Tree Improvement Branch

Cone and Seed Improvement Program
BCMoF Tree Seed Centre

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Germination: Definitions, Assumptions, Implications

Germination is an important seedlot attribute that can impact seed pricing, seed use, and the success of your crop. Germination is intuitively understood, but I would like to provide a condensed review on the Definitions, Assumptions, and Implications behind the word. The discussion will focus on conifers, but some of the quantitative information is equally applicable to angiosperms (any discussion regarding dormancy mechanisms may not be!). It certainly isn't the last word on the subject, but I think it is a worthwhile review and if it initiates some lively, pro-active discussions that would be a bonus.

DEFINITIONS:

Germination is generally associated with emergence of the radicle through the seed coat. The International Seed Testing Association (ISTA 2004) defines germination as "*the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether it is able to develop further into a satisfactory plant under favourable conditions*". This broad definition is intended to allow for a variety of testing methods used through the world to be acceptable, but a certain level of subjectivity is involved in judging whether a germinant is able to develop into a satisfactory plant. Questions arise regarding what is a satisfactory plant? with implications to seedling specifications, but that is beyond the scope of this review.

In BC, the criterion for counting a normal and healthy germinant is the ratio of the radicle length to the seed coat. For all species (except Ba and BN) a seed is classified as germinated once the radicle is 4X the length of the seed coat. For Ba and BN the criteria is 2X the length of the seed coat due to the larger size of these seeds. In some parts of Canada, germination vigor classes are used in the classification of germinating seeds. This method categorizes germinants into various classes based on how far seedling development has progressed. It will not be discussed further here, but if interested the following paper is worth reading (Wang 1973).

THE GERMINATION TEST

A germination test is composed of four replicates of 100 seeds that have been randomly selected and are representative of the seedlot being tested. Proper sampling cannot be over-emphasized – the test result is only as good as the sample taken! The seeds are pre-treated with a soak (except Cw and some hardwoods), and generally a period of stratification. For information on species specific treatments refer to the Seed Handling Guidebook (Kolotelo et al 2001). Following treatment, each 100-seed replicate is transferred to a germination dish containing a piece of 22-ply wadding paper, 50 ml of water and filter paper on which the seeds are placed. Each dish is labelled with the seedlot number, test type, test identification number and replicate number.

On Monday, Wednesday and Friday germination counts are performed in order to calculate the germination capacity (GC) and the Peak Value (PV) of the seedlot (Figure 1). The germination capacity is the percentage of seeds that have germinated during the test (21 or 28 days depending

on species) based on the average of all four replicates. The Peak Value is an estimate of germination rate and the point whose tangent has the steepest slope on the germination curve. The Peak value is presented as the peak germination percent / peak count day (e.g. 89/10 indicates that the peak value corresponds to 89% germination in 10 days). For Statistical analysis or use in formulas the integer (i.e. 8.9 in above example) should be used for evaluating differences in germination rate. Both the GC and the PV are available on SPAR and on sowing request labels. The GV was formerly used, but this variable combine both GC and PV together in one variable making it difficult to assess each independently. A more thorough example of the calculations can be found in the Seed Handling Guidebook (Kolotelo et al 2001).

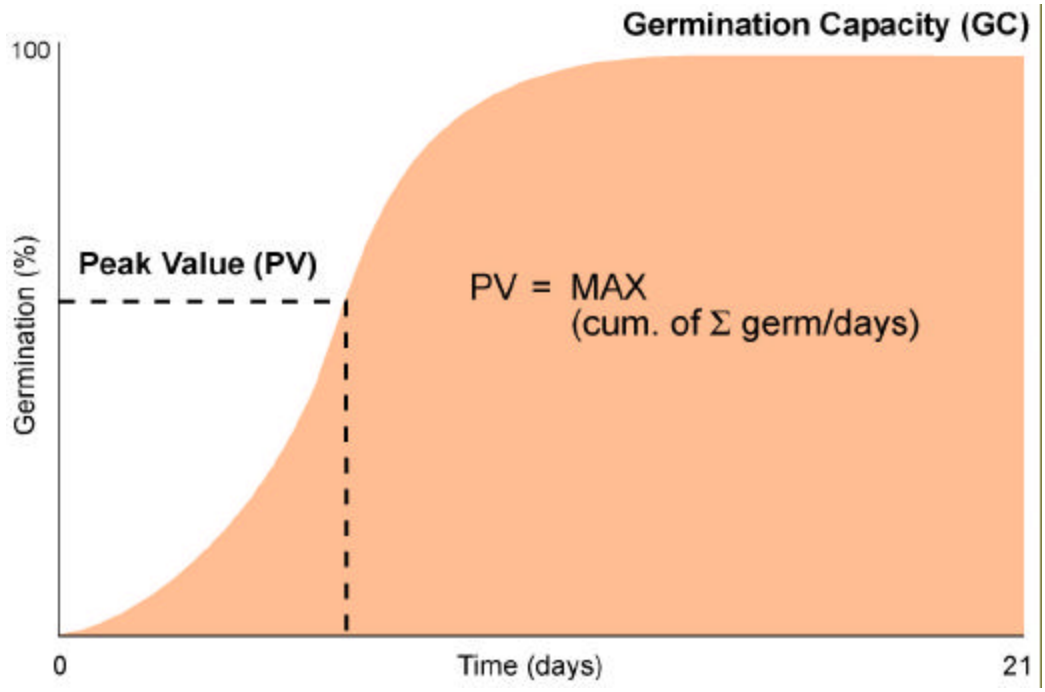

Figure 1. The germination curve illustrating the Germination Capacity (GC) and Peak Value (PV).

Germinants categorized as 'abnormal' are not included in the GC or PV of a seedlot and are tallied separately by abnormal class. The most common abnormal germinants are where the cotyledons emerge first (reversed = easy to recognize) and germinants with stunted radicles (a more subjective classification). Reversed germinants generally are only a fraction of a percent of the seed in a seedlot, but the percent of stunted radicles can be much higher for some seedlots. Stunted radicles appear to be associated with seedlot age and species. In general, *Abies* spp. tend to have higher proportions of stunted radicles even on new seedlots, but older seedlots of Cw, Hw, and Lw also have higher levels of these abnormals. No study has clearly determined the cause of these abnormals, but it appears related to ageing and possibly damage to the root cap protecting the root apical meristem. Other less common abnormal classes are: stunted hypocotyl, thickened hypocotyl, thickened radicle, twin; rotten, weak and megagametophyte collar.

GERMINATION TEST ACCURACY AND PRECISION

The germination test is composed of four replicates and ISTA provide tolerances for the maximum tolerated range between the four replicates (two-way test at 2.5% significance level). If one of these replicates is outside the tolerances then the test is repeated. Formerly the Association of

Official Seed Analysts (AOSA) allowed calculation of germination parameters based on the remaining three replicates if one replicate was out-of-tolerance. This is no longer the case and both ISTA and AOSA indicate that tests should be repeated if a single replicate is out of tolerance. This is a method improvement that ensures all tests have the same sample size involved in the calculation of germination parameters.

The germination test also allows one to compare the precision of the test result based on the variability between the four replicates (*see* Kolotelo 2002 *for more details*). Based on the four replicates one can calculate the standard error and any confidence interval for a germination test. An example is two seedlots both with a GC of 85%, but seedlot A has replicate data of 84, 84, 88 and 83%, while seedlot B has replicate data of 87, 77, 88, and 87. Seedlot A would have a 95% confidence limit of germination between 82.4% to 87.2%, but seedlot B would have a much wider confidence limit of 76.6% to 93.0% indicating less precision in the GC estimate. This is not currently reported and although some nurseries have shown interest in this data there is no clear indication from nurseries that this would be operationally useful. I suggest the standard error is the best statistic to report allowing nurseries to calculate confidence intervals to their specific significance level of interest.

SPECIES AVERAGE GERMINATION

The species average (SA) germination is used when a seedlot is required for sowing during the same production year as it was collected and there is insufficient time to complete processing and testing. Each species has a SA germination on SPAR, but this germination is not specific to the genetic class and GC can vary by genetic class for some species (i.e. Yc, Pw or Lw). When additional information is available, such as historic performance from a specific orchard, then the owner can request an estimated germination (SE) value be used as the GC to calculate grams required for sowing requests if there is insufficient time to complete processing and testing.

ASSUMPTIONS

The primary assumption of a germination test is that it is performed on a representative sample of the seedlot. A thorough sampling design that is outlined by ISTA (ISTA 2004) is used to sample seedlots for testing. Depending on seedlot size, the rules indicate the number of boxes to sample and the number of samples per box. Is the estimate perfect – No, conifers are extremely variable and we are using 400 seeds to estimate the GC of a seedlot that may be many kilograms (hundreds in some cases) in size. I don't foresee us increasing the number of replicates we use in testing and I think the way to deal with this is to provide the variation between the replicates as an estimate of how variable the result is. Everyone wants to be dealing with a uniform product, but I would feel much less comfortable with our reforestation program if there was no variability present in the products we are putting on the landscape for most of the next century!

Another assumption of the germination test is that it is conducted under optimal conditions. In reality, the conditions we use in germination tests (temperature, light levels, and moisture levels) are international standards, applied to a variety of species. Are they optimal for everything – probably not, but imagine the job of arriving at the optimal combinations of these factors for all seedlots in storage. We have adjusted conditions when there is good evidence that it is justified. Good examples are the use of a constant 20° C germination temperature for Hw germination (Bientjes 1954) and the use of 25:15 temperatures for *Abies* spp. (Leadem 1989). I think the most important factor is that the testing method and procedures are consistently applied over time.

Individual nurseries need to determine how well the standardized germination tests compare in their unique set of nursery conditions.

OPERATIONAL IMPLICATIONS

How well do germination tests in a laboratory relate to germination in the nursery? The results are surprisingly good and I thank all nurseries that have provided feedback on actual nursery germination. The last summary I forwarded to nurseries summarized nursery falldowns from 1999 to 2003. Our big four reforestation species had the following average operational falldowns in germination: Pli =1%; Sx = 0%; Fd = 3% and Cw =3% (what I want to show is that the – should be replacedc with = as the former may be confused with a minus sign) There is still room for improvement in Bl, Dr, SxS and Pw, but I am surprised at how close nursery results are to test results. Nurseries can take a great deal of this credit as they realise the importance of starting a crop off quickly and uniformly and they have improved their facilities to make sure this happens. Updated germination falldowns will be forwarded to nurseries and placed on our website prior to 2006 sowing request entry.

An area that still requires some improvement is recognition of the need to be highly efficient in seed use when we have a deficit situation for orchard produced seed. This refers primarily to Pli, but BC-produced Fdc is also in a deficit situation. Lodgepole pine has the best germination characteristics (capacity and rate) of any species in BC. Nurseries should have good data of the amount of seed required to produce Pli crops to virtually eliminate excess seed. This is preferred to having to hardwire more stringent sowing guidelines for orchard-produced Pli into SPAR. This past year a few nurseries that have reduced grams in the past have not. Everyone should be aware that seed is never the same once it has been soaked and stratified. It is much better to reduce grams up front. I'd like to identify several nurseries who have made efforts to reduce Pli gram for 2005 sowing: PRT Armstrong, PRT Vernon; Eaglerock, Silva Gro and Juniper Beach. Several forest companies are also restricting the amount of seed used for Pli sowing and we appreciate their efforts in ensuring that orchard produced Pli seed is used as efficiently as possible. Thank you.

If you have additional comments concerning the topic of germination I would appreciate your feedback. I can be reached at (604) 541-1683 extension 228 or at Dave.Kolotelo@gems7.gov.bc.ca.

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