Photo by Dale Simpson

Five years' storage of seeds from three willow species

J Dale Simpson and Bernard I Daigle

ABSTRACT

Seeds of *Salix bebbiana* Sarg., *S. discolor* Muhl., and *S. eriocephala* Michx. (Salicaceae) were stored at 2 moisture contents (low, 5.1 to 7.3% and high, 8.5 to 9.8%) and 4 temperatures (4, –20, –80, and –145 °C [39, –4, –112, –229 °F]) for 60 mo. Seeds stored at 4 °C lost most or all viability by 24 mo. We observed no significant difference in germination between the 2 seed moisture contents for each species. After 60 mo of subzero storage, germination of *S. bebbiana* seeds declined from 89 to 83%, *S. discolor* from 60 to 54%, and *S. eriocephala* from 71 to 54%.

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KEY WORDS

Salix bebbiana, *S. discolor*, *S. eriocephala*, Salicaceae, germination, moisture content

NOMENCLATURE

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The National Tree Seed Centre (NTSC) collects, processes, tests, and stores seeds of Canadian tree and shrub species. The NTSC's mission is to provide seeds for research and to store seeds for genetic conservation. The NTSC has been actively fulfilling this mandate for the past 40 y with about 10 000 seed collections of Canadian tree and shrub species in storage.

The Salicaceae family includes *Populus* L. (poplars) and *Salix* L. (willows) genera. Poplar and willow seeds—which consist of an embryo, seedcoat, and almost no endosperm—are small (< 1 to 3 mm long [0.04 to 0.12 in]), short-lived in nature, and must germinate soon after dispersal. Storage temperature is a well-known factor affecting the storage life of seeds. Douglas (1995) reported that *S. setchelliana* C.R. Ball seeds stored at room temperature lost all viability after 20 d. Mean germination of seeds from 4 *Salix* species stored for 36 mo at –10 °C declined from 96 to 75% (Zasada and Densmore 1980). Storing *Salix* seeds cryogenically in liquid nitrogen is also feasible. Maroder and others (2000) reported that dry seeds of *S. alba* L. and *S. matsudana* Koidzumi survived immersion in liquid nitrogen without loss of viability. Moisture content is another storage factor that must be considered when storing seeds. Optimal seed moisture content for *Populus* and *Salix* seeds was reported to be between 4.0% and 7.5% (Buch 1960), and Tauer (1979) recommended that *Populus deltoides* Bartram ex Marsh. seeds be dried to a moisture content of 6 to 10%.

Recent germination results from *Populus* and *Salix* seeds stored at –20 °C at the NTSC show little loss in viability after several years in storage. Seeds of *Populus* species showed similar results. Mean germination of seeds from 7 seedlots of *P. tremuloides* Michx. declined from 93.6 to 90.8% after 10 y whereas mean germination of 9 *P. grandidentata* Michx. seedlots stored for 9 y increased from 90.3 to 94.1% (Simpson and Daigle, unpublished data).

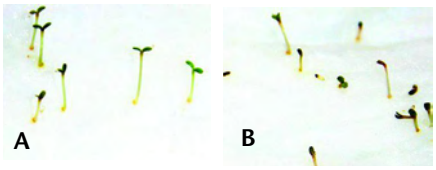


Figure 1. Normal (A) and abnormal (B) willow germinants. Photos by George Fanjoy

Temperature Conversions

°C	°F
103	217
30	86
20	68
4	39
–10	14
–20	–4
–80	–112
–145	–229

An experiment was initiated at the NTSC using seeds from 3 common willow species (*S. bebbiana* Sarg., *S. discolor* Muhl., and *S. eriocephala* Michx.) to evaluate viability following storage of the seeds at 2 moisture contents (high and low) and 4 temperatures (4, –20, –80, and –145 °C). Results from these experiments should be applicable to other willow species and will provide guidance for *ex situ* conservation.

METHODS AND MATERIALS

Catkins were collected in late May 1999 from a single clone of *S. discolor,* and bulk collections were made from 4 clones of *S. bebbiana* and 10 clones of *S. eriocephala.* The catkins were brought into the lab, processed following Daigle and Simpson (2009), and moisture content (MC) was determined by drying 2 samples of seeds in a forced-draft oven for 16 h at 103 °C (ISTA 1996). Each seedlot was halved, and one of the subsamples dried to a lower moisture content by air-drying in a forced-draft oven at 30 °C for 1 h. Germination tests were carried out for each species on the high and low MC subsamples. The high and low MC subsamples were further divided into storage samples weighing 0.35 g each. The seeds were placed in 1.8-ml cryogenic vials and stored at 4 °C. After 24 h, samples destined for subzero storage were transferred to a –20 °C freezer. Following 24 h at –20 °C, samples destined for storage at colder temperatures were removed and stored in a freezer at –80 °C or in the vapor phase of liquid nitrogen at approximately –145 °C.

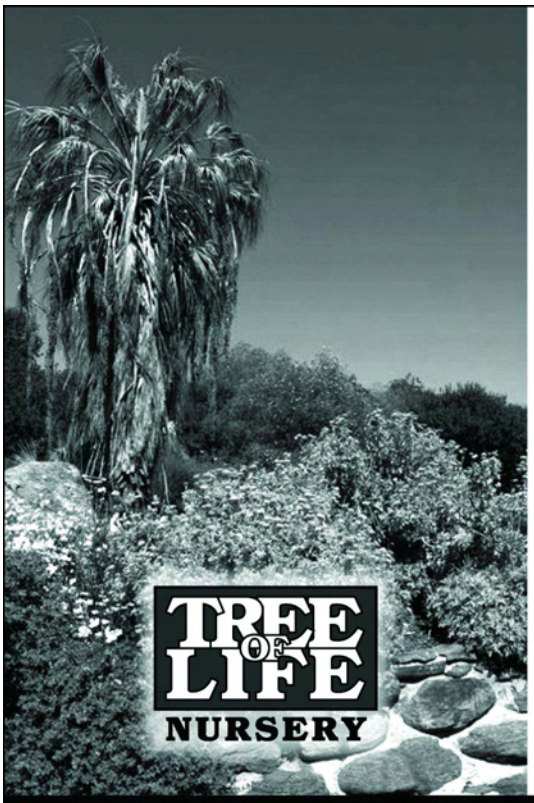
In addition to the initial germination test, seeds were tested after storage for 6, 12, 24, and 60 mo. Two replicates of 100 seeds each were used for the initial tests, and 4 replicates of 100 seeds each were used for the 6-, 12-, 24-, and 60-mo tests. Vials of seeds stored cryogenically and at –80 °C were removed and placed in –20 °C storage for 24 h, then these vials along with those stored solely at –20 °C were placed at 4 °C for 24 h before being removed and placed at room temperature for 4 h to allow for gradual warming of the seeds. Seeds were placed on moistened Kimpak™ in Petawawa germination boxes (Wang and Ackerman 1983) and germinated for 10 d in a Conviron™ (Controlled Environments Ltd, Winnipeg, Manitoba) G30 germination cabinet set at 20 °C for 16 h without light and 30 °C for 8 h with light and at a constant relative humidity of 85%. Germination was considered normal when germinants had chlorophyll and were erect, seedcoats had shed, cotyledons were open, and hypocotyl hairs were capable of firmly anchoring the germinant on the substrate as described by Simak (1982) (Figure 1A). Abnormal germinants were stunted and poorly anchored to the substrate, and cotyledons were sometimes fused together (Figure 1B).

RESULTS AND DISCUSSION

After processing, seed MC ranged from 8.5 to 9.8%, and declined to 5.1 to 7.3% after drying. The greatest range in MC occurred with *S. discolor*, with high and low MCs of 9.8% and 5.1%, respectively. The difference in MC for *S. bebbiana* and *S. eriocephala* ranged from 8.6 to 7.2% and from 8.5 to 7.3%, respectively. Although we hoped that MC of all 3 species would be similar, the use of the forced-draft oven to lower MC did not produce the desired results. The *S. discolor* seeds were dried on 25 May, whereas the *S. bebbiana* and *S. eriocephala* seeds were dried 1 June. We suspect relative humidity differed at the time the 2 sets were dried and that this affected seed MC. Germination test results of the freshly collected seeds (tested after processing and before drying) were 89.0% for *S. bebbiana*, 60.5% for *S. discolor*, and 71.5% for *S. eriocephala.*

We did not detect a significant difference in germination between MCs within each species after 60 mo storage at the 3 subzero temperatures. Therefore, for each species, data from the 2 MCs were combined for each storage temperature. Germination of seeds after storage for 60 mo was not significantly different among the 3 subzero temperatures for each species (Table 1). Storage at –20 °C was best for *S. bebbiana* and *S. discolor,* whereas storage at –80 °C was best for *S. eriocephala* seeds. Previous analyses showed that seeds stored at 4 °C for 24 mo rapidly lost viability and had the highest proportion of abnormal germinants (Daigle and Simpson 2002). Seeds stored for 60 mo at 4 °C did not germinate for any of the species. Seed viability was generally maintained for only 6 or 12 mo at 4 °C. Germination of seeds in subzero storage declined slowly over the 60-mo period, with the least decline in *S. bebbiana* (89.0 to 83.3%) and *S. discolor* (60.5 to 54.4%) and the greatest decline in *S. eriocephala* (71.5 to 54.3%).

The presence of abnormal germinants can be an indicator of immature seeds and (or) seeds damaged during storage. A germinant was scored as abnormal if it possessed one or more of



the following traits: no hypocotyl elongation; bent hypocotyl; no hypocotyl hairs, causing germinants to lean; cotyledons did not separate; seedcoat not shed; or radicle failed to fully develop, resulting in a stump root (Simak 1982). *Salix bebbiana* had the lowest percentage of abnormal germinants compared with the other species (Table 2); and *S. bebbiana* seeds displayed no difference among storage temperatures at either MC. There were more abnormal *S. discolor* germinants in seeds with a MC of 9.8% stored at either –80 °C or –145 °C than those stored at a MC of 5.1%. It is possible that 9.8% MC is the level at which an impact on seed quality begins to occur. By and large, storing seeds at the 2 MCs did not affect *S. eriocephala*. Seeds at the lower MC stored at –80 °C had significantly fewer abnormal germinants.

The mean germination of seedlots used in this experiment suggests that seeds may have been collected too early. This is especially true for *S. discolor* and *S. eriocephala* as germination results were mediocre and a high number of abnormals were present, which suggests immature seeds. The mean loss in germination over the 5-y period was much greater than that witnessed for other *Salix* and *Populus* seedlots stored for the same or longer duration at the NTSC. The experiment was designed, however, to compare MC and storage conditions, and although germination may not have been optimal, the results do demonstrate the storage potential of seeds from these species at subzero temperatures.

CONCLUSIONS AND RECOMMENDATIONS

1. Storage of willow seeds at 4 °C was not effective in maintaining seed viability.
2. Storage at –20 °C was sufficient for *S. bebbiana* and *S. discolor* seeds, but –80 °C was best for *S. eriocephala* seeds. Generally, good viability was maintained at –20 °C.
3. Seed moisture contents between 5% and 10% did not have an impact on storability.
4. Higher proportions of abnormal *S. discolor* and *S. eriocephala* germinants may indicate that the seeds were immature when collected.
5. Seeds should be stored in several small vials rather than in a single container to avoid possible damage to the entire seedlot when removing samples from storage for testing or distribution.

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TABLE 1

Mean germination percentage of seeds from 3 willow species stored at 4 temperatures for 60 mo.

Storage temperature °C	*S. bebbiana*	*S. discolor*	*S. eriocephala*
4	0.0 a[z]	0.0 a	0.0 a
–20	85.6 b	54.8 b	49.5 b
–80	81.6 b	54.5 b	57.9 b
–145	82.8 b	53.8 b	55.4 b

[z] Means significantly different at $P = 0.05$ determined by a Duncan's Multiple Range Test.

TABLE 2

Percentage of abnormal germinants from seeds of 3 willow species stored at 3 temperatures and different moisture contents for 60 mo.

Storage temperature °C	*S. bebbiana* Moisture content		*S. discolor* Moisture content		*S. eriocephala* Moisture content	
	8.6%	7.2%	9.8%	5.1%	8.5%	7.3%
–20	4.5 a[z]	5.5 a	13.8 a	13.8 a	12.8 a	17.0 a
–80	6.8 a	7.3 a	22.8 b	17.5 a	10.8 a	9.5 b
–145	5.5 a	8.0 a	24.0 b	12.8 a	10.4 a	15.5 a

[z] Means significantly different at $P = 0.05$ determined by a Duncan's Multiple Range Test.

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