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Invited paper

SEED STORAGE POTENTIAL FOR DWARF BIRCH (*BETULA GLANDULOSA* MICHX.)

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Abstract

Seed from 27 Dwarf birch (*Betula glandulosa* Michx.) shrubs were collected from the one remaining *B. glandulosa* population in New Brunswick. Average initial germination was 69% for the 27 shrubs, ranging between 38 to 96%, and the mean germination time (MGT) in days was 5.8. Stratification (seed imbibed with water 5°C in the dark for 3 weeks) did not improve germination or decrease the MGT, suggesting that the seed was not initially dormant. Seed from a selection of 7 shrubs tolerated 2-week low temperature treatments to -20, -80 and -196°C and a 1-year -196°C treatment. The -196°C treated seed appeared to develop dormancy. Non-stratified -196°C treated seed germination averaged 30% for the 7 shrubs, and germination increased to 67% with stratification and was not significantly different from the germination of the control seed. The MGT decreased in the stratified -196°C treated seeds as compared to the non-stratified -196°C seed. In the -20 and -80°C 2-week treated seed stratification decreased germination and increased MGT. Seed from the 27 shrubs tolerated storage for up to 6 years at 5°C, with only 2 shrubs (shrub 1 and 19) exhibited a significant decrease in germination ion after 6 years. The MGT significantly increased in all seed 6 years after storage, suggesting that seed deterioration was occurring and the seed was reaching their maximum storage potential.

Key words: *Betula glandulosa, ex situ* conservation, mean germination time, percent germination, storage

INTRODUCTION

Dwarf birch (*Betula glandulosa* Michx.) is a sub-alpine woody shrub found across North American and Greenland. In Canada, the eastern range of Dwarf birch extends from the boreal forests of Cape Bretton and Matagami area of Quebec to Southern Baffin Island (Andrews et al. 1980). In southern areas such as the province New Brunswick (NB) this species is found at elevations between 600-800 m, typically on the summits of mountains (Powell and Beardmore 2002). In the 1950's three populations of *Betula glandulosa* were identified at Big Bald, Caribou and Carleton Mountains in northern NB (Hinds 2000), unfortunately only the Big Bald Mountain populations remains, the others were lost to fire. Big Bald Mountain is part of the Appalachian Mountains, a large system of mountains in eastern North America.

Betula glandulosa is relatively small compact straggling shrub-like tree ranging from 10 to 50 cm in height that frequently has gnarled twisted branches and an irregular crown (Powell and Beardmore 2002). It is known to grow in large clonal patches at the northern limit of its range (Hermanutz et al. 1989). It is monoecious with pistillate (emerge beyond expanding leaves from terminal buds if short shoots) and staminate (at the ends of long shoots over winter) catkins borne on different positions of the shrub (Fryxell 1957). The pistillate catkins contain many flowers, each of which can develop a seed. These flowers consist of bracts (small leaves) and a small fruit, which are spiral along a central axis. The fruit is a broadly ovoid nutlet that has two narrow lateral wings, and two tiny, hair-like, stigma remnants at its tip (Powell and Beardmore 2002). Each fruit can contain a single seed. Seed matures late summer or early fall and are usually wind dispersed.

Weis and Hermantutz (1988) studying the *B. glandulosa* grown in cultivation in the United Kingdom found that germination was approximately 70%, while seed collected from natural population in northern Quebec, in Canada was over 60% following cold stratification (Weis and Hermanutz 1988). Another dwarf birch, *B. nana* produces dormant seed which required either cold stratification or gibberellic acid treatment for breaking dormancy (Polosova 1962, Junttila 1997). Non-dwarf *Betula* spp. also produce dormant seed (reviewed by Karrfalt 2008) and often there are latitudinal

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differences where species in more southernly location produce non-dormant seed, while in a more northern location the seed is dormant (Bevington 1986, Dirr and Heuser 1987).

Information concerning seed storage of *Betula glandulosa* or any other dwarf *Betula* spp. is lacking. In general, seeds can be classified based on their storage potential as orthodox, which are seeds tolerant of low moisture content and low temperature or as recalcitrant, which are seeds intolerant of drying and are intolerant of low temperatures (Roberts 1973). Typically seed is classified as orthodox can be stored for relatively long durations. Non-dwarf *Betula* spp. seed storage behaviour is classified as orthodox with exceptions (Hong et al. 1998). The recommended storage temperature for non-dwarf *Betula* spp. is 3 to 5°C (Heit 1967). *Betula microphylla* seeds can remain viable in storage after 26 years (Hong et al. 1998) at 3 to 5°C, and Simpson et al. (2004) observed out of 185 *Betula* spp. stored seed lots two remained viable after 10 and 28 years of storage at -20°C. However, Clausen (1975) found that *B. alleghaniensis* and *B. papyrifera* seed could be stored for up to 8 years between 2 to 5°C (Clausen 1975) but viability was declining suggesting that this was close to the maximum storage duration for this species. Wang et al. (1994) assessed the ability to store *B. alleghaniensis* at below freezing temperatures and found that germination declined in seed stored at -18°C for 15-23 years, however the decline was much greater in seed stored at -4°C. These studies suggest that there is the potential for long-term seed storage; however this may be species specific, and while 3 to 5°C is the recommended storage temperature, seeds may be able to tolerate below freezing temperatures.

A selection of dwarf *Betula* spp. are available through North American and European nurseries as ornamental plants and seed is used for propagation (Dirr and Heuser 1987). This interest in using these species as ornamental may result in propagation-related research for these species. Dwarf *Betula* spp. are also of interest as indicators of climate change since they are often found in northern transitional zones, such as in the Arctic (Jacobs et al. 1997, Alsos et al. 2003). Research of this type addresses their long-term stability north of the tree line, focusing on identifying minimum number and size of populations necessary for ensuring species survival. Their presence in environments of this type has also contributed to their increasing vulnerability. In NB, Canada, *B. glandulosa* was identified as a species of concern by the NB Gene Conservation Association, given that it is extremely rare in the province and current knowledge pertaining to its health is inadequate (Canadian Forest Service 1997). This species has no official provincial, territorial, or national risk designation in Canada; while in the US, 3 states have officially designations for *B. glandulosa* (Maine, endangered (Maine Natural Areas Program 1999), New Hampshire, threatened (New Hampshire, Natural Heritage Bureau 2006) and New York, endangered (New York Department of Environmental Conservation 2000)). The goal of this work was conserve material from this one population in NB, specifically to determine if *ex situ* conservation in the form of seed storage was an appropriate strategy for *B. glandulosa* and to identify the optimum storage temperatures.

MATERIAL AND METHODS

Site description

Big Bald Mountain (47.11.41 latitude and 66.25.24 longitude) is one of New Brunswick's highest elevations (674.5 m) (Service New Brunswick 1998). The terrain is rugged, exposed to prevailing winds and has a climate typical of a subalpine location. *Betula glandulosa* surrounds the summit of Big Bald Mountain (Figure 1A). These shrubs are low lying, growing on a granite rock with a shallow organic material, with no over story protection. Other species associated with the location are jack pine (*Pinus banksiana*), blueberry (*Vaccinium boreale*), lambkill (*Kalmia augustifolia* L.), and various mosses (*Cladina stellaris* and *C. rangiferina*) and lichens (personnal communication Vince Zelanzy, New Brunswick Department of Natural Resources and Energy Ecologist Crown Lands Branch).

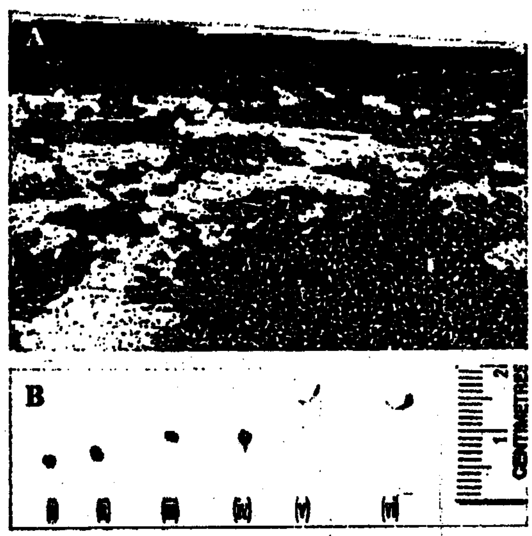

Fig. 1. *Betula glandulosa* at Big Bald Mountain (A) and seed germination (B). A) summit of Big Bald Mountain with scattered *Betula glandulosa* shrubs, B) progressively germinating seeds (i-iii) and early seedling emergence (iv-vi). In A, the arrows identify the shrubs.

Seed collection and processing

Catkins were collected from 27 shrubs in October 2001. The bracts remained closed on all catkins collected. Approximately one half of the total catkins on each shrub were collected. Catkins were collected in paper bags and were processed within 1 month after collection.

Shrubs were given a final identification number 1 to 27 and these numbers were assigned based on initial germination following seed collection (control germination); the shrub which had seed with the lowest percent germination was given the identification number of 1, shrub number increased with increasing percent germination.

For processing, catkins were first rubbed gently, sieved, then debris was aspirated off. Bracts and central axis of the catkins were separated from the seed at this initial processing step. Samples were further partitioned using an alcohol separation technique to separate filled from empty seeds. Samples of seed from the empty and filled fraction of each shrub were subjected to X-ray analysis in order to ensure that the separation technique was successful. These tests verified that the separation technique was successful (results not shown). Seeds were placed on mesh screens and air-dried for 7 days at 25°C and then stored at 5°C in sealed glass vials for the storage duration experiment or exposed to various low temperatures, as described below. After seeds were dried water content (WC) determinations were made using the International Seed Testing Association high constant temperature oven-drying method (ISTA 1985) and WC was expressed on a fresh weight basis. For each WC determination, 3 replicates with 0.5 gm seed/replicate were used.

Germination testing

Germination tests were conducted on all collected seed immediately after seed was processed (control germination) and after various storage durations. For the control germination, seeds were germinated with and without stratification. For seed stratification prior to germination, seeds were placed on Kimpak moistened with 120 ml distilled water in a Petawawa germination box (Wang and Ackerman 1983) at 5°C, in the dark for 3 weeks in a Conviron germinator, (Winnipeg, MB, Canada). Stratification did not alter percent germination of the control for each of the 27 shrubs (results not shown). For germination seeds were placed on Kimpak moistened with 120 ml distilled water in a Petawawa germination box (Wang and Ackerman 1983) at 30/20°C day/night temperature, 8 h photoperiod for 3 weeks in a Conviron germinator, (Winnipeg, MB, Canada). There were 4 replicates of 20 seeds per replicate for each shrub. Germination counts were made twice weekly and seed was considered germinated when the root and hypocotyl were well developed and healthy (Figure 1C (i-ii)). Mean germination time (MGT), the average time required for seeds to germinate, was determined using the formula (Ellis and Roberts 1980):

$$MGT = \sum(t \times n)/\sum n,$$

where: t = day t of the germination test, and n = number of seed attaining vigour class four (Wang 1973) on day t.

Low temperature tolerance

Short term low temperature tolerance experiment

The seeds from 7 shrubs that had either low (shrubs 1 and 2), medium (shrubs 9 and 10) or high control germination (shrubs 22 and 26) were selected for assessing low temperature tolerance. For low temperature tolerance testing seed was placed in 1.5 ml cryovials (Nalgene, Rochester, New York, USA) and placed at either -20, -80 and -196°C for 2 weeks or for 1 year at -196°C. Vials for the -196°C treatment were placed in the vapor of liquid nitrogen, providing an approximate storage temperature of -120 to -150°C (Walters et al. 2005). There was insufficient seed for testing all temperatures after 1 year storage. Seeds were removed from the low temperatures and germinated with or without stratification as described above. There were four replications with 20 seeds per replicate.

Long-term low temperature tolerance experiment

Seeds were stored at 5°C in sealed glass vials and percent germination was evaluated after 1, 2, 4 and 6 years for storage at 5°C by germinating seed as described above. There were four replications with 20 seeds per replicate. We were concerned about the possibility of dormancy being induced during storage, but given that we had a limited amount of seed, dormancy was evaluated after each storage duration by randomly selecting the seed from 5 shrubs and by stratifying seed as previously described. Stratification did not increase germination following any storage durations at 5°C (results not shown).

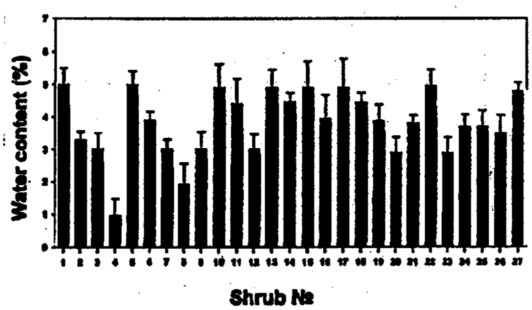

Fig. 2. Water content of *Betula glandulosa* seeds collected from 27 shrubs at Big Bald Mountain.
Values are the mean of 3 replicates with 15 seeds per replicate ± Standard Error.

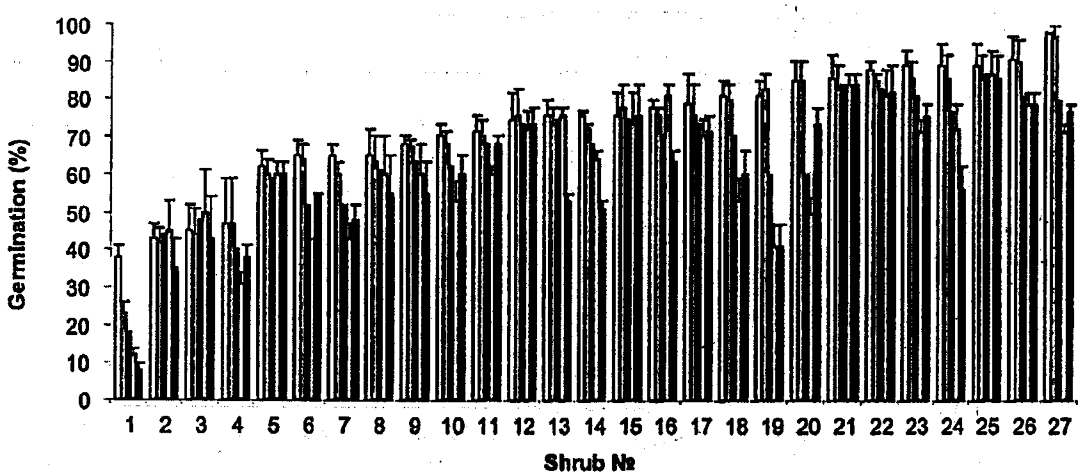

Fig. 3. Percent germination and mean germination time (B) of the seed collected from *Betula glandulosa* shrubs stored for 1, 2, 4, 5 and 6 years at 5°C. Values are the mean of 4 replicates with 20 seeds per replicate ± Standard Error.
For each shrub in order of presentation the results of 0 ☐, 1 ☐, 2 ■, 4 ☐ and 6 ■ years of storage. 0 year of storage is the control seed, germinated after seeds were cleaned and separated.

RESULTS

Germination studies

Seed WC ranged between 1 to 5% for the seed of the 27 shrubs (Fig. 2) and over all mean WC for the seed of 27 shrubs was 3.8% (Table 1). The average percent germination for the seed of all the 27 shrubs was 69% (Table 1) and ranged from 38 (shrub 1) to 96% (shrub 27) (Fig. 2). The average MGT was 5.8 days (Table 1), and ranged from 4.4 (shrub 25) to 6.9 days (shrub 26) (Fig. 3). Control germination was moderate to high for most of the shrubs (Fig. 2). Over 80% germination was evident in the seed collected from 10 shrubs (shrubs 18-22), while 60-79% germination occurred in the seed from 13 shrubs (shrubs 5-17) and in 4 shrubs (shrubs 1-4) the germination was less than 60% (Fig. 2).

Low temperature tolerance

The percent germination of seed from each shrub exposed to -20 and -80°C 2-week treatment was not significantly different than that of the control, however, MGT had increased after treatment for all shrub seed (Table 2). The combination of -20 or -80°C treatment followed by stratification decreased mean germination and increased MGT in seed from all shrubs. There was a significant decline in the mean germination for all shrubs from 61% for the -20°C treated non-stratified seed to 38% for the -20°C treated stratified seed. Following -80°C stratification treatment there was a decline in the mean germination for all shrubs from 61% to 38% and there was a significant increase in MGT (Table 2). This trend was reversed following exposure to 196°C; non-stratified seed exhibited a decline in mean germination for all shrubs to 30%, while mean germination increased following stratification to 67%. For each shrub, percent germination after -196°C stratification treatment was not significantly different from that of the controls. Mean germination time also decreased in the -196°C stratified seed, with all seed exhibited a decrease in MGT, as compared to the -196°C non-stratified seed (Table 2). Germination following 1 year -196°C treatment yielded similar results, with seed from all shrub that were stratified exhibiting a higher percent germination than seed, which was not stratified (Table 3). The mean percent germination for the -196°C treated stratified seed was not significantly different from that of the control and mean MGT was significantly lower than that of the control seed.

Table 1. Average seed water content, germination (%) and mean germination time (MGT) of the seed from the 27 shrubs immediately following seed processing.

Water content (%)	3.8 ± 0.7
Germination (%)	69.0 ± 4.5
MGT (days)	5.8 ± 0.4

Germination (%), water content (%), and MGT values are the mean for the 27 shrubs.
Values are the mean of 4 replicates with 20 seeds per replicat ± Standard Error.

For assessing the effect of storage duration at 5°C, percent germination did not change with the increasing durations of storage for the seed of 10 shrubs (shrubs 2, 3, 5, 8, 12, 13, 15, 17, 21, and 25) (Fig. 2). The remaining 17 shrubs had seed, which exhibited a decrease in germination at some point during the increased storage duration. The seed from only 2 shrubs had over a 50% decline in germination 6 years after storage (Fig. 2). These were the seed shrub 1, which exhibited low control germination and seed from shrub 19, which had

Table 2. Germination (%) and mean germination time (MGT) of *Betula glandulosa* seed storage seed exposed to -20, -80 and -196°C for 2 weeks.

Shrub №	Control 4°C			
	Germination		MGT	
	Non-Stratifed seeds	Stratified seeds	Non-Stratifed seeds	Stratified seeds
1	38 ± 3.0 a, A	36 ± 2.4 a, A	5.7 ± 0.3 ad, A	5.6 ± 0.3 ace, A
3	45 ± 3.0 b, A	46 ± 2.7 b, A	5.8 ± 0.1 b, A	5.8 ± 0.2 ade, A
9	65 ± 2.0 c, A	67 ± 2.3 c, A	5.1 ± 0.1 c, A	5.1 ± 0.1 b, A
11	71 ± 3.3 c, A	70 ± 4.0 c, A	5.4 ± 0.1 a, A	5.4 ± 0.1 c, A
16	74 ± 2.0 cd, A	76 ± 1.7 d, A	6.0 ± 0.0 d, A	6.0 ± 0.0 d, A
22	86 ± 4.4 e, A	81 ± 3.6 de, A	5.8 ± 0.0 ab, A	5.9 ± 0.1 a, A
26	87 ± 1.7 e, A	86 ± 0.4 e, A	5.4 ± 0.0 a, A	5.4 ± 0.2 e, A
Mean value	66 ± 7.1 A	66 ± 6.9 A	5.6 ± 0.1 A	5.6 ± 0.1 A
	-20°C treatment			
	Non-Stratifed seeds	Stratified seeds	Non-Stratifed seeds	Stratified seeds
1	26 ± 1.2 a, A	17 ± 1.8 a, B	6.1 ± 0.2 a, A	7.0 ± 0.2 a, B
3	48 ± 6.0 b, A	26 ± 1.2 b, B	6.9 ± 0.2 b, A	7.6 ± 0.1 b, B
9	52 ± 4.8 b, A	23 ± 1.6 b, B	5.9 ± 0.3 a, A	6.8 ± 0.3 a, B
11	70 ± 5.0 c, A	48 ± 4.8 c, B	6.1 ± 0.1 a, A	7.5 ± 0.1 b, B
16	62 ± 1.7 d, A	51 ± 3.0 c, B	7.7 ± 0.1 c, A	8.4 ± 0.0 c, B
22	82 ± 4.4 e, A	58 ± 2.4 c, B	6.9 ± 0.2 b, A	7.9 ± 0.0 d, B
26	63 ± 4.4 cd, A	43 ± 2.6 c, B	6.9 ± 0.2 b, A	7.3 ± 0.1 e, B
Mean value	61 ± 7.9 A	38 ± 5.9 B	6.6 ± 0.2 A	7.5 ± 0.1 B
	-80°C treatment			
	Non-Stratifed seeds	Stratified seeds	Non-Stratifed seeds	Stratified seeds
1	25 ± 3.0 a, A	13 ± 2.1 a, A	7.5 ± 0.1 a, A	8.6 ± 0.1 a, B
3	47 ± 4.0 a, A	31 ± 1.5 b, B	6.7 ± 0.3 b, A	7.5 ± 0.0 b, B
9	55 ± 3.6 c, A	40 ± 2.0 c, B	5.8 ± 0.1 c, A	8.1 ± 0.1 c, B
11	67 ± 6.0 d, A	43 ± 2.8 c, B	6.4 ± 0.3 b, A	7.6 ± 0.0 d, B
16	68 ± 1.7 cd, A	43 ± 3.1 c, B	7.8 ± 0.0 a, A	8.4 ± 0.3 a, B
22	87 ± 3.3 e, A	54 ± 1.6 d, B	6.7 ± 0.2 b, A	8.3 ± 0.1 e, B
26	80 ± 0.0 f, A	49 ± 2.4 d, B	6.4 ± 0.2 b, A	8.7 ± 0.0 f, B
Mean value	61 ± 7.9 A	39 ± 5.1 B	6.8 ± 0.3 A	8.2 ± 0.2 B
	-196°C treatment			
	Non-Stratifed seeds	Stratified seeds	Non-Stratifed seeds	Stratified seeds
1	18 ± 3.3 a, A	39 ± 2.1 a, B	8.2 ± 0.1 a, A	5.3 ± 0.3 ac, B
3	15 ± 0.0 a, A	44 ± 3.5 a, B	7.8 ± 0.2 b, A	5.9 ± 0.1 b, B
9	26 ± 5.8 b, A	64 ± 3.0 b, B	7.1 ± 0.2 c, A	5.1 ± 0.1 c, B
11	33 ± 3.0 c, A	70 ± 2.4 c, B	7.8 ± 0.1 b, A	4.8 ± 0.1 d, B
16	42 ± 6.7 cd, A	75 ± 1.3 d, B	8.3 ± 0.0 d, A	5.0 ± 0.0 c, B
22	41 ± 1.7 d, A	87 ± 3.1 e, B	8.3 ± 0.1 d, A	5.3 ± 0.1 ac, B
26	36 ± 3.0 cd, A	88 ± 2.7 e, B	7.6 ± 0.1 b, A	5.5 ± 0.1 ac, B
Mean value	30 ± 4.1 A	67 ± 7.2 B	7.9 ± 0.2 A	5.2 ± 0.1 B

Values for each shrub are the mean of 3 replicates ± Standard Error.
Mean values are the average of shrubs 1, 3, 9, 11, 16, 22, and 26 ± Standard Error.
Values followed by the same small letter in either the Germination or MGT column for each temperature treatment are not significantly different based on a Tukey's multiple comparison test at $p \leq 0.05$.
Values followed by the same capital letter in a row in either the Germination or MGT column for each temperature treatment are not significantly different based on a Tukey's multiple comparison test at $p \leq 0.05$.

Table 3. Germination (%) and mean germination time (MGT) of *Betula glandulosa* seed storage seed stored at -196°C for 1 year.

Shrub №	Control 4°C			
	Germination		MGT	
	Non-Stratifed seeds	Stratified seeds	Non-Stratifed seeds	Stratified seeds
1	24 ± 2.0 a, A	16 ± 2.4 a, B	5.9 ± 0.1 a, A	11.6 ± 0.3 a, B
3	41 ± 5.2 b, A	30 ± 4.1 b, B	5.8 ± 0.1 b, A	12.2 ± 0.1 b, B
9	64 ± 2.1 c, A	34 ± 3.2 b, B	5.2 ± 0.1 c, A	12.8 ± 0.1 c, B
11	70 ± 3.0 d, A	49 ± 6.3 c, B	6.0 ± 0.1 d, A	11.4 ± 0.1 d, B
16	76 ± 1.4 e, A	42 ± 3.7 cd, B	5.8 ± 0.2 b, A	12.0 ± 0.0 e, B
22	85 ± 1.7 f, A	35 ± 4.1 d, B	5.5 ± 0.1 e, A	11.6 ± 0.1 a, B
26	90 ± 3.5 f, A	49 ± 3.0 c, B	5.7 ± 0.1 b, A	12.5 ± 0.0 f, B
Mean values	64 ± 9.0 A	36 ± 4.4 B	5.7 ± 0.1 A	12.0 ± 2.0 B
	One year storage at -196°C			
	Non-Stratifed seeds	Stratified seeds	Non-Stratifed seeds	Stratified seeds
1	7 ± 1.5 a, A	35 ± 3.6 a, B	9.1 ± 0.3 a, A	5.8 ± 0.3 a, B
3	9 ± 1.2 a, A	43 ± 2.9 b, B	8.3 ± 0.4 b, A	5.1 ± 0.1 b, B
9	13 ± 3.1 ab, A	65 ± 2.4 c, B	9.8 ± 0.1 c, A	4.7 ± 0.1 c, B
11	14 ± 2.8 b, A	69 ± 1.8 c, B	10.2 ± 0.1 c, A	5.1 ± 0.1 b, B
16	13 ± 4.5 b, A	71 ± 4.6 cd, B	8.9 ± 0.2 a, A	4.9 ± 0.0 d, B
22	26 ± 1.2 c, A	79 ± 2.5 de, B	9.2 ± 0.3 a, A	5.2 ± 0.1 b, B
26	11 ± 2.0 b, A	81 ± 3.0 f, B	9.7 ± 0.2 c, A	5.6 ± 0.1 a, B
Mean values	13 ± 2.3 A	63 ± 6.7 B	9.3 ± 0.2 A	5.2 ± 0.2 B

Values for each shrub are the mean of 3 replicates ± Standard Error.
Mean values are the average of shrubs 1, 3, 9, 11, 16, 22, and 26 ± Standard Error.
Values followed by the same small letter in either the Germination or MGT column for each temperature treatment are not significantly different based on a Tukey's multiple comparison test at $p \le 0.05$.
Values followed by the same capital letter in a row in either the Germination or MGT column for each temperature treatment are not significantly different based on a Tukey's multiple comparison test at $p \le 0.05$.

had high control germination. There were no trends with regard to seed with high percent germination being able to maintain this high germination over the various storage durations or, seed with low germination being unable to tolerate the increase in storage duration. The MGT increased for the seed from all shrubs as the storage duration progressed (Fig. 3). The largest increase for all shrubs was after 6 years where MGT increased by at least 1/3 over that of the control seeds.

DISCUSSION

The results suggested that seed with a WC ranging between 1-5% can be stored at 5°C for 6 years. However, there was evidence that seed germination was decreasing in seed collected from approximately 1/3 of the shrubs 6 years after storage at 5°C, as well as the MGT was increasing in seed from all shrubs indicating that seed deterioration may have been occurring. This suggests that 6 years may be approaching the maximum storage duration for 5°C.

Below freezing temperature testing showed the seeds from shrubs with high, medium and low control germination will tolerate -20, -80 and -196°C. For the -20 and -80°C treated seed, MGT increased indicating that even after a two week exposure the seeds were starting to deteriorate suggesting that these temperatures may not be optimal for this species. Interestingly, the response to stratification altered, with the -196°C treatment appearing to induce dormancy. Initially *B. glandulosa* seed was non-dormant and over 6 years of storage at 5°C, the seed from 5 randomly selected shrubs (results not shown) did not exhibit any dormancy. Stratification of the -196°C 2-week and 1-year treated seed significantly improve germination and decreased MGT as compared to the non-stratified seed -196°C stored seed, suggesting that dormancy was induced. There was no evidence for this occurring at -20 or -80°C, and stratification after exposure to these low temperatures decreased germination. Previous work has shown that -196°C exposure can increase germination by functioning as a scarification treatment, weakening seed coats that are impermeable to water (Pritchard et al. 1988, Martin and De al Cuadra 2004). It is unlikely that this occurred since *B. glandulosa* seed coat could be removed with ease and germination was high in the control non-stratified seed. This phenomenon of dormancy induction during storage was reported to occur in black spruce seed stored at -196°C (Beardmore et al. 2008), and the cycling into and out of dormancy has been reported for species stored at above and below freezing temperatures (Douglas fir, Jensen and Noll 1959, Edwards and El-Kassaby 1988; rape seed, Pekrun et al. 1996; Sitka spruce, Jones et al. 1997). This phenomenon is usually associated with storing moist seed (Jones et al. 1997) and the *B. glandulosa* seed water content was low (average for all 27 shrubs was 38% water content). The ability to store *B. glandulosa* for longer durations at -196°C and the phenomenon of inducing dormancy warrants further study.

Seed WC can significantly impact longevity during storage with a lower seed WC correlated to increasing longevity if the seed is classified as orthodox (Ellis and Roberts 1981). This seed storage experiment show for most shrubs seed germination had decreased after 6 years of storage at 5°C, the recommended storage temperature for *Betula* spp. These results suggest that *B. glandulosa* produces seed that can be classified as sub-orthodox. Given that the Big Bald Mountain *B. grandulosa* population is the last in NB we will initiate further storage experiments, with the goal to optimise the seed storage requirements for this species. It would be highly beneficial to conduct long-term seed storage experiments for assessing longevity and dormancy induction at -196°C, and for continuing to evaluate stage at 5°C and at below freezing temperatures for longer durations (e.g. 15 years).

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