Tree Improvement Branch

Cone and Seed Improvement Program
BCMoF Tree Seed Centre

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New Retesting Frequencies and Deterioration Rate Estimates from the BCMoF Tree Seed Centre

Seedlot retesting is considered to be a Quality Assurance (QA) function with the goal of providing accurate, up-to-date information on the germination capacity (GC[1]) of a seedlot. The GC will guide seed requirements and how this seed is allocated into styroblocks (# seeds/cavity) to produce at least one germinant per cavity. The seed testing conditions are 'considered' optimum, but operational nursery results may differ. It is my opinion that lab test results cannot always accurately predict the germination of seed under the variety of growing conditions that it may be exposed to. Proactive nurseries should track germination (through their own QA programs) and have a good handle on how lab test results relate to germination under their specific germination environment. The GC of a seedlot will change over time as seed deteriorates in long-term storage. An estimate of a species deterioration rate aids in the decision of how often seedlots should be retested as part of our stewardship function.

This analysis of the germination history of seedlots in storage at the BCMOF Tree Seed Centre (TSC) was performed to:

1) Provide individual seedlot linear deterioration rate estimates.
2) Provide an average estimate of a species' linear deterioration rate in storage.
3) Identify seedlots that are deteriorating faster than the species average.

METHODOLOGY

To determine the linear deterioration rate of a seedlot only three pieces of information were required a) initial germination germination capacity (GC) b) current GC and c) the time between the two tests. The seedlot deterioration rate was calculated using the following formula:

Deterioration Rate = $(GC_{current} - GC_{initial})$ /(time interval) = ΔGC / Δtime

Results are presented as change in GC per year and species averages were obtained by averaging all seedlots of that species. The standard error of the deterioration rate estimate is also presented to provide a measure of precision. The only conditions placed on seedlots were that they had more than one germination test (of the same type) and were in storage at least 500 days. Deterioration rates calculated using shorter intervals are likely to give high estimates that are based more on sampling variation than deterioration. For example, a 5% difference in germination over 5 years is equivalent to a deterioration of 1%/year, but over 6 months this same GC difference inflates to an estimate of 10%/year.

Although seedlots may not deteriorate in a linear fashion through their entire lifespan, the construction of non-linear deterioration equations or pooled seedlot regression analysis is not

[1] Germination capacity (GC) is used to indicate the percent germination of a seedlot with a specific pretreatment and count duration.

practical with the available data. Some data limitations are few data points per seedlot, irregular testing intervals, and relatively few long-term results. The presented method provides a simple means of estimating and discussing deterioration and provides a biological basis for the recommended germination retesting frequencies. Calculation of seedlot deterioration rates also allows one to identify and retest more frequently the seedlots that are deteriorating faster. The working assumption is that estimation of deterioration rates using a linear procedure will not produce significantly different species deterioration rankings than a true average of the non-linear deterioration rate (i.e. *Thuja plicata* is going to be a priority no matter what the exact deterioration rate is).

RESULTS

It is TSC policy that the germination retesting frequencies will be examined at 5-year intervals with the last review occurring in 1997. The 1997 and 2002 species deterioration estimates are presented in Table 1. Sample sizes for most species have increased considerably. The exceptions are the *Abies* spp. as in 1997 the total sample size was based on several germination test types.

Table 1. The 1997 and 2002 estimates of species deterioration rates (DET) in germination %/year, standard errors (s.e.) for 2002 estimates; sample sizes, recommended retest frequencies in months; average seedlot age (S Age), maximum seedlot age (MAge), and age of oldest seedlot used in deterioration rate calculations (DAge) in years.

	1997			2002						
Species	**#**	**DET**	**Retest**	**#**	**DET**	**s.e.**	**Retest**	**SAge**	**MAge**	**DAge**
		%/year	months		%/year		months	years	years	years
Abies amabilis	254	-0.78	24	165	0.06	0.15	26	7.8	24.6	**8.9**
Abies grandis	79	-0.24	24	49	-0.72	0.22	22	11.3	22.4	**12.5**
Abies lasiocarpa	150	0.67	24	107	0.17	0.54	20	8.9	26.7	**4.9**
Thuja plicata	248	-1.44	18	370	-1.24	0.11	18	8.5	33.9	33.9
Pseudotsuga menziesii var. menziesii	264	0.03	36	349	-0.1	0.03	44	11.7	41.8	41.8
Pseudotsuga menziesii var. glauca	402	-0.07	39	609	-0.21	0.02	40	13.2	43.2	43.2
Tsuga heterophylla	272	-1.22	20	366	-1.13	0.07	20	12.1	34.1	34.1
Tsuga mertensiana	33	-0.36	24	47	-0.46	0.10	30	14.1	23.5	23.5
Larix occidentalis	95	-1.06	22	173	-0.67	0.07	24	7.8	25.2	25.2
Pinus contorta var *latifolia*	756	-0.01	36	1495	-0.08	0.01	44	8.5	29.7	29.7
Pinus contorta var *contorta*	34	0.08	30	49	-0.15	0.06	42	8.5	14.6	14.5
Pinus monticola	77	-1.03	30	95	-0.19	0.26	26	8.6	21.0	**6.4**
Pinus ponderosa	126	-0.28	30	150	-0.46	0.11	32	6.6	40.8	**17.2**
Picea sitchensis	97	0.1	42	194	0.03	0.03	48	14.7	34.5	32.8
Picea glauca/ engelmannii[2] complex	820	-0.07	36	1233	-0.22	0.02	40	13.3	41.5	41.5
Picea lutzii	62	-0.25	30	50	-0.28	0.07	38	11.5	28.8	28.8
Chamaecyparis nootkatensis	15	0.46	36	35	-2.16	0.79	18	4.6	10.2	10.2
Total	**3784**			**5610**						

[2] In British Columbia, *Picea glauca*, *Picea engelmannii* and hybrids between these species are not differentiated and commonly referred to as "interior spruce"

The 2002 estimates for all species are based solely on one germination test type. For the 2002 analysis the average seedlot age (Age), maximum seedlot age (MAge) and the maximum amount of time used in the calculations of deterioration rate (DAge) are included in Table 1. The DAge is less than the MAge when a change in germination test type has occurred during a seedlots lifetime. This effects the results of *Abies amabilis*, *A. grandis*, *A. lasiocarpa*, *Pinus monticola* and *P. ponderosa*.

Some species also have positive estimated rates of deterioration and these are considered to be artifacts of the process and more likely related to sampling variation, lack of appreciable deterioration and improvements in seed testing rather than increases in GC during storage. The use of only one test type per species, a decreased number of species with positive deterioration rate estimates and an increase in seedlot information, both in numbers of lots as well as in maximum seedlot age, increase my confidence in the 2002 results.

The deterioration rate played a large role in the recommended retesting frequency, but other factors such as the precision of this estimate, the sample size, degree of change in estimated deterioration rate since 1997, number of seedlots currently in storage, age of seedlots in sample, and the number of problem seedlots per species all influenced the frequency recommendations.

The largest change in estimated deterioration rate was with *Chamaecyparis nootkatensis*. This species had a 2.3X increase in the sample size, but it still had the smallest sample size[3], largest standard error and youngest seedlots of any species included here. The retesting frequency of this species has therefore been doubled from testing every 36 months to every 18 months.

Abies amabilis and *A. lasiocarpa*. have several unique characteristics that deserve discussion. Standard test types have changed more frequently for these species as the research into an optimum dormancy breaking treatment progressed. Therefore long-term data with any one test type is limited in these species. Seedlots of these species, and especially *A. lasiocarpa*, sometimes display highly variable germination results between subsequent tests. This is reflected in the positive deterioration estimates and large standard errors associated with these estimates. This is problematic for assigning seedlot quality. These species also possess resin vesicles and the 'condition' of these will impact seed longevity. A more conservative retesting frequency has been assigned to *Abies* spp. compared to other species with similar deterioration rates. The other non-*Abies* species with resin vesicles are *Thuja plicata*, *Tsuga mertensiana* and *Tsuga heterophylla* which have relatively high estimated deterioration rates.

The other species showing a large change in deterioration estimates is *Pinus monticola*. In 1994 the standard pretreatment for this species was changed and treatment significantly increased the GC of this species. The data available using this test type was extremely limited in 1997. The 2002 estimate is substantially reduced, but there is a large standard error associated with this estimate. A more conservative retesting frequency is recommended for this species compared to its low estimated deterioration rate. The remaining species have reasonably low deterioration rate estimates and standard errors.

FURTHER CONSIDERATIONS

It has been suggested that the species datasets should be broken down into categories based on **seedlot age** or based on **initial seed quality**. To investigate the data distribution of initial seedlot

[3] Deterioration rate estimates are available for other species, but sample sizes are quite small and they have therefore not been presented. Contact the author for additional information on a BC tree species of interest if it is not presented.

quality and seedlot age, these factors are plotted against the seedlots estimated linear deterioration rate for *Pseudotsuga menziesii* var. *glauca* (Figures 1 and 2). These plots appear fairly typical of the species examined.

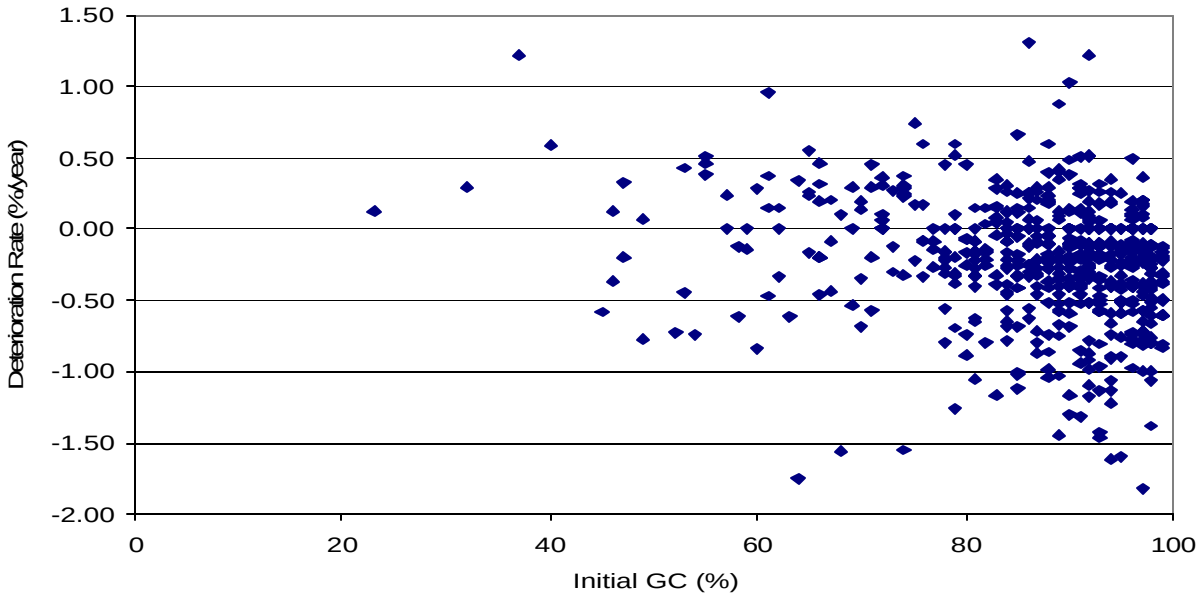

Figure 1. The relationship between initial germination capacity (GC) and estimated deterioration rate for *Pseudotsuga menziesii* var. *glauca* (n=609).

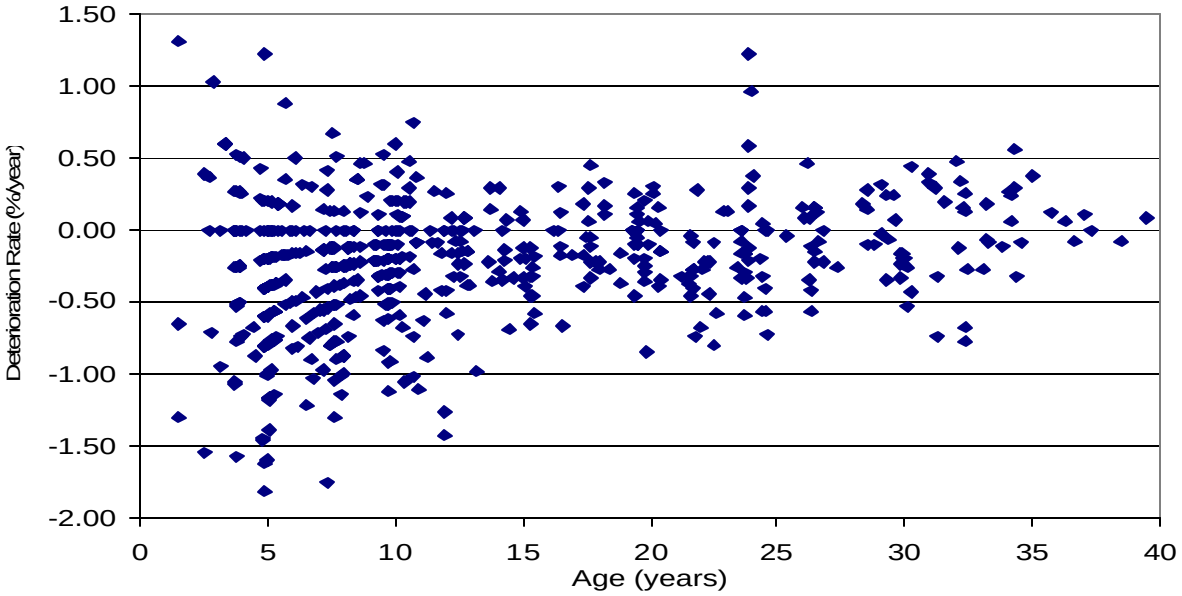

Figure 2. The relationship between seedlot age and estimated deterioration rate for *Pseudotsuga menziesii* var. *glauca* (n=609).

In Figure 1 the data illustrates that most seedlots are initially of high quality, but the deterioration rate does not increase with decreasing initial seed quality. In Figure 2 the range of deterioration estimates is greater for young seedlots and appears to be reduced with seedlot age. These observations are initially counter-intuitive – let's look at some possible reasons. The dataset is

from an operational facility – it does not represent the results of a controlled experiment. Individual seedlots will differ in basic attributes such as moisture content, seed size, level of dormancy etc..,. Testing frequencies have fluctuated over the 40-year time period we have data on for some species and for other species the retesting history with one test type is minimal.

Initial seed quality is intuitively something we believe is important to seed longevity. Seedlots with a higher GC should store better, but this is not always the case (see Seed Handling Guidebook page 50; Kolotelo *et al.* 2001). A seedlot can have a low GC for various reasons:

1) Seed is viable, but dormancy has not been completely overcome
2) Non-viable seed is present in the seedlot
3) A combination of the above

If a low GC is caused mainly by the presence of non-viable seed, then although the GC may be low it doesn't necessarily mean that the remaining viable seed will deteriorate faster than average. The presence of non-viable seed is generally not significant for our main reforestation species today, but it can be substantial in *Abies* spp., *Chamaecyparis nootkatensis* or older seedlots. Are these non-viable seeds empty or deteriorated? and What is the condition of the viable seed? These questions are relevant to seedlot storability, but are not adequately addressed by the GC estimate.

There is much more data available on young (<10 years old) seedlots with generally high GC values. The reasons are of a practical nature as seedlots are consumed to grow seedlings and most do not remain in storage for long lengths of time. For tree seeds the quality of all steps throughout the seed handling system have dramatically changed for the better in the past 40 years. The deterioration rates of our oldest seedlots are therefore probably greater than current collections.

Initial GC and seedlot age may not be good general predictors of deterioration. Other factors such as collection timing, post-collection handling, cone and seed processing techniques and seed moisture content may have a large impact on the deterioration rate. The long-term effects of these factors may not be evident in the initial GC estimate. It has been suggested that since deterioration is generally slow it would be better to investigate speed of germination that is generally thought to decrease prior to seed death and the resulting drop in GC. This suggestion has merit, but unfortunately germination rate data is only readily available for the past 10 years.

The deterioration estimates are generally quite small on a species basis, but there is a wide range of variability between seedlots. To investigate the proportion of variation attributable to species a random effects model using PROC VARCOMP in SAS was used to determine the proportion of variance that can be accounted for by species. This analysis showed that only 6% the variability could be accounted for by species with the remaining variability being accounted for within-species or at the individual seedlot level. This emphasizes the point that although it may be operationally useful to look at species differences the greatest amount of variability resides at the individual seedlot level.

DISCUSSION

The method used for quantifying deterioration in this article has several advantages including providing a simple, easily understood procedure for calculating and discussing seedlot deterioration rates. The method also allows one to identify seedlots that deteriorate significantly faster than the species average enabling one to test these seedlots more frequently and maintain the same level of confidence in the results. Species average deterioration rates, and their precision,

play a key role in determining the retesting frequency, but other factors such as the sample size, degree of change in estimated deterioration rate since 1997, number of seedlots currently in storage, age of seedlots and the number of problem seedlots per species all influenced the frequencies. There is no mathematical formula for this.

The main disadvantages of this method is that it is a linear estimate of the deterioration rate, but we expect that deterioration over time, measured in terms of GC loss, is not linear. The number of tests available on each seedlot is a major obstacle in employing more sophisticated analytical techniques to the data. The expectation is that GC will be relatively stable or decrease linearly until the majority of seeds fail to germinate and a larger decrease in GC will occur. At what age and how quickly individual seedlots enter this rapidly deteriorating phase is not known. Some seedlots (even some species) appear to have a stable GC over a fairly long (decades vs. years) timespan.

Species retesting frequencies are used to identify pending retests for a species. The actual decision to perform a retest depends on other factors such as seedlot size, seedlot usage, and genetic class. The retesting frequencies act as a filter, but not a determinant of when retesting of an individual seedlot should be performed. The results clearly show that most species store very well, but the amount of variability within a species dictates that decisions should be made on an individual seedlot basis. As deterioration is generally low with conifers it is at least equally important to have earlier identification of the exceptions (seedlots with high deterioration rates) as it is to predict average seedlot performance. What to measure and how to efficiently integrate this result within a scheduling tool is a current challenge.

The revised deterioration rates and recommended retest frequencies will allow lab staff to focus on retesting rapidly deteriorating species (priorities) and individual seedlots deteriorating faster than average. The more rapid retesting of species that do not store as well provides an accurate estimate of germination and also adds more datapoints to the database to improve the estimate of deterioration in future reviews. These methods are not perfect (and neither is the database) and our quantification of deterioration may change in the future. Maybe feedback on this article will spur that change?

The seedlot and species deterioration estimates are currently our best biological estimates of change in germination capacity in long-term storage. I don't believe that we will suddenly discover a method of quantifying deterioration at the species level that is better than at the individual seedlot level. There are too many unquantified features of collection, processing, handling and year-to-year variability to predict the relatively small change in GC over time on a species basis with accuracy. It is time to recognize the unique characteristics of the individual seedlot. The key to the future of retesting lies in using the information on how an individual seedlot performs to guide how often it should be retested. The species average provides a benchmark for the individual seedlot.

Comments are graciously welcomed.

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