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Impact of Storage on Viability of White Spruce Seed

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Abstract

Samples of seed of white spruce (*Picea glauca* [Moench] Voss) from 36 individual trees, collected in 1974 from 5 provenances in Ontario, Canada, were placed in frozen storage at -20 ˚C (-4 ˚F) in 1977 and in storage at 4 ˚C (39 ˚F) in 1982. Seed was removed from both storage temperatures and tested in 2002 and 2005. Seed moisture content increased on average, whereas average germination remained the same or declined slightly after storage at -20 ˚C (-4 ˚F) for 25 and 28 yr. For the same seedlots stored at 4 ˚C (39 ˚F) for 20 and 23 yr, seed moisture content also increased on average, whereas mean germination declined drastically. Seed moisture content exceeding 8.5 percent negatively impacted germination of seed stored at both temperatures. Seed stored at -20 ˚C (-4 ˚F) germinated faster than seed stored at 4 ˚C (39 ˚F). These results demonstrate the long-term storage potential of white spruce seed stored at -20 ˚C (-4 ˚F).

Introduction

Storage of tree seed is advantageous for reforestation programs, research, and genetic conservation. Maintaining the initial genetic and physiological quality of seed is one objective of storage (Wang and others 1993).

Several studies have shown that long-term storage of tree seed is possible. Simpson and others (2004), examining seed storage for 15 tree species, found that storage ability varied among species and suggested that seed of a number of species, including white spruce (*Picea glauca* [Moench] Voss.), could store well for 100 yr. Successful long-term storage also depends on storage conditions and treatment and quality of the seed (Wang 1976).

The major factors affecting seed longevity and viability in storage are storage temperature and moisture content (Bewley and Black 1994). Hansen and others (2005) found that germination of white spruce seed stored for 23 yr at -18 ˚C (0 ˚F) declined from 92 to 86 percent. Walters and others (2005) quantified germination data from seed of 276 agricultural and other plant species stored for 16 to 81 yr. They found that some species tended to survive longer than others during storage at both 5˚ (41 ˚F) and -18 ˚C (0 ˚F). The benefit of low-temperature storage at -18 ˚C (0 ˚F) on seed longevity was progressively lost if seed was first stored at 5 ˚C (41 ˚F) (Walters and others 2004). Wang and others (1993) reported that subfreezing temperatures to -20 ˚C (-4 ˚F) are considered better than above-freezing temperatures for long-term storage, provided the seed moisture content is less than 10 percent. Under these conditions, physiological activity is minimal (Leadem 1996).

Results are presented here from a white spruce seed storage experiment set up over 25 yr ago, using seed collected from individual trees from five provenances and stored at 4 ˚C (39 ˚F) and -20 ˚C (-4 ˚F).

Methods

In 1974, staff of the Tree Breeding Unit at the Petawawa Forest Experiment Station (now Petawawa Research Forest) collected white spruce cones from five locations in Ontario for a series of provenance trials. Samples were taken at Antrim (45˚19'; 76˚11'), Bancroft (45˚06'; 78˚58'), Petawawa (46˚00'; 77˚26'), Renfrew (45˚28'; 76˚44'), and Whitney (45˚32'; 78˚27'). Cones were collected 27 August–3 September from 10 to 47 individual trees spaced 20–100 m (65–325 ft) apart at each location. Seed was extracted and cleaned on an individual-tree basis during the autumn, tested for moisture content and germination, and stored in glass jars with threaded lids at 2 ˚C (35 ˚F). [Threaded lids with rubber seals enhance the hermetic qualities of a container best (Manager and others 2003).]

In February 1977, 10-g (0.36-oz) samples from 36 collections were set aside for germplasm preservation and evaluation of the storage ability of white spruce seed. Each sample was subdivided into four samples of 2.5 g (0.09 oz) each and placed into small, heat-sealed 5-mil thick poly bags;

the four subsamples were stapled together such that the staple did not penetrate the portion of the packets containing the seed. The packets of seed were placed in 1 L (1.06 qt) mason jars with a screw cap and stored at -20 ˚C (-4 ˚F). In 1982, seed samples from the original collections were prepared for storage at 4 ˚C (39 ˚F). Seed was placed in 5-ml (0.17-oz) vials with two vials per seedlot, and the two vials were placed in a small poly bag that was heat sealed. All bags from one provenance were placed into a larger poly bag that was also heat sealed.

Seedlots initially tested for moisture content and germination in late 1974 or early 1975 were selected for evaluation of seed storage ability. In 2002, 1 sample from each of 25 seedlots from the Whitney provenance was removed from each storage temperature and tested for moisture content and germination. In 2005, samples from the 4 other provenances (a total of 11) were evaluated to determine whether the trends found with the Whitney provenance were consistent.

Seed moisture content was determined by placing approximately 1 g (0.036 oz) of seed into each of two aluminum containers. The seeds were then dried for 17±1 h in a force-draft oven at 103±2 ˚C (217±4 ˚F), and moisture content was calculated on a fresh-weight basis [ISTA (International Seed Testing Association) 2006].

For the germination tests, seed was placed on moistened Versa Pak™ (Kimberly-Clark, Neenah, WI) in Petawawa germination boxes with a vacuum plate. Four replicates of 50 seeds were placed in each box. The boxes were moist-chilled for 21 d in a cooler maintained at 3 ˚C (37 ˚F). After 21 d, the boxes were placed in a Conviron G30 germinator at 8 h light at 30 ˚C (84 ˚F), followed by 16 h darkness at 20 ˚C (78 ˚F), with a constant relative humidity of 85 percent. Germinants were monitored at 7 d and every 3–4 d thereafter until day 21. Seed was considered germinated when cotyledons, hypocotyl, and a developing radicle had appeared.

Data were analyzed with SAS (SAS Institute, Inc., Cary, NC). Before analyses of variance, arcsine transformation was applied to the percentages.

Results

Seed moisture content and germination showed the same trends for the stored seed from all five provenances. Seed moisture content increased significantly from when the seed was initially tested to when it was removed from storage (table 1). The increase was less, however, for the seed stored at -20 ˚C (-4 ˚F).

Germination of seed stored at -20 ˚C (-4 ˚F) was unchanged from its original value for seed stored for 25 yr and declined only 1.2 percentage points in seed stored for 28 yr (table 2). Germination of seed stored at 4 ˚C (39 ˚F) for 20 and 23 yr, however, declined drastically. Germination of seed stored at -20 ˚C (-4 ˚F) varied less than of seed stored at 4 ˚C (39 ˚F), as indicated by the standard errors (SE).

Germination declined sharply for seed stored at 4 ˚C (39 ˚F) when seed moisture content exceeded 8.5 percent (table 3). Germination was also substantially lower for seed stored at 4 ˚C (39 ˚F) with moisture content between 4.5 and 6.9 percent than for seed stored at -20 ˚C (-4 ˚F). Indeed, with the exception of two seedlots, germination of seed stored at 4 ˚C (39 ˚F) was always lower, regardless of seed moisture content. Even in the case of four seedlots with similar moisture contents at both storage temperatures [6.0 percent at 4 ˚C (39 ˚F) and 6.2 percent at -20 ˚C (-4 ˚F)], the seed stored at -20 ˚C (-4 ˚F) had higher mean germination. These findings illustrate the impact of storage temperature on seed longevity.

Table 1. Comparison of seed moisture content of 25 white spruce seedlots from Whitney provenance with that of 11 seedlots from 4 other provenances after collection and after storage at -20 ˚C (-4 ˚F) and 4 ˚C (39 ˚F).

	Whitney[1]			Other provenances[1]		
Storage condition	**Mean**	**SE**	**Range**	**Mean**	**SE**	**Range**
Initial	4.6a	0.08	3.6–5.3	4.2a	0.13	3.5–5.0
– 20 ˚C (4 ˚F)	5.8b [2]	0.11	4.9–6.9	6.4b [3]	0.23	5.5–8.5
4 ˚C (39 ˚F)	7.5c [4]	0.34	5.6–10.0	8.2c [5]	0.45	5.2–9.5

[1] Within a provenance or provenance group, means followed by different letters differ significantly at p=0.05 by Duncan's test. SE=standard error.

[2] Seed was stored for 25 yr.

[3] Seed was stored for 28 yr.

[4] Seed was stored for 20 yr.

[5] Seed was stored for 23 yr.

Table 2. Comparison of percent germination of 25 white spruce seedlots from Whitney provenance with that of 11 seedlots from 4 other provenances after collection and after storage at 4 ˚C (39 ˚F) and -20 ˚C (-4 ˚F).

	Whitney			Other provenances		
Storage conditions	Mean[1]	SE	Range	Mean[1]	SE	Range
Initial	95.8a	0.75	83.8–99.2	91.4a	3.34	64.2–99.5
– 20 ˚C (4 ˚F)	95.8a [2]	0.71	84.5–99.5	90.2a [3]	2.46	71.0–98.5
4 ˚C (39 ˚F)	33.2b [4]	6.23	0.0–98.5	5.1b [5]	3.50	0.0–38.5

[1] Within a provenance or provenance group, means followed by different letters differ significantly at p=0.05 by Duncan's test.

[2] Seed was stored for 25 yr.

[3] Seed was stored for 28 yr.

[4] Seed was stored for 20 yr.

[5] Seed was stored for 23 yr.

Table 3. Germination (percent) by seed moisture content (MC) class for 36 white spruce seedlots stored at 4 ˚C (39 ˚F) or -20 ˚C (-4 ˚F).

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

Storage conditions also impacted germination vigor. Seed stored at 4 ˚C (39 ˚F) germinated later and more slowly than seed stored at -20 ˚C (-4 ˚F) (figure 1). Germination was complete by day 14 for seed stored at -20 ˚C (-4 ˚F), but continued up to day 21 for seed stored at 4 ˚C (39 ˚F). At day 21, seed that had not completed germination was counted and classified as being of low vigor. Seed stored at -20 ˚C (-4 ˚F) exhibited 0.9 percent low vigor germination, but this value almost doubled to 1.7 percent for seed stored at 4 ˚C (39 ˚F).

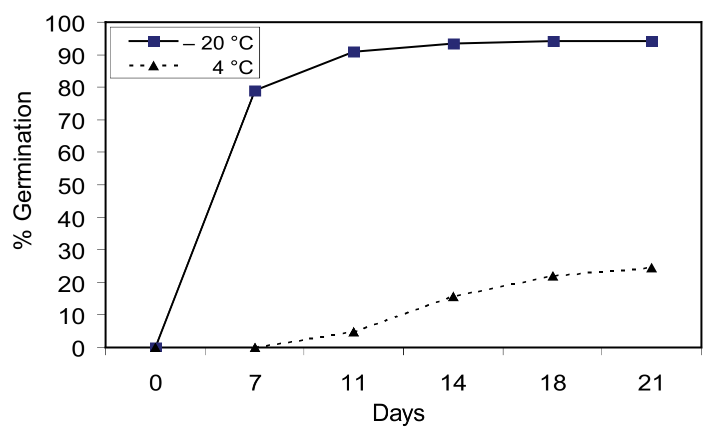

Figure 1. Germination speed of white spruce seed stored at 4 ˚C (39 ˚F) and -20 ˚C (-4 ˚F).

Discussion

The cone crop on white spruce in Ontario was heavy in 1974, as substantiated by the large number of accessions in the National Tree Seed Centre's database. This implies that the genetic and physiological quality of the seed crop that year was high as a result of presumed abundant pollen production and trees diverting a significant proportion of their resources to seed production. These factors should positively impact storage ability of seed collected that year. Willan (1985) stated that, in a good crop year, seed collected from trees with high production is likely to have the best longevity in storage. Caron and others (1993), however, reported that seed maturity at collection time varied between heavy and light crop years for white spruce. This points out that seed quality varies from year to year, and assessing seed quality before committing seed to storage is important.

Seed moisture content was higher after storage than shortly after seed processing. It is unlikely that the seed acquired moisture while in storage because of the manner in which it was packaged. More likely, the seed acquired moisture before storage because of improper seed handling. The seed was repeatedly taken from and returned to storage as seed was provided to cooperators to establish provenance trials. Possibly it acquired moisture from the air if seed containers were not allowed to equilibrate to room temperature before they were opened, or the seed lots were handled in high ambient relative humidity. The seed stored at 4 ˚C (39 ˚F), in particular, may have had a higher moisture content before being placed in long-term storage, because the samples were prepared after all seed requests from cooperators establishing provenance trials had been satisfied.

Seed moisture content has been considered a critical factor for the life of seed in storage (Holmes and Buszewicz 1958; Bewley and Black 1994; Hong and Ellis 2002). Storage temperature also interacts with seed moisture content. Barton (1961) pointed out that the higher the storage temperature, the faster the rate of seed deterioration for seed with a given moisture content; at a lower storage temperature, there is greater tolerance for higher moisture content. A storage temperature above freezing is not sufficient to maintain viability of seed with low moisture content because the seed continues to metabolize and deplete its energy reserves, resulting in death. When seed tissues are frozen, metabolic activity is substantially decreased, and the reserves stored in the megagametophyte remain intact. In this study, moisture content of the two groups of seed stored at 4 ˚C (39 ˚F) increased on average, with a corresponding decline in mean germination. Although the moisture content of seed of the same two groups stored at -20 ˚C (-4 ˚F) also increased, germination remained unchanged or declined only slightly. Seed with a moisture content above 8.5 percent had consistently lower germination. Daigle and Simpson (2003) reported that seed moisture content above 9 percent had an increasingly negative impact on germination of white spruce seed stored at -20 ˚C (-4 ˚F). A moisture content of 5±1 percent is recommended for long-term storage for gene conservation (FAO/IPGRI 1994).

Vigor of germinating seed is a useful trait to evaluate impact of storage. As seed ages, vigor declines, and eventually the seed dies. Total germination is also important, but does not take vigor into account. At the completion of the germination test, the number of low-vigor germinants was twice as high for seed stored at 4 ˚C (39 ˚F) as for seed stored at -20 ˚C (-4 ˚F). The impact of storage temperature on seed vigor was also evident in the germination speed.

Conclusions

Results presented in this paper illustrate the potential long-term storage ability of white spruce seed, which is advantageous when storing seed for *ex situ* gene conservation.

1. Germination of seed stored for 25 and 28 yr at -20 ˚C (-4 ˚F) exhibited little or no change.

2. Germination of seed stored for 20 and 23 yr at 4 ˚C (39 ˚F) declined by up to 82 percent.

3. Germination of seed stored at -20 ˚C (-4 ˚F) started sooner, occurred faster, and reached its maximum sooner.

4. Seed moisture content greater than 8.5 percent negatively impacted germination, particularly in seed stored at 4 ˚C (39 ˚F).

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