Seed Matters 3

Sowing five-needle pines from Alberta seed

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Executive summary

The following are recommendations only, above and beyond the Alberta regulations (Alberta Forest Genetic Resource Management and Conservation Standards, [FGRMS]) and only apply to handling seeds from whitebark pine and limber pine: two species listed provincially as *Endangered* in Alberta. For a copy of the latest FGRMS regulations, please visit Alberta.ca.

- The thick seed coats, which help to prevent moulds from penetrating to the seed inside, require a 48-hour water soak before stratification can begin. Sterilization of seed to prevent mould growth has limited effectiveness and should not be necessary with healthy, vigorous seeds that are sown using the methods described here, combined with accepted greenhouse methods.
- The recommended pretreatment medium is clean International Seed Testing Association (ISTA) approved sand. This provides better surface area water contact for large tree seeds and limits the spread of seed mould and bacteria for improved staff health and safety.
- To sow limber pine seeds: 48-hour water soak, 10-week cold stratification on sand at 1-2°C (to limit pre-germination), and sowing at a 1.5” depth in order to remove the seed coat upon emergence. Emergence should be seen within seven days and complete in 21 days.
- To sow whitebark pine seeds: 48-hour water soak, 12-week warm stratification at 20°C, 16-week cold stratification at 1-2°C (to limit pre-germination), and sowing at a 1.5” depth in order to remove the seed coat upon emergence. Emergence should be seen within seven days and complete in 21 days.
- Size 512A copper polystyrene blocks or similar are recommended for growing larger numbers of seedlings. Both species grow very slowly, but put on roughly twice the root-to-shoot growth in the first season. A shortened season, due to lower light quality in many northern Alberta greenhouses, means that early spring sowing and two seasons are often required for growth to enable lifting and to ensure high survival rates after outplanting.
- Biodiversity loss should be considered before deviating from the prescribed best practice methods for germination and sowing provided in this document. Insufficient planning or time constraints should be weighed against loss of genetic diversity. Losing specific genetic groups, for example lighter or more dormant seed, can hinder genetic disease resistance testing programs and cause domestication, thereby reducing resilience and survival of outplanted seedlings.

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Background

The Alberta Tree Improvement & Seed Centre (ATISC) has run trials designed to obtain better germination and sowing methods for growing Endangered limber and whitebark pine seedlings. This document is a distillation of trials and experience obtained from growing over 25,000 seedlings over the past decade as part of Alberta's Five-Needle Pine Recovery Programs. ATISC staff collect cones and store seed, as well as extract and store seed collected from Alberta's National Parks.

These methods retain the high genetic diversity inherent in these species, while minimizing time and specialized equipment.

For more information on collecting seed and growing five-needle pines in Alberta, please see the other technical documents in the Seed Matters series.

Figure 1: Whitebark pine seeds

Seed

Limber and whitebark pine produce seeds with diverse sizes, shapes, weights and air pockets, meaning it is practically impossible to remove empty seed from seed lots without also removing good seed containing potentially valuable genetics. This diversity exists both amongst populations and within individual tree seed lots.

Therefore, the majority of seed lots will contain approximately 50% empty seeds. It is recommended that any group processing seed for use in disease screening and reclamation projects, does not attempt to remove these empty seeds. These attempts will result in the removal of genetic traits and will not help to maintain resilience and survival on the landscape. Retaining high genetic diversity is a necessary goal when testing for genetically controlled white pine blister rust resistance (*Cronartium ribicola*) and growing seedlings for outplanting in order to maximize resilience in their harsh mountainous habitats.

Please see 'Seed Matters 2: Extracting and Processing 5-needle Pine Seed in Alberta' for further discussion of seed extraction and processing techniques.

Sterilization and imbibition

Testing at ATISC has shown that sterilization is both largely ineffective and unnecessary if appropriate pretreatments are applied before germination, using the methods discussed in this document. Methods such as the use of highly concentrated hydrogen peroxide solutions (30%) are not widely used now, nor are they considered effective for general seed sterilization. The sterilizing properties are not strong enough and peroxide does not penetrate seed coats well, which is why it was historically chosen to treat thin-coated species, such as birch and alder seed.

Using newly collected whitebark pine seed from various populations and a widely accepted bleach sterilization method (10% bleach solution for five minutes with rinsing), there was very little to no difference in the quantity of seed mould growth inside or outside the seed coat when compared to untreated seed. As well, no difference was found in seedling survival after sowing. These seeds have evolved thick, dense seed coats that prevent most moulds from reaching the healthy seed tissue inside. Repeated cut tests have shown that the proportion of whitebark or limber pine seeds that were mouldy inside after germination treatments on sand medium was the same as those that were mouldy before stratification(s) started. In other words, any mould was already inside the seed before treatment began.

Due to the physiological structure of their seed coat, these seeds imbibe water very slowly but, they are not classed as 'physically dormant', so they do not have impermeable seed coats. Full imbibition occurs around 48 hours after soaking in water. Because stratification reactions will not begin until the seed is fully imbibed, it is best to do this soak prior to beginning any stratification treatments. Seeds can be soaked in clean tap, distilled or deionized water. No aeration or running water is required as long as the duration is no more than 48 hours.

Stratification medium

Warm or cold stratification should be done in trays of clean, washed, medium-grain sand. Sterilized sand is unnecessary. Follow International Seed Testing Association guidelines (ISTA) when using sand as a medium (ISTA 2019). Sand stratifies large tree seeds (>5mm) more effectively than paper or agar due to the increased surface area contact with water (personal comm. J. Adams, Royal Botanic Gardens, Kew 2008) and it will also inhibit the spread of mould amongst the seeds (Figure 2). A sand depth of ten millimetres is sufficient in the container. Very little water is required when using sand for large tree seeds (10ml per 50g sand). However, since these are relatively large seeds, it is difficult to 'drown' them by using too much water. Therefore, it is simpler to use a rule of thumb instead of measuring exact amounts. Use only as much water as will not pour off when the container is tipped (Figure 3). Containers with tight fitting lids or sealable bags will help prevent evaporation during the long pretreatment period.

Press the seeds lightly into the sand to ensure they do not roll during transport. If sand is not available, Kimpak (cellulose wadding) can be used. However, as this is a food medium, there will be increased mould growth and spread (Figure 2). ATISC has not tried the ‘peat mix in a bag’ method, but this may produce other moulds, especially during the warm stratification period required by whitebark pine seeds and this medium may provide inconsistent moisture and oxygen if not done carefully. For sowing large numbers of these species, ATISC recommends using sand. This process for sowing is quick and the sand is relatively cheap.

Figure 2: Limber pine seeds on the left grown on Kimpak (cellulose wadding) showing fungal and bacteria growth and spread. Limber pine seeds on the right grown on ISTA-quality approved clean (not sterile) sand showing some mould growth but little spread. Most of the mouldy seeds are unharmed inside and will produce quality seedlings.

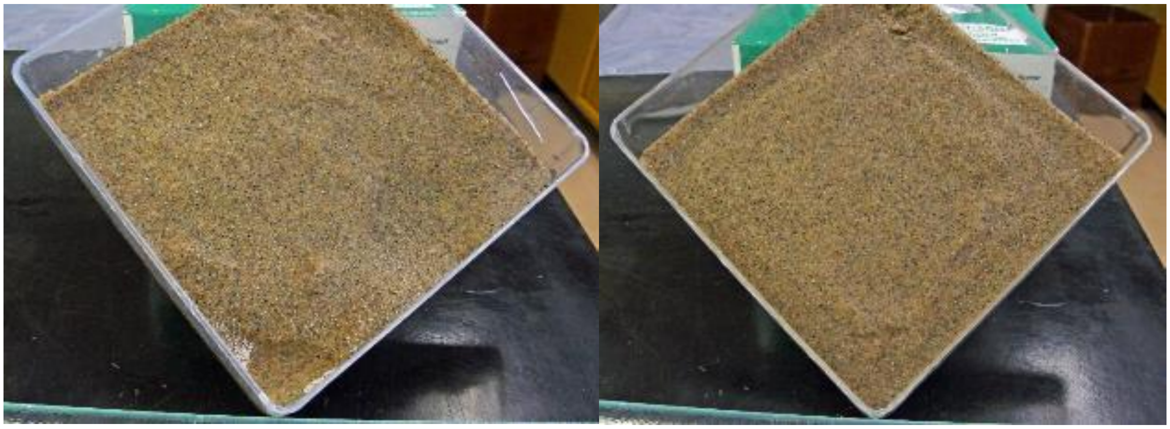

Figure 3: ISTA-approved clean sand medium showing too much water on left and correct water amount on right. When the correct amount of water is added, particles of sand should have the capacity to hold sufficient water to provide continuous movement of water to the seeds, but also provide sufficient pore space for aeration.

Stratification treatments

Seed lots from multiple provenances spread across the species' ranges in Alberta have been fully tested and analyzed in order to establish one method that works for all seed lots. As discussed, these species possess a large amount of genetic diversity and the following methods have proven to be highly effective on seeds collected from across Alberta.

There is a wide spread of dormancy variation within any given seed lot for both these species. Further testing and experience has shown that a small portion of any seed lot (usually 1-5%) may complete stratification early and germinate before sowing. In 10 years, ATISC has encountered only one genetically abnormal whitebark pine seed lot that contained 18% non-dormant seeds that germinated during warm stratification. The warm stratification for whitebark pine may be done at various temperatures from 10-25°C. However, 20°C was chosen to minimize the time required and minimize mould growth for health and safety. Changing the temperature for warm stratification may require a change to the time necessary to complete the chemical process.

The pregerminant numbers can become more problematic if the cold stratification temperatures are above 2°C. Since stratification occurs at 0-5°C, early sprouting can be minimized by simply using a colder stratification temperature of ~2°C. This is achieved by using a modern refrigerator, adjusting the cold setting and checking with a thermometer. For further discussion on dealing with pregerminants, refer to the 'Germination considerations' section .

Both species also have relatively large germination temperature ranges. Germination testing at 15°C and 25°C showed no difference in the final germination percentage in either species but the lower temperature did slow germination by 2-4 days.

The methods described below facilitate fast germination or emergence, maximum seedling numbers, and evenly aged seedlings for easier greenhouse care. Emergence should be seen within seven days after sowing and complete by 21 days.

Limber Pine (Robb 2012)

- 48-hour water soak
- Cold stratification on sand – 10 weeks (1-5°C but 2°C is recommended)
- Sow 1.5" deep in cavities and cover with grit

Whitebark Pine (Robb 2014)

- 48-hour water soak
- Warm stratification on sand – 12 weeks at 20°C
- Cold stratification on sand – 16 weeks (1-5°C but 2°C is recommended)
- Sow 1.5" deep in cavities and cover with grit

Greenhouse trays/blocks

A shortened season due to lower light quality means that in many northerly Alberta greenhouses, conifer tree growth will stop in September without special supplemental lighting. Both species grow very slowly, but put on roughly twice the root-to-shoot growth in the first season. This shortened season means that in most Alberta greenhouses, an early spring sowing and two seasons of growth will be required to enable lifting. In general, larger seedlings with more roots help to ensure survival after planting, especially in harsh habitats.

One method of ensuring the highest quality seedlings requires the use of a dormancy conditioner or environmentally controlled room. A cycling method can be used to ‘trick’ the plants into a new flushing period by giving them fake winter conditions. In this way, ATISC puts two additional flushes on our seedlings during the winter months, meaning the seedlings are only two summers old, but have nearly four seasons of growth on them. However, this option is too expensive for most private nurseries and making early plans for October dormancy breaking treatments and an early spring sowing is much easier.

ATISC has trialed greenhouse sowing in Root Makers, individual yellow tubes, and two copper style blocks – 415D and 512A – and because of the increased root growth, the 512A size is preferred. There were no differences between the two types of copper blocks during the first season. However, in the months before the seedlings were due for lifting and outplanting, the seedlings in the 415D cavities became root bound, causing watering issues. Using the larger width cavities also allows for sowing of multiple seeds and no thinning, which is more efficient for these seed lots where the number of empty seeds may be high. For these reasons, ATISC recommends using 512A copper blocks for growing five-needle pines in central and northern Alberta. We also highly recommend a two-year seedling for increased transplant survival into their harsh mountain habitats.

Seed owners wanting to pull or transplant seedlings in their first year might trial using smaller cavity sizes, but should understand the survival risks after planting and monitor if possible. These seedlings grow extremely slowly and the trees will typically not start to produce cones until around 50 years of age. Winter grafting has been very successful at ATISC (99%), with rootstock being cross-compatible between the species. Grafted limber and whitebark pine trees can begin producing cones after only three years.

Sowing

At ATISC, seeds are sown into a two parts peat moss to one part Perlite mix using large forceps to a 1.5” depth, then covered with grit to reduce moisture loss and fungus gnats. Ignoring the 1.5” sowing depth and sowing seeds on the surface will result in higher seedling deaths and increased staff labour. These five-needle pine tree seeds are adapted for being sown at 1.5” depth by their natural primary disperser, Clark’s Nutcrackers (*Nucifraga colombiana*).

This method has been tested at ATISC and is recommended for good reason. If the seeds are sown too shallow, most seedlings will not be able to slough off the hard seed coat regardless of humidity conditions. This will eventually kill them within the first few months or require numerous staff hours carefully sliding coats off the young seedlings, which will invariably kill a small portion (Figure 4).

The depth of seeding and the depth of the cavity are not related. Both species will form a 1” collar below the soil surface where no fibrous roots develop (Figure 5), regardless of sowing depth. The root ball will grow at the same depth whether sown on the surface or at 1.5”.

Figure 4: Whitebark pine seedlings showing seeds sown on the surface and covered with grit as per regular tree growing practices in Alberta. Very few seedlings will shed the seed coat if still attached. The blue circle shows a seedling that has had the seed coat slipped off by hand but the inner coat (endocarp) is still trapping the cotyledons and will eventually kill the seedling.

Figure 5: Two whitebark pine seedlings from the same seed lot early in their second season of growth. Seedling on the left was grown in a 512A copper block and the right was grown in a 415D copper block.

Germination considerations

As discussed, within Alberta the seed dormancy is high in these two species and the required stratification treatments are long but do not require intensive labour. Unlike other germination methods that claim to speed up germination, for example using acid, rock tumblers or seed chipping, stratification does not destroy or damage seeds.

There is a small but significant portion of any seed lot that may germinate during the cold stratification treatment if temperatures above 2°C are used. The problems associated with choosing to stratify at higher temperatures are threefold. Firstly, these seeds are germinating at the very low end of their preferred temperature range and any germinants usually produce abnormal roots. Secondly, any healthy germinating seedlings can produce large tap roots before detection during treatments, making transplant difficult or they often die before discovery. Finally, there are often not enough seedlings to fill a single block and make transplanting economical.

However, before discarding these few seedlings as too difficult or expensive to manage, seed owners should consider the cost of the seed loss and whether it is worth the risk. This lost portion of the seed lot contains genetics that may be linked to low dormancy. By discarding non-dormant or shallowly dormant seed, genetics required for improved competitiveness, blister rust resistance or drought tolerance could be lost. These types of genetic links (pleiotropy, linkage disequilibrium, etc.) are common in plants, but they are often not intuitive and are not well understood for these two species. Seed owners should consider these implications and discuss their requirements with greenhouse growers before a project begins.

For all these reasons, if a greenhouse facility would like to produce these seedlings in significant numbers in the future, it is worth a small investment in a modern refrigerator that can achieve 2°C in order to minimize the loss of seedlings and genetics.

For more information, please contact the Alberta Provincial Seed Specialist at the Alberta Tree Improvement & Seed Centre:

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