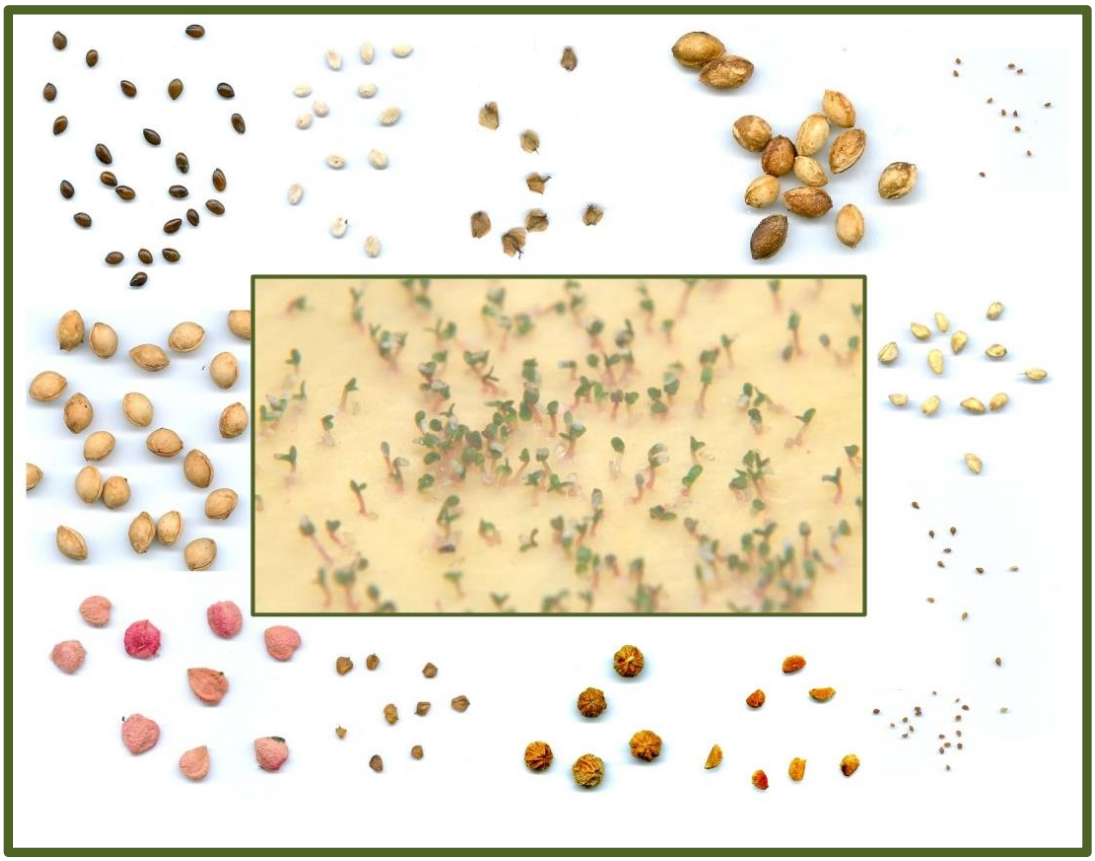
SEED VIABILITY, GERMINATION AND LONGEVITY OF SELECTED BOREAL SPECIES: A LITERATURE REVIEW



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INTRODUCTION

The oil sands industry has been, and continues to be, an important economic driver of the Alberta and Canadian economies and associated activities continue to modify large tracts of land in the boreal forest of northeastern Alberta. To mitigate disturbances following extraction, industry is required, by approvals, to progressively reclaim, establish native boreal plant species and create functional, self–sustaining ecosystems. To ensure adaptability of plant materials and to maintain genetic integrity of individual species, plant material must be from local sources as per the *Forestry Genetic Resource Management and Conservation Standards* (FGRMS) (Alberta Government 2016). Historically, individual oil sands operators in the mineable oils sands region of northeastern Alberta carried out seed collection, as required, to meet their reclamation needs. While this model is sufficient to meet the needs of an individual operator, it became apparent that a collaborative effort could greatly enhance the supply of native plant propagules to meet and exceed the reclamation targets of the present and preserve some for the future. The Oil Sands Vegetation Cooperative (OSVC), established in 2009, is comprised of 12 member companies that collectively harvest and bank plant propagules for current and future needs. They recognize the benefit of advance seed harvest to take advantage of high production years and to have seeds available when required with little lead time. In their 2014 year-end report, they recognized that, as closure continues, significantly greater amounts of plant material will be required annually and that efficient use of banked material is necessary (Wild Rose Consulting, Inc. 2014).

In discussions at the Oil Sands Research & Information Network (OSRIN) 'Future of Shrubs in the Oil Sands Reclamation Workshop' (2013) concerns were expressed that shrub seed harvest from natural sites is expensive, both time and labour intensive, and thus this valuable resource must be used in the most efficient and effective manner. Among the recommendations were 1) development of criteria for evaluating seed quality (cut tests/germination tests), 2) research into germination and growth, 3) increasing understanding of dormancy and how to break it, and 4) development of an Alberta oil sands based 'Seed Collection, Preparation and Storage Manual'. Another concern expressed by attendees was that much of the pertinent information is spread throughout the grey literature and not always accessible. This concern is also reflected in the OSVC knowledge gap matrix, which extends beyond seed to nursery production and establishment.

About this document

For this document, we have considered three areas that are vital to the effective and efficient use of the seed resources. Firstly, viability – whether a seed is alive and capable of germinating under appropriate conditions. Secondly, treatments required to overcome dormancy and the conditions under which germination can be optimized, and finally, storage and longevity – conditions under which seed can be best stored to maintain its viability for as long as possible.

This review compiles accounts from disparate sources regarding 16 species commonly used in oil sands revegetation (Table 1).

Table 1. Species included in this review.

Alnus incana	*Lonicera involucrata*	*Symphoricarpos albus*
Alnus viridis	*Populus tremuloides*	*Vaccinium myrtilloides*
Arctostaphylos uva-ursi	*Prunus pensylvanica*	*Vaccinium vitis idaea*
Betula nana	*Prunus virginiana*	*Viburnum edule*
Betula pumila	*Rosa acicularis*	
Dasiphora fruticosa	*Shepherdia canadensis*	

An attempt was made to trace paths back through the literature to find the earliest information (often upon which later recommendations were based). In the interest of completeness, and to avoid ambiguity, all references for each species (anecdotal and empirical) have been included. The searches uncovered a significant amount of information regarding germination requirements and/or incubation environments and much less concerning viability testing and storage conditions. As expected, germination and pretreatment information is primarily anecdotal, often conflicting and, rarely are results backed up by viability testing (either by cut-tests, TZ or x-ray). For each species, information was divided into recommended protocols (usually anecdotal) and results of empirical studies. This information provides a starting point for an extensive study being undertaken by the Alberta Tree Improvement and Seed Centre, Government of Alberta (ATISC).

For the most part, information included refers to specific shrub species; however, in a few cases more general information regarding genera is included. Babb (1959) is a reference that was encountered in numerous citations (especially those used by the revegetation community) and his work is included here although it primarily refers to genera rather than individual species. Some of the literature regarding *Vaccinium* germination does not identify species (Crossley 1974, Young & Young 1992) and has not been included in this review. Only references that specifically identify *V. myrtilloides* or *V. vitis idaea* were cited.

Although generally in this document, information is compiled and presented by species, a few references are notably comprehensive and/or rigorous and are discussed below.

Densmore's 1979 thesis on seed ecology of woody plants in Alaska sheds light on germination of several species included in our review (*Alnus incana, Alnus viridis, Dasiphora fruticosa, Shepherdia canadensis*, *Vaccinium vitis-idaea*). She compared various stratification pretreatments under light and dark conditions. During incubation, to encourage germination, she compared long versus short days and for some species, fluctuating diurnal temperatures based on local growing conditions (*Shepherdia canadensis* and *Dasiphora fruticosa)*. Her results were analyzed for significance across all treatments, providing for unambiguous results.

Likewise, King *et al.* (1983) evaluated germination of two species, *Rosa acicularis* and *Arctostaphylos uva-ursi*, for which he has a comprehensive experimental design: 0, 60, 90 or 120 days warm stratification followed by 0, 60, 90 or 120 days cold stratification. Their data were not statistically analyzed but they point out some obvious trends.

Bai *et al.* (unpublished) included many of the species covered in this document. He examined viability and germination following various storage conditions (temperature, moisture content and nitrogen gas versus air). Although statistical analyses were conducted, they are confounded by variation in experimental design and seed quality. Germination was rarely optimized (i.e., fell short of viability expectations) so results are somewhat ambiguous but provide trends that should be further examined.

There are several more sources that are less rigorous in their analysis, often comparing a single treatment over several species, or summarizing earlier work. These include: Nichols (1934), Swingle (1939), Schopmeyer (1974a), Belcher (1985), Young & Young (1992), Marchant & Sherlock (1984), and Hudson & Carlson (1998).

Species specific information is presented below. We have provided an overview of information in an initial table (which includes primarily anecdotal information and/or limited empirical studies). An in-depth analysis of the more comprehensive and/or rigorous studies follows.

Viability

Seed viability is the ability of a seed to germinate into a vigourous, normal seedling that can thrive given appropriate pretreatments and growing conditions. The term is often used to represent the proportion of live seeds in a seedlot or sample. This metric is important to propagators to estimate seeding rates, and reduce the risks associated with sowing poor quality seeds. The most reliable way to evaluate viability is to germinate seeds under ideal conditions, however this is often time consuming. Several quick tests have been developed to estimate seed viability. For cut-tests, seeds can be cut open to reveal the embryonic tissue. This test is often used in field assessments to determine maturity and quality. A second test uses x-rays to evaluate seed health. As with the cut tests, x-rays can be used to determine the quality of seed. Both tests can identify filled (with a developed embryo), empty, immature, and insect- or mechanically-damaged seeds. X-ray techniques were developed as early as 1903 but up until the advent of digital x-rays, this methodology was difficult, time-consuming and often resulted in poor quality images. We have included information published by Belcher (1985) for those species which he included in his work; however, his methodologies all refer to photographic plate radiography. Digital x-ray methods, developed in the 1990's, are much faster and results can be obtained in a few minutes. This non-destructive method does not appear to be widely used, likely due to the need for expensive equipment. A more accurate evaluation of viability, is the use of tetrazolium (TZ) staining tests, where seeds are rendered permeable and soaked in a colourless tetrazolium chloride solution. In the presence of active respiration within tissues, tetrazolium chloride is reduced to a red insoluable formizan. This occurs when hydrogen radicals, produced by respiration, are transferred to the tetrazolium chloride. (Porter *et al.* 1947). Staining patterns within the tissue are examined to determine germinability (AOSA 2010). This method requires intimate knowledge of seed morphology and must be undertaken by well-trained technicians.

Generally, information regarding viability testing is limited for the species covered in this document.

Pretreatments and Germination Conditions

This section explains common treatments required for seeds to overcome various types of dormancy and discusses incubation conditions under which germination is optimized. Seed dormancy describes the condition where viable seed does not germinate when placed in suitable germination conditions. In natural situations, dormancy is a mechanism by which seeds are protected from germinating during inappropriate seasons. Artificial pretreatments attempt to emulate conditions found in environments where each species thrive.

Common seed germination pretreatments include:

- Various stratification regimes where seeds are imbibed (rehydrated) and placed in warm or cold moist conditions (or a combination of both) for various durations – mimicking natural seasonal variation. Soaking seeds in chemical solutions (e.g. gibberellic acid) can sometimes be used to replace stratification requirements (Hartmann & Kester 1983).
- Scarification is generally used on 'hard seeds' where the seed coat is a barrier to water absorption. In natural environments, this is accomplished through soaking, freeze-thaw cycles, natural abrasion or the passage of seed through animal digestive tracts. Artificial scarification treatments can be mechanical (seed coat abrasion), acid digestion (where seeds are soaked in a concentrated acid for a specific period) or by soaking (seeds are placed in warm or hot water for a period of time to soften the seed coat). This latter method is also used to leach chemical inhibitors.

Other less common seed pretreatments include:

- Soaking seeds in an organic solvent (e.g. acetone) to eliminate waxy coatings,
- Heating seeds to emulate fire conditions, or
- Soaking seeds in 'smoke water' where chemicals produced during the burning of cellulose stimulate seeds to germinate (Light *et al.* 2004).

Conditions under which seeds are incubated following pretreatments also affect germination. Seeds of some species germinate over a wide range of conditions whereas others have very limited tolerance ranges. Optimum incubation temperatures vary among species but generally range from just above 0°C to as high as 30-35°C and some species germinate best under alternating temperatures. Light is a second factor affecting germination. Some seeds have a light requirement whereas other germinate best in the dark (in natural situations seeds either germinate on the soil surface or when covered). Some seeds sprout best under alternating light/dark conditions that emulate day and night. Moisture (too much or too little) can affect germination of seeds as well.

Storage and Longevity

Storing seeds can assist in preserving genetic diversity and optimize the use of harvested seed. This allows for harvest of areas prior to disturbance and to maximize harvest in prolific seed producing (mast) years. Storage of temperate commercial tree species is well documented (Palamarek pers. comm.) however storage conditions for most native shrub species has not been as well studied. Generally, seeds of temperate species are considered 'orthodox' which implies that mature seeds can

survive desiccation and are stored best dry at low temperatures (Hong *et al.* 1998). Longevity, the length of time a seed can survive in storage, has not been examined in detail for most of the north temperate (boreal) shrub species.

Alnus incana – river alder

Viability Testing

The only reference found regarding viability testing was that of Schalin (1967). Although he tested viability by x-raying seeds, he gave no detail regarding methodology.

Pretreatments and Germination Conditions

Numerous references for river alder recommend cold stratification. This can range from 28 days to 180 days (Smreciu *et al.* 2008, Hudson & Carlson 1998, Forest Research nd, Nichols 1934 and Harrington *et al.* 1999). Several others (Swingle 1939, Eulert & Hernandez 1980, Haeussler & Coates 1986 and Young & Young 1992) state that stratification is not necessary and Berry & Torrey (1985) don't recommend stratification but suggest the use of gibberellic acid (Table 2). King (1980) provides his own literature review, which contains many personal communications that we could not verify but have included in the following table. Most of the actual germination results are low (below 50%).

Table 2. Cited germination protocols for *Alnus incana.*

Cold Stratification (days)	Author	Germination (%)
0	Berry & Torrey 1985*	
	Sen Gupta 1937 **	24
	Eulert & Hernandez 1980	
	Haeussler & Coates 1986	
	Young & Young 1992	28
	DenHeyer pers. comm. ***	
	Emery 1964***	
	Shoemaker & Hargrave 1936***	
	Stark 1966***	
21-63	Forest Research nd	
28	Smreciu *et al.* 2008†	15.6
30-60	Benson pers. comm.***	
	Lohmiller pers. comm. ***	
60-90	Hudson & Carlson 1998	
	Babb 1959	
112	Nichols 1934†	11.5
180	Schopmeyer 1974b	34
	Harrington *et al.* 1999	49
	Young & Young 1986	

* Giberillic acid; ** Reported by Swingle 1939; *** Reported by King 1980;
† Empirical results.

Young & Young (1986) report that seeds of alder species (not specifically *A. incana*) become dormant if dried and that a longer stratification is required (up to 180 days). Babb (1959) also reports on alder generically and recommends fall sowing or stratifying seeds for 60-90 days.

In one of two detailed empirical studies, Schalin (1967) examined the effect of drying, cold stratification period, freezing temperatures and pH on germination of river alder seeds (Table 2). Extracted in cool temperatures, some seeds were dried (no moisture content was recorded) at 30°C and stored at 8°C whereas others were placed in cold stratification or grown fresh. Prior to testing, seed was x-rayed and sterilized with 20% hydrogen peroxide. Tests consisted of 500 seeds and all germination tests were performed in light. Approximately 30% of fresh seed germinated compared to 15% for seed dried and stored for 6 months. 30-50% of stratified seed germinated with the best results if seeds were frozen at (-20°C) for 3 days following the stratification. pH was also evaluated. *When all results were examined best results followed 3 days of freezing at a pH of 5 (52% germination) although standard deviations suggest that variability was high.* The averages across treatments are presented below.

Table 3. Selected germination results for *A. incana* (Schalin 1967). All seed was stratified 180 days and germinated under light conditions.

Freeze (days)	Germination Temperature (°C)	Average Germination (%)
1	25	29.7
3		37.3
7		30.0
14		21.2
pH		
3	25	28.7
4		32.4
5		42.4
6		27.7
7		16.8

The second extensive study was conducted by Densmore (1979). She cold stratified seed for approximately 70 days prior to germination in the light and 107 days prior to germination in the dark. 100% of stratified seed germinated in light at a constant temperature (either 10, 15, 20, or 25°C) and 98% germinated without stratification at 25°C (Table 4). Several daylengths were examined with the result that in either long days of short days significantly greater germination was recorded at 25°C than at any of the other temperatures (95-96%). By excising seeds following testing, Densmore reports percentages based on filled/viable seed. Her conclusions were:

1. Seeds require light for complete germination.
2. Warm incubation temperatures promote more complete germination.
3. If seeds are stratified complete germination will occur at lower temperatures.

Storage and Longevity

Recommended storage conditions for this species are somewhat confusing, as various authors refer to one or other of the subspecies of *A. incana*. Royal Botanic Gardens (RBG Kew nd) cites Schopmeyer (1974b) that seed is likely orthodox and that air-dried seeds can be stored at 2-5°C in hermetically sealed containers. Forest Research (nd) confirms this and recommends a moisture content of 8-10%.

We found no comprehensive studies examining longevity under various storage conditions. Schopmeyer (1974) and Young & Young (1992) both state that when stored as described above, seeds of *A. rugosa* (= *Alnus incana* subsp. *rugosa*) suffered no loss in viability after 10 years.

Table 4. Selected germination results for *Alnus incana* (Densmore 1979).

Stratification (days)	Light Regime	Germination Temperature (°C)	Germination (%)
0	Light	20	16
		25	98*
	Dark	25	5
70	Light	10, 15, 20 or 25	100*
107	Dark	20	13

* Statistically better than other results.

Alnus viridis - green alder

Viability Testing

Belcher (1985) notes that TZ testing can be conducted on seed soaked for 24 hours prior to clipping the coat and placing in a 1% tetrazolium chloride solution. No TZ results were discussed. A study by Bai *et al.* (unpublished) tested viability of this species using TZ as per the 'Seed Vigour Testing Handbook' (Baalbaki *et al.* 2009). He found viability was generally >60%.

To distinguish among full, empty and abnormal seeds, x-ray technology can be utilized. Shots are taken using 12 kV for 20 seconds on Kodak film and Industrex paper (Belcher 1985).

Pretreatments and Germination Conditions

Results for green alder (*Alnus viridis*) closely resemble those for river alder (*A. incana*), with most authors recommending cold stratification. Much of the information referenced here is anecdotal or the result of an evaluation of a single stratification treatment. Recommended stratification durations range from 3 weeks (Wood pers. comm.), 1 month (Smreciu *et al.* 2008, Swingle 1939), to 2-3 months (Schopmeyer 1974b (reprinted in Young & Young 1992), Belcher 1985, Formaniuk pers. comm. and Nichols 1934) and up to 120 to 180 days (Marchant & Sherlock 1984). Germination percentages in the above cited references never exceeded 40% (Table 5). Bai *et al.* (unpublished) exposed seed to 2 weeks cold stratification (4°C) and incubated at either 15/5°C or 25/15°C on 12 hour days to reach 60% germination. In few cases was germination a reflection of viability, likely indicating that a different pretreatment or germination condition is required.

Table 5. Cited germination protocols for *Alnus viridis.*

Cold Stratification (days)	Author	Germination (%)
14	Bai *et al.* unpublished	60
21	Wood pers. comm.	
28	Smreciu *et al.* 2008	14-17
30	Swingle 1939	29
30-60	Belcher 1985	30-40
60	Schopmeyer 1974	28
60	Formaniuk pers. comm.	
71	Nichols 1934	40
180*	Young & Young 1986	

* seeds had been dried and stored (secondary dormancy?).

Two extensive empirical studies were available. Densmore (1979) examined a variety of incubation conditions with seed harvested from Fairbanks, Alaska (Table 6). Using 8 replicates of 50 seeds per treatment, she statistically compared germination of stratified (at 2-5°C) and unstratified seeds under light and dark conditions at 5 temperatures (5, 10, 15, 20, and 25℃). Stratification periods were 70 days in light and 107 days in dark. She found that stratified seeds germinated completely at all temperatures except 5°C in the light, whereas unstratified seeds germinated completely only at 25°C in the light.

Regardless of stratification treatment, seeds did not germinate fully in the dark. Her results were based on filled seeds. From her results the following are recommendations:

1. Seeds require light for complete germination.
2. Warm incubation temperatures promote more complete germination.
3. If seeds are stratified, complete germination will occur at lower temperatures.

Table 6. Selected germination results for *Alnus viridis* (Densmore 1979).

Stratification (days)	Light/dark	Germination Temperature (°C)	Germination (%)
0	Light	25	98
	Dark	20 and 25	9-11
70	Light	10, 15, 20 or 25	100
107	Dark	20	2

Kaur *et al.* (2016) recently published a study on cold stratification requirements of *A. viridis* seeds (which had been stored dry (5.4-8.6 MC) at -18 ℃ for several months) from three boreal populations. Treatments consisted of an unstratified control and seeds that had been stratified at 4°C for periods of 2, 6 or 12 weeks. Using 4 replicates of 50 seeds for each treatment, germination was carried out in a greenhouse at ambient temperatures of 19-26°C. Germination percentages varied among seedlots from different populations but no statistical differences were observed among treatments (Table 7). However, germination rate increased (significantly) with 2 and 6 weeks stratification.

Table 7. Selected germination results for *Alnus viridis* (Kaur *et al*. 2016).

Stratification (days)	Light/dark	Germination Temperature (°C)	Germination (%)
14	Light	19-26	61.5
42			62
84			50

Storage and Longevity

Seeds are viable for at least 2 years when stored dry at room temperature (Smreciu *et al.* 2008). RBG Kew (nd) recommends seed be stored at low moisture content (5-8%) in hermetically sealed containers and kept at low temperatures (2-5°C). This is corroborated by Young & Young (1992), Schopmeyer (1974b) and Wang *et al.* (1994) although Wang recorded some (0% to 34%) viability loss after 4 years in low temperature storage.

Bai *et al.* (unpublished) tested storage conditions over three variables: air versus nitrogen, three temperatures (-20°C, 4°C and 23°C) and moisture content (high versus low). The authors determined that viability, germination and seedling growth decreased with increased storage temperature. Seed viability and germination at 25/15°C (12 hour days) decreased over storage time.

***Arctostaphylos uva-ursi* –** bearberry, kinnikinnick

Bearberry or *Arctostaphylos uva-ursi* seed (nutlet) has a relatively hard seed coat. Nutlets are generally united into a stone which can be split when fresh into 6-8 nutlets, each with a separate embryo.

Viability Testing

To test viability, seeds can be soaked for 48 hours and individual nutlets separated from the stone with a knife, and each cut at the peak of the seed (top of wedge) and twisted open. The inner coat can then be removed or nicked and the seed placed in 1% TZ solution. The seed can be sliced along the long axis for evaluation (Belcher 1985). Alternatively, seed can be x-rayed to show filled and unfilled seed cavities using 12 kV for 90 seconds on Kodak film and Industrex paper or for 3 minutes on Polaroid film (Belcher 1985). A study by Bai *et al.* (unpublished) tested viability of this species using TZ as per the 'Seed Vigour Testing Handbook' (Baalbaki *et al.* 2009). Initial viability was >75%.

Pretreatments and Germination Conditions

Some sources report germination results for the entire stone because of the difficulty in breaking the nutlets apart. Other sources report results for individual nutlets. Most references indicate that acid scarification and/or warm stratification are required to break dormancy. All references include a cold stratification period (Table 8).

Table 8. Cited germination protocols for *Arctostaphylos uva-ursi*.

Acid Scarification (Conc. H_2SO_4) (hours)	Warm Stratification (days)	Cold Stratification (days)	Author	Germination (%)
0	60	60	Belcher 1985	40-60
0	0	140	Chadwick 1935*	
0	63	49	Bai *et al.* unpublished	23
0.5-2	60	60	Luna *et al.* 2008	80
2-5	60-120	60-90	Millstein & Millstein 1976	
3-5	0	84	McKeever 1938	6
3-6	60-120	60-90	Young & Young 1986	
2-5	Sow in spring	Natural cold	Young & Young 1986	
6	60	60	Glazebrook 1941	61
6	60	60	Berg 1974	61
7	0	90	McLean 1967†	34

* Reported by Swingle (1939); † Empirical results – only a single stratification period tested.

Belcher (1985) suggests no scarification treatment but warm/cold stratification for 60 days each. Luna *et al.* (2008), Glazebrook (1941) and Berg (1974) recommend acid scarification in addition to the warm/cold stratification period as cited above. Luna *et al.* (2008) suggest 0.5-2 hours in acid while Glazebrook (1941) and Berg (1974) both used 6 hours. The difference here may be that nutlets rather than whole stones were used, but the anecdotal records do not specify. Acid scarification for 2-5 hours is recommended by Millstein & Millstein (1976) followed by 60-120 days warm stratification and 60-90

days cold stratification. McKeever (1938) and Young &Young (1986) suggest using 3-5(6) hours acid scarification and the latter advises sowing in the fall to stratify naturally over winter. Bai *et. al.* (unpublished) exposed seed to 9 weeks warm stratification (30/20°C) and 7 weeks cold stratification (4°C) prior to germinating at either 15/5°C or 25/15°C. This was insufficient to completely break dormancy as germination percentages were <25%.

Three detailed studies testing an array of pretreatments were reviewed. The oldest and most detailed source is Giersbach (1937), but it lacks statistical analysis. She tested whole stones, partially separated nutlets and single nutlets, as well as conditions ranging from: 0-5 hours of acid scarification (in concentrated H_2SO_4), 5-15 months at 25°C warm stratification, and/or 4-18 months at 5-10°C cold stratification and/or placed outdoors in Long Island, New York (Table 9). Her best results (76%) occurred when entire stones were sown in flats following 3-5 hours of scarification and placed outdoors in a mulched frame in June and maintained until the following spring (*i.e*. exposed to natural warm and cold stratifications). All other treatments in her numerous experiments resulted in germination percentages under 40%.

Table 9. Selected germination results for *Arctostaphtlos uva-ursi* (Giersbach 1937). Whole stones were treated and germinated on granulated peat moss.

Acid Scarification (hours)	Warm Stratification (months)	Cold Stratification (months)	Germination (%)
3	Outdoors in June	Overwinter outdoors	70
4			65
5			76

King *et al.* (1983) tested mechanical scarification followed by 0, 60, 90 or 120 days each of warm and cold stratification. Detailed methods regarding scarification were not given, only that sandpaper was used to remove part of the seed coat without exposing the embryo. No germination is reported when either the warm or the cold period was 0 days and they didn't achieve >10% germination with any treatment. The best germination (10%) was observed with mechanically scarified seed, stratified for 120 days at 25°C and 90 days at 5°C (Table 10). Although their experiments were set up in a factorial design (4 replicates of 50 seeds per treatment), data were not presented with statistical comparisons.

Table 10. Selected germination results for *Arctostaphylos uva-ursi* (King *et al.* 1983).

Mechanical scarification (y/n)	Warm (25°C) Stratification (days)	Cold (5°C) Stratification (days)	Germination (%) (estimated from graph)
No	60	60, 90 or120	3/3/0
	90		2/0/9
	120		0/9/4
Yes	60		9/1/0
	90		3/0/0
	120		0/10/6

Smreciu & Gould (2009) tested fresh and year old seed after 3, 4.5 and 6 hours of acid scarification and 8, 12 and 16 weeks warm stratification (20°C) followed by 8 or 12 weeks cold stratification (4°C). They report >50% germination (statistically significant) following 4.5 hours scarification of single year-old nutlets with any of the tested combinations of stratifications (Table 11). This is the only reference that includes statistical analysis.

Table 11. Selected germination results for *Arctostaphylos uva-ursi* (Smreciu & Gould 2009). Year-old nutlets stored dry at ambient room temperatures. Results presented are those that were significantly better than all other treatments.

Acid Scarification (hours)	Warm (20°C) Stratification (days)	Cold (4°C) Stratification (days)	Germination (%)
4.5	56	56	56
		84	52
	84	56	48
		84	52
	112	56	57
		84	50

The greatest germination percentages for this species over all the studies and recommendations were obtained following acid scarification and a combination of warm and cold stratification. The highest reported germination of this species was by Luna *et al.* (2008) who recommended the following germination temperatures: 21-25°C during the day and 16-18°C at night (no reported photoperiod).

Longevity and Storage

Although Smreciu & Gould (2009) had good results using seed stored dry in ambient conditions for 1 year, RBG Kew (nd) recommends sealing dry seed in hermetic containers and freezing at low temperatures (-18°C).

Bai *et al.* (unpublished) tested storage conditions over three variables: air versus nitrogen, three temperatures (-20°C, 4°C and 23°C) and moisture content (high versus low) over a three-year period. They found storage condition did not affect viability or germination. Although viability remained high over the three years, germination declined, suggesting that the pretreatments and germination conditions became less effective over time. Seedling vigour remained constant over all storage treatments, but vigour at 25/15°C (warmer incubation regime) decreased with age.

Betula nana – bog birch, dwarf birch

Viability Testing

No information was found regarding testing seed viability of this species.

Pretreatments and Germination Conditions

Although some sources suggest cold stratification for germination of *Betula nana* seeds (Black & Wareing 1955, Densmore 1979), far more information is available regarding light exposure, light quality, and day length. Sarvas (1950) found that exposure to light improved germination. Bliss (1958) used common conditions on several species and found seed stored at -15°C for 6-7 months, with no other pretreatment germinated at 38% in the dark and 52% in light.

Black & Wareing (1955) compared germination at two temperatures (15°C and 20°C) and a variety of light intensities, single light exposure times (15 minutes to 24 hours), two photoperiods (8 and 20 hour), and exposure to infra-red light. In addition, various temperatures were applied in combination to a 12/12 hour day. Unfortunately, this study does not provide statistically analysed results (Table 12). They found that light intensity had a small effect on germination. When given a reasonably long photoperiod (8 or more hours) and warm conditions (20°C) they achieved germination percentages of 74-93%. They also tested 4 weeks cold stratification and found 95% of seeds germinated either in the dark or exposed to 8 hour photoperiod at 14°C. This latter result suggests that stratification reduced/eliminated the light requirement.

Table 12. Selected germination results for *Betula nana* (Black & Wareing 1955). Each experiment is separated by a solid line.

Stratification (days)	Day length or Single exposure (hours)	Germination Temperature (°C)	Germination (%)
0	8/16	15	34
		20	93
	20/4	15	90
		20	93
0	4 (single)	20	55
	8 (single)		74.
	12 (single)		91
	16 (single)		89
	20 (single)		90
	24 (single)		93
0	12/12	15 light/15 dark	55
		15 light/20 dark	97
		20 light/15 dark	95
		5 light/20 dark	94
28	8/16	14	95
	dark		95
0	8/16		16
	dark		0

Densmore (1979) conducted a rigorous experiment comparing cold stratified seed (66 days) and incubation daylengths (long days of 22 hours, short days of 13 hours, dark conditions) (Table 13). The greatest germination was observed when seed was stratified, exposed to a long day, and germinated at 15-25°C. Seed that wasn't stratified also germinated at 97% after long days at 25°C, although this was statistically less than stratified.

Table 13. Selected germination results for *Betula nana* (Densmore 1979).

Stratification (days)	Day length (hours)	Germination Temperature (°C)	Germination (%)
0	13	20 or 25	21/81
	22		46/97
	0		5/55
66	22	15, 20 or 25	100
	0	20 or 25	40/47

Junttila (1970) used factorial analysis to compare germination of seed stratified for 0-20 days and those incubated in gibberellic acid in the presence of light (Table 14). Generally, stratification improved germination, although this difference wasn't always significant, particularly when seed was germinated at high temperatures (24°C). Gibberellic acid hastened germination.

Table 14. Selected germination results (Junttila 1970). All tests were incubated in 24 hours light.

Stratification Duration (days)	Germination Temperature (°C)	Germination (%)
20	15, 18, 21 or 24 (constant)	100
10	12, 15, 18, 21 or24 (constant)	80-100
10	15-12, 18-15, 21-18 or 24-21 (alternating)	90-100
1000 mg/l GA_3 (0 days)	15, 18 or 24 (constant)	90-100

Storage and Longevity

RBG Kew (nd) recommends drying seed of *Betula nana* to 15% relative humidity and storing in hermetically sealed containers at freezing temperatures (-20°C). Young & Young (1992) report that most birch species can be stored at 1-3% moisture content at 2-5°C.

Betula pumila – bog birch, dwarf birch

Very little information was available for this plant. We recommend that information regarding *B. nana* be a starting point for this closely related species.

Viability Testing

No information was obtained regarding testing seed viability of these species.

Pretreatments and Germination Conditions

Fedkenheuer (1979) reports 12-14% germination in his greenhouse trial although no details of pretreatment or germination conditions are given. Mandel & Gibson (2011) germinated their seed under light, with bottom heat to maintain a constant temperature of 21°C, but they do not report emergence results. Brinkman (1974a) reports that stratification is not required. He obtained 31% germination after 30 days incubation at 24/18°C.

Storage and Longevity

No information.

Dasiphora fruticosa – shrubby cinquefoil

This species has recently undergone some name changes depending on the authority used. We have followed International Taxonomic Information System (ITIS nd) as an authority and included information based on older names (*Potentilla fruiticosa*, *Dasiphora floribunda*).

Viability Testing

Belcher (1995) published the following protocols for testing viability of *Dasiphora fruticosa* seeds. Seeds can be soaked overnight in water, cut or nicked longitudinally off-centre and placed in a 1% TZ solution. Seeds are then sliced length-wise to evaluate. Alternatively, X-rays can also be used to distinguish filled or empty cavities or abnormally developed embryos using 12 kV for 30 sec on Kodak and Industrex paper or 1 min on Polaroid film (Belcher 1985).

Pretreatments and Germination Conditions

Baskin & Baskin (2001) state that stratification is unnecessary and Fedkenheuer (1979) obtained 53-82% germination under standard greenhouse conditions without pretreatment. Others recommend a short cold stratification (Walsh *et al.* 2003, Emery 1988, Densmore 1979, Prairie Moon nd, Clothier nd). Walsh *et al.* (2003), as part of their propagation protocol, recommend 20 days stratification and report 81% germination. Prairie Moon (nd) stratifies their seed for 60 days. Clothier (nd) recommends 12 weeks of cold stratification. Belcher (1985), using a procedure cited by King *et al.* (1980), found that soaking seeds in hot water (28-30°C) for 18 hours improved germination without the need for cold stratification (80-90%). Other sources referenced by King indicate no pretreatments are necessary. Babb (1959) makes a recommendation for *Potentilla* (as a genus) to sow as soon as seed is ripe. Recommended protocols are cited in Table 15.

Table 15. Cited germination protocols for *Dasiphora fruticosa.*

Cold Stratification (days)	Additional Treatments or Conditions	Author	Germination (%)
0		Fedkenheuer 1979	53-82
0		Babb 1959 (*Potentilla sp.*)	
0		Anonymous 1970**	76%
0		Baskin & Baskin 2001	
0, 14 or 70		Stidham *et al.* 1980*	~65
0, 28, 56 or 84		Smreciu *et al.* 2008	37-61
0	18 hr hot (25°C) water soak	Anonymous 1979**	80-100
0	18 hr hot (25°C) water soak	Belcher 1985	
20		Walsh *et al.* 2003	81
30 †		Meshinev 1973*	94
60		Prairie Moon nd	
90		Clothier nd	

* Empirical study; ** King cites two anonymous sources from 1970 and 1979; † Meshinev does not make it clear in his work that the 'storage' time was truly a stratification.

Four empirical germination studies of *Dasiphora fruticosa* were found in the literature although two of these were somewhat limited in their scope. Meshinev (1973), as part of a study into the benefit of light

on germination, stored seeds for a month at 0°C followed by 5 months at room temperature (unclear as to whether this was a stratification or dry storage). When seeds were incubated at 18-26°C and 30-35% relative humidity, up to 94% germinated. His conclusions were that at low light levels or in complete darkness, seeds exhibited 'weaker germination energy' but overall germination percentages remained similar.

Stidham *et al.* (1980) tested 0, 2 and 10 weeks of cold stratification but primarily examined the use of Thiourea (an ammonium compound – $(NH_2)_2CS$) and potassium nitrate (KNO_3) as a treatment on seeds of forage species. Their results indicate that all cold stratification periods were equally effective in germinating ~65% of seeds if distilled water was used but if KNO_3 solution was used, germination was inferior. It is unclear as to the effect that Thiourea had on germination.

Two more rigorous germination studies are available for this species (Table 16 and 17). One of these studies was undertaken by Densmore (1979). She compared germination under long days, short days, and in the dark at a variety of germination temperatures on cold stratified (107 days) seeds and an unstratified control. 100% of stratified and unstratified seeds germinated when exposed to long days (22 hours). Stratified seed germinated equally well at a range of temperatures (10-25°C) whereas unstratified seed required warm conditions to achieve complete germination (20-25°C). Incubation in total darkness resulted in significantly lower germination percentages at any temperature.

Table 16. Selected germination results for *Dasiphora fruticosa* (Densmore 1979).

Stratification (days)	Photoperiod (hours light/dark)	Germination Temperature (°C)	Germination (%)
0	13/11	20/25	98/100
	22/2		100
	0/24		13/33*
107	22/2	15/20/25	100
	0/24	25	98

* statistically significant.

Smreciu *et al.* (2008) tested germination at ambient room temperatures with seeds harvested from northeast Alberta with 0, 4, 8, and 12 weeks of cold stratification. Stratification did not significantly improve germination in either fresh seeds or after one year of dry, room temperature storage. Although not statistically significant, marginally more year old seeds germinated than fresh and 12 weeks of stratification was slightly better than none. No indication was given as to whether ungerminated seeds were viable.

Table 17. Germination results for *Dasiphora fruticosa* (Smreciu *et al.* 2008).

Stratification (days)	Age (fresh or one year ambient storage)	Germination (%)
0	0/1	37/44
28	0/1	56/46
56	0/1	52/54
84	0/1	48/61

Storage and Longevity

RBG Kew (nd) reports that seeds are orthodox and retained 90% viability after drying to 15% relative humidity and stored frozen (-20°C) for one month. Walsh *et al.* (2003) report seed could be stored for 6-7 years when frozen (-18°C). When stored dry at 1 to 5°C, Rose *et al*. (1998) report that seeds can remain viable for up to 5 years.

Lonicera involucrata – bracted honeysuckle

Viability Testing

No information was located regarding viability testing for this species.

Pretreatments and Germination Conditions

Most of the germination information available regarding bracted honeysuckle (*Lonicera involucrata*) is anecdotal and part of either a literature review or a propagation protocol (Table 18). Most sources recommend a cold stratification to overcome dormancy. The oldest information comes from Swingle (1939) and Brinkman (1974b) who cite a USDA test (1928-1942). They report 83% germination, but no pretreatment was identified. Marchant & Sherlock (1984) recommend cold stratification for 45-60 days to obtain 56-80% germination (this is also cited by Haeussler & Coates (1986)). Hudson & Carlson (1998) provide a protocol that includes cleaning in 5-10% peroxide for 15 minutes and then cold stratifying seed for 3 months. Following this, seeds are incubated at 24/20°C on 20 hour days. Vories (1981) also recommends germinating at warm temperatures (constant 18°C). Mytty (2003) suggests cold stratification for 2-6 months following a 12 hour soak in water, but doesn't report germination percentages. Likewise, Darris (2011) suggests 30-60 days of cold stratification but gives no indication of success. Trindle & Flessner (2003) provide a protocol to cold stratify seed for 90 days and report 55% germination using this treatment.

Table 18. Cited germination protocols for *Lonicera involucrata.*

Cold Stratification (days)	Additional Treatments	Author	Germination (%)
Unknown		Brinkman 1974b, Swingle 1939	83
30-60		Darris 2011	
45-60		Marchant & Sherlock 1984	56-80
90		Trindle & Flessner 2003	55
90	15 min in 5-10% peroxide	Hudson & Carlson 1998	
60-180	12 hr water soak	Mytty 2003	

Storage and Longevity

RBG Kew (nd) state that seed is orthodox, indicating that it can be stored frozen in hermetically sealed containers once the relative humidity has dropped below 15%. Brinkman (1974b) reported that *L. tartarica* seeds maintained viability for 15 years, but longevity specific to *L. involucrata* is not included.

Populus tremuloides - trembling aspen

Viability Testing

No information is available regarding viability testing for this species. As aspen seeds are very small, viability testing is usually done by merely germinating seeds under suitable conditions as they germinate quickly and completely with little intervention (Robb pers. comm.).

Pretreatments and Germination Conditions

In their propagation protocols, Vines (1960), Hudson & Carlson (1998) and Harrington *et al.* (1999) all mention that trembling aspen seed does not exhibit any dormancy, requiring no special treatment prior to germination. More interesting are the conditions recommended for optimal germination by other authors (Table 19). Fedkenheuer (1979) obtained 81-100% germination under his standard (unspecified) greenhouse conditions. Haeussler & Coates (1986) report better germination and seedling survival on mineral soils, citing Steneker (1976) and McDonough (1979). Hudson & Carlson (1998) as part of their protocol, recommend surface sowing *(i.e.* in light conditions) and keeping seed in a warm (24/20°C) greenhouse using a 20 hour photoperiod. Faust (1936) and Schreiner (1974) reported their best results at 29°C and 30°C respectively. Citing ASOA (1985), Young and Young (1992) report a germination protocol that includes incubation at 23/30°C for two weeks while exposing seeds to light. Rose *et al.* (1998) report optimal germination at 15-25°C.

Table 19. Cited germination protocols for *Populus tremuloides.*

Germination temperature (°C)	Photoperiod (hrs)	Author	Approximate Germination (%)
5-25		Shreiner 1974*	
5-30		McDonough 1979	80-100
15-25		Rose *et al* 1998	
24/20	20	Hudson & Carlson 1998	
23/30	>1	Young & Young 1992	
30		Shreiner 1974	

* Cooler temperature for more robust seedlings, higher temperature for more complete germination.

McDonough (1979) specifies that germination of aspen seed in generally very good (~90%) at temperatures of 2-30°C but starts to decline at higher temperatures. Abnormal seedlings start to occur at 35°C, and, at 40°C, 100% of germinants are abnormal. Light did not affect germination; germination was complete in dark or light. This author also noted that germination was more rapid at higher temperatures (*i.e.* faster at 20°C than 5°C).

Zasada & Densmore (1977) report 100% germination with seeds harvested in Alaska and germinated at 5°C, 15°C or 20°C with no pretreatment (Table 20).

Table 20. Selected germination results for *Populus tremuloides* (Zasada & Densmore 1977).

Stratification (days)	Germination Temperature (°C)	Germination (%)
0	5	100
0	15	100
0	20	100

Storage and Longevity

RBG Kew (nd) describes aspen seed as orthodox, indicating that dried seed can be stored frozen in hermetically sealed containers. Shreiner (1974) reported 97% germination after 1 year in dry, cold sealed storage (5°C, 6-10% moisture content or 15-25% relative humidity) and McDonough (1979) found that aspen seeds frozen at -5°C retained 90% or more viability through 48 weeks. He found that fluctuating temperature and humidity in field storage facilities was detrimental to longevity.

Zasada & Densmore (1977), in a study of storage conditions, found that aspen seeds stored at -10°C or 3°C maintained their viability (~100%) for up to 15.5 months provided they were dried (6-10% moisture content) and stored in sealed, watertight containers.

Palamarek (unpublished) reported a reasonably steady annual decline in germination of dried seed stored frozen (-20°C). Some seed lots germinated well (>75%) after 12 years storage but for most, germination was <75% after 3-5 years.

Prunus pensylvanica – pin cherry

Viability Testing

Belcher (1985) describes TZ tests for this species. He states that the seeds are very hard to open but suggests using a hammer on the seed turned to the suture. Soak the open seed for 1-2 hours in water and slice the edge to expose the embryo tissue. Re-soak in 1% tetrazolium chloride and slice lengthwise to evaluate staining. A study by Bai *et al.* (unpublished) tested viability of this species using TZ as per 'Seed Vigour Testing Handbook' (Baalbaki *et al.* 2009). He found initial viability was generally >90%.

X-rays can also be used to distinguish filled or empty cavities or abnormally developed embryos using 12 kV for 2.5 min (150 sec) on Kodak and Industrex paper. Alternatively, one can apply 18 kV for 3 minutes on Polaroid film (Belcher 1985).

Pretreatments and Germination Conditions

Sources agree that cold stratification is required to break dormancy in pin cherry seeds (Table 21). The length of stratification, and the need for prior warm stratification, differs among sources. Grisez (1974) recommends 60 days warm followed by 90 days cold and incubated at 25/10°C (8/16 hour day). Belcher (1985) suggests 60-90 days cold stratification and then germinating seed at 20-30°C, but he does not give an indication of success. Other sources studied seed in the soil bank or following digestion. Auchmoody (1979) and Marks (1974), look at seed in the soil bank and examine the effect of fertilizer and disturbance respectively. Rogers & Applegate (1983) study germination of pin cherry seeds that had passed through the digestive tract of black bears and found a slight improvement in germination (22-25% vs 35-41%) when seeds were subsequently given a cold stratification of 50 days. Two local nurserymen (Wood pers. comm. and Formaniuk pers. comm.) cold stratify their seed for 60 or 120 days respectively.

Table 21. Cited germination protocols for *Prunus pensylvanica*.

Warm Stratification (days)	Cold Stratification (days)	Author	Germination (%)
0	50	Rogers & Applegate 1983*	35-41
	60	Wood pers.comm.	
	60-90	Belcher 1985	
	120	Formaniuk pers.comm.	
14	140	Simpson 2016	27-63
60	90	Grisez 1974	62
63	70	Bai *et al.* unpublished	20

* Following passage through the digestive tract of black bears.

Simpson (2016) tested germination of pin cherry seeds from 5 distinct populations (4 from Alberta, 1 from New Brunswick). He immersed seeds in water for 72 hours prior to testing nine pretreatments. He exposed soaked seeds to 2, 4 or 6 weeks warm stratification in the dark (21°C) followed by 16, 20 or 24 weeks cold stratification also in the dark (4°C). Seeds were germinated at 25/20°C at 12 hour days at 85% relative humidity. His results were variable among populations. He describes the ideal treatment as

2 weeks of warm stratification followed by 20 weeks cold stratification, although he does not report all of his data. Bai *et al.* (unpublished) exposed seed to 9 weeks warm stratification (30/20°C, 12/12 hour light) and 10 weeks cold stratification (4°C) prior to incubation at either 20/10°C or 30/20°C in the dark. Germination was very low (<30% and most <10%) despite high viability.

Laidlaw (1987) published one of the best studies into germination pretreatments and incubation conditions for pin cherry (*Prunus pensylvanica*) (Table 22). The study treatments included 30 days of both static and alternating temperatures during a warm stratification followed by 60, 90, 120 or 150 days of cold stratification. The incubation period was 10 days at 30°C. He observed splitting of the endocarps and some subsequent germination. The best treatment was 5-30°C (alternating every 5 days or every other day) as a warm stratification, followed by a 150 days cold stratification. This resulted in approximately 80% splitting of the endocarps and a subsequent germination of 42-44% of these seeds. In a later experiment, he found that fluctuating temperatures during the incubation period increased germination to nearly 100% of seeds whose endocarp had split and 34% of seeds with intact endocarps. Laidlaw also tried puncturing or clipping the endocarp and found this increased germination rate, but was inefficient and resulted in disease.

Table 22. Selected germination results for *Prunus pensylvanica* (Laidlaw1987).

Warm stratification (°C)	Warm Stratification	Cold stratification (days)	Approximate germination* (%)
30	Constant temperatures	150	25
5/20	5 days each (alternating)	150	10
5/30	5 days each (alternating)	150	42
5/20	1 day and 1 day each (alternating for 10 days)	150	30
5/30	1 day and 1 day each (alternating for 10 days)	150	44

* Germination of seeds whose endocarps had split during cold stratification

Storage and Longevity

RBG Kew (nd) describes pin cherry seed as orthodox, suggesting that dry seed can be frozen in hermetically sealed containers. Hardy BBT (1989) also recommends frozen storage (-18°C). Belcher reports seed can be stored 3-5 years under cool conditions (3-5°C). Vilkitis (1974) recommends storing stones dry at 1°C which can maintain viability up to 10 years. Bai *et al.* (unpublished) tested storage conditions over of three variables: air versus nitrogen, three temperatures (-20°C, 4°C and 23°C) and moisture content (high versus low). Seed viability, germination and vigour did not vary among storage conditions over three years. However, seed germination was extremely low throughout the study.

Prunus virginiana – chokecherry

Viability Testing

Belcher (1985) describes TZ tests for *Prunus virginiana* similar those of *Prunus pensylvanica*. He recommends soaking seeds for 24 -48 hours in water then turning the seed on edge to tap the suture with a hammer. Re-soak for a further 1-2 hours in water. Slice the edge to expose the embryo and soak in 1% tetrazolium chloride. Slice lengthwise to evaluate staining. A study by Bai *et al.* (unpublished) tested viability of this species using TZ as per the 'Seed Vigour Testing Handbook' (Baalbaki *et al.* 2009). Initial viability was generally >90%.

As with *P. pensylvanica*, X-rays can also be used to distinguish filled or empty cavities or abnormally developed embryos using 12 kV for 2.5 min (150 sec) on Kodak and Industrex paper or, 18 kV for 3 minutes on Polaroid film (Belcher 1985).

Pretreatments and Germination Conditions

Sources agree that chokecherry seed benefits from a period of moist, cold stratification (Table 23). The length of this stratification, the addition of warm stratification, and incubation conditions vary. The oldest reference we found was Swingle (1939), who reported unpublished results from Soil Conservation Service. He suggested stratifying seed in a cellar from November to March and reported 84% germination. Grisez (1974) recommended 120-160 days of cold stratification, including reference to Swingle and Kreftling & Roe (1949). 100-120 days cold stratification is suggested by Marchant & Sherlock (1984) and they recommend trays be monitored for germination while in stratification. Belcher (1985) recommends 60-90 days of cold stratification, like his recommendation for *P. pensylvanica*. Dirr & Heuser (1987) achieved 52% germination during a 6 month cold stratification. Fourteen days warm stratification followed by 3 months cold is recommended by Emery (1988). St-Pierre (1993) warns against over drying seed and found he could break dormancy with a 16-24 week cold stratification. Hudson & Carlson (1998) acid scarified seed for 15-90 minutes prior to a 2 month warm stratification and 4 month cold stratification. They then germinated seed at 24/20°C with 20 hour days. All the above references are presented as protocols, not investigations into germination. In a study comparing storage conditions, Bai *et al.* (unpublished) exposed seed to 9 weeks warm stratification (30/20°C, 12/12 hour light) and 8 weeks cold stratification (4°C) prior to incubation at either 15/5°C or 25/15°C in the dark. Although seeds maintained almost 100% viability, maximum germination of 69% was achieved in one case, but most germination results were much lower.

Simpson (2015) immersed seeds in water for 72 hours prior to testing nine treatments including: 2, 4 or 6 weeks warm stratification in the dark (21°C) followed by 16, 20 or 24 weeks cold stratification in the dark (4°C). Seeds were germinated at 25/20°C (12 hour days) at 85% relative humidity. This test was conducted on seeds from four harvest locations in northeastern Alberta with varied results, likely due to seed lot quality. The ideal treatment was 2 weeks of warm stratification followed by 20 weeks cold. Results were not compared to an unstratified control nor were data statistically analysed.

Table 23. Cited germination protocols for *Prunus virginiana.*

Scarification	Warm Stratification (days)	Cold Stratification (days)	Author	Germination (%)
from bear scat	0	50	Rogers & Applegate 1983*	26-37
	63	56	Bai *et al.* unpublished*	69
		60-90	Belcher 1985	
from bird ingested seeds	0	90	Meyer & Witmer 1998*	60-80
	14	90	Emery 1988	
	0	100-120	Marchant & Sherlock 1984	
	0	112-168	St-Pierre 1993	
15-90 min in H_2SO_4	60	120	Hudson & Carlson 1998	
from pheasant droppings	0	120-160	Kreftling & Roe 1949*	77
soak in water for 72 hrs	14	140	Simpson 2015	19-85
	0	150	Soil Conservation Service Data 1938**	84
	0	120-160	Shaw 1984	
	0	180	Dirr and Heusser 1987	52

* Empirical studies; ** Reported by Swingle (1939);

There is another group of references that examine digested fruit and seed (Table 23). Kreftling & Roe (1949) cold stratified seed for 120 days (22%) and 160 days (14%) after seeds were recovered from pheasant droppings. Rogers & Applegate (1983), reported on seed that had been digested by black bears. 37% and 26% of seed germinated with and without 50 days cold stratification respectively. These were significantly better than percentages for unprocessed seeds (17% and 9%). Meyer & Witmer (1998) tested cleaned seed (pulp removed) and bird ingested seed following 3 months of cold stratification and found no significant difference between the two (60-80%).

Probably the most detailed result, which still lacks statistical analysis, is Lockley (1980) (Table 24). This study examined 10, 16 and 24 weeks of cold stratification followed by incubation at alternating temperatures (10/16°C, 16/21°C, 21/27°C). Seeds that didn't germinate within 29 days were cold stratified for a further 9 weeks. When exposed to high temperatures (21/27°C) following the 24 weeks cold stratification, an average of 82% of seed germinated.

Table 24. Selected germination results for *Prunus virginiana* (Lockley 1980).

Cold stratification (days)	Germination Temperature (°C)	Approximate Germination (%)
168	10/16	60-100
168	16/21	77-100
168	21/27	80-100

Storage and Longevity

RBG Kew (nd) describes chokecherry seed as orthodox, indicating that dry seed can be stored frozen in hermetically sealed containers. St-Pierre (1993) stored seeds in cool temperatures (-3 to 3°C), whereas Belcher (1985) stored seeds at 3-5°C for 3-5 years and Rose *et al.* (1998) sealed dry seed in containers at 1°C for up to 5 years

Bai *et al.* (unpublished) tested storage conditions over three variables: air versus nitrogen, three temperatures (-20°C, 4°C and 23°C) and moisture content (high versus low). Seed viability was consistently high over all storage conditions for up to three years. Germination and vigor at lower incubation temperature (15/5°C) decreased over storage time. Germination results decreased after two years of storage, but increased again after three years. These authors conclude that their results reflect a secondary dormancy. Although Bai *et al.* (unpublished) found that seed stored at 23°C or in nitrogen maintained viability, their germination was extremely poor.

Rosa acicularis - prickly rose

Viability Testing

Belcher (1985) published information regarding estimating viability of *Rosa acicularis* seeds using TZ or by x-raying seeds. To test viability with TZ, he suggests soaking seeds overnight in water and opening the seed coat by cutting with pressure on the suture. Scratch or cut the inner seed coat and place the seed in 1% tetrazolium chloride solution; remove the embryo to evaluate staining. X-raying seeds can differentiate filled or empty cavities or abnormally developed embryos and Belcher (1985) recommends using 12 kV for 90 seconds on Kodak and Industrex paper or 12 kV for 3.5 minutes on Polaroid film.

Pretreatments and Germination Conditions

Table 25 summarizes recommended protocols for *Rosa acicularis*. Two early works (Babb 1959, Gill & Pogge 1974a) give recommendations for *Rosa* species in general, or species other than *R. acicularis*. Babb (1959) suggests acid scarification for 1-2 hours followed by 3-4 months of cold stratification. Gill & Pogge (1974a) reference the seed coat condition as a contributor to dormancy in many *Rosa* species (citing McKeever 1938) but point to cold stratification as the best method to achieve germination rather than acid scarification. Young & Young (1986) do not specify *R. acicularis* either but recommend cold stratification for seeds of *Rosa* species. Two local nurserymen (Wood pers. comm. and Formaniuk pers. comm.) agreed that 120 days of cold stratification is required prior to sowing. Belcher (1985) provides the following protocol: 60 days warm stratification, 120 days cold stratification and incubation at 20°C with 16 hour days, which is based on King *et al.* (1983) (see below). Three months warm stratification and 5 months cold stratification is the protocol cited by Hudson & Carlson (1998). They also recommend soaking seed for 24 hours prior to stratification and germinating seed on 20 hour days at 24/20°C. King (1980) conducted a literature review, which included an anonymous source suggesting 48 hours in

Table 25. Cited germination protocols for *Rosa acicularis.**

Scarification or Other Treatments	Warm Stratification (days)	Cold Stratification (days)	Incubation Conditions	Author
1-2 hrs in concentrated H_2SO_4	0	90-120		Babb 1959**
	0	120		Wood pers. comm. Formaniuk pers. comm.
48 hr GA^3	60	90		Anonymous (in King 1980)
	60	120	20°C with 16 hr days	Belcher 1985 King *et al.* 1983
Soak seeds	90	150	24/20°C with 20 hr days	Hudson & Carlson 1992
		150		DenHayer *et al.* 1980 (in King 1980)

* Protocols do not cite any germination percentages; ** *Rosa* sp. not specifically *R. acicularis*.

gibberallic acid (3.46g/1000 cc) prior to 60 days warm and 90 days cold stratification, as well as DenHeyer *et al.* (nd) who recommended 5 months of cold stratification.

Densmore & Zasada (1977) tested several methods to break dormancy of *Rosa acicularis* seeds (Table 26).

1. Acid scarified for 1 hour then cold stratified for up to 1 year.
2. Warm stratified for 118 days and then cold stratified for up to 1 year. 2 weeks after germination began (107 days), a third of the replicates dishes were placed at 20°C, another third were placed at 20/10°C (16/8 hrs); the remaining third were left at 5°C.
3. Cold stratified seeds at 5°C for up to 1 year.

They recorded their best results (>80%) by warm stratifying seed for 118 days followed by a cold stratification of 107 days and transferring seed to warmer incubation temperature (either alternating or constant). Germination was rapid following this procedure. Seed left at 5°C reached comparable germination percentages, but took 180 days total (approx. 2 ½ months longer). Acid scarification was included in this study, and they report that seeds with half or more of the pericarp removed germinated quicker. Unfortunately, there is no statistical analysis of these data.

Table 26. Selected germination results for *Rosa acicularis* (Densmore & Zasada 1977). All tests were conducted at 16 hour daylength.

Acid (hr)	**Warm Stratification at 25° C (days)**	**Cold Stratification at 5°C (days)**	**Germination Conditions** (°C)	**Approximate Germination [#] (%)**
0	0	365	5	55*
1	0	365	5	60*
0	118	107	5	85 [†]
			20/10	85
			20	85

[#] Estimated from a graph; *Germination began at 107 or 110 days but increased slowly - final counts after a year; [†] germination slower (approximately 80 days longer) than at higher incubation temperatures.

In their study, King *et al.* (1983) tested germination of seeds taken from (i) fruit not fully ripe (hips that remain yellowish-orange) and (ii) fully ripe (deep red hips). Seeds of both fruit types were at the hard dough stage and were dried following extraction to 6% moisture content and stored in sealed containers at 0°C until tested. Other variables in the King *et al.* study were length of warm and cold stratification (0, 60, 90 and 120 days each). Four replicates of 50 seeds were exposed to each treatment. For both fruit types, the best germination was achieved by stratifying seeds at 25°C for 60 days and 5°C for 120 days (>50% not ripe, >30% ripe). Incubation conditions were 16 hrs of light at a constant 20°C. They found that longer warm stratification suppressed germination, contradictory to Densmore & Zasada (1977) discussed above. Unfortunately, King *et al.* didn't statistically analyse their data (Table 27).

Table 27. Selected germination results for *Rosa acicularis* (King *et al.* 1983).

Warm Stratification (days)	Cold Stratification (days)	Fruit Ripeness	Germination[#] (%)
0	120	Not ripe	40
60	120	Not ripe	50
90	120	Not ripe	40
60	90	Ripe	35
60	120	Ripe	35

[#] estimated from a graph.

Storage and Longevity

RBG Kew (nd) lists 21 species of *Rosa* as having orthodox seed, meaning seed should be dried to low moisture content prior to storing frozen in hermetically sealed containers. However, they have no data specifically for *R. acicularis*. King *et al.* (1983) stored their seed dry at low temperatures prior to initiating their germination tests. Belcher (1985) dried seed to 6% moisture content and stored at -7°C, but suggests 3°C may be acceptable as well. Gill & Pogge (1974a) store seed cool and dry, and assert that germination remains fair after 2-4 years.

Shepherdia canadensis – Canada buffaloberry

Viability Testing

To test viability, Belcher (1985) recommends either TZ tests or x-ray. For TZ evaluations, soak seeds overnight in water, remove the cotyledon end to expose the embryo. Soak in 1% tetrazolium chloride for less than 24 hours and slice the seed lengthwise to evaluate the stain. A study by Bai *et al.* (unpublished) tested viability of this species using TZ as per the 'Seed Vigour Testing Handbook' (Baalbaki *et al.* 2009). Initial viability was generally >90%, however results were inconsistent, perhaps due to difficulty in interpretation of staining results. For x-ray, Belcher (1985) recommends using 12 kV for 60 sec on Kodak and Industrex paper or 12 kV for 2.5 minutes on Polaroid film.

Pretreatments and Germination Conditions

According to Babb (1959) fall sowing or 60-90 days cold stratification promotes germination of *Shepherdia* species (Table 28). More specifically, McLean (1967) obtained 68% germination following 60 days of cold stratification in an empirical study. However, McLean doesn't provide results from other stratification periods, nor does he analyse his results statistically. Thilenius *et al.* (1974) cite McLean as well as Heit (1967). The latter was primarily an essay on breaking hard seed coats, resulting in a recommendation to scarify seed in acid for 20-30 minutes.

The following are taken from protocols and not from empirical testing. Belcher (1985) recommends 60 days cold stratification or 15-30 minutes in acid (scarified seed do not require stratification). Young & Young (1992) call for acid scarification (length unknown) followed by 2-3 months cold stratification and incubation at 20/30°C. McTavish & Shopnik (1983) recommend 5-15 minutes of acid scarification followed by 30 days of cold stratification. Remlinger & St-Pierre (1995) plant in the fall or cold stratify 3 months prior to spring sowing. Hudson & Carlson (1998) suggest washing in peroxide (5-10%) for 15 minutes. They follow this with a 24 hour soak and 5 months of cold stratification with germination occurring at 20/15°C, 20 hours days. Luna & Wick (2008) mechanically scarified their seed (tumbled with gravel) for five days prior to 90 days in cold stratification. Bai *et al.* (unpublished) exposed seed to 3 weeks cold stratification (4°C) and incubated at either 15/5°C or 25/15°C in the light (12 hour days). Germination varied widely over the storage conditions applied and generally the pretreatment was not sufficient to optimize germination. Wood (pers. comm.) recommend 30-60 days of cold stratification whereas Formaniuk (pers. comm.) uses 90 days.

King (1980) published a literature review that included sources we were unable to obtain. They suggest acid scarification up to 30 minutes and/or cold stratification for up to 60 days (Table 29).

Table 28. Cited germination protocols for *Shepherdia canadensis.*

Misc. pretreatments	Scarification (min)	Cold Stratification (days)	Author	Germination (%)
	0	21	Bai *et al.* unpublished	0-83 extremely variable
		30-60	Wood pers. comm.	
		60	McLean 1967*	68
		60	Belcher 1985	
		60-90	Babb 1959 (*Shepherdia sp.*)	
		90	Remlinger & St-Pierre 1995	
		90	Formaniuk pers. comm.	
15 min peroxide wash followed by 24 hr H_2O soak		150	Hudson & Carlson 1998	
	5-15 acid	30	McTavish & Shopnik 1983	
	20-30 acid	0	Heit 1967	72-80
	30 acid	0	Belcher 1985	
	acid	60-90	Young & Young 1992	
	tumble 5 days with gravel	90	Luna & Wick 2008	49-75

* Empirical study, not comprehensive.

Table 29. Cited germination pretreatments for *Shepherdia canadensis* (King 1980).

Acid Scarification (minutes)	Cold Stratification (days)	Author	Germination (%)
	30-60	Lohmiller pers. comm.	
	60	Benson pers. comm.	
	56-84	Simonson 1976	11-88
	90	DenHeyer *et al.* (unpub)	
15	30	Cram 1978	89
20-90	60	Dick 1979	

Densmore (1979) provided one of the most detailed study of buffaloberry germination, examining various lengths of warm and cold stratification as well as several combinations of light and dark conditions (Table 30). Light did not significantly affect germination. The most complete germination followed a warm stratification or an alternating warm period (Table 30) for 64 days prior to 127 days cold stratification. After cold stratification (2-5°C), incubation at 24/2°C or constant 20°C resulted in >80% germination.

Table 30. Selected germination results for *Shepherdia canadensis* (Densmore 1979).

Warm stratification (days)	Cold stratification (days)	Light or Dark	Germination Temperature (°C)	Germination (%)
0	191	Light	20	29
			25	50
		Dark	17/0	56
64	127	Light	24/2	95
		Dark	20	83
Alternating*		Light	24/2	85

* Alternating stratification: 27/7°C for 42 days and 17/3°C for 22 days

Smreciu & Gould (2009) examined 0, 4, 8 and 12 weeks of cold stratification and obtained significantly higher germination percentages (68-77%) with 8 or 12 weeks of stratification over 4 weeks or none (Table 31).

Table 31. Selected germination results for *Shepherdia canadensis* (Smreciu & Gould 2009).

Warm stratification (days)	Cold stratification (days)	Light Regime	Germination Temperature (°C)	Germination (%)
0	0	ambient	ambient	1
	28			20
	56			72*
	84			68*

*Germination significantly greater than those recorded at 0 or 4 weeks stratification.

Rosner & Harrington (2002) conducted a study examining acid scarification of 5 minutes prior to 0, 45 or 98 days cold stratification (Table 32). They found the short acid scarification significantly improved germination, as did the longer cold stratification period (90% of viable seed). Analysis compared stratification periods within scarification treatments therefore comparisons between the two acid treatments cannot be made. Germination times were significantly reduced by acid scarification (from 9 days to 5 days). *A most practical observation was that 1 week of imbibition and recording the change in weight can give a good indication of the degree of physical dormancy, (i.e., requirement for scarification).*

Table 32. Selected germination results for *Shepherdia canadensis* (Rosner & Harrington 2002).

Acid Scarification (minutes)	Cold Stratification (days)	Approximate Germination* (%)
0	45	35 a
	98	60 b
5	45	70 a
	98	90 b

* Germination without stratification was negligible. Each acid treatment was considered a separate experiment ergo significance presented here is valid only within each acid treatment.

Storage and Longevity

Remlinger & St-Pierre (1995) stored seed in sealed containers at 13.1% relative humidity and 5°C. After 42 months of storage, 97% of seed remained viable. Five years is the longevity claimed by Belcher (1985) when stored dry at 3°C (corroborated by Luna & Wick (2008)). RBG Kew (nd) states that based on seed size the species may show orthodox storage behavior (*i.e*. storing dry at -18-20°C).

Bai *et al.* (unpublished) tested storage conditions over three variables: air versus nitrogen, three temperatures (-20°C, 4°C and 23°C) and moisture content (high versus low). Seeds with high moisture content lost more viability over the same time when compared with low moisture content. Germination and vigour at each of two incubation temperatures (15/5°C and 25/15°C), decreased with increased storage temperature. Although their viability results were quite variable, they concluded that seed viability decreased with increased seed moisture content and storage temperatures, particularly notable after three years of storage and most notable when seeds were stored in nitrogen at room temperature. These authors recommend storing dry seed (low moisture content) at low temperatures.

Symphoricarpos albus – snowberry

Viability Testing

To test viability, seeds should be soaked overnight in water, then punctured in the centre with a needle or cut at the cotyledon end. Soak in a 1% tetrazolium chloride solution until stained. Seeds can be sliced along the flat axis for evaluation (Belcher 1985). Alternatively, seed can be x-rayed to show filled and unfilled seed cavities. This can be accomplished using 12 kV for 60 seconds on Kodak film and Industrex paper or for 3 minutes on Polaroid film (Belcher 1985). Use of newer x-ray technology was not found in the literature.

Pretreatments and Germination Conditions

Most sources focusing on propagation of snowberry from seed provide anecdotal protocols rather than empirical studies. Almost all recommendations suggest a combination of warm and cold stratifications with or without acid scarification (Table 33). According to Evans (1974), the first warm period softens the coat whereas the cold period overcomes embryo dormancy. Alternatively, acid scarification can be used to soften the seed coat (Glazebrook 1941). Flemion (1934) (cited by Swingle 1939) recommends 75 minutes acid scarification followed by 21 days warm stratification and 180 days cold stratification. Unable to access Flemion's original study, we did locate Flemion (1942) which uses the warm/cold stratification treatments in association with fertilizer. Swingle also records Chadwick (1935) who used 90 days warm followed by 180 days cold stratification. Vories (1981) includes the later reference to Flemion (1942) as well as Glazebrook (1941), who recommends an hour of acid scarification in addition to 100-140 days of cold stratification. Evans (1974) recommends 60 minutes acid scarification followed by 60 days warm and 180 days cold stratification. Marchant & Sherlock (1984), Haeussler & Coates (1986) and Dirr & Heuser (1987) all recommend 90-120 day warm stratification followed by 120-180 cold stratification. Belcher (1985) reports that acid scarification for 60-75 minutes can be used in combination with 60 days cold stratification, or in the absence of acid, 30 days warm and 180 days cold stratification. 24 hours soaking in water is recommended by Hudson & Carlson (1998) prior to 4 months warm and 6 months cold stratification. They then germinated seed with 20 hour days at 24/20°C. Young & Young (1992) suggest 20-60 days of warm stratification followed by 180 days cold stratification. Doucette *et al.* (2001) examined germination of seeds ingested by cattle. 69% of snowberry seeds recovered from cattle feces germinated using AOSA standard procedures (1993) however no comparisons were made with undigested seeds. Most of these sources are prescriptive. The exceptions are Flemion (1942) and Doucette *et al.* (2001) which are empirical although neither was very robust.

Flemion (1942) (who in an older publication (1934) had shown that seed coats need to be broken down prior to cold stratification) placed duplicate lots of 200 seeds into bottles of peat moss and kept them at 25°C. Every 5 or 6 days she would add 5 mL nitrogen in solutions (KNO_3, NH_4NO_3, $(NH_4)_2SO_4$). After 49 days, 70 days or 91 days, seeds were washed and moved to fresh peat moss for six months (180 days) of cold stratification. Germination began before seeds were removed from cold treatment. Data were not analysed and no controls were used but the greatest germination percentages (up to 88%) were found with seeds exposed to 70 or 91 days of warm stratification with added N compounds although the form of nitrogen does not appear to affect germination. She did note however that Pfieffer (1934) had shown

that the benefit of exposing seeds to a warm stratification period in peat was that fungal elements began to penetrate the seed coat thereby causing thinning.

Table 33. Cited germination protocols for *Symphoricarpos albus.*

Acid Scarification (minutes)	Warm Stratification (days)	Cold Stratification (days)	Author	Germination (%)
0	20-60	180	Young & Young 1992	
0	30	180	Belcher 1985	
0	90	180	Chadwick 1935	
0	90-120	120-180	Marchant &Sherlock 1984	
0	120	180	Hudson & Carlson 1998	
60	0	100-140	Glazebrook 1941	
60-75	0	60	Belcher 1985	
60	60	180	Evans 1974	35
75	21	180	Flemion 1934 (cited by Swingle)	74
Digested	0	0	Doucette *et al.* 2001*	69

* Empirical study.

Storage and Longevity

Evans (1974) reports 45% germination after 2 years stored dry and cold (5°C). Belcher (1985) concurs that seed remained viable for two years stored dry at 3-5°C. Shaw (1984) maintained seed viability for 7-10 years when stored cold and dry however no storage temperatures nor moisture contents were listed.

Vaccinium myrtilloides – dwarf blueberry

Viability Testing

A study by Bai *et al.* (unpublished) tested viability of blueberry using tetrazolium staining as per the 'Seed Vigour Testing Handbook' (Baalbaki *et al.* 2009). The authors noted some concerns about underestimating viability using TZ (likely due to the small seed size). Initial viability was measured at >75%.

Pretreatments and Germination Conditions

van der Kloet & Hall (1981) and Smreciu *et al.* (2008) report seed of dwarf blueberry did not need stratification, but that germination was bimodal with some seeds germinating in the first few weeks and a second group germinating after 2-3 months (Table 34). Smreciu *et al.* (2008) compared 4 weeks of cold stratification with a control (no stratification) and found no significant difference between them. Likewise, Carter & St-Pierre (1996) do not recommend pretreatment for blueberries generally (*V. angustifolium* and *V. myrtilloides*). Bai *et al.* (unpublished) exposed seed to 5 weeks cold stratification (4°C) and incubated at either 15/5°C or 25/15°C in the light (12 hour days). Their best germination was achieved at higher incubation temperatures and initial germination was often lower than that attained after a year of storage. Our local nursery (Smoky Lake Forest Nursery) cold stratifies their seed for 30-60 days (Durago pers. comm.).

Table 34. Cited germination protocols for *Vaccinium myrtilloides.*

Cold Stratification (days)	Author	Germination (%)
None	van der Kloet & Hall 1981	
None	Carter & St-Pierre 1996	
None	Smreciu *et al.* 2008*	10
30	Durago pers. comm.	
45	Bai *et al.* unpublished	71

* Empirical study (comparing 4 weeks stratification versus a control)

A comprehensive germination evaluation was conducted by McKechnie (2009) (Table 35). In 2006, she compared freshly extracted seed, those air-dried for 48 hours and those stored for 1 year. Although she doesn't provide specific conditions under which seed were stored, based on the refences she cites (Darrow & Scott 1954, Shafii & Barney 2001) it was likely dry at 0-5°C. Five replicates of 20 seeds were surface sown and maintained at 20/10 °C for 15/9 hour days. Although germination ranged from 5-20% she reported no significant differences among treatments. In 2007, she compared harvest locations, and although there was a significant difference among sites, the time to reach 50% of the germination attained was the same (44-63 days). This test included 5 replicates of 100 surface sown seeds maintained as described above. Seeds from superior sites germinated 14-18% compared with only 7% of seeds from the inferior site. In 2008, the same comparison between sites was conducted, but rather than on soil in greenhouse conditions, these were laboratory tests at 20-23°C. Three replicates of 100 seeds were maintained at room temperature in ambient light for 4 months. As in the greenhouse trial

above, there was a significant difference in total germination among sites, but the time to 50% of the germination total was similar (27-56 days).

Table 35. Selected germination results for *Vaccinium myrtilloides* (McKechnie 2009).

Storage Conditions	Germination Temperatures	Germination Daylengths (hrs)	Germination (%)
None (fresh -2006)	20/10 °C	15/9	20
None (air dried)	20/10 °C	15/9	19
1 year (dry cold) *	20/10 °C	15/9	5
25 days (dry cold) *	20/10 °C	15/9	13
None (fresh - 2008) *	20-23°C	ambient light	28.5

* Storage conditions are not specified, based on the refences she cites (Darrow & Scott 1954, Shafii & Barney 2001) it was likely dry at 0-5°C.

In each of the studies quoted here, germination percentages were low. No indication was given as to whether ungerminated seeds were viable.

Storage and Longevity

RBG Kew (nd) found 94% of seed remained viable following drying to 15% relative humidity and freezing (-20°C) for 17 months. They describe seed as orthodox, indicating that longevity in this state could be longer. Carter & St-Pierre (1996) and McKechnie (2009) do not specify *V. myrtilloides* but suggest blueberry seed can be stored cold (0-5°C) for up to 6 years.

Bai *et al.* (unpublished) tested storage conditions over three variables: air versus nitrogen, two temperatures (-20°C and 4°C) and moisture content (high versus low). Blueberry seed viability and germination generally decreased with age regardless of storage conditions. The exception was that when stored at 4°C with low moisture seeds maintained viability. Standard orthodox seed storage conditions (-20°C at low moist) were not included in this study.

Vaccinium vitis-idaea – lingonberry or bog cranberry

Viability Testing

A study by Bai *et al.* (unpublished) tested viability of this species using tetrazolium staining as per the 'Seed Vigour Testing Handbook' (Baalbaki *et al.* 2009). Initial viability was generally >90%, however tests performed on stored seed of this species were less conclusive as germination exceeded some viability test results. TZ on these small seeds is likely problematic.

Pretreatments and Germination Conditions

A few references record germination results for a single treatment. Nichols (1934) observed 37% germination in 160 days after an 83 day cold stratification. Hall & Beil (1970) found 76.5% germination when fresh seed was sown into pots and kept at 21°C. This compared with 6% germination of seeds that had been stored for 37 days at -2°C and no germination of seed which had been scarified for 5 min in 0.1 N H_2SO_4. Hall & Shay (1981) observed even more complete germination (87%) by surface sowing seeds (which had been stored at 1°C for approximately 3 months) and placing them in a greenhouse at 26/24°C on 16/8 hour days. Bai *et al.* (unpublished) exposed seed to 13 weeks cold stratification (4°C) and incubated at either 15/5°C or 25/15°C in the light (12 hour days).

van der Kloet & Hill (1994) kept berries overwinter in a cold frame and found 81% germination when seeds were extracted and sown. This compared with 18% germination of freshly extracted seed. Both treatments were incubated in a greenhouse at 25/10°C for 14/10 hour days. They also examined the germination of seed buried for up to six years and found germination following this treatment varied; it ranged from 15-31%.

Lehmushovi (1975) compared germination of seeds stratified for 1 -12 months. Seeds were sown on a milled peat substrate which he determined to be better than sand, mineral or heath soil. He was unable to draw any specific conclusions because seeds germination was extremely variably through the year (e.g. 2 months – 40% germination, 6 months - >10%, 9 months – 25%). Table 36 summarizes results discussed in the previous three paragraphs.

Table 36. Cited germination protocols for *Vaccinium vitis-idaea.*

Cold Stratification (days)	Incubation Period (days)	Author	Germination (%)
83	160	Nichols 1934*	37
0	(at 21°C)	Hall & Beil 1970	77
90	(26/24°C)	Hall & Shay 1981	87
overwinter	25/10C	Van der Kloet & Hill 1994	81
30-360		Lehmushovi 1975	>10-40

* Empirical study with a single stratification treatment.

Densmore (1979) provides a detailed treatise of lingonberry germination (Table 37). She studied germination of seeds from fruit frozen *ex situ* (-19°C in berries for 70 days) and harvested fruit

Table 37. Germination results for *Vaccinium vitis-idaea* (Densmore 1979). Individual experiments are separated by a bold, solid line, however, significance is based on an analysis of all results. Shaded cells indicate best results.

	Stratification (days)	Photoperiod (hours light/dark)	Germination Temperature (°C)	Germination (%)	Significance
Extracted seeds	0	light	5	0	a
			10	0	a
			15	88	efg
			20	100	h
			25	88	efg
	60	light	10	0	a
			15	71	e
			20	100	h
			25	88	efg
Seeds extracted from frozen *ex situ* fruit	0	light	10	0	a
			15	91	fg
			20	100	h
			25	85	efg
Extracted seeds	0	light	10	0	a
			15	45	d
			20	92	fg
			25	77	ef
		dark	10	0	a
			15	4	a
			20	70	e
			25	26	bc
Seeds extracted from fruit harvested *in situ* following 1 winter	0	light	15	77	ef
			20	97	gh
			25	96	gh
		dark	15	37	cd
			20	83	ef
Extracted seeds	0	22/2	10	2	a
			15	78	ef
			20	95	gh
			25	72	e
	0	13/11	10	0	a
			15	79	ef
			20	95	gh
			25	76	ef
	0	dark	10	0	a
			15	18	b
			20	53	c
			25	29	bc
Seeds from bear droppings	0	22/2	10	0	a
			15	68	e
			20	92	fg
			25	47	d
Seeds from vole dropping	0	22/2	15	84	efg
			20	94	fg

overwintered *in situ* (June the following year). She compared germination of these seeds with a control (stratified and unstratified). Within this experiment, she included a light versus dark treatment over an array of germination temperatures. Generally light conditions and 20°C resulted in the most complete germination. Digestion of seeds by black bears and red-backed voles did not have a significant affect, but increased damage to seeds.

Mallik & Gimingham (1985) examined the effect of heat, such as from fire, on germination of several species, including lingonberry (Table 38). Three temperatures (50, 75 and 100°C) for three durations (0.5, 1 and 2 minutes) were tested. Germination of *V. vitis-idaea* was minimal in all treatments except 30 seconds of 100°C (dry oven heat). In that case 98% germination was observed. Another examination compared heat exposure following 12 weeks of cold stratification (0°C). This time two temperatures (50 and 100°C) were applied for three durations (1, 2 and 3 minutes). Germination ranged from 52% to 76%, both resulting from exposure to 100°C (1 and 2 minutes respectively) but there was no statistical analysis. Stratification appeared to be beneficial for germination however a single heat treatment of 100°C for 30 seconds was superior to any of the stratified treatments.

Table 38. Selected germination results for *Vaccinium vitis-idaea* results (Mallik & Gimingham 1985).

Stratification (days)	Heat treatment (°C)	Heat (minutes)	Germination (%)
0	0	0	0
	50	0.5	6
		1	8
		2	4
	100	0.5	98
		1	0
		2	0
84	0	0	62
	50	1	72
		2	66
		3	60
	100	1	52
		2	76
		3	54

In a very confusing paper, Ripa (1993) describes a study of germination under various conditions and after numerous pretreatments. He conducted an examination of:

- cold stratification (4, 6 or 7 months),
- pH (3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5),
- warm stratification (at 15, 23 and 30°C) for 6 months.
- seed preparation (cleaning mesocarp, 24 hours soak in 10% Na_2CO_3 solution, soaked in hot (80°C) water and tepid water (20°C) for two minutes each),
- light regimes (light and dark conditions), and
- drying of seed after imbibition and stratification.

He does not present all of his results but rather states that 'Seeds stored at 4°C in their berries undergo complete stratification in 4 months and give a good germination rate. The highest germination (84-92%) was observed on acidic media (pH 3.5-4.5).'

Baskin *et al.* (2000) examined three *Vaccinium* species including *V. vitis-idaea* (Table 39). They tested stratification, light versus dark, and 3 germination temperatures. The most complete germination was observed across all temperature regimes (15/5, 20/10 and 25/15°C) when seed was cold stratified (1°C) for 12 weeks and germinated in the light (35.5-82%). The best germination temperature was 25/15°C (all using 12/12 hour days). As a secondary study, berries set in the same year, but kept overwinter on plants *in situ* were harvested in May and germinated under the same temperature regimes. Seed incubated for only 2 weeks failed to germinate at most temperatures (26% at 25/15°C). The best results (93-100%) were found when berries overwintered *in situ* incubated for 6 weeks (although no statistical analysis was provided).

Table 39. Selected germination results for *Vaccinium vitis-idaea* (Baskin *et al.* 2000).

Stratification (days)	Incubation time (days)	Light Regime	Germination temperature (°C)*	Germination (%)
84	28	Dark	15/5	4
			20/10	37
			25/15	42
	14	Light	15/5	36
			20/10	59
			25/15	72
	28	Light	15/5	50
			20/10	79
			25/15	82
0	112	Light	15/5	10
			20/10	3
			25/15	4
Overwinter**	14	Light	15/5	0
			20/10	0
			25/15	26
	28	Light	15/5	36
			20/10	88
			25/15	92
	42	Light	15/5	93
			20/10	99
			25/15	100

* 12/12 hour days; ** berries overwintered *in situ* then seed extracted

Harvesting seed from northeastern Alberta, Smreciu *et al.* (2008) tested germination of *V. vitis-idaea* seed following several stratification lengths (0, 4, 12 and 16 weeks at ~4°C). Seed were germinated in ambient conditions (~20°C, normal light) (Table 40). They found both 12 and 16 weeks cold stratification adequate to achieve >80% germination. Seeds stored dry at ambient temperatures for 1 year germinated as well as fresh, however, by the end of a second year of storage, germination and had declined significantly and after 6 years, almost none of the seed germinated.

Table 40. Selected germination results for *Vaccinium vitis-idaea* (Smreciu *et al.* 2008).

Stratification (days)	Age	Germination (%)
0	0	15
	1	12
28	0	40
	1	36
84	0	89
	1	89
112	0	88
	1	88

Storage and Longevity

89% viability was maintained by RBG Kew (nd) following drying to 15% relative humidity and freezing (-20°C) for one month. As orthodox seed, it is anticipated that seed will remain viable much longer. Smreciu *et al.* (2008) stored dry seed in ambient conditions and reported a decline in germination after two years.

Bai *et al.* (unpublished) tested storage conditions over three variables: air versus nitrogen, two temperatures (-20°C and 4°C) and moisture content (high versus low). Seeds stored for one year generally germinated well, but both viability and germination, regardless of incubation conditions, decreased in subsequent years. Viability, germination and seedling vigour did not vary among storage conditions.

Viburnum edule – low-bush cranberry

Viability Testing

To distinguish between full and empty seeds, Belcher (1985) used tetrazolium staining. Seeds were sliced longitudinally along the flat face. The endosperm was scratched or punctured to expose the tiny embryo or alternatively, the embryo was removed. The seed (or excised embryo) was soaked in 1% TZ solution and staining of the embryo was evaluated. No results were reported. A study by Bai *et al.* (unpublished) evaluated viability of this species using tetrazolium staining as per the 'Seed Vigour Testing Handbook' (Baalbaki *et al.* 2009) and he found initial viability (of fresh seeds) was generally >90%.

According to Belcher (1985) x-ray technology can be used to differentiate filled or empty cavities or abnormally developed embryos. He recommended using 12 kV for 80 sec on Kodak and Industrex paper or 12 kV for 3 minutes on Polaroid film. Technological advances have likely rendered this method obsolete and new digital x-rays technology is probably a better option.

Pretreatments and Germination Conditions

Much of the information in the literature regarding germination treatments for *Viburnum edule*, indicates a requirement for two distinct stratification periods; warm stratification followed by a cold period (Babb 1959, Hauessler & Coates 1986, Moore *et al.* 2004, Luna 2008, Smreciu & Gould 2009). Moore's method yielded the most complete germination but is taken from a propagation protocol and does not compare other treatments. They also state that sowing seeds immediately after harvest, prior to drying and storage, yield better germination percentages. Gill & Pogge (1974b) working with a related species (*V. opulus*) state that the warm stratification is required to release dormancy of the radicle whereas the ensuing cold stratification is responsible for breaking dormancy of the plumule. Duration of each of the warm and cold treatments varies depending on the author (Table 41).

Wood (pers. comm.) and Formaniuk (pers. comm.) recommend 1 year and 180 days cold stratification respectively. They do not employ a warm stratification period nor do they indicate ultimate germination percentages. Taylor suggests 60-90 days stratification at 30/10°C followed by 30 to 60 days cold period and incubation at 30/20°C to obtain approximately 60% germination.

Smreciu & Gould (2009), in an empirical study comparing germination of an unstratified control with seeds warm stratified for 0, 8, 12, or 16 weeks and 0, 4, 8 or 12 weeks cold period, attained a maximum germination of 14% following 16 weeks warm and 12 weeks cold. Although germination was poor, this treatment resulted in significantly higher germination than all other treatments.

Table 41. Cited germination protocols for *Viburnum edule*.

Warm Stratification (days)	Warm Stratification Temperature (°C)	Cold Stratification (days)	Cold Stratification Temperature (°C)	Germination Temp. (°C)	Author	Germination (%)
0		365	2-4		Wood pers. comm.	
0		180	2-4		Formaniuk pers. comm.	
	163	14	4		Bai *et al.* unpublished**	71 (max)
60-90	30/10	30-60	5	30/20	Taylor 1997	
60-90	20	30-60	1-6	86/68	Pogge & Gill* 1974b	60
90	20	90	5	Ambient greenhouse	Moore *et al*. 2004	75
112	Ambient room temperature	84	2-4	Ambient room temperature	Smreciu & Gould 2009**	14
90-150	22	90	5		Luna 2008	
120		60			Babb 1959	

* For *V. opulus;* ** Indicates an empirical evaluation.

Bai *et al.* (unpublished) exposed seed to 16 weeks warm stratification (30/20°C) and 2 weeks cold stratification (4°C) prior to incubation at either 20/10°C or 30/20°C in the dark. Generally, these were insufficient to obtain >50% germination, the exception is a single accession which reached 71%.

Babb (1959), indicates that if this species is to be direct-sown, it should be seeded in spring to ensure the two stratification periods. Smreciu *et al.* (2008) and Smreciu and Gould (2015), in studies of direct seeding, found that emergence was significantly better if fruit were previously frozen (for up to 1 year) and sown whole (as compared to extracted seeds). No emergence was observed in the first year after sowing; some emergence was observed in the second year and more in year 3. Generally spring sowing was better than fall.

Viereck & Schandelmeier (1980) mention that *Viburnum edule* belongs to a class of species with thick-coated hard seeds that are fire resistant but that perhaps their germination is stimulated by fire whereas Hauessler & Coates (1986) also wrote about the thick-coat of these seeds but indicated that seed coat treatments were not effective in breaking dormancy.

Storage and Longevity

RBG Kew (nd) indicate that seeds of *Viburnum edule* are orthodox. They have attained 75% viability following drying to moisture contents in equilibrium with 15% relative humidity and storage for approximately 2 years at -20°C. Bai *et al.* (unpublished) tested storage conditions over three variables: air versus nitrogen, three temperatures (-20°C, 4°C and 23°C) and moisture content (high versus low). Viability remained consistent (~90%) over three years with no differences among storage treatments for

some seedlots. However, viability decreased for a single seedlot. The authors of this study did not provide an interpretation of this result. Generally, germination however, was consistently low and continued to decrease over storage time.

Moore *et al.* 2004 state that germination is best if seeds are not dried and stored but sown immediately following harvest and extraction.

REFERENCES

Alberta Government. 2016. Alberta Forest Genetic Management and Conservation Standards. 158 pages. Available at: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/formain15749/$FILE/FGRMS_Stream1_2Dec2016.pdf

Anonymous. 1970. Germination of twenty-one species collected from a high elevation rangeland in Utah. American Midland Naturalist 84(2): 551-554.

Anonymous. 1979. Native shrub production project. USDA Forest Service, SEAM Program. 40 pages.

Association of Official Seed Analysts. 1993. Rules for testing seeds Vol. 16(3). Bozeman, Montana.

Association of Official Seed Analysts. 2010 edition. AOSA/SCST Tetrazolium Testing Handbook.

Auchmoody, L.R. 1979. Nitrogen fertilization stimulates germination of dormant pin cherry seed. Canadian Journal of Forest Research. 9:514-516.

Baalbaki, R., Elias, S., Marcos-Filho, J. and McDonald, M. B. 2009. Seed vigour testing handbook. Ithaca, New York, Association of Official Seed Analysts.

Babb, M. 1959. Propagation of woody plants by seed. IN: E. Peterson and N. Peterson, eds. Revegetation information applicable to mining sites in northern Canada. Indian & Northern Affairs, Environmental Studies, No.3 Pages 6-9.

Bai, Y., Y. Wai, Y. Wang, R. Chibbar, C. Walters and L Ren. 2016. Unpublished. Improving seed longevity of native shrubs during storage for reclamation of oil sands mines. Final Report prepared for COSIA. University of Saskatchewan. 139 pp.

Baskin, C.C. and J.M. Baskin. 2001. Seeds: Ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego, CA. 666 pages.

Baskin, C., P. Milberg, L. Anderson and J. Baskin. 2000. Germination studies of three dwarf shrubs (*Vaccinium*, *Ericaceae*) of northern hemisphere coniferous forests. Canadian Journal of Botany 78:1552-1560.

Bai, Y., Y. Wai, Y. Wang, R. Chibbar, C. Walters and L Ren. Unpublished. Improving seed longevity of native shrubs during storage for reclamation of oil sands mines. Final Report prepared for COSIA. University of Saskatchewan. 139 pp.

Belcher, E. 1985. Handbook on seeds of browse-shrubs and forbs. Prepared by Browse-shrub and forb committee or the Association of Official Seed Analysts, USDA Forest service. Atlanta, Georgia. 145 pages

Benson, D.A., Nursery Superintendant Coeur d'Alene Nursery, Coeur d'Alene, Idaho. Personal communication, cited by King 1980

Berg, A. 1974. *Arctostaphylos* Adans. IN: Seeds of Woody Plants, C. Schopmeyer (Technical Coordinator). USDA Forest Service, Agriculture Handbook. No. 450. Pages 228-231.

Berry, A. and J. Torrey. 1985. Seed germination, seedling inoculation and establishment of *Alnus* spp. in containers in greenhouse trials. Plant and Soil 87:161-173.

Black, M. and P.E. Wareing. 1955. Growth studies in woody species VII. Photoperiodic control of germination in *Betula pubescens* Ehrh. Physiologia Plantarum 8:300-316.

Bliss, L. 1958. Seed germination in arctic and alpine species. Arctic 11:180-188.

Brinkman, K. 1974a. *Betula* L. IN: Seeds of Woody Plants, C. Schopmeyer (Technical Coordinator). USDA Forest Service, Agriculture Handbook. No. 450. Pages 252-257.

Brinkman, K. 1974b. *Lonicera* L. IN: Seeds of Woody Plants, C. Schopmeyer (Technical Coordinator). USDA Forest Service, Agriculture Handbook. No. 450. Pages 515-519.

Carter, P. and R.G. St-Pierre. 1996. Growing blueberries in Saskatchewan. Department of Horticulture Science, University of Saskatchewan. Saskatoon, SK. 24 pages.

Chadwick, L.C. 1935. Practices in propagation by seed. Stratification treatments for many species of woody plants. American Nurseryman 62(12): 3-9.

Clothier, T. No date. Seed Germination Database. Available at: http://tomclothier.hort.net/

Cram, W.H. 1978. PFRA Tree Nursery Annual Report, 1978. Department of Regional and Economic Expansion, Prairie Farm Rehabilitation Administration. 77 pages.

Crossley, J.A. 1974. *Vaccinium* L. IN: Seeds of Woody Plants, C. Schopmeyer (Technical Coordinator). USDA Forest Service, Agriculture Handbook. No. 450. Pages 840-843.

Darago, H. 2013. Seed Plant Manager, Smoky Lake Forest Nursery.

Darris, D. 2011. Plant fact sheet for twinberry honeysuckle (*Lonicera involucrata*). USDA Natural Resources Conservation Service, Corvallis, OR. 2 pages.

Darrow, G.M and D.H. Scott. 1954. Longevity of blueberry seed in cool storage. Proceedings of the American Society for Horticultural Science 63: 271.

DenHeyer, J. Production Supervisor, Provincial Tree Nursery, Oliver, Alberta. Personal communication, cited by King 1980.

DenHeyer.J. G. Grainger. P. Flinn and H. Oosterhuis. No date. Propagation and production of wordy ornamentals in a small nursery. Horticulture Branch, Plant Industry Division, Alberta Agriculture, Adex 275/16. 65 pp. Cited in King (1980).

Densmore, R. 1979. Aspects of the seed ecology of woody plants of the Alaskan Taiga and Tundra. A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Botany in the Graduate School of Arts and Sciences of Duke University. Duke University, Durham, NC. 285 pages.

Densmore, R. and J.C. Zasada. 1977. Germination Requirements of Alaskan *Rosa acicularis*. Canadian Field Naturalist 91(1):58-62.

Dick, J.H. 1979. A Management plan for the rehabilitation of surface mined coal lands in the East Kootenays, BC. MSc. Thesis, Department of Forestry, University of British Columbia, Vancouver, BC. 338 pages.

Dirr, M. and J. Heuser. 1987. The reference manual of woody plant propagation: from seed to tissue culture: a practical working guide to the propagation of over 1100 species, varieties and cultures. Varsity Press, Athens, Georgia. 239 pages. (selected pages)

Doucette, K., K. Wittenberg and W. McCaughey. 2001. Seed recovery and germination of reseeded species fed to cattle. Journal of Range Management 54:575-581.

Emery, D.E. 1964. Seed Propagation of Native California Plants. Leaflets of the Santa Barbara Botanic Garden, 1(10)81-96. Cited in King (1980).

Emery, D.E. 1988. Seed Propagation of Native California Plants. Santa Barbara Botanic Garden, Santa Barbara, California. 115 pages.

Eulert, G.K. and H. Hernandez. 1980. Synecology and autecology of boreal forest vegetation in the Alberta oils sands environmental research program study area. Prepared for the Alberta Oil Sands Environmental Research Program by interdisciplinary systems Ltd. AOSERP Report 99. 184 pages.

Evans, K.E. 1974. *Symphoricarpos* Duham. IN: Seeds of Woody Plants, C. Schopmeyer (Technical Coordinator). USDA Forest Service, Agriculture Handbook. No. 450. Pages 787-790.

Faust, M.E. 1936. Germination of *Populus grandidentata* and *P. tremuloides* with particular reference to oxygen consumption. Botanical Gazette 97: 808-821.

Fedkenheuer, A.W. 1979. Propagation and establishment of native woody plants on oil sands reclamation areas. Prepared for Syncrude Canada Ltd., 1979 Minerals Waste Stabilization Liaison Committee meeting. Eveleth, Minnesota. 22 pages

Flemion, F. 1934. Physiological and chemical changes preceding and during the after ripening of *Symphoricarpos racemosus* seeds. Contributions from the Boyce Thompson Institute 6: 91-102.

Flemion, F. 1942. Effect of the addition of nitrogen upon germination of seeds of *Symphoricarpos racemosus*. Contributions from Boyce Thompson Institute 12:485-490.

Forest Research, UK Forestry. No date. Seed storage and pretreatment for *Alnus incana*. Available at: http://www.forestry.gov.uk/fr/infd-7fab2l (Accessed 12 December 2016).

Formaniuk, S. 2013. Tree Time Services. Personal communication.

Gill, J.D. and F.L. Pogge. 1974a. *Rosa* L. IN: Seeds of Woody Plants, C. Schopmeyer (Technical Coordinator). USDA Forest Service, Agriculture Handbook. No. 450. Pages 732-737.

Gill, J.D. and F.L. Pogge. 1974b. *Viburnum* L. IN: Seeds of Woody Plants, C. Schopmeyer (Technical Coordinator). USDA Forest Service, Agriculture Handbook. No. 450. Pages 844-850.

Giersbach, J. 1937. Germination and seedling production of *Arctostaphylos uva-ursi*. Contributions from Boyce Thompson Institute 9:71-78.

Glazebrook, T.B. 1941. Overcoming delayed germination in the seed of plants valuable for erosion control and wildlife utilization. MSc Thesis, University of Idaho, School of Forestry. 97 pages.

Grisez, T. 1974. *Prunus* L. IN: Seeds of Woody Plants, C. Schopmeyer (Technical Coordinator). USDA Forest Service, Agriculture Handbook. No. 450. Pages 658-673.

Haeussler, S. and D. Coates. 1986. Autecological characteristics of selected species that compete with conifers in British Columbia: a literature review. BC Ministry of Forests, Vancouver, British Columbia. 180 pages.

Hall, I. and C. Beil. 1970. Seed germination, pollination, and growth of *Vaccinium vitis-idaea* var. *minus* Lodd. Canadian Journal of Plant Science 50:731-732.

Hall, I. and J. Shay. 1981. *Vaccinium vitis-idaea* L. var. *minus* Lodd. Supplementary Account. Canadian Field-Naturalist 95(4):434-464.

Hardy BBT Ltd. 1989. Manual of plant species suitability for reclamation in Alberta -- 2nd Edition. Alberta Land Conservation and Reclamation Council Report #RRTAC 89-4. 436 pages.

Harrington, C., M. McGrath and J. Kraft. 1999. Propagating native species: experience at the Wind River Nursery. Western Journal of Applied Forestry 14(2):61-64.

Hartman, H.T. and D.E. Kester. 1983. Plant Propagation: Principles and Practices (4th Edition). Prentice Hall Inc, Englewood Cliffs, NJ.

Heit, C.E. 1967. Propagation from seed, Part 6: Hard seededness—a critical factor. American Nurseryman 125(12): 5.

Hong, T.D., S. Linington and R.H. Ellis. 1998. Compendium of information on seed storage behavior. Royal Botanic Gardens, Kew. 400 pp.

Hudson, S. and M. Carlson. 1998. Propagation of interior BC native plants from seed. British Columbia Ministry of Forests, Research Program. Victoria, British Columbia. 30 pages.

International Taxonomic Information System. No date. Available at: http://ITIS.gov

Junttila, O. 1970. Effects of Stratification, Gibberellic acid and germination temperature on the germination of *Betula nana*. Physiologia plantarium 23:425-433.

Kaur, J., A.L. Schoonmaker and J-M Sobze. 2016. Length of cold stratification period affects in green alder (*Alnus viridis* (Chaix) DC. Subsp. *crispa* (Aiton) Turrill) seed collected from northwestern Alberta. Native Plants Journal 17(2): 95-102.

King, P.J., 1980. Review of seed pretreatments required for germination of candidate native tree and shrub species in the Eastern slopes of the Rocky Mountains and foothills of Alberta. Alberta Energy and Natural Resources, Alberta Forest Service, Edmonton, Alberta. ENR Report Number 154. 56 pp.

King, P., G. Grainger and A. Straka. 1983. Testing of seed pre-germination treatments for selected native shrub species. Alberta Energy and Natural Resources, ENR Report No T/43. Edmonton, AB. 80 pages.

Kreftling, D. and E. Roe. 1949. The role of some birds and mammals in seed germination. Ecological Monographs 19(3):271-286.

Laidlaw, T. 1987. Drastic temperature fluctuation - the key to efficient germination of Pin cherry. Tree Planters' Notes 30-32.

Lehmushovi, A. 1975. Methods of propagating the cowberry. Annales Agriculturae Fenniae 14:325-333.

Light, M.E., J. Van Stade and C.H. Bornman. 2004. He potential of smoke in seed technology. South African Journal of Botany 97(1):97-101.

Lockley, G.C. 1980. Germination of chokecherry (*Prunus virginiana*) seeds. Seed Science and Technology 8:237-244.

Lohmiller, R.G. Plant Materials Specialist, Soil Conservation Service, Bozeman Montana. Personal communication, cited by King (1980)

Luna, T. 2008. Propagation protocol for vegetation production of container *Viburnum edule* (Michx.) Raf. plants (800ml containers); Glacier National Park, West Glacier, Montana. IN: Native Plant Network, University of Idaho, College of Natural Resources, Forest Research Nursery, Moscow,

Idaho. http://npn.rngr.net/renderNPNProtocolDetails?selectedProtocolIds=caprifoliaceae-viburnum-56

Luna, T. and D. Wick. 2008. Propagation protocol for production of container *Shepherdia canadensis* Nutt. plants (160mL containers). Available at: http://www.nativeplantnetwork.org/Network/ViewProtocols.aspx?ProtocolID=311

Luna, T., J. Evans and D. Wick. 2008. Propagation protocol for production of container (plug) *Arctostaphylos uva-ursi* (L.) Spreng plants 172 mL container; USDI NPS - Glacier National Park West Glacier, Montana. In: Native Plant Network, Propagation Protocol Database

Mallik, A.U. and C.H. Grimingham. 1985. Ecological effects of heather burning: II. Effects on seed germination and vegetative regeneration. Journal of Ecology 73:633-644.

Mandel, R.H. and C. Gibson. 2011. Suncor wetland seed collection – nursery protocols. Prepared for Suncor Energy and C. Daly. Prepared by Golder Associates Ltd. 7 pages.

Marchant, C. and J. Sherlock. 1984. A guide to selection and propagation of some native woody species for land rehabilitation in British Columbia. BC Ministry of Forest Research. Report RR84007-HQ. 117 pages

Marks, P.L. 1974. The role of pin cherry (*Prunus pensylvanica* L.) in the maintenance of stability in northern hardwood ecosystems. Ecological Monographs 44(1):73-88.

McDonough, W.T. 1979. Quaking aspen – Seed germination and early seedling growth. Intermountain Forest and Range Experiment Station, Forest Service, USDA. Ogden, UT. 13 pages.

McKechnie, I. 2009. Propagation methods and the effectiveness of fungal inoculation on *Vaccinium* species native to central British Columbia. MSc Thesis. University of Northern British Columbia, Biology, Natural Resources and Environmental Studies. Prince George, BC. 117 pages.

McKeever, D.G. 1938. The effects of various methods of treatment on the germination of seeds of some plants valuable for game and erosion purposes. MSc thesis (unpublished) University of Idaho, School of Forestry. Moscow, Idaho. 132 pages.

McLean, A. 1967. Germination of forest range species from southern British Columbia. Journal of Range Management 20:321-322.

McTavish, B. and T. Shopik. 1983. Propagation and use of native woody plants in northern latitudes. IN: Reclamation of lands disturbed by mining. Proceedings of the Seventh Annual British Columbia Mine Reclamation Symposium. Technical Research Committee on Reclamation, Mining Association of British Columbia, Victoria. pp.159-181.

Meshinev, T. 1973. The effect of light on the germination of *Potentilla fruticosa* L. seeds. Biologie botanique 26(5):691-693

Meyer, G. and M. Witmer. 1998. Influence of seed processing by frugivorous birds on germination success of three north American shrubs. American Midland Naturalist 140:129-139.

Milstein, D. and G. Milstein. 1976. Water, Light & Love: A guide to Growing Plants from Seeds. Applewood Seed Company. Lakewood, Colorado. 96 pages.

Moore, N., D. Ross and P. Hunt. 2004. Propagation protocol for production of container (plug) *Viburnum edule* (Michx.) Raf. Plants. Alaska Plant Materials Center, Palmer, Alaska. IN Native Plant Network

Mytty, M. 2003. Plant Data Sheet. Available at: http://depts.washington.edu/propplnt/Plants/Lonicera%20involucrata.htm

Nichols, G. 1934. The influence of exposure to winter temperatures upon seed germination in various native American plants.

OSRIN (Oil Sands Research and Information Network). 2013 Future of Shrubs in Oil Sands Reclamation Workshop. Oil Sands Research and Information Network, University of Alberta, School of Energy and the Environment, Edmonton, Alberta. OSRIN Report No. TR-43. 71 pp. https://era.library.ualberta.ca/collections/44558s16n

Palamarek, D. 2011. Reference Seedlot Testing. Unpublished.

Palamarek, D. 2016. ATISC – Provincial Seed Officer. Personal Communication.

Pfieffer, N.E. 1934. Morphology of the seed of *Symphoricarpos racemosus* and the relation to fungal invasion of the coat to germination capacity. Contributions to the Boyce Thompson Institute 6:103-122. Cited by Flemion (1942).

Porter, R.H., M. Durrell and H.J. Romm. 1947. The use of 2,3,5-triphenyl—tetrazolium chloride as a measure of seed germinability. Plant physiology 22(2): 149-159.

Prairie Moon Nursery. No date. Prairie Moon Nursery website. Available at: https://www.prairiemoon.com/plants/bare-root/trees-shrubs-vines/potentilla-fruticosa-bush-cinquefoil.html

Ripa, A. 1993. Introduction of the cowberry (*Vaccinium vitis-idaea*) into cultivation. Aquilo: Ser. Botanica 31:55-58.

Remlinger, B. and R.G. St-Pierre. 1995. Biology and culture of the buffaloberry. Department of Horticulture Sciences, University of Saskatchewan. Saskatoon, SK. 22 pages.

Robb, L.A. 2016. Seed Specialist, Alberta Tree Improvement and Seed Centre. Personal communication.

Rogers, L. and R. Applegate. 1983. Dispersal of fruit seeds by black bears. Journal of Mammalogy 64(2):310-311.

Rose, R., C.E.C. Chachulski and D.L. Haase, 1998. Propagation of Pacific northwest native plants. Oregon State University Press, Corvallis, Oregon. 248 pp.

Rosner, L. and J. Harrington. 2002. Propagation protocol for production of container *Shepherdia canadensis* (L.) Nutt. plants (164mL container). Available at: http://www.nativeplantnetwork.org/Network/ViewProtocols.aspx?ProtocolID=2345

RBG Kew (Royal Botanic Gardens - Kew). No date. Seed Information Database. Webpage available at http://data.kew.org/sid/

Sarvas, R. 1950. Effect of light on the germination of forest tree seeds. Oikos 2:109-119.

Schalin, I. 1967. Germination analysis of *Alnus incana* (L.) Moench and *Alnus glutinosa* (L.) Gaertn. Seeds. Oikos 18(2):253-260.

Schopmeyer, C. 1974a. Seeds of Woody Plants. USDA Forest Service, Agriculture Handbook. No. 450. 6883 pp.

Schopmeyer, C. 1974b. *Alnus* B. Ehrh. IN: Seeds of Woody Plants, C. Schopmeyer (Technical Coordinator). USDA Forest Service, Agriculture Handbook. No. 450. Pages 206-211.

Schreiner, E.J. 1974. *Populus* L. IN: Seeds of Woody Plants, C. Schopmeyer (Technical Coordinator). USDA Forest Service, Agriculture Handbook. No. 450. Pages 645-655.

Sen Gupta, J.N. 1937. Seed weight, plant percents, etc. for forest plants of India. Indian Forest Records Indian Forest Rec. (NS) Silviculture 2(5): 1-221.

Shafii, B. and D.L. Barney. 2001. Drying and cold storage affect germination of huckleberry seeds. HortScience 36(1): 145-147.

Shaw, N. 1984. Producing bareroot seedlings of native shrubs. In: Murphy, P. M., compiler. The challenge of producing native plants for the Intermountain area: Proceedings, Intermountain Nurseryman's Association conference. Gen. Tech. Rep. INT-168. Ogden, UT: USDA, Forest Service, Intermountain Forest and Range Experiment Station: 6-15.

Shoemaker, J.S. and P.D. Hargrave. 1936. Propagating trees and shrubs from seed. Department of Extension, University of Alberta. Circular 21, 22 pp. Cited in King (1980).

Simonson, G. 1976. Seed technology study. Revegetation Research Progress report 1975. Alberta Oil Sands Environmental Research Program. 350 pp. Cited in King (1980).

Simpson, J.D. 2016. National Tree Seed Centre – Annual Report 2015. National Tree Seed Centre, National Resources Canada, Canadian Forest Service. Fredrickton, N.B. 17 pages.

Simpson, J.D. 2015. National Tree Seed Centre – Annual Report 2014. National Tree Seed Centre, National Resources Canada, Canadian Forest Service. Fredrickton, N.B. 16 pages.

Smreciu, A. and K. Gould. 2009. Priority shrub species: propagation and establishment. Prepared for Cumulative Effects Management Association. 50 pp.

Smreciu, A. and K. Gould. 2015. Field emergence of native boreal forest species on reclaimed sites in northeastern Alberta. Native Plants Journal 16(3): 204-226.

Smreciu, A., M. Pahl, K. Gould and M. Fung. 2008. Native Plants for Revegetation: Propagation and Establishment: Final report. Prepared for Syncrude Canada Inc. 26 pages + appendix

St-Pierre, R. 1993. The chokecherry: a guide for growers. Department of Horticulture Sciences, University of Saskatchewan. Saskatoon, SK. 30 pages.

Stark, N. 1966. Review of highway planting information appropriate to Nevada. University of Nevada Bulletin B-7. Reno, Nevada 208 pp. Cited by King (1980).

Steneker, G.A. 1976. Guide to the silvercultural management of trembling aspen in the prairie provinces. Canadian Forest Service. INF. REP. Nor-X-164. IN: Haeussler, S. and D. Coates. 1986. Autecological characteristics of selected species that compete with conifers in British Columbia: a literature review. BC Ministry of Forests, Vancouver, British Columbia. 180 pages.

Stidham, N.D., R.M. Ahring, J. Powell and P.L. Claypool. 1980. Chemical scarification, moist prechilling and thiourea effects on germination of 18 shrub species. Journal of Range Management 33(2): 115-118.

Swingle, C.F. 1939. Seed propagation of trees, shrubs and forbs for conservation planting. Prepared for USDA Soil Conservation Service, Washington, D.C. 212 pages

Taylor, S. 1977. The genus Viburnum in British Columbia. Davisonia Volume 8.

Thilenius, J., K. Evans and E. Garrett. 1974. *Shepherdia* Nutt. Buffaloberry. IN: Seeds of Woody Plants, C. Schopmeyer (Technical Coordinator). USDA Forest Service, Agriculture Handbook. No. 450. Pages 771-773.

Trindle, J.D.C. and T.R. Flessner. 2003. Propagation protocol for production of container *Lonicera involucrata* Banks ex Spreng. plants (1-gallon containers); USDA NRCS - Corvallis Plant Materials Center, Corvallis, Oregon. In: Native Plant Network. URL: http://www.nativeplantnetwork.org (accessed 6 January 2010). Moscow (ID): University of Idaho, College of Natural Resources, Forest Research Nursery.

Soil Conservation Service. 1938. (USDA) Data region 1, 2,3,4,5,6,7,8,9,10, 11. Cited in Swingle (1939).

van der Kloet, S.P. and I.V. Hall. 1981. The Biological Flora of Canada. 2. *Vaccinium myrtilloides* Michx., velvet-leaf blueberry. Canadian Field-Naturalist 95(3): 329-345.

Van der Kloet, S.P. and N.M. Hill. 1994. The paradox of berry production in temperate species of *Vaccinium*. Canadian Journal of Botany 72:52-58.

Viereck, L.A. and L.A. Schandelmeier. 1980. Effects of fire in Alaska and adjacent Canada: A literature review. Bureau of Land Management, US Department of the Interior. Anchorage, Alaska. 124 pages.

Vilkitis, J.R., 1974. Cherries. USDA Forest Service, General Technical Report NE 9: 23-25.

Vines, R.A. 1960. Trees, shrubs and woody vines of the Southwest. University of Texas Press, Austin, Texas. 1104 pages.

Vories, K.C. 1981. Growing Colorado plants from seed: A state of the art. Volume I: Shrubs. USDA Forest Service General Technical Report INT-103. 87 pages.

Walsh, D., S. Waldren and J. Martin. 2003. Monitoring seed viability of fifteen species after storage in the Irish threatened plant genebank. Biology and Environment: Proceedings of the Royal Irish Academy. Vol 103B (2):59-67.

Wang, B.S.P., P.J. Charest and B. Downie. 1994. *Ex situ* storage of seeds, pollen and *in vitro* cultures of perennial woody plant species. FAO Forestry Paper 113. Food and Agriculture Organisation of the United Nations, Rome. (found on Kew)

Wild Rose Consulting, Inc. 2014 Final Report for 2014 – Oil Sands Vegetation Cooperative. Prepared for COSIA Land EPA. 109 pp.

Wood, B. 2014. Head Grower Smoky Lake Forest Nursery. Personal communication.

Young, J. and C. Young. 1986. Collecting, Processing and Germinating seeds of wildland plants. Timber Press. Portland, Oregon. 236 pages. (selected pages scanned)

Young, J. and C. Young. 1992. Seeds of Woody Plants in North America. Dioscorides Press. Portland, Oregon. 407 pages.

Zasada, J.C. and R.A. Densmore. 1977. Changes in seed viability during storage for selected Alaskan *Salicaceae*. Seed Science and Technology 5: 509-518.