Seed Matters 7

Lodgepole pine cone classes and impacts on seed quality

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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 lodgepole pine cone collection and seed lots. For a copy of the latest FGRMS regulations, please visit Alberta.ca.

- For general reforestation purposes, only Class 1 cones are recommended for collection.
- If circumstances dictate the collection of Class 2 cones, it is recommended that these are extracted separately from Class 1, as a distinct seed lot to be used first.
- For seed lots that have special conservation or research purposes or where there are not yet any plans for use or use of the seed lot is expected to surpass 10 to 15 years, it is recommended that seed owners only accept cone Class 1.
- While seed germination was significantly higher in Class 1 cone seeds compared to Class 3, a fresh germination test in itself does not give a full indication of overall seed lot quality.
- There was a significant decline in potential usable lifespan in storage among Classes 1, 2 and 3. Lifespans of seed from Classes 2 and 3 declined by 61% and 87%, respectively.
- Given the high costs of cone collection, this increased use time should already be highly attractive to seed owners. The implication of these results in this study is that it is feasible that by harvesting older cone Classes, a seed owner could be reduced to only 10 to 20 years of use out of a lodgepole pine seed lot.

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Background

Lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) normally produces serotinous cones in Alberta, meaning that they have cones that are held closed on the tree at maturation. These cones will open in the heat of a fire event, which breaks the resin binding the scales, allowing the seeds to fall out later. Without fire, the bonds will also break eventually over time, usually after more than ten years. The cones are medium-to-light brown in colour when 'new' and, as they age and weather on the tree, they gain more grey patches, eventually becoming completely grey over time. It is difficult to tell the exact age of the cones, as the weathering will differ depending on the local climate and position on the tree.

Because the cones can be held on the tree for long periods of time, cone harvesters have the ability to collect cones from many different years of cone production. Many seed owners will contract a company to collect wild cones from Classes 1 and 2 of the four cone classifications used in forestry in British Columbia and familiar to foresters in Alberta (Eremko *et al* 1989). However, cone collectors often use discretion to choose to include Class 3 cones to fill a volume requirement in a contract. The cone Classes and approximate ages in the wild, based on experience (J. Quinn, RPF, personal communication 2013), are as follows:

- Class 1 cones are brown, bronze or gold coloured on all faces and tightly closed. They are this year's cones or up to approximately three years old.
- Class 2 cones are partially weathered (up to 66%), but closed. The cones are brown, bronze or gold on one face and grey on other faces. The cones are approximately two to seven years old.
- Class 3 cones are closed and mostly or all grey (weathered) on all faces (67-100%). The cone age is estimated at over five years old.
- Class 4 cones are partially open, fully open or damaged cones.

The reason for rejecting Class 3 cones is an assumption that the seeds may be of poorer quality due to their age, based on germination test results. Seeds age and die over time and, since the cones are held on the tree, the speed at which this happens is unpredictable in these wild conditions where moisture and temperature - two factors that directly affect seed ageing - are highly variable.

The seed viability monitoring program at the Alberta Tree Improvement & Seed Centre (ATISC) has shown that high quality lodgepole pine seed lots in controlled long-term cold storage can have extremely long lifespans if collection and handling are optimal (Robb 2018). The potential use of high quality lodgepole seed is over 100 years when handling is ideal and the seeds are stored at accepted seed banking conditions of 15-40% eRH (equilibrium relative humidity) and -18°C. This inherent lifespan differs between species. In comparison, cold stored aspen seed can only be used for about two to five years. In Alberta, the use time of any seed lot varies directly due to the initial seed quality at collection, handling practices after collection and seed moisture during cold storage.

This trial was conducted by ATISC to identify any large differences in seed quality between 'newer' (Class 1) and 'older' lodgepole pine cones (Classes 2 and 3). This included germination tests on fresh seed and also estimates of storage times and use for seeding by performing artificial ageing tests. The artificial ageing test, also called a 'comparative longevity test', enables comparison between seed lots and ranking of seed lot longevity. Although it cannot be directly converted to exactly how long a seed lot will survive in cold storage since the conditions vary, it can be used to compare to other seed lots with known storage lifespans.

These results may be used to support the alteration of lodgepole pine cone collection practices in the future and help to ensure higher quality seed lots going forward, resulting in longer use times and lower costs to industry.

Seed lots

The bulk wild harvested cones used in the trial were from Sibbald Meadows (+51.04946, -114.9006), collected in January 2013. Thirty cones were randomly chosen from each of Classes 1, 2 and 3. All the cones included in Class 2 were estimated at 50-66% weathered/grey (Figure 1). Seeds were extracted from the cones at ATISC in the usual manner for small extractions, namely a brief 30 second dip in 80°C water to break the resin seals and then kilned at a ramping temperature for eight hours that peaks at 60°C. This wet/dry combination flexes the cone scales open, enabling the seed to fall out in a tumbler. The seeds were then dewinged and cleaned using a seed blower, making efforts to severely limit any discarded filled seed.

Figure 1: Three cone Classes used in the trial. (from left to right) Class 1 – closed and 100% brown. Class 2 – closed and 50-66% grey. Class 3 – 100% grey.

Method

There was a large variation in the available seed amounts obtained from the 30 cones from each Class, which affected the germination test sizes. The initial germination test on fresh seed (day 0) was conducted on each cone Class, using 200 seeds for Class 1 (in four replicates), 100 seeds for Class 2 (in four replicates), and 50 seeds for Class 3 (in two replicates).

All germination tests were set up in Petawawa boxes on moist cellulose wadding (Kimpac) according to Alberta Seed Testing Standards and then cold stratified at +2°C for 21 days. Once stratified, seeds were germinated at 25°C with a 12/12 light regime. Germinated seeds were counted and removed every seven days. The tests were ended after 21 days in germination conditions and any remaining seeds were cut tested to identify empty seeds.

To detect differences in storage lifespans, artificial ageing tests were conducted following a standard protocol for wild seeds (Newton *et al* 2009) by rehydrating seeds for two weeks at 47% RH and 20°C and then ageing at 60% RH and 45°C. A sample of 50 seeds was taken from each cone Class at intervals of 1, 2, 5, 9, 20, 30, 50, 75, 100 and 125 days under these ageing conditions. Standard single replicate germination tests were conducted on each 50 seed sample following the above procedures. In this way, eleven data points (including day 0 fresh) were obtained for each Class of cones over time.

To enable analysis and comparison, empty seed numbers were removed from any statistical calculations. The results from the initial germination tests were compared among the three cone Classes using chi^2 statistical tests. Longevity results were analyzed using probit analyses. All statistical analyses were performed using Genstat 12.0.

Results

There was a small yet significant difference in fresh seed viability between Class 1 cones (98%) and Class 3 cones (88%). However, seed from all three Classes displayed high, acceptable levels of germination for using seed to produce reforestation seedlings (Figure 2).

In all three cone Classes, seed viability significantly declined with increasing age (Figure 3), showing a large difference in p85 values (time for viability to fall to 85%) among the three Classes (Table 1). The longevity of the seed declined by 61% between Classes 1 and 2 and declined by 87% between Classes 1 and 3.

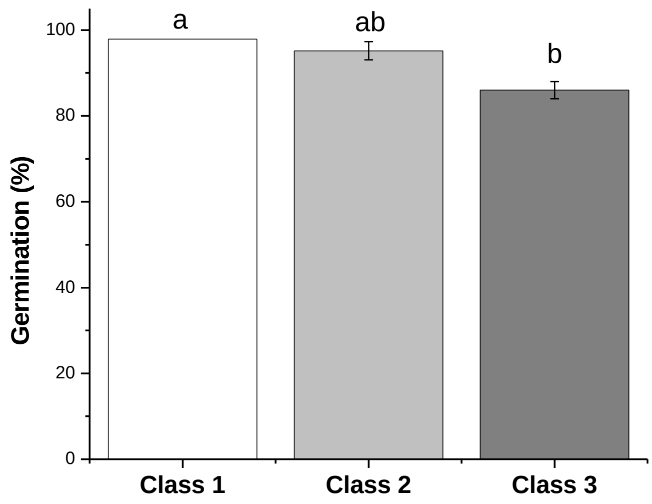

Figure 2: Initial germination (± S.E.) of seeds from cone Classes 1, 2 and 3. Different letter designations indicate significantly different groups at p<0.05.

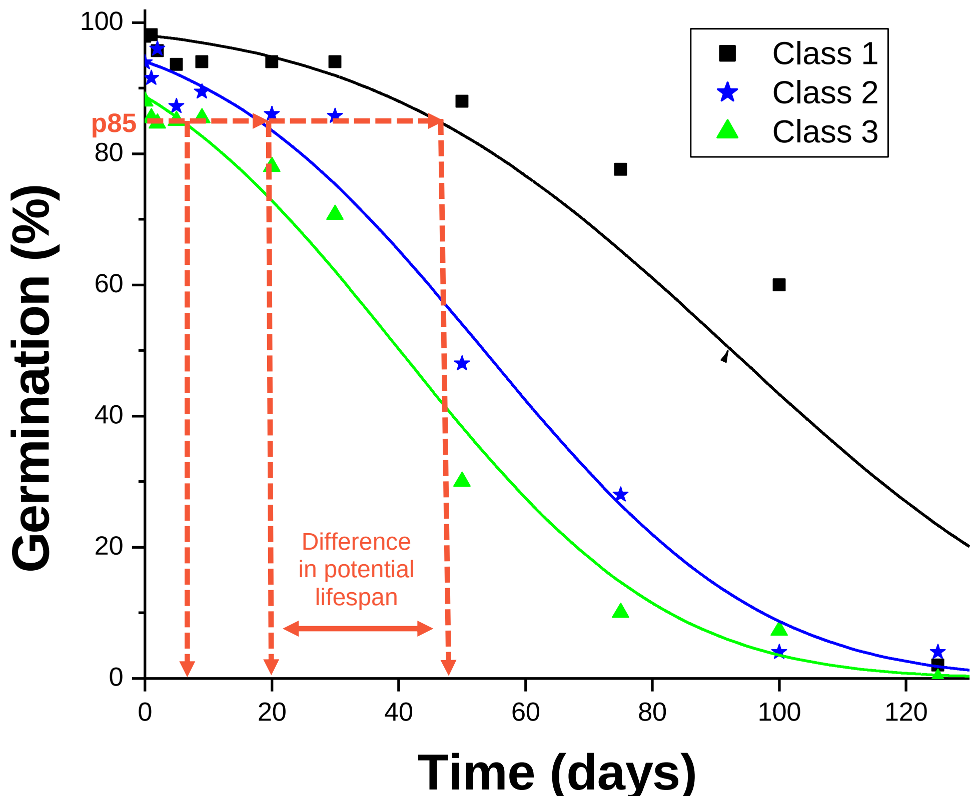

Figure 3: Survival curves fitted by probit analyses for each of the three cone classes tested over time under ageing conditions. Each point is the result of a germination test conducted on seed that is artificially aged at high humidity and temperature. The p85 dashed lines are an indication of the useable lifespan of the cone Classes. There is a 61% loss of lifespan between seeds from Class 1 and Class 2 cones.

Table 1: The p85 value (time to 85% germination) for each cone Class under artificial ageing conditions. There is a 61.2% loss and an 87.4% loss of potential storage lifespan between Class 1 and Classes 2 and 3 respectively.

Cone Class	P85 value (days)	% loss of possible lifespan
1	46.2	n/a
2	17.9	61.2
3	5.8	87.4

Conclusions

Results showed that there was little difference in the viability of fresh seed between the cone Classes. This supports past testing results at ATISC. However, basing an evaluation on just the fresh seed germination results, all 88% and above, an assumption could (and has in the past) be made that all three seed lots are high quality and relatively equal.

The rapid decline in germination once ageing tests began, indicates that even just after harvest the two older Classes were already beginning to age. They would have been experiencing a drop in vigour, which precedes a loss of germination and can make it difficult to propagate healthy trees. The Class 3 cone seeds had already passed the point of low vigour and were showing a decline in viability at harvest (Figure 2).

The p85 value used in the analysis indicates the time required for viability to fall to 85% germination under artificial ageing conditions. Since the ageing is artificial, the value is measured in days not years, and the numbers are estimations of the effects of storage on a seed lot. This allows comparisons between any seed lots that undergo the same longevity testing. Any viability point may be chosen for the statistic but 85% was established as being important to seed owners because viability below this level can have negative impacts on seeding operation costs by tree nurseries. It is also the global standard set for wild seed, to avoid the risk of excessive genetic erosion during storage, which affects plant resilience and survival (FAO 2013).

The difference in storage longevity among the seed lots is important, even for a species with inherently long-lived seeds like lodgepole pine. As noted, properly harvested and handled lodgepole pine seeds in cold storage can last well over 100 years. Seeds from cone Class 1 could be used for cost effective seeding for more than twice as long as seeds from Classes 2 and 3 (Table 1). Given the high costs of cone collection, this increased use time should already be highly attractive to seed owners. The implication of these results in this study is that it is feasible that by harvesting older cone Classes, a seed owner could be reduced to only 10 to 20 years of use out of a lodgepole pine seed lot.

ATISC's viability monitoring program also showed that in some lodgepole pine seed lots the storage time can decline from 90% germination at harvest to 50% within 30 to 50 years (Robb 2018). If the seed lot is already of declining quality at harvest, for example Class 3 cones, then the decline can happen even more quickly. The inherent longevity of a seed lot does not vary within a species (Leprince et al. 2016), and so any differences are due to outside influences that can start with harvest timing, through cone storage conditions and extraction procedures, and finally end with the moisture level when the seeds go into freezer storage. Therefore, it is conceivable that if collectors include the Class 2 or 3 cones, then in addition to the declining viability at harvest, there could also be other factors after harvest that will decrease the viability and longevity even further.

The difference in seed quality between cone ages may not be an issue if an owner knows that the seed will be used quickly; a 10-year turnaround is not uncommon for the majority of operational reforestation seed in Alberta. However, approximately one quarter of lodgepole pine seed still in reforestation use by companies is over 20 years old. By taking small steps to ensure that only new cone Classes are collected, seed owners can avoid the possibility of dying seed and wasted recollection costs.

It is important to understand that these harvest and handling processes affect seed aging in all Alberta native plant seed. Although lodgepole pine is a long-lived seed species that is forgiving of mistakes, other shorter-lived Alberta species such as white spruce, black spruce and aspen will show cause and effect more quickly and therefore, usage plans are highly recommended before collecting huge quantities of any Alberta seed.

Since the ultimate use of long-term conservation or research seed collections is usually unclear, these are especially important factors to consider when collecting seed for this type of use.

Therefore, ATISC recommends that lodgepole pine reforestation collections avoid Classes 2 and 3, especially when the use timeline is unclear. The nature of contracting work and collecting from felled trees in logged populations means that this may not always be practical. A second suggestion is to keep cones from Classes 2 and 3 separate from Class 1 cones, to be extracted separately and used first.

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

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