Tree Improvement Branch

Cone and Seed Improvement Program
BCMoF Tree Seed Centre

Direct distribution to internal and external SPAR distribution lists **September 30, 2013**

Western White Pine Germination Review

Executive Summary

This note is intended to review the recent performance of western white pine (*Pinus monticola*) germination in response to nursery concerns. The issue of white pine (PW) germination is not new and various efforts have been put in place to improve germination and hopefully this review will help with further improvements to increase seed use efficiency in this species. I will provide some background into the PW stratification process, how it has changed over time and review in detail the germination and moisture content results for the 2008 to 2013 sowing seasons.

The primary recommendation is that due to the consistent reduction in germination using operational quantities of PW seed that the allocation to nurseries should be increased by 5%. The other nursery specific recommendation is based on the observation that the use of a hydrogen peroxide treatment at the nursery appears to reduce the germination falldown. The other recommendations are Tree Seed Centre (TSC)-specific and include the recalibration of our moisture content targets, a slight increase in QA sampling intensity and a return to more frequent sowing request monitoring.

Background

Dormancy in PW is generally considered deeper than most commercial conifers and a prolonged stratification method (90 to 120 days) to break dormancy has been used by most facilities in the Pacific Northwest. The dormancy mechanism has been shown to be complicated and variable due to various tissues restricting germination (Hoff 1987). Although Pw is generally deeply dormant at the seedlot level, some individual seeds are non-dormant or have varying degrees of dormancy. Treatments to maximize germination must provide enough stratification to overcome dormancy in the most dormant seeds, but not provide conditions enabling the non-dormant seeds to germinate during stratification.

In 1995 a change of stratification method was implemented at the TSC to address fungal proliferation and pre-germination of non-dormant seed - issues that seemed primarily the result of the 28-day warm stratification phase. A 14-day running water soak replaced the warm stratification and cold stratification was increased from 56 to 98 days. This is basically the procedure that is in place today, but other minor changes, mainly in operational implementation, are presented in Table 1. Further elaboration on these changes can be found in the articles found on this page:
http://www.for.gov.bc.ca/hti/treeseedcentre/tsc/csio1-westernwhitepine.htm

Table 1. A review of historical changes to the stratification procedure used for western white pine.

Year	Test Type	Definition/ Change
Pre-1996	**G52**	**48-hour soak, 28 days warm stratification and 56 days of cold stratification**
1996 ➔	**G55**	**14-day Running water soak and 98 days of cold stratification**
1999 ➔	G55	Quality Assurance (QA) testing increased to 4 X 100 seeds from 4 X 50 seeds used for other species
2000 + 2001	G55	Attempts to mimic tray-type stratification system (similar to lab) attempted, but abandoned and stratification in polyethylene bags re-introduced.
2001 ➔	G55	Eliminate surface drying – target seed moisture content between 34% and 40% (Goal= 37%) and introduce monitoring 3X per week to alleviate non-uniform moisture distribution issues and identify and treat any observed fungal problems.
2003 ➔	G55	Maximum stratification unit size (bag size) reduced from 1000 to 750 grams to allow for better moisture content control and monitoring.
2003 ➔	G55	Extended stratification made available to clients – initial 5 week extension too long (pre-germination in several facilities) and current 3 week extension recommendation adopted by most nurseries.
2004 ➔	G55	Destructive QA moisture content monitoring abandoned and replaced by target moisture content monitoring in all species. Larger bag sizes used in Pw requests (vs. other species) to further promote aeration. QA program to sample and test every seedlot (Not every SRQ) for germination capacity prior to shipping each season.
2012 ➔	G55	Switch from 3X per week monitoring and amelioration to 2X per week

Data Sources

The data for this review was supplied by the Tree Seed Centre Standard and Quality Assurance tests and from nurseries as part of their QA programs. Germination information is supplied by the Standard G55 lab result on SPAR which is used with seeds per gram to calculate grams of seed required for a sowing request (SRQ). This standard test is generally scheduled for retesting at a 26-month interval, but implemented primarily in the spring to ensure up to date results from this long test are available for placing sowing requests in the autumn. The TSC SRQ-QA program is designed to quantify quality of seed as it leaves our facility and since 2004 we have attempted to sample and test each Pw seedlot that we stratify. If seed was shipped early (=abbreviated stratification by more than three weeks) the QA tests may not have been performed as seed would not correspond to standard lab protocols. These two data sources provide a seedlot-specific estimate of the falldown experienced by going from standard lab testing with 400 seeds to a bulk SRQ bag of 750 grams (approximately 36 000 seeds). Both the standard and QA tests are performed under controlled conditions with a standardized evaluation process.

One of the main differences in the standard laboratory testing was the increased moisture content measured during stratification. Moisture content is currently measured non-destructively using the storage moisture content and weighing the seed to arrive at a total sample moisture content estimate

for that sowing request. Fungal assay testing is a priority for *Fusarium* spp and *Caloscypha fulgens* in PW as both can reduce germination. *Caloscypha* is purely a seed-borne issue, but although *Fusarium* spp. can be seed-borne it can also contaminate seed via contaminated soil, water, sowing equipment, styroblocks or pallets (Kolotelo *et al.* 2001).

The final important piece of data is the actual germination obtained in the nursery to convert seeds to seedlings. This germination estimate is much less standardized and controlled compared to the lab test results. Aspects that add to the variability include actual stratification duration, actual germination environment conditions, timing and method used to quantify germination. This last factor adds a great deal of variability simply due to time as standard testing is based on the radicle being 4X the length of the seed coat while most nurseries report germination at the point of seed coat shedding, significantly later in the crop cycle than what germination test results are based on.

Comparisons between Standard lab tests, Quality Assurance tests at shipping and nursery results are intended to determine degree and direction of germination differences to prioritize areas of improvement. One limiting factor is the balance of the comparisons – all sowing requests would have a standard lab test result, some will have QA results and others will have nursery results with a smaller subset having all three results.

Tree Seed Centre Results

The standard germination results used to calculate seed requirements and the operational sowing request germination results, sampled just prior to shipping, have been a challenge to synchronize. Figure 1 illustrates the changes over time with relatively poor germination overall before the introduction of the G55 treatment in 1996, followed by a period of great difficulty duplicating these standard lab results, and achieving better agreement from 2003 onwards. The annual germination results are based on an average of 17 sowing requests and range from seven (2009) to 29 (2001).There is still a falldown in germination by going from our four 100-seed replicates to stratification units of up to 36 000 seeds in size. The focus of the remainder of this section will be a more detailed look at sowing requests sown in the last six sowing seasons (2008-2013).

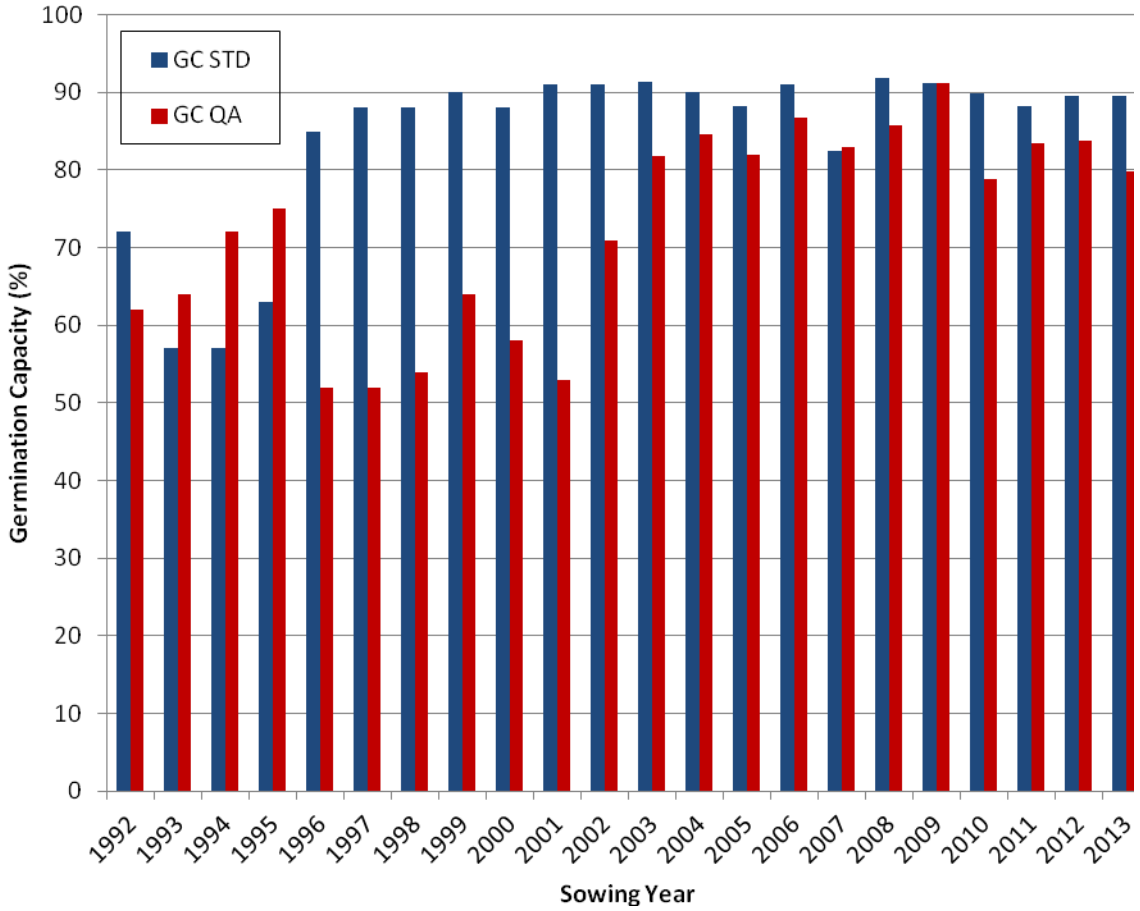

Figure 1. Comparison of average Standard germination capacity results (GC STD) with average Quality Assurance results (GC QA) for western white pine between 1992 and 2013.

Sowing Year Tree Seed Centre Results

The Tree Seed Centre results for 2008 to 2013 are based on 81 sowing requests which received QA testing prior to shipping (Table 2). During this period there was a 6.6% average decrease in germination going from standard test samples to operationally sized sowing requests. The variability in germination presented as the standard deviation for the same seedlots was also much higher for the sowing requests than Standard tests. Moisture content levels were within range, although a little on the high side, and stratification durations until shipping were within current recommendations.

Table 2. Characteristics of western white pine sowing requests prepared for the 2008-2013 sowing seasons.

Sowing Year	# Sowing Requests	Standard GC%	s.d.[1] of Standard GC%	QA GC%	s.d. of QA GC %	GC Difference	QA Moisture Content	Average Days of Stratification
2008	15	91.9	3.9	85.8	8.3	-6.7	38.6%	113
2009	6	91.2	2.2	91.2	4.9	0.0	39.1%	110
2010	13	89.9	5.0	78.8	14.6	-11.1	39.2%	113
2011	15	88.2	9.8	83.4	11.5	-4.8	39.1%	113
2012	12	89.5	5.5	83.8	6.9	-5.7	38.0%	113
2013	20	89.6	5.2	79.9	9.1	-9.7	38.2%	120
Mean[2]	**13.7**	**89.9**	**6.0**	**82.9**	**10.3**	**-6.6%**	**38.6%**	**114**

Genetic Class and Seedlot Tree Seed Centre Results

Almost 100% of PW seed sown in the province is from seed orchard seedlots. Out of the 81 sowing requests there were four representing wild stand seed and the actual germination falldown was not greater than orchard seed, but the initial quality was much lower. The sampled orchard seedlots averaged 91% germination while wild stand seedlots averaged 69% germination. The wild stand seedlots are also much older, so it may not simply be a production environment effect.
Western white pine is planted in both the coastal and interior portions of BC. In storage we have 33 seedlots representing each area and both have an average germination of 89%. The interior destined seedlots germinate consistently faster, but in general seedlot characteristics are quite similar for both. In terms of orchard production facility the costal facilities produced the extremes in standard GC% and operational falldown (Table 3). The Saanich produced seedlots had the lowest falldown, but they also had the longest stratification duration and the fewest sowing requests sampled. The other four orchards were represented by seedlots displaying the four largest germination falldowns indicating no consistent orchard pattern is evident. Not all current production facilities are represented here as Mt Newton (orchard 403) and Dorena, OR (Orchard 998) are not represented in this analysis.

The request size or more importantly the actual stratification unit size did not appear to influence the operational SRQ falldown as illustrated in Figures 2. In Figure 3 there appears to be an inclination for the smaller stratification units to have higher moisture contents, but these did not appear to result in increased germination falldowns. To be clear, the stratification unit size is the individual bag used to stratify seed. The maximum size is 750 grams, so all requests below this would only be one bag in size. For a 1400 gram request we would have two bags of 700 grams and for a 1600 gram request we would have three bags of 533 grams of seed. A larger sowing request above 750 grams does not necessarily mean a larger stratification unit size.

[1] s.d. – abbreviation for standard deviation – easiest way to make table fit.
[2] Mean represents the average of all sowing requests during this period, not the average of the yearly total. Difference is due to yearly variation in number of sowing requests.

Table 3. Characteristics of western white pine orchard production facilities prepared for the 2008-2013 sowing seasons.

Orchard (#)	SPU	# Sowing requests	Standard GC %	s.d. Standard GC	QA GC %	s.d QA GC %	ΔGC	QA Moisture Content	Strat. Length (days)
Sechelt (174)	MGL	16	87.0	5.6	79.0	6.5	-8.0	38.9	116
Saanich (175)	MGL	2	94.5	3.5	94.5	5.0	0.0	39.1	129
Bailey (335)	KQ	17	90.4	2.6	87.7	6.0	-2.6	38.3	115
Skimikin (609)	KQ	21	93.0	2.1	84.9	9.5	-8.0	38.2	120
Moscow, ID (999)	KQ	21	90.5	4.7	82.7	9.7	-7.8	38.9	113
Mean		**15.4**	**90.5**	4.4	**83.9**	**8.7**	**-6.6**	**38.6**	**116**

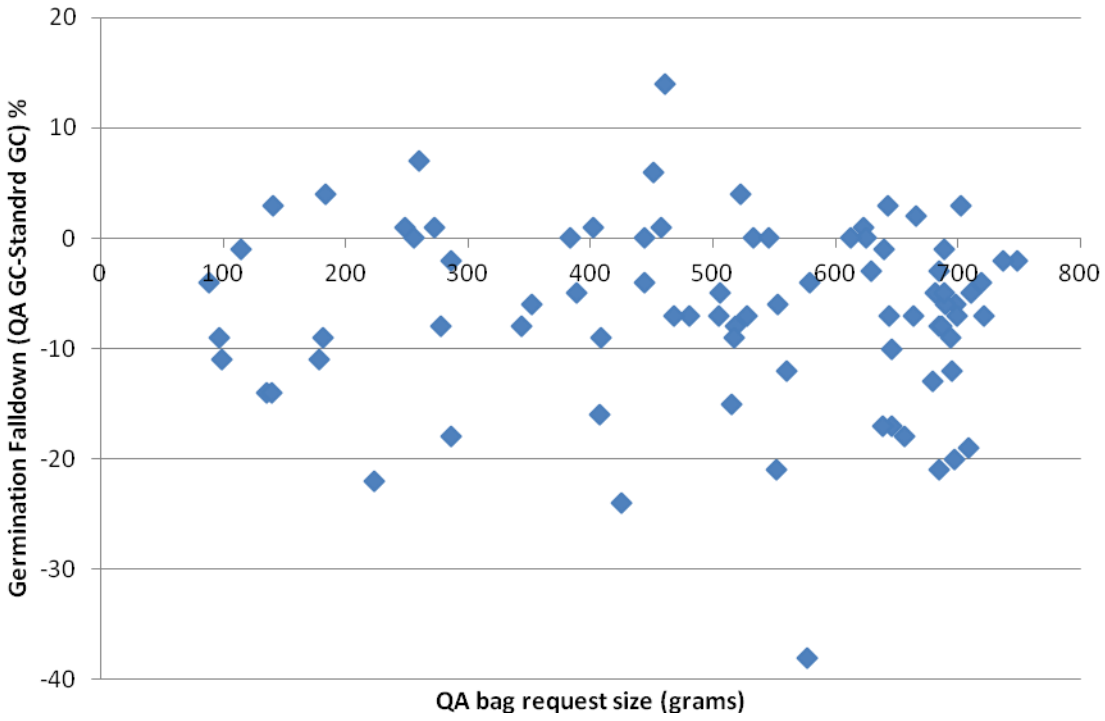

Figure 2. The Germination falldown from Standard to QA tests at the Tree Seed Centre as affected by stratification unit size.

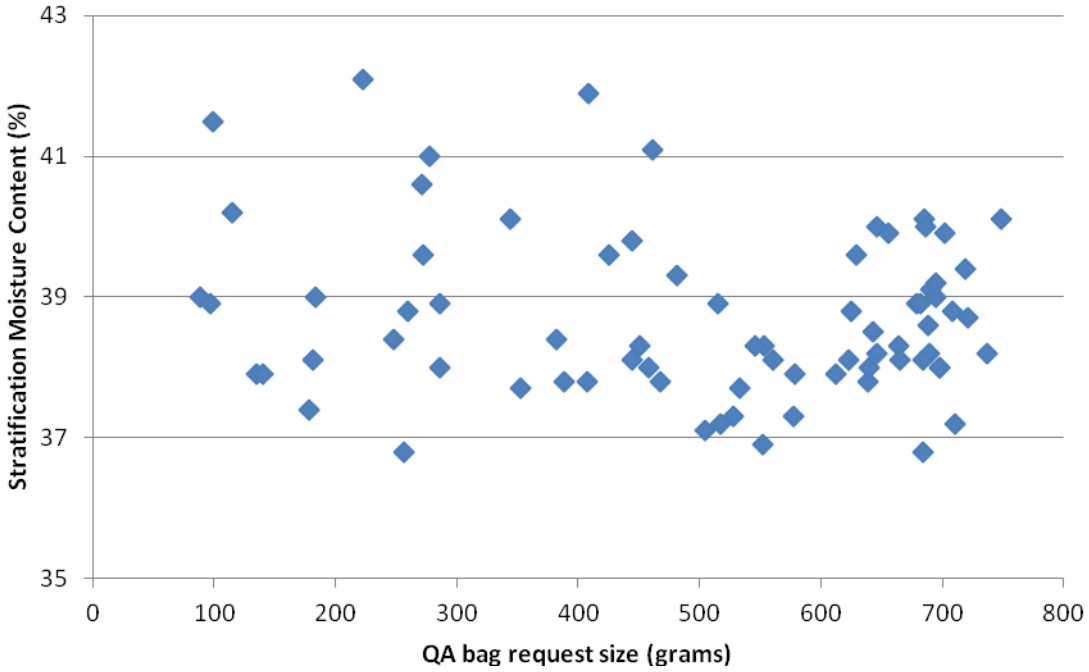

Figure 3. The Stratification moisture content achieved as affected by stratification unit size.

Nursery Results

Nursery germination results are available for 156 of a possible 218 sowing requests for the period 2008-2013. Thank you to all the nurseries that supplied this information. As noted earlier in the Background section this is the most unstandardized germination evaluation, but highly valuable in ensuring germination is close to that reported on SPAR and that seed is being allocated efficiently for seedling production.

Variation between nurseries for germination falldown compared to the Standard test was large ranging from -18 % to +7%, although that large increase was only based on one sowing request (Figure 4). This is not a perfectly clean comparison as all seedlots are not allocated equally to all nurseries, but it seems clear that there are varying degrees of success in germinating western white pine in the nursery. As has been standard practice we do not publicly disclose nursery identity when presenting nursery germination information.

For 43 SRQ's we had all three germination evaluations: Standard, QA at the TSC, and feedback from the nursery. For these requests the Standard germination achieved 90.3% germination and when these requests were bulked up to operational quantities the resulting germination was 82.0%, a falldown of 8.3%. The feedback for these same SRQ's from the nursery indicated an average of 83.8% germination equivalent to a 6.5% falldown from standard test results and a 1.8% improvement over operational seed quantities. This increase compared to germination at shipping is relatively small and most reasonably explained by the extended stratification that these requests would have experienced prior to sowing in the nursery. In terms of variability, indicated by the standard deviation in brackets, the operational QA (9.27) and nursery results (9.65) had almost twice as much variability compared to the standard results (4.87) and this is consistent with other results presented.

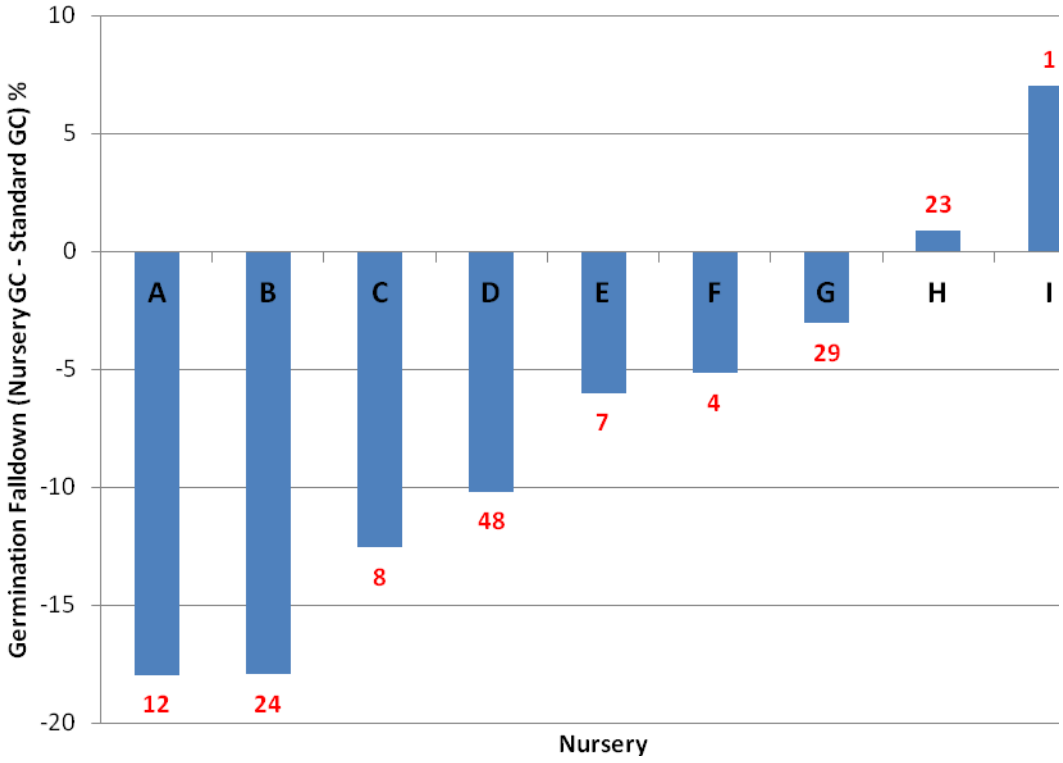

Figure 4. Nursery variation in germination falldown for sowing requests grown during the 2008-2013 sowing seasons. Data labels (in red) indicate the number of sowing requests reported by nursery.

Nurseries also provided comments on seed treatments performed prior to sowing which fall into three main groupings: A. No treatments (or no indication provided); B. Hydrogen peroxide soak prior to sowing and C. Soak prior to sowing, and the results were very interesting. For those that performed a hydrogen peroxide treatment the germination falldown was only 3.8% (67 SRQ's), but the soak only (65 SRQ's) and No Treatment (24 SRQ's) options produced falldowns of 12.9% and 12.6% respectively. The same nursery specific caveats exist regarding seedlot distribution and the specific means of estimating germination.

The timing of germination evaluation is also important, especially with a species like Pw that displays a large variability in dormancy and germination speed. An early assessment may quantify a large falldown, but the same seed evaluated later may indicate a much smaller or no falldown. Some of the pictorial feedback received also points to issues far beyond initial germination, although the scope of results we provide are to the point of the radicle being 4X the length of the seed coat. The nursery germination is a vital component of the reforestation program, but a large part of germination variability appears to be at the nursery level.

Discussion

Germination of western white pine is maximized under standardized conditions with a minimum quantity of seed. This provides the germination result presented on SPAR and used to calculate grams of seed required to produce a given quantity of seedlings. The germination results based on operational quantities of seed (SRQ's) prior to shipping and germination at the nursery, have not (on average) been able to meet these standard test results. This isn't new and various operational adjustments have been

put in place to try and synchronize the effectiveness of the stratification treatment including non-destructive moisture content estimation and targeting, incorporating an intensive monitoring program, reducing stratification unit size, and prioritizing the species for QA testing prior to shipment.
For the 43 SRQ's in which we had all three germination estimates the difference between our operational SRQ results at shipping and nursery germination results were quite similar. Continued improvement efforts to synchronize germination will focus on how to provide similar environmental conditions for small units (100 seeds) in the Standard tests compared to up to 36 000 seeds on average for an operational stratification unit. The germination falldown did not seem related to the stratification unit size and I see no reason to reduce this.

For the 2004-2008 sowing season the average nursery falldown for all species based on 978 sowing requests was 2.5%. This is certainly what I would consider acceptable and well within sampling variability. Conifer seedlots are highly variable; not surprising as our seed is close to the wild state compared to most nursery crops and the species are long-lived. It is unreasonable to expect that there is no error in the estimate of germination. A quantification of germination test precision was done in 2002 and for PW the average germination was 82.4% but the 95% confidence interval ranged from 76.1% and 88.1% or a GC value plus or minus 6.3%. Even our most uniform species, PLI has a GC% plus or minus 3.8%!

No consistent trends were observed in germination falldown by seedlot or orchard facility, but large variability was observed at the nursery. Based on feedback received from nurseries the use of hydrogen peroxide appeared to reduce the germination falldown. It should be noted that some nurseries performing hydrogen peroxide treatments still had large germination falldowns with some sowing requests – it isn't a guarantee, but it sure seems to help based on data provided. Currently this treatment seems more like a nursery philosophy decision as we have some seedlots without any *Fusarium* being treated at some nurseries whereas at other facilities seedlots with as high as 21.4% contamination have not been treated at all. I don't think the treatment can be harmful, although cost effectiveness can be debated, but seedlots with high *Fusarium* levels being sown untreated is not good practice. *Fusarium* is emphasized as the hydrogen peroxide will only reduce this, and other, seed-borne contaminants, but will not reduce internal fungi like *Caloscypha* or *Sirococcus*. *Caloscypha* is relatively rare in PW (5.4% of seedlots infected/ 1.8% average of infected lots/ 4.8% maximum infection rate observed), but its ability to grow under the cool, long stratification conditions of PW make it a fungus of concern. *Sirococcus* is not considered a problem with PW.

The Tree Seed Centre will continue to strive to improve the germination of operational quantities of Pw seed. A set of recommendations is provided below as the minimum effort we will put into this project. It should be recognized that this valuable species accounts for less than 0.5% of the seedlings sown each year in BC.

Recommendations

1. To compensate for the consistent reduction in germination using operational quantities of seed, at the TSC and at the Nursery, it is recommended that we increase the quantity of PW seed allocated to nurseries by 5% (i.e. a 1000 gram request will receive 1050 grams of seed). Note that this will change our recommended sowing guidelines, which many follow, but it is not a rule. If grams allocated are controversial, final decisions are based on discussions between the seedling request agency and the nursery.

2. Based on the apparent benefit of using hydrogen peroxide to reduce germination falldowns in PW this treatment is recommended. General recommendations for all species are a one to four hour soak in 3% Hydrogen Peroxide. Specific instructions are included in Appendix 4 (pages 102-104) of the Seed Handling Guidebook (Kolotelo *et al.* 2001). This is not a service the TSC offers or is planning on offering. The greatest benefit will be obtained by applying the treatment just prior to sowing.
3. A large part of our operational pretreatment is based on targeting the moisture content to values obtained from lab evaluations performed in 2002. This moisture target (target =37%:- 34% to 40% being the acceptable range) should be reviewed to confirm this target with current standard tests. Some of you may receive seed requests to help perform this review.
4. We have been testing approximately 38% (19% to 39%) of the sowing requests over the past 6 sowing seasons. It's a high sampling rate compared to the <1% applied to most other species, but we will strive for 40% of sowing requests moving forward with an emphasis on those seedlots from facilities not tested to date – Mt Newton and Dorena. This will be a general increase over the current "test every seedlot" guideline.
5. In 2012 we scaled back monitoring efforts to 2X per week and we will return to 3X per week monitoring. This includes observing for fungal growth, pre-germination, dessication, or water pooling and the redistribution of free moisture through stratification unit agitation with the bag closed.

References

Hoff, R.J. 1987. Dormancy in *Pinus monticola* seed related to stratification time, seed coat and genetics. Can. J. for. Res. 17:294-298.

Kolotelo, D. 2008. 2008 Sowing request Quality Assurance. TicTalk Vol 9(1):9-11.

Kolotelo, D.,E. Van Steenis, M. Peterson, R. Bennett, D. Trotter and J. Dennis. 2001. Seed Handling Guidebook. BC Tree Improvement Branch Publication. 106 pp.
also available as a pdf at bottom of this link: http://www.for.gov.bc.ca/hti/treeseedcentre/index.htm

David Kolotelo, RPF
Cone and Seed Improvement Officer
Dave.Kolotelo@gov.bc.ca
(604) 541-1683 extension 2228