Seed Testing

Tests are performed on seedlots to quantify their germinability, purity, size, and moisture content. For additional details on the methods and procedures for testing tree seeds in Canada the reader should consult Edwards (1987). A key aspect of good seed testing is proper sampling. The test result for a seedlot is only as good as the sample taken to represent the seedlot. Keywords for sampling are random and representative. Guidelines for sampling, which account for seedlot size, are provided by the International Seed Testing Association (ISTA 1999). Sample sizes used in each of the four tests described apply regardless of seedlot size (see Figure 56).

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A seedlot is required to pass two tests for registration and reforestation use on Crown land in BC—purity and moisture content. The purity of a seedlot is the weight of pure seeds divided by the weight of pure seeds plus debris and is presented on a percentage basis. Debris commonly found in seedlots includes cone fragments, pitch, needles, seed wing remnants, pollen cones, insects, damaged seed, rocks, and other inert matter. The purity of a seedlot must be 97% or better for Crown land reforestation in BC. Exceptions to this rule may occur if it is thought that further processing will adversely affect seedlot quality.

$$\textbf{Purity (\%)} = \frac{\text{pure seed weight}}{\text{pure seed weight + debris weight}} \times 100$$

The moisture content of seeds is calculated on a fresh weight basis[3] as the fresh weight minus the oven dry weight (i.e., weight of water). This result is divided by the fresh weight. Moisture content is commonly expressed on a percentage basis by multiplying this result by 100. The ovendry weight is the weight of seeds with 0% moisture and is obtained by placing the seeds in an oven at 103°C for 17±1 hours and weighing the resulting seeds. Seeds must have moisture content between 4.9 and 9.9% to be registered and stored for use for Crown land reforestation in BC.

$$\textbf{Moisture content (\%)} = \frac{\text{fresh weight} - \text{ovendry weight}}{\text{fresh weight}} \times 100$$

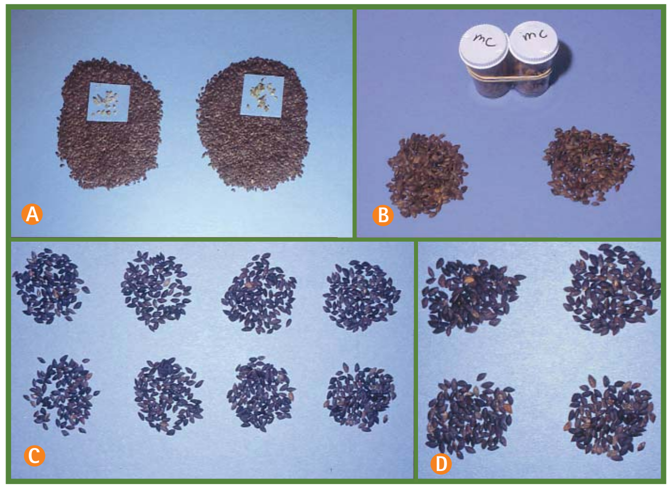

Figure 56 The sample sizes used for seed testing a) purity, b) moisture content, c) seed weight, and d) germination.

Seed size is a general term used to describe the relative size of tree seeds. The weight of 100 or 1000 seeds is the test used to quantify the weight of tree seeds. For BC species the weight of 100 seeds is based on eight 100-seed replicates and varies from 0.13 g for western redcedar to 5.26 g for ponderosa pine. The term seeds per gram (SPG) is the test result that appears on the seed planning and registry (SPAR) system and is used in sowing calculations. The SPG value is a function of seed weight and the purity of a seedlot relative to its storage moisture content.

$$\textbf{Seeds per gram} = \frac{100}{\text{weight per 100}} \times \frac{\text{purity (\%)}}{100}$$

The SPG varies from an average of 19 for ponderosa pine to 792 for western redcedar. Obviously, the seeds that weigh most have the fewest SPG and vice versa. Seed orchard seeds in the Pinaceae are generally larger by approximately 15%, although differences exist between species, families, and years. In the Cupressaceae the difference in seed size between wild and seed orchard seeds appears negligible (Kolotelo 2000).

Seeds must have a purity of 97% and a moisture content between 4.9 and 9.9% for registration and use in Crown land reforestation

[3] The moisture content of **wood** is generally calculated on a dry weight basis (i.e., divided by dry weight versus fresh weight) to indicate the amount of water relative to the solid substance and can be greater than 100%. However, for seeds and many other living plant tissues, the weight of water is relative to the total weight of water + solid substance and lies between 0 and 100%.

The germination test is probably the test that first comes to mind when thinking of seed testing as it has immediate implications for seed quality and estimating the number of seedlings that can be obtained from a quantity of seeds. The germination test is based on four replicates of 100 seeds that have been treated with a specific protocol considered optimum, on average, for the species. The main features of a seed pretreatment protocol are the soak duration and the cold stratification duration. These are presented in Table 5 for conifers planted in BC. Cold stratification is thought to be most effective at temperatures between +2 and +5°C.

In a few species two protocols are initially used for germination testing, and the one producing the highest germination capacity is recommended for operational pretreatment and subsequent retesting. In particular, for lodgepole pine and the spruces, a soak only and a soak + stratification protocol are used as initial tests. The stratification treatment generally produces the higher germination capacity and rate, but problems with poor overall quality, mechanical damage, or fungi may result in the soak-only treatment being superior. ISTA recommends[4] double testing with a dry and a soak + stratify test, but this comparison confounds the effect of soaking and stratifying. Sowing dry seeds in the nursery is not recommended as the time required to uptake moisture in the seedling cavities will delay germination and probably decrease the uniformity of emergence. This will result in a greater input of energy during germination at the nursery.

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Several species have specific modifications to the testing protocols due to complex dormancy mechanisms. For yellow-cedar, a 28 day period of warm stratification is also required to increase the germination capacity, although chemicals may be able to replace this warm stratification period (Xia and Kermode 2000). For Amabilis fir, subalpine fir, and Noble fir, a method which incorporates a split stratification regime, is generally implemented (Edwards 1986). This protocol includes four weeks of cold stratification at a high moisture content (≈45% = no surface drying of seeds) followed by surface drying the seeds to between 30 to 35% moisture content

4 ISTA (International Seed Testing Association) is a large international body mainly concerned with the testing of agricultural seeds and when reasonable evidence is available indicating that we should deviate from their guidelines we may choose to do so. The AOSA (Association of Official Seed Analysts) guidelines are also used as a reference, but in general these two organizations have similar guidelines.

Table 5 The germination test codes, associated species, and testing protocols employed at the BC Ministry of Forests Tree Seed Centre

Code	Species[a]	Soak (hours)	Stratification (days)	Additional protocol components	Germination[b] temp (°C)	Count days
G10	Douglas-fir, spruce, western larch	24	21		30–20	21
G20	Lodgepole pine, ponderosa pine	24	28		30–20	21
G31	Western hemlock, mountain hemlock	24	28		20–20	28
G32	Grand fir	48	28		30–20	28
G44	Amabilis fir, subalpine fir, noble fir	48	56		25–15	28
G52	Yellow-cedar	48	84 (28 warm/ 56 cold)	28 days at 20°C followed by 56 days at 2–5°C	30–20	28
G55	Western white pine	336	98		30–20	28
G64	Amabilis fir, subalpine fir, noble fir	48	84 (split)	28 days at ≈45% moisture followed by 56 days at 30–35% moisture	25–15	28
D1	Western redcedar	0	0		30–20	21
W1	Lodgepole pine, spruce	24	0		30–20	21

a Scientific names, common names, and abbreviations of BC conifer species are presented in Appendix 1.
b Eight hours at higher temperature with illumination and 16 hours at lower temperature in the dark.

Figure 57 The contents of a germination test dish.

Figure 58 Germination testing a) environmentally controlled germinator and b) counting and removing germinants from dishes.

and placing the seeds back into cold stratification for an additional eight weeks. The majority of true firs mentioned here perform best with the split stratification regime, but a protocol involving 56 days of cold stratification without surface drying is also performed and the superior treatment for each seedlot is recommended for operational use.

Germination testing procedures vary slightly between facilities. The key is to have a well documented, reproducible regime that is based on accepted testing protocols (ISTA or AOSA). This guidebook will use the procedures employed at the BC Ministry of Forests Tree Seed Centre as an example.

Sowing dry seeds in the nursery is not recommended

For each of the four 100-seed replicates, the seeds will be soaked (except western redcedar) for the proper duration in a vial (**Table 5**). To prepare the germination box, place one layer of 22-ply kimpack in the dish, add 50 ml of water and compress the kimpack to evenly distribute water and provide a flat surface onto which you place one piece of filter paper (**Figure 57**). The water will be drained from the soaked seeds and they will be spaced equally onto the filter paper in the germination dish. The four germination dishes that encompass a stratified test will then be put into the cooler at 2°C for the appropriate stratification duration (**Table 5**). For yellow-cedar, the period of warm stratification is conducted at a constant 20° in a germination cabinet.

After the appropriate stratification period, the germination dishes are transferred to the germination cabinet (**Figure 58**) under conditions considered optimal. An assessment of germinated seeds will continue for up to 28 days and will be performed on Mondays, Wednesdays, and Fridays, with germinants counted and removed from the dish to estimate germination rate. A seed is considered germinated when its radicle is four times the length of the seed coat and no abnormalities are displayed by the germinant. The ISTA germination definition for tree species having **epigeal** germination is when the primary root and hypocotyl together exceed four times the length of the seed (ISTA 1999). Common abnormalities in conifers include reversed germinants (**Figure 59a**) and stunted radicles (**Figure 59b**). However, stunted hypocotyls and seeds in which the megagametophyte constricts the germinant (megagametophyte collar), not allowing germination to progress normally, are also seen. Abnormal germinants are recorded but not included in the estimate of germination capacity. In other regions of Canada the germination-vigour classes proposed by Wang (1973) are used to quantify germination capacity.

Abnormal germinants are recorded but not included in the estimate of germination capacity

Figure 59 a) Examples of reversed germinants, with cotyledons emerging first, in a variety of species and b) stunted radicles in Amabilis fir.

The germination capacity (GC) is the main criteria used to define seedlot quality. The GC is the percentage of seeds that have germinated during a germination test (21 or 28 days depending on species – see Table 5). Germination capacity is useful in quantifying seedlot quality and in determining the amount of seeds required to produce a given quantity of seedlings, but it should be supplemented with a variable describing the germination rate (faster germination usually equates to a more uniform crop).

The germination value (GV) has historically been used to define 'vigour' (Czabator 1962) and is the other variable in addition to GC that *was* available on SPAR. The GV has now been replaced by PV on SPAR. The GV is a product of two additional variables: mean daily germination (MDG) and peak value (PV) GV=MDG * PV. Figure 60 presents a graphical representation of GC, PV, and GV. The MDG is simply the germination capacity divided by the number of days in test [MDG=GC/#days in test]. For example, an interior spruce seedlot which is tested for 21 days, with a GC of 91% would have a MDG of 4.3. MDG is a linear description of germination, but germination is not linear and this parameter alone is not very useful. The PV is the point at which the cumulative germination percent divided by the number of days is maximum. The PV describes germination rate and is best understood with an example from a germination test sheet

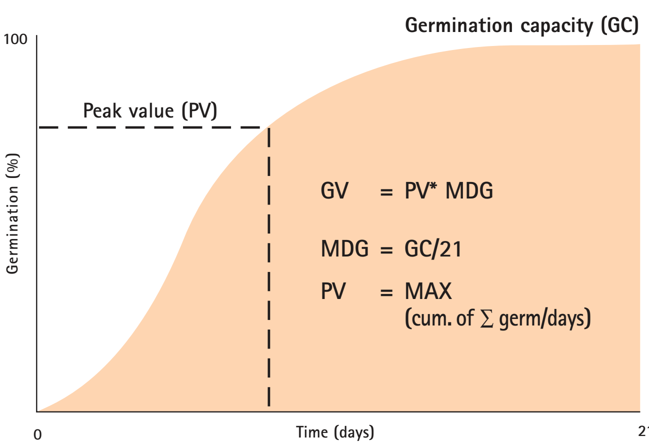

Figure 60 A graphical representation of germination capacity (GC), peak value (PV), and germination value (GV), also termed vigour.

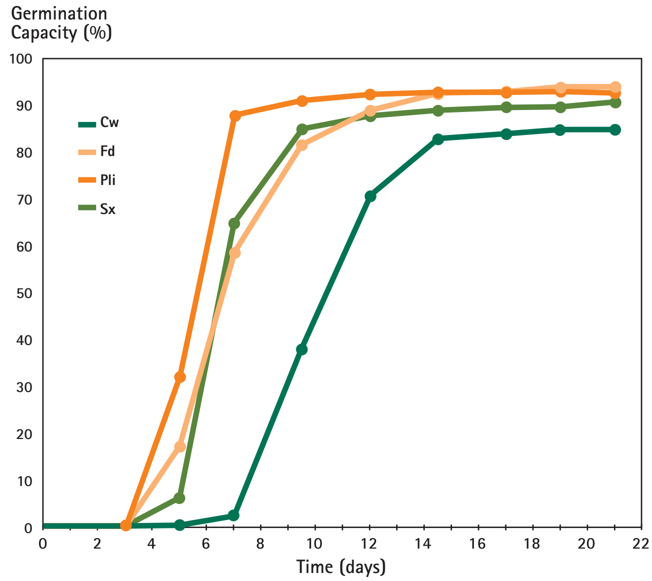

Figure 61 Representative germination curves for interior lodgepole pine (Pli), interior spruce (Sx), Douglas-fir (Fd), and western redcedar (Cw).

as illustrated in Table 6. The first step is to obtain the mean germination for each test date (i.e., for day 7: (52+57+55+60)/4 = 56). For each test date we will then calculate the average cumulative germination (i.e., for day 7: 20.8+56=76.8). The cumulative germination is then divided by the test days and the maximum value for the cumulative germination divided by test days is the peak value (i.e., for day 7: 76.8/7 =11.0). In this example, the peak value is 11.0 and it occurs on day 7. The PV is more informative if presented as cumulative germination and days to arrive at this level (77%/7 days) rather than simply a single number (11). The GC is equal to 91% and the MDG is equal to 4.3.

A comparison of the germination patterns for our four main species are illustrated in Figure 61. The high and rapid germination of lodgepole pine is typical of this species and aids in producing a uniform crop. The other end of the spectrum includes western redcedar which has a lower germination capacity and a more drawn out germination curve. This curve represents the testing of dry seed, but pelleting will move this curve even further to the right—delaying germination. Douglas-fir and interior spruce are similar and intermediate in their average germination patterns.

Table 6 The raw germination data used in the calculation of the germination parameters

Test Day	3	5	7	10	12	14	17	19	21
Rep	Number of Normal Germinants Counted								
1	0	20	52	10	8	0	0	0	0
2	0	21	57	10	4	2	0	0	0
3	0	23	55	8	5	1	0	0	0
4	0	19	60	7	3	1	2	0	0
Mean	0	20.8	56.0	8.8	4.0	1.0	0.5	0.0	**0.0**
Cumulative	0	20.8	76.8	85.5	89.5	90.5	91.0	91.0	**91.0**
Cum./Day	0	4.2	11.0	8.6	7.5	6.5	5.4	4.8	**4.3**