Tree Improvement Branch

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Imbibition Trial

A study of seed imbibition in germination dishes was carried out in three laboratories: Alberta Land and Forest Service Seed Centre (AB), BC Ministry of Forests Tree Seed Centre (BC), and the Canadian Forest Service, National Tree Seed Centre (NB) with two germination dish types (PNFI and WEST[1]). The objective of the trial was to investigate the uptake of moisture in the two germination dishes commonly used in Canada. Specific questions were 'How long does seed imbibition take in germination dishes?'; 'Is there a significant difference between dish types?'; and 'Is there a significant difference between laboratories?'. Information on the distribution of the variance across the remaining random factors (e.g. units of replication, number of seedlots etc..,) should also indicate where improvements could be made in testing efficiency.

Many facilities do not soak seed prior to stratification or germination testing and the time required for moisture uptake in dishes was not well documented. The moisture status of seed imbibed in germination dishes will also be compared to seed soaked in vials for 24 hours. This study is the first part (imbibition) comparing specific germination test methods used throughout Canada. It is anticipated that a subsequent study will focus on the differences between the two main criteria used to quantify germination in Canada (vigour classes *vs*. seed length ratios).

Materials

The study was conducted on two species: interior lodgepole pine (*Pinus contorta* Dougl. ex Loud. Var. *latifolia* Engelm.) and white spruce (*Picea glauca* (Moench) Voss) and analyses is presented separately for each species. The trial incorporated four seedlots of each species (two from BC and two from AB) to sample geographic variability in each species. The two germination dishes were prepared at all labs according to procedures used in BC (West dishes) and AB and NB for PNFI dishes (Wang and Ackerman 1983) as presented below:

<u>WEST Germination Dish Preparation</u>
1) place one piece of kimpack (cellulose wadding paper) in germination dish
2) add 50 ml of water
3) squish kimpack to distribute moisture and provide uniform surface
4) place filter paper on kimpack

<u>PNFI Germination Dish Preparation</u>
1) pour 125 ml of water into bottom tray
2) place grid into bottom tray
3) put pre-cut kimpack on top of grid
4) pour 125 ml of water evenly over kimpack

[1] West dish is the terminology used to define a square dish 4 5/16" X 4 5/16" X 1 1/8"used to hold one germination test replicate. The PNFI dish is the dish marketed by Spencer-Lemaire Ltd. and developed by Ben Wang which houses four germination replicates. These two dishes were the two most commonly used for germination testing in Canada (see Tree Seed Testing Survey article in this issue).

Methods

Seed was counted into 100-seed replicates and initial fresh weight was measured and recorded. Replicates were randomly assigned to dish type (i.e. four replicates per dish type) and position at each laboratory and unimbibed seed spread onto each dish (or position within dish for PNFI dishes) so that adjacent seeds were not touching. Seed was removed from the dishes and weighed at intervals of 24, 48, 72 and 96 hours and promptly returned to the dish after weighing to avoid drying the seed. After the 96–hour measurement the seed was surface dried to provide an estimate of internal moisture content. After final measurements were recorded the seed was placed into a convection oven at 103º C for 17 hours to obtain the oven-dry weight of seed. The moisture content (MC) was calculated using the fresh weight at each time interval and the final oven-dry weight. Due to differences between seedlots in initial seed moisture content the variables to be analysed were the amount of moisture taken up after 24, 48, 72 and 96 hours (i.e. MC after 24 hours imbibition minus initial MC).

To compare seed imbibed in dishes with those imbibed in vials an additional four replicates were weighed and then imbibed in vials for 24 hours. The seed was then drained to remove excess moisture, weighed and placed into a convection oven at 103º C for 17 hours and then weighed to determine the oven-dry weight. Moisture content of the seed was then calculated.

Analysis

The trial is considered a split-split plot design as there are two restrictions on randomization: first the eight replicates are randomly assigned to the two dish types within each laboratory, then the four seedlots are randomized within each combination of laboratory, dish type and replicate. For more information on split-split plot designs refer to Hicks (1982) page 273. The trial was designed to determine the significance of laboratory, dish and the interaction of these two factors on moisture uptake and are considered fixed effects in the model. All other effects are considered random effects and the proportion of variance each source contributes to the total variance is presented. Percentage of variance for each effect is calculated as the estimated variance component divided by the sum of all variance components, multiplied by 100. The replicate factor was considered nested within laboratory as replicates are specific to laboratory. The model for the analysis is therefore:

$$MC_{@\ x\ hours} = \mu+L_i+R_{j(i)}+D_k+LD_{ik}+DR_{kj(i)}+S_l+LS_{il}+SR_{lj(i)}+DS_{kl}+LDS_{ikl}+DSR_{klj(i)}$$

where L_i is the effect of the ith Lab; $R_{j(i)}$ is the effect of the jth rep nested within the ith Lab; D_k is the effect of the kth dish; LD_{ik} is the effect of the interaction between the ith Lab and kth dish; $DR_{kj(i)}$ is the effect of the interaction between the kth dish and jth Rep within the ith Lab; S_l is the effect of the lth seedlot; LS_{il} is the effect of the interaction between the ith Lab and lth Seedlot; $SR_{lj(i)}$ is the effect of the interaction between the lth seedlot and jth rep within the ith Lab; DS_{kl} is the effect of the interaction between the kth dish and lth Seedlot; LDS_{ikl} is the interaction between the ith Lab, kth dish and lth Seedlot; $DSR_{klj(i)}$ is the effect of the interaction between the kth Dish, lth Seedlot and jth Rep within the ith Lab.

The results are presented in terms of significance of effects for fixed factors (L, D and LD) and in terms of proportion of remaining variance explained by the remaining random factors. Analysis was performed using PROC MIXED in SAS (SAS Institute Inc. 1997) Probability values are given for fixed effects, but $\propto$ =0.05 was chosen as the level to specify statistical significance of

differences among the means. The help of BC Ministry of Forests Biometrician Peter Ott with the statistical analysis is graciously acknowledged.

Statistical Results

The probability values for the fixed effects are presented in Table 1 for both species and all time intervals. The Lab effect was statistically significant for all variables examined and the dish effect was not statistically significant for any of the variables. The interaction term (LD) was statistically significant for all variables except for the estimate of internal moisture following 96 hours of imbibition (MCSD). The two species had identical patterns in terms of statistical significance of the variables and effects.

Table 1. The probability values for the statistical significance of fixed effects. Results are presented for lodgepole pine and white spruce after 24 (MC24), 48, 72, and 96 hours of imbibition and following surface drying the seed after 96 hours of imbibition (MCSD). Results significant at α =0.05 are bold and shaded.

	Lodgepole Pine					White spruce				
	MC24	MC48	MC72	MC96	MCSD	MC24	MC48	MC72	MC96	MCSD
Lab	**.0165**	**.0089**	**.0209**	**.0059**	**.0004**	**.0088**	**.0028**	**.0024**	**.0063**	**.0055**
Dish	.0886	.4480	.8855	.2261	.4600	.5175	.4057	.1914	.1068	.9859
LD	**.0014**	**.0005**	**.0013**	**.0003**	.0804	**.0011**	**.0015**	**.0010**	**.0019**	.7560

The majority of the non-residual variance is explained by the Rep*Dish interaction for the variables which were not surface dried, but disappeared (estimated at zero) for the surface dried moisture content (Table 2). The total amount of variance was about one-third for the surface dried samples (2.50 vs. 7.65) on average. The error term explained a large percentage of variation for all variables ranging from 24.0% to 78.7%. The seedlot component explained a small proportion or none of the variance in the non-surface dried estimates. For the surface dried samples the variance percentage estimates for seedlots were 7.9 % for white spruce and 50% for lodgepole pine (Table 2).

Table 2. Percentage of variance for each of the random effects. Results are presented for lodgepole pine and white spruce after 24 (MC24), 48, 72, and 96 hours of imbibition and following surface drying the seed after 96 hours of imbibition (MCSD). Sum of Variance components is bold and shaded.

	Lodgepole Pine					White spruce				
	MC24	MC48	MC72	MC96	MCSD	MC24	MC48	MC72	MC96	MCSD
Rep	0.0	0.0	0.0	0.0	0.0	18.6	0.0	9.0	0.0	2.0
RD	37.0	40.5	44.0	24.3	0.0	27.7	34.4	28.1	27.7	0.0
S	0.0	0.0	0.0	4.1	50.0	3.9	0.0	0.0	0.0	7.9
RS	5.6	4.8	10.0	2.4	3.4	0.0	0.0	2.5	0.0	0.0
DS	7.8	1.7	0.3	3.0	0.0	0.1	0.0	0.0	0.0	0.0
LDS	13.4	10.3	21.7	17.4	0.0	0.0	15.3	9.2	7.7	11.3
Error	36.2	42.7	24.0	48.8	46.6	49.7	50.3	51.1	64.6	78.7
Total	**8.94**	**7.23**	**8.03**	**7.40**	**2.94**	**11.13**	**6.54**	**5.66**	**6.28**	**2.05**

Graphical Results

To further examine the significant Lab*Dish interaction the averages were plotted by dish type over all three labs for white spruce (Figure 1) and lodgepole pine (Figure 2). Data on surface dry seed, after 96 hours imbibition, are not included in these averages as the interaction was not significant for this variable.

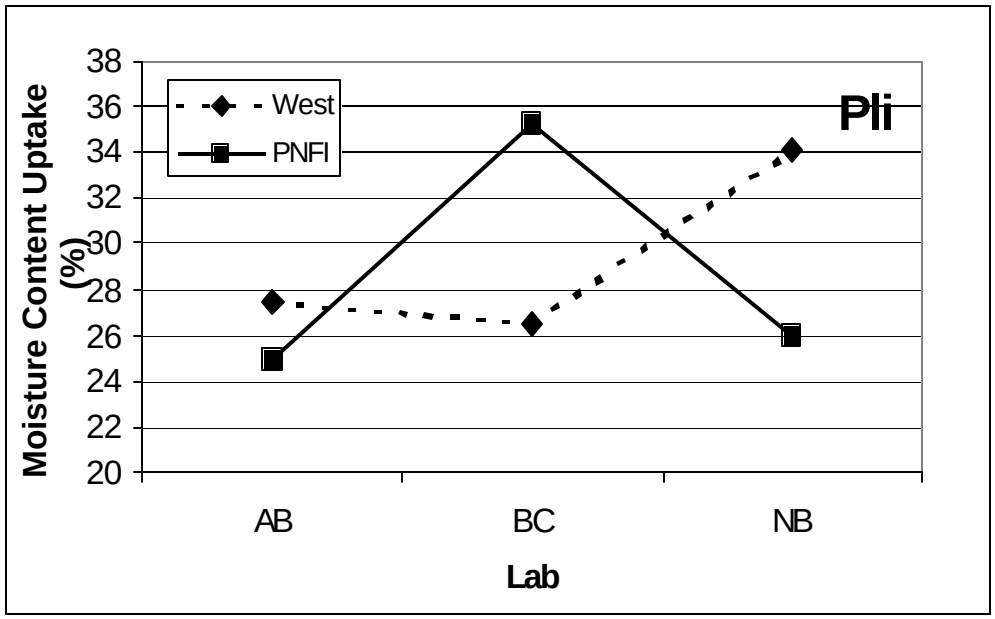

Figure 1. The graphical representation of the average Lab*Dish interaction for lodgepole pine.

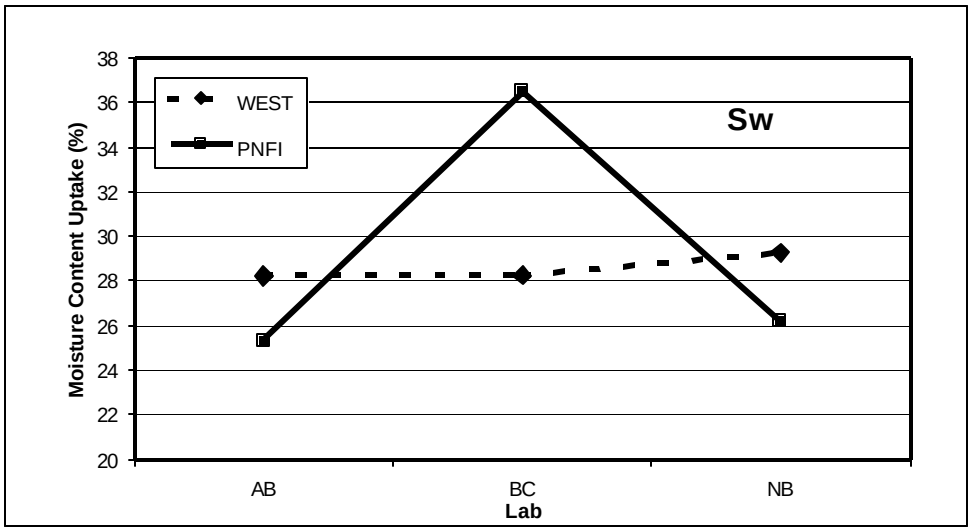

Figure 2. The graphical representation of the average Lab*Dish interaction for white spruce

The moisture uptake curves for lodgepole pine and white spruce, for each lab, are presented in Figures 3 and 4, respectively. Imbibition from germination dishes was rapid reaching levels of approximately 30 to 35% after 24 hours. The corresponding moisture contents following 24-hour vial soaking were 36.8% for lodgepole pine (lab estimates ranged from 34.3 to 38.7%) and 35.7% for white spruce (ranging from 33.6 to 38.6%).

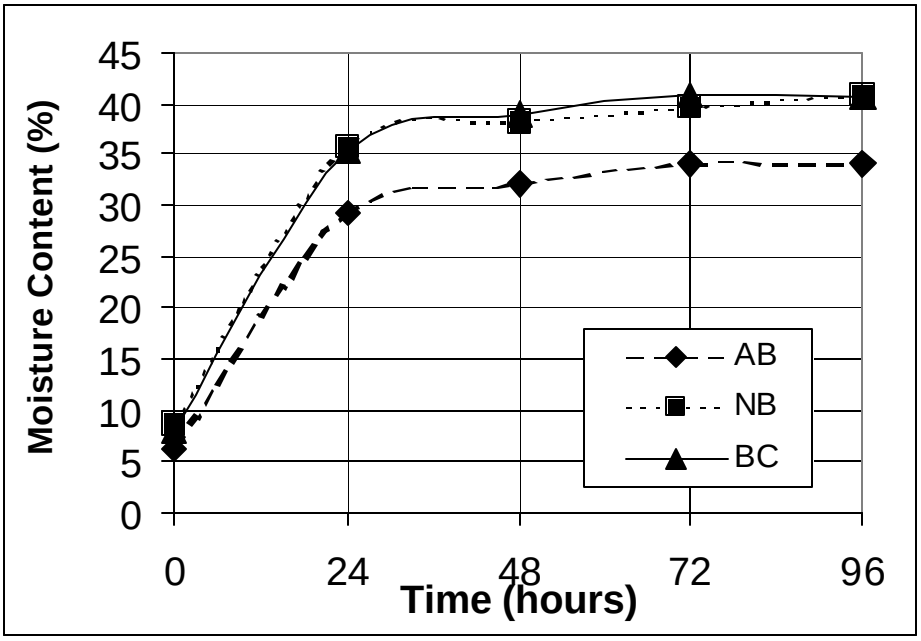

Figure 3. The moisture uptake curves for lodgepole pine over all three laboratories.

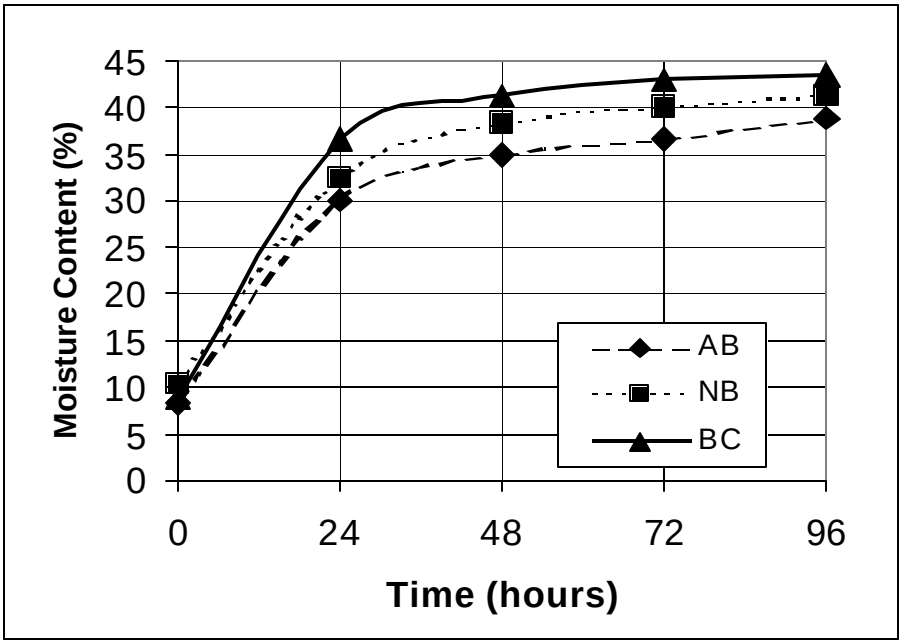

Figure 4. The moisture uptake curves for white spruce over all three laboratories.

Discussion

The Lab effect was statistically significant for both species at all time intervals (including surface dried seed). The Dish effect was not statistically significant, but the interaction of the Lab*Dish was significant for all time intervals that did not incorporate surface drying making it difficult to clearly interpret the main effects. A further examination of this interaction showed that for lodgepole pine the interaction was mainly the result of high moisture contents in the BC lab for PNFI dishes and high in the NB lab for WEST dishes (Figure 1). These dishes are not the regular dishes used in the respective labs and this is the probable reason for the interaction. For white spruce the Lab*Dish interaction seemed solely caused by the high moisture content for the PNFI dish at the BC lab. For both species the surface drying of the seed following 96 hours of imbibition resulted in the interaction effect being non-significant and greatly reducing the total amount of random variation (Table 2).

The interaction appears to be mainly accounted for by different handling practices of the labs over the two dishes. Although the statistical significance of the dish is unclear, due to the significant Lab*Dish interaction, the practical significance of differences between the two dishes appears quite small. For lodgepole pine the average moisture content for the WEST dish is 29.3% and 28.7% for the PNFI dish. For white spruce, the average moisture content for the WEST dish is 28.6% and 29.3% for PNFI dishes. These differences are relatively small compared to lab differences presented in Figures 3 and 4. Laboratories can provide quite different estimates of moisture content using very similar techniques. This is partly explained by familiarity with certain techniques and the difficulty in quantifying something that is changing: the moisture content of the seed as it equilibrates to ambient conditions. Differences between labs in quantifying moisture content have also been observed to occur when more precise and sophisticated methods are used (i.e. liquid distillation methods) (Ben Wang, pers. comm. Aug. 2000).

For the non-surface dried moisture contents, the Rep*Dish interaction accounted for a large proportion of the variation. When seed was surface dried the Rep*Dish interaction was estimated to have a variance component equal to zero for both species. Surface drying the seed greatly reduced the total amount of random variation for both species. It was uncertain before the experiment began on whether we should surface dry seed for each time interval. These results certainly indicate that surface drying the seed (with reasonable guidelines) could reduce the total amount of variation found and ‘clarify’ the statistical significance of the fixed effects. It is recommended for future studies on moisture uptake that surface drying occur to reduce the apparently large amount of variation attributed to surface moisture content. Variability is due to differences in moisture on the seed coat as well as moisture within the seed coat. We are mainly interested in moisture imbibed into the living tissues of the megagametophyte and embryo and surface drying the seed would provide a more accurate estimate of this.

Species differences were large when the seedlot variance was examined at the surface dried condition (Table2). For lodgepole pine this term accounted for half of the variance, but in white spruce seedlot only accounted for 7.9% of the variance. Interactions (Rep*Seedlot , Dish*Seedlot and Lab*Dish*Seedlot) also accounted for variability in moisture content, but clear trends across species or time intervals was not found. The replicate effect did explain 18.6% of the variation in white spruce after 24 hours imbibition, although it was estimated at zero for lodgepole pine. Further work looking at early imbibition may benefit by increasing the number of replicates used to increase the efficiency of the trial. The amount of residual variation was quite high and indicates a great deal of variability between cells (moisture content of a seedlot within a replicate,

dish type and lab). This may be due to different individuals within a lab performing the measurements, or differential drying of cells prior to fresh weight determination.

The amount of moisture taken up directly from the media was slightly lower than vial soaking after 24 hours. The moisture content after 24 hours is adequate to initiate dormancy breaking mechanisms and the imbibitional delay is considered not important from a practical point-of-view. The BC lab currently soaks seed prior to testing and for lodgepole pine and white spruce it appears that this step can be eliminated and the seed allowed to imbibe moisture directly from the media. This would also allow for the use of the seed vacuum to count and place seeds into germination dishes. Prior to changing the methodology in the BC lab, the imbibitional delay will need to be quantified for other species routinely tested in BC, especially species with soak durations greater than 24 hours (Amabilis, grand and subalpine fir; yellow-cedar; and western white pine).

References

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