee for Agriculture and Council for adoption. This will mean that the Scheme will be equivalent to the European Union Directive for seed certification for the two lower categories and with these updates it is hoped that other countries will want to join the Scheme.

PRESENTATIONS / PUBLICATIONS

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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 communication with general public, tours of the lab facility, participation in events that promote our organization, and special events when opportunities present themselves. Some of the more notable events of 2006 are mentioned below.

On February 21, delegates attending an Access and Benefit Sharing Workshop were given a tour of the Seed Centre.

On April 25 a delegation from Jilin province in China.

On May 24 the Seed Centre was visited by two scientists from Harbin province China. They were in Fredericton visiting Dr. Fen Rui Meng (UNB).

On May 26 the Honourable Andy Scott, MP for Fredericton and Mr. Andrew Holland, Mr. Scott's assistant, visited the Seed Centre.

In July, delegates attending the IUFRO Tree Seed Symposium visited the Seed Centre. This group consisted of managers and scientists from Canada, United States, and Europe.

On September 26, the Deersdale Advisory Group to J. D. Irving, Limited visited the Seed Centre.

SEED CENTRE STAFF

The amount of work carried out by the Seed Centre during 2006 was increased through “extra” work weeks that were funded through FSWEP/ASEP and the Federal Public Sector Youth Internship Programs. These programs enabled the Seed Centre to acquire employees without having to cover their wages.

Jessica Deluney was hired through FSWEP/ASEP funding and worked part-time (20 hours/week) from January to the end of April. Her work during this period focussed on testing and compiling germination results of Tree Breeding seedlots (tamarack). Jessica had been a summer student in 2005 and was able to work well with minimal supervision.

Shelley Young was also hired through FSWEP/ASEP. She was employed from May 1 to August 29. Shelley assisted with seed collections and processing.

James Bird started work on October 23 and worked to the end of December. He was hired through the Federal Public Sector Youth Internship Program. His term will end in April 2007. His work consisted mainly of seed processing and testing.

These employees provided an “extra” 36 weeks of work to the Seed Centre in 2006. This is just above the average of 34 weeks/year of “extra” work weeks that the Seed Centre has benefited from over the past 9 years (Figure 27).

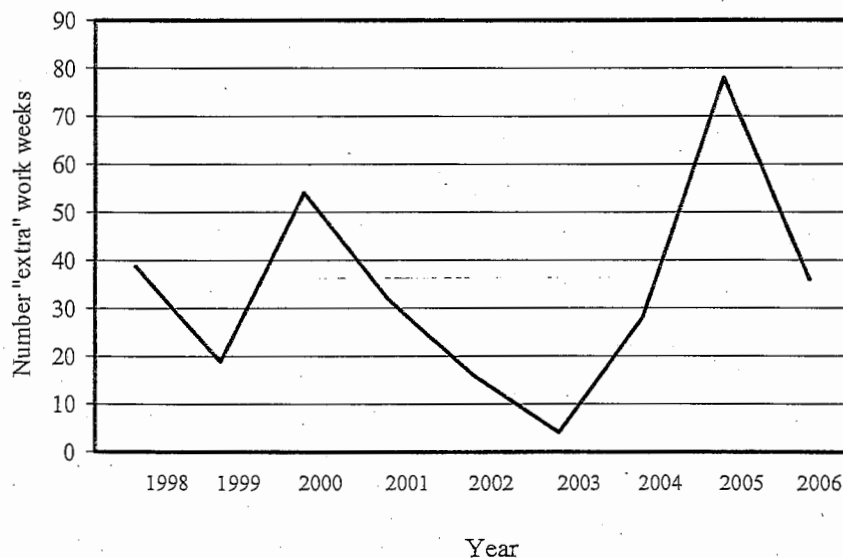


Figure 27. Number of “extra” work weeks provided to the NTSC between 1998 and 2006.