Tree Improvement Branch

Cone and Seed Improvement Program
BCMoF Tree Seed Centre

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Yellow Cypress Germination Review

Executive Summary

This note is intended to review the recent performance of yellow cypresss (*Callitropsis nootkatensis*) due to substantial germination differences between standard lab test and Quality Assurance tests on sowing requests in 2013. Yellow cypress has had a variety of trials attempting to improve germination and these will be briefly reviewed. This species is unique in that it currently is not producing seed orchard seed and its inventories are relatively low at 3 to 4 years at current planting rates, but some of this demand is offset by rooted cuttings with attached genetic gain at a substantially greater cost. Research into appropriate orchard locations is progressing well and it is expected that seed orchard seed will be the main deployment vehicle in the future.

In contrast to western white pine (PW), I am not recommending an increase in seed allocation for yellow cypress at this time. I think that the G57 treatment is still relatively new (two seasons) and that we are still in the operational adjustment phase of introducing this pretreatment. I think there is a need to review our target moisture content levels, adjust maximum request stratification bag size from 600 to 450 grams, and to increase our QA sampling and adjust moisture maintenance practices in warm stratification. Seed owners are also encouraged to review inventories as only 2.7% of YC seed is indicated as surplus and many very small seedlots exist. An area being offered up to nurseries on a trial basis is the incorporation of a running water soak for 18 hours (overnight) for a single sowing request bag per request during cold stratification to test the effectiveness in leaching out chemical inhibitors that prevent germination.

Background

Yellow cypress (YC) has been the subject of a variety of trials to try and improve its germination due to its high value and relatively deep dormancy compared to most conifers. Unique among this species is its failure-to-date of providing good quality seed orchard produced seed and rooted cuttings have been the main deployment method for genetic gain. Research has identified temperatures at the low elevation orchard sites to be a limiting factor in producing good quality pollen and subsequently viable seed (Hak and Russell 2004). Research continues to move forward to produce yellow cypress seed in a more natural seed orchard production environment and improvements in pretreatment methodology still remain a priority. Seed inventories are relatively low at 3.2 million potential seedlings worth of seed distributed over 48 sedlots and having an average germination of only 53%.

In 1992, YC and western white pine (PW) had the same G52 pretreatment, but some trial work had already indicated some pretreatment requirement differences. As a PW germination review has just been completed and due to the similarity in original test, some comparisons with PW will be included in the text. The largest difference appears to be the greater importance of maintaining a warm stratification phase in the YC pretreatment. Removal of this warm phase resulted in poor germination even when providing interrupted warm treatment (8 hours per week) as a substitute. Similar to PW, YC trials indicated that the greatest germination benefit could be obtained by extending cold

stratification (Table 1). In 1995 we double-tested 17 seedlots with a new procedure that included 17 weeks of cold stratification, but this pretreatment was not adopted due to the high amount of pre-germination (26%) experienced during testing.

Starting in about 1996, yellow cypress, and its problems, became a good research topic for various trials. We managed to get some anatomical seed coat research done at the University of Oulu, Finland as they had demonstrated some expertise and interest in seed coat related problems (Tillman-Sutela and Kappi 1998). Simon Fraser University (SFU) also initiated some work to determine the underlying basis of seed dormancy in this species and specifically if embryo immaturity was an issue (Xia and Kermode 1998; Ren and Kermode 1999). The SFU group also initiated some work on chemical treatments and recommended a much shorter pretreatment, although results were based on only the very best seedlot available at the time (Xia and Kermode 2000). Chemical treatments were 'reproduced' at the TSC with traditional extended stratification and the chemical treatments performed relatively poorly, although pregermination was virtually absent in these treatments. Discussions with Dr. Kermode at SFU indicated chemical treatments need to be duplicated exactly, including the use of rotary shakers and greater temperature control – areas that are not easy to reproduce on the operational scale required.

We also tested the use of Accelerated Aging (AA) dishes which are currently used in this species with standard kimpack used for most other species. The total germination was the same, but the kimpack had slightly more pregermination and testing continues to be performed on AA trays. A meeting at SFU with the YC working group representing seed owners, nurseries and other interested parties was organized to discuss current status of research activities. The group was not advocating chemical treatments as they would be difficult to replicate operationally and may have WSBC implications at nurseries. It was also noted by those stratifying the species themselves that pregermination can occur and that YC germinants are relatively easy to transplant compared to most conifer species, but it is an additional cost.

Seed dormancy is only one part of the poor germination story with this species as many seedlots have a high proportion of non-viable seeds. These are rarely 'empty' and usually composed of a woody, resinous or tar substance that makes the density of these seeds similar to viable seeds and more difficult to separate during final cleaning. Most YC collections are older with only 7 of 48 seedlots collected after the year 2000, although three seedlots were collected in 2012. Most of the older seedlots have been processed by other facilities and we have not had many opportunities to improve processing efficiency with the species. There is a need for developing better processing methods with this species for excluding non-viable seed and improving seed-use efficiency in this valuable species. This may be relevant to new seedlots or the upgrading of existing seedlots.

Table 1. A historical overview of stratification changes and research efforts designed to improve dormancy breakage in yellow cypress.

Year	Test Type	Definition / Trial Findings / Changes
Pre-2011	**G52**	**48-hour soak, 28 days warm stratification and 56 days cold stratification**
1987	Trial	Initial research into long soak durations (up to 28 days) performed by Dr. George Edwards was promising, only one seedlot explored at this time

1992	Trial	Contract research indicated potential for Density Separation Processing (DSP) for germination upgrades. Hot water soaks attempted, but 50°C was too hot!
1992	Trial	TSC Pretreatment Trial 1: Keep Total treatment Time at 3 months - greatest improvements found by extending cold stratification; warm stratification is a requirement and interrupted stratification was a poor substitute for the warm phase. http://www.for.gov.bc.ca/hti/publications/misc/YcSSET93.pdf
1993	Trial	TSC Pretreatment Trial 2: Increase Treatment Time to up to 150 days – increased cold stratification (up to 120 days) produced germination gains. Recommendation for new pretreatment G54 comprised of 48-hour soak, 4 weeks of warm stratification and 17 weeks of cold stratification
1995	Trial G52/G54	Double Testing G52/G54 – 17 seedlots were tested with both treatments. Unfortunately the treatment was too good and resulted in a large amount of pregermination. Germination Results: G52=50%; G54 (without counting pregerminants) =38%; G54 (including pregerminants) = 64%. **New test type not implemented.**
1997	Trial	Seed Coat anatomy work with experts in Finland – passage of water mainly restricted by internal membranes rather than seed coat proper.
1997➔	Trial	Research at SFU – variety of trials dealing with underlying basis of seed dormancy in YC; chemical pretreatments and some associated work on seedling performance.
1999/2000	Trial	TSC Pretreatment Trial 3: Investigate placing seed directly on Kimpack vs. Accelerated Aging dishes and to test a few of the more promising chemical treatments advocated by SFU.
2002	Operations	Although a new test type was not implemented, operations switched to a 72-hour running water soak and moved warm stratification from the variable seed preparation area to a dedicated germination cabinet (20° C) with the targeting of seed moisture content to 44%. Monitoring of seed is performed 3X per week during warm stratification and twice weekly during cold stratification.
2011	Trial G52/G57	Double Testing G52/G57 – 41 seedlots tested with G52 and G57 treatments with a G57 gain of 17.3% in germination.
2011	Operations	Late in 2011 sowing season (for YC) the use of kimpack to maintain high moisture contents was replaced with bar towels. Reduces labour associated with monitoring and provides a cheap, more durable and reusable (after washing) media for YC pretreatment. Bar towels washed and bleached weekly.
2012	**G57**	**72-hour soak, 28 days warm stratification, 77 days cold stratification** introduced operationally.

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 G52 (up to 2011) and G57 (2012 and 2013) germination test result on SPAR which is used along 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 24-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 we try to sample each YC 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. For Yc, a few nurseries perform their own stratification and we have stratified only 48% of the sowing requests, but we have stratified 68% of the seed based on potential trees. Quality Assurance sampling is 25.3% of the entire YC seedling program and 53% of the sowing requests that we stratified in the period of 2008 to 2013.

The Standard and Quality Assurance germination tests provide a seedlot-specific estimate of the falldown experienced by going from standard lab testing with 100 seeds per dish to a bulk SRQ bag of up to 600 grams (approximately 125 000 seeds). Both the standard and QA tests are performed under controlled conditions with a standardized evaluation process. Fungal assay testing is not a priority for any of our significant pathogens in YC and although one in four seedlots (26.1%) showed *Fusarium* spp contamination the mean of the contaminated seedlots was only 0.4%. *Fusarium* spp. are seed-borne, but 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 the degree and direction of germination differences to prioritize areas needing improvement. One limiting factor is the imbalance 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

Yellow cypress Standard and Quality Assurance (QA) germination results are presented in Figure 1 with an estimated falldown of 16.4% in 2013. This is the main rationale for this review at this time. There were no operational pretreatment changes to explain this increase from the 7% falldown value in 2012 which is close to the six-year average falldown of 7.6%. Only four quality assured seedlots were common between these two years and looking at them only increases the yearly falldown difference with 7.5% estimated in 2012 and 19.2% estimated in 2013. The number of sowing requests sampled is relatively low due to the low number of total requests and many of them are stratified at the nursery. The increased germination results for 2012 as compared to 2011 are indicative of the change from our G52 to G57 pretreatment (Figure 1).

The moisture content varied very little with yearly averages ranging from 44.2% to 45.9% between 2008 and 2013. There does not seem to be a relationship between stratification moisture content and germination capacity at least with moisture contents above 44% which is our current target (Figure 2). It is much easier to uniformly dry than to moisten a sowing request and the 44% target is implemented as a minimum. Similar to PW it will be worthwhile reviewing this target moisture content in relation to

moisture levels obtained during standard testing where higher germination is obtained. It is most likely that this variation in stratification moisture content is related to the proportion of viable seeds as this varies much more in YC than in other species. This high moisture level requirement is a consistent factor with everyone who has had success pretreating the species. The actual stratification unit (bag) size did not appear to impact germination capacity, but it seemed easier to maintain higher moisture levels in the smaller quantities of seed. This observation was further reinforced when stratification bag size was plotted against TSC germination falldown (Figure 3) indicating that the largest germination falldowns were associated with stratification units above 450 grams in size (red line).

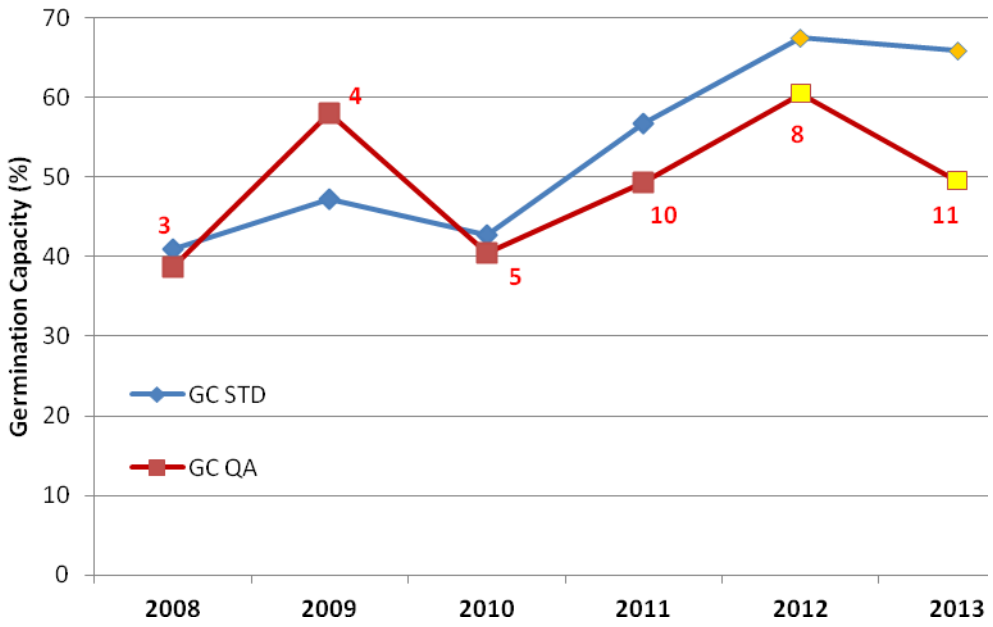

Figure 1. Comparison of average Standard germination capacity results (GC STD) with average Quality Assurance results (GC QA) for yellow cypress between 2008 and 2013. Numbers in red indicate the number of sowing requests tested each year; Yellow points indicate G57 and blue or red points are G52 results.

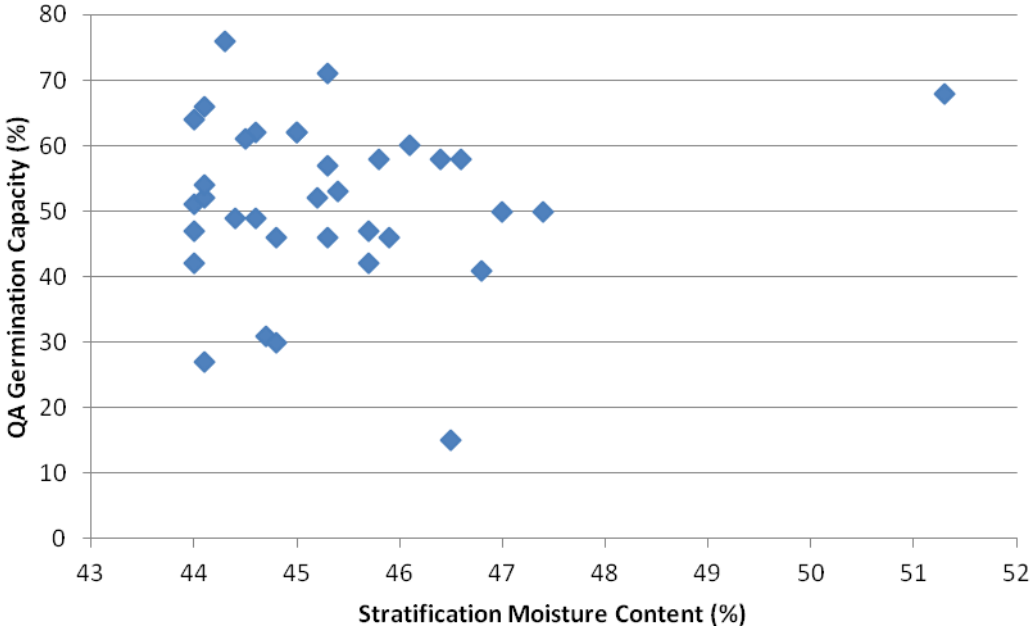

Figure 2. The relationship between Stratification Moisture Content (%) and QA germination capacity for sowing requests tested between 2008 and 2013.

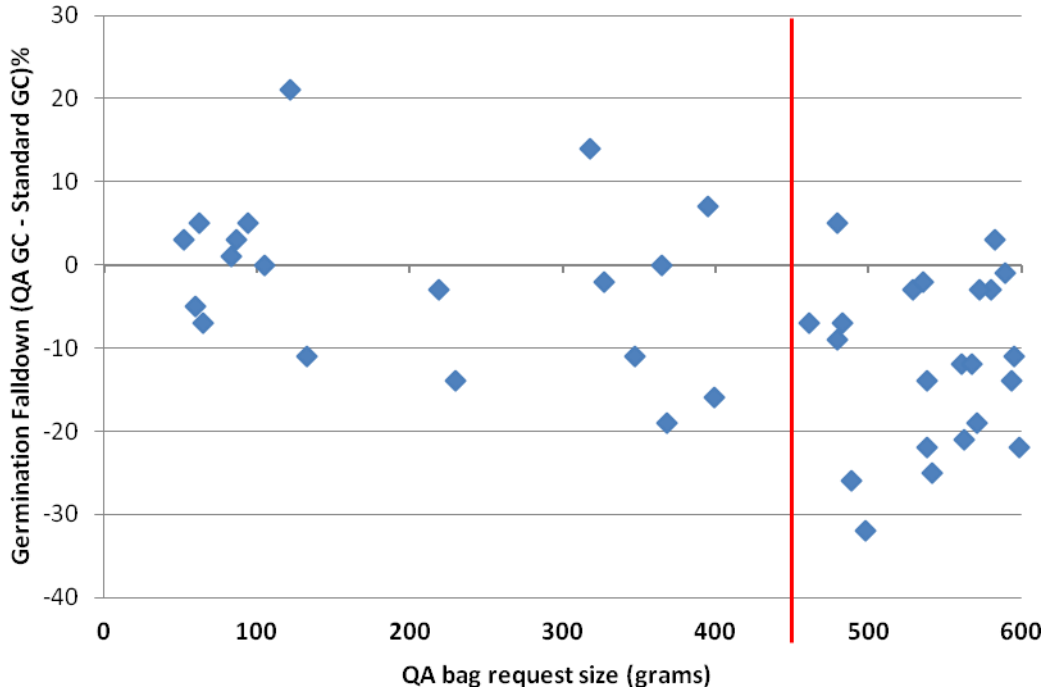

Figure 3. The relationship between sowing request bag size and germination falldown at the Tree Seed Centre (QA minus Standard GC%).

Nursery Results

Nursery germination information was supplied for 56 out of a total of 162 sowing requests between 2008 and 2013. The average nursery falldown on these SRQ's was 10% lower than Standard GC result. For the 24 SRQ's (only 15% of 2008-2013 requests) with all three germination results the average results are Standard GC = 61.0%; QA GC = 47.9% and Nursery GC = 54.8%. Put another way, the falldown from Standard results of these requests was 13.2% going to operational quantities of seed, but only 6.2% in comparison with nursery germination. This 6.9% increase in germination at the nursery compared to when leaving the TSC is most likely the result of extended stratification prior to sowing. No nurseries indicated the incorporation of additional seed treatments like hydrogen peroxide which is commonly employed with PW. YC does not have any significant pathology issues.

Variation in germination falldown between nurseries was high, although this could simply be a function of the specific seedlots being grown at specific nurseries as distribution is not random. It may also be due to differences in timing of germination counts with one nursery indicating they probably performed their counts too early before germination had completed. As has been standard practice, we do not publicly disclose nursery identity when presenting nursery germination information. I am grateful for the information they have been willing to share for this review – Thank you.

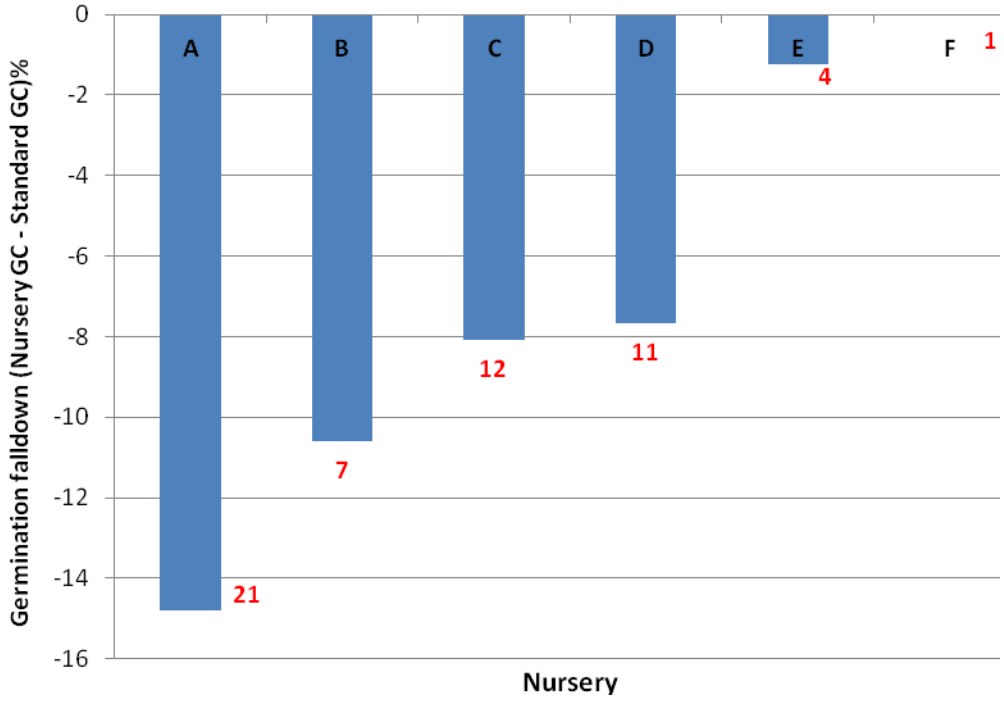

Figure 3. Nursery variation in germination falldown for sowing requests grown during the 2008-2013 sowing seasons. Data labels (in red) indicate the number of sowing requests reported by nursery.

Discussion

There is a significant falldown (>10%) in germination by increasing seed quantities from lab quantities to operational quantities of seed used for meeting sowing requests. For most species this increase does not impact germination, but most species also have limited seed dormancy which is usually overcome with 3 to 4 weeks of cold stratification. For our deeper dormant species (YC, PW, some *Abies* spp.) there appears to be more exacting stratification requirements in terms of moisture regimes, maximum unit size and in the case of YC the need for a warm phase.

The perplexing issue for us is why the 7% 2012 TSC falldown increased to 16.4% in 2013 as there were no changes introduced to operational stratification for YC. This was not simply a case of different seedlots being grown as nine of the thirteen seedlots sown in 2013 were sown in 2012. The actual stratification moisture content itself did not appear to have an impact on germination and the stratification unit size did not appear to effect moisture content or germination capacity. The interesting result was that most, but not all, of the germination falldowns occurred with stratification units above 450 grams in size (Figure 3). Our deeply dormant species seem to need greater control of actual conditions which appear to be facilitated by reducing stratification bag size. For most species stratified for 3 to 4 weeks stratification units can be as large as 3000 grams.

I do not see chemical treatments as being a practical solution in the near term. We have very little information on how these treatments perform over a range of seedlots and the required controls (i.e. rotary shakers, water temperature control to 1°C) are not practical at this point in time. Current stratification procedures are expensive at $118 per request (http://www.for.gov.bc.ca/hti/treeseedcentre/tsc/fees.htm) and it is expected this cost would increase substantially if we needed to incorporate these modifications into operations. The one area that has some promise and is cheap is the introduction of a soak treatment during cold stratification to leach inhibitors from the seed and overcome dormancy. I think this is worthwhile investigating, but I have two concerns:
1) That it will work too well and germination will be initiated prior to the intended sow date.
2) That testing this on a small germination test scale will not be indicative of gains achieved with operational quantities of seed.

In relation to the second concern, I am at this time suggesting that we test this additional opportunity on operational sowing requests. I understand that this brings some uncertainty to nurseries, but I'm not convinced that small lab scale results will be indicative of operational germination rates. Additional YC trial work is not planned (other than moisture content quantification) in the near future and this is a potential area for gain, and so the offer presented in the recommendations is being made available to nurseries.

Recommendations

1. At this point I am not recommending an increase in seed allocation for YC. This is based on the recent introduction of a new pretreatment in 2012 and the lag associated with adapting it to operational SRQ's , a relatively small sample size available for this analysis and probably most significantly the lack of a substantial seed inventory for this species and expectation that we will not see seed orchard crops for several years.
2. A large part of our operational pretreatment is based on targeting the moisture content to values obtained from lab evaluations performed in 2000. This moisture target (target =44%) should be

reviewed to confirm this target with current standard tests. Some of you may receive seed requests to help perform this review.
3. The maximum sowing request bag size should be adjusted from 600 grams to 450 grams to reduce the germination falldown by allowing greater control of stratification conditions (see Figure 3).
4. During operational warm stratification the water should be exchanged between seedlots for the re-soaking of bar towels used to maintain moisture levels. Although pathology has not been indicated as a concern, this is a fairly simple risk-reduction technique.
5. The Tree Seed Centre should review existing processing information and available techniques to increase the proportion of viable seeds in YC seedlots. This may be relevant to new collections or to attempts to upgrade existing seedlots.
6. Seed owners should review inventories as only 2.7% of the seed is designated as SURPLUS and available for sale. There are many very small seedlots and if the current owners don't plan to use them they will stay there forever reducing the amount of seed available for reforestation efforts.
7. This last recommendation may be more of an opportunity than a recommendation. There may be a benefit to introducing a soak treatment during cold stratification to leach out germination inhibitors. I'm suggesting that for one stratification bag per sowing request we would perform a running water soak overnight (18 hours) after seven weeks of cold stratification. This offer is for a maximum of three sowing requests per nursery. This will allow an operational comparison on whether this treatment has any operational benefit. The cost to you – the results of a minimum of three germination blocks for each treatment type. The associated risk would be a substantial increase in the amount of pregermination encountered.

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