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Understanding lodgepole pine seed germination for improved utilization

Y.A. EL-KASSABY¹, D. KOLOTELO² AND D. REID³

¹ Department of Forest Sciences, Faculty of Forestry, The University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada (E-mail: y.el-kassaby@ubc.ca)

² Tree Seed Centre, Tree Improvement Branch, British Columbia Ministry of Forests and Range, Surrey, British Columbia, V4P 1M5 Canada

³ Saanich Seed Orchards, Tree Improvement Branch, British Columbia Ministry of Forests and Range, Saanichton, British Columbia, V8M 1W4 Canada

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Summary

Germination behaviour of 18 lodgepole pine (*Pinus contorta* var. *latifolia*) seed orchard clones collected over two years were assessed to evaluate the extent of variation among clones and between years. For each year, seed from individual clones were tested using a modified International Seed Testing Association (ISTA) germination protocol that consisted of control (unstratified) and one stratification (4 weeks pre-chilling) treatment. Stratification effect was overwhelming and shadowed the effect of clone, year and their interaction. The role of clones, years, and their interaction were assessed on unstratified and stratified tests separately. The subsequent analyses highlighted the significant role of clones (genetics) and the non-significant effect of years (environment), indicating that seed source genetic background plays a major role in controlling germination behaviour and the possibility of predicting clonal performance over years. The results demonstrated the effectiveness of stratification on minimizing clonal dormancy differences resulting in the production of higher and faster germination. The impact of stratification treatment length (1 to 5 weeks) was further investigated on composite seedlots from each collection year and the results were contrasted and compared to the procedure recommended by the ISTA (3 weeks). Extending the stratification treatment to 5 weeks resulted into a 2% increase in germination capacity and produced more prompt germination. The observed increase in germination could be translated to additional seedling production if nursery sowing factors accommodate this increase.

Introduction

The lodgepole pine (*Pinus contorta* Douglas ex Loudon var. *latifolia* Engelm.) natural stand seed inventory in British Columbia^{*} is expected to satisfy most of the artificial regeneration program needs for a period of up to 17 years. However, specific seed planning zones (SPZ) such as the Thompson Okanagan Arid (TOA) and Cariboo Transition (CT) zones have under less than a 10-year supply (Kolotelo, 2006). There has been a significant increase in planting of lodgepole pine resulting from a recent mountain pine beetle (*Dendroctonus ponderosae* Hopk. (Coleoptera: Scolytidae)) epidemic. Between

* Author for correspondence

1993 and 2003, average planting of lodgepole pine was 96 million seedlings per year and this increased to an average of 123 million seedlings between 2004 and 2007.

Future natural stand seed requirement estimates have a large degree of uncertainty due to the unpredictability of harvest levels, success of seed orchard crops, alternative species choices, degree of reliance on natural regeneration and the need to possess a seed supply to address potential catastrophic losses, especially from fire. Natural stand cone collections are also becoming more expensive due to the loss of serotiny in many dead stands causing the canopy “seed bank” to be liquidated before it can be collected. These uncertainties and increased costs point to the need for an efficient seed use utilization strategy of improved seed in order to maximize the use of available seed inventory, specifically for zones with critical seed supply as well as precious seed orchard seed.

All aspects of seed inventory (Kolotelo, 2006), seed biology such as germination environment (El-Kassaby *et al.*, 2002; Krakowski and El-Kassaby, 2005) and seed pre-treatment (ISTA, 2006), commonly applied nursery sowing factors (Richter and Switzer, 1982; British Columbia Ministry of Forests and Range, 2008), and nursery risk management require re-evaluation, so the maximum number of plantable seedlings is produced. In the present study, the commonly prescribed seed pre-treatment for lodgepole pine is evaluated and a modification associated with germination improvement is recommended.

Materials and methods

Seed source and germination

Individual wind-pollinated seedlots from 18 lodgepole pine (*Pinus contorta* var. *latifolia*) orchard parents (families) from the 2001 and 2002 crops provided the seed for this study. Seed germination protocols used followed those established by the International Seed Testing Association (ISTA 2006) with one modification involved extending the recommended stratification period from three to four weeks. Seed, stratified and unstratified, received one day soak in water followed by surface drying prior to germination. Stratification treatment consisted of storage at 4°C for 4 weeks and germination for both stratified and unstratified (pre-soaked) treatments was run at alternating temperature between 30°C for 8hr day and 20°C for 16hr night (light, at $\approx 13.5 \mu\text{mol.m}^{-2}\text{s}^{-1}$ was provided during the day by cool-white fluorescent tubes). For each family within year, a dual germination test (i.e., unstratified (control) and stratified) were performed and each test was presented by four replications of 100 seeds. Germinants count was conducted daily and cumulative germination was determined for each replication-family-treatment-year combination. A seed was considered as a germinant when the emerging radicle was four times the length of the seed. The germination test and seed count continued until the end of the 21-day testing period. To explore the role of stratification period on seed germination, variations of the ISTA's recommended 3-week stratification treatment was conducted on two bulk seedlots representing a composite sample of equal representation of the 18 families' yearly collections with stratification periods ranging from 1 to 5 weeks.

Germination parameters and data analyses

Cumulative germination data for every replication-family-treatment-year combination were curve fitted to the 4-parameter Hill function following El-Kassaby *et al.* (2008). Four germination parameters; namely, GC (asymptote = germination capacity), R_{50} (time required for 50% of viable seeds to germinate which is a representative of germination speed (Thomson and El-Kassaby 1993)), lag (time at germination onset (El-Kassaby *et al.*, 2008)), and D_{lag-50} (duration between lag and R_{50} and is a representative of promptness and germination uniformity (El-Kassaby *et al.*, 2008)), and DI (dormancy index: quantitative expressions of dormancy (Richter and Switzer 1982)) were also estimated from the curve fitting data following El-Kassaby *et al.* (2008). The significance and percent contribution of the experimental variables were determined using ANOVA following this additive linear model:

$$Y_{ijkl} = \mu + F_i + S_j + Y_k + FS_{ij} + FY_{ik} + SY_{jk} + FSY_{ijk} + \epsilon_{(ijk)l} \quad [\text{Eq. 1}]$$

where:

Y_{ijkl} is the observation within the l^{th} replication, i^{th} family, j^{th} stratification, and k^{th} year,

μ is the overall mean,

F_i is the effect of the i^{th} family ($i = 1$ to 18, random),

S_j is the effect of the j^{th} stratification treatment ($j = 1$ to 2, fixed),

Y_k is the effect of the k^{th} year ($k = 1$ to 2, random),

FS_{ij} is the effect of the i^{th} family x the j^{th} stratification interaction,

FY_{ik} is the effect of the i^{th} family x the k^{th} year interaction,

SY_{jk} is the effect of the j^{th} stratification x the k^{th} year interaction,

FSY_{ijk} is the effect of the i^{th} family x the j^{th} stratification x the k^{th} year interaction, and

$\epsilon_{(ijk)l}$ = the residual term ($l = 4$).

With the exception of germination capacity (square-root arcsine transformation), all parameters were analysed using their original values. Expected mean squares were calculated, components of variance were estimated and percentage contributions of random effects to the total variation were determined (table 1). With the exception of stratification (seed pre-treatment) effect, all variance components were directly tested using the appropriate error term (see E.M.S. table 1); however, testing the effect of seed pre-treatment, required the use of a pseudo F -test to construct the appropriate error term as follows:

$$\begin{aligned} \text{stratification error term (MS}_{EP}) &= MS_{SY} + MS_{FS} - MS_{FSY} \\ &= \sigma_e^2 + 4\sigma_{fsy}^2 + 72\sigma_{sy}^2 + 8\sigma_{fs}^2 \end{aligned}$$

with degrees of freedom (1 and r), where $r = (MS_{EP}) / (MS_{SY} + MS_{FS} - MS_{FSY})$

The results from the above linear model indicated that the effect of stratification treatment (fixed effect) and family (random effect) were highly significant ($P < 0.01$) while year (random effect) was not significant across all germination parameters studied (table 1). Twelve out of the 16 first and second order interactions were either significant

or highly significant (table 1), creating a situation where the main effects (family, stratification and year) could not be effectively evaluated. Thus, additional complementary analysis was conducted using an abbreviated version of the original linear models [Eq. 1], after the removal of the stratification (separate analyses for the unstratified and stratified germination tests (table 2)) as follows:

$$Y_{ijk} = \mu + F_i + Y_j + FY_{ij} + \epsilon_{(ij)k} \quad [\text{Eq. 2}]$$

where:

Y_{ijk} is the observation within the i^{th} family, j^{th} year and k^{th} replication,

μ is the overall mean,

F_i is the effect of the i^{th} family ($i = 1$ to 18, random),

Y_j is the effect of the j^{th} year ($j = 1$ to 2, random),

FY_{ij} is the effect of the i^{th} family \times the j^{th} year interaction, and

$\epsilon_{(ij)k}$ = the residual term ($l = 4$).

Expected mean squares were calculated, components of variance were estimated, and percentage contributions of the experimental variables to the total variation were determined (table 2).

Yearly dormancy index analysis was conducted using a simple 1-way ANOVA that intended to test family dormancy variation. For each family, dormancy index was estimated using the derived 4-parameter Hill function of both stratified and unstratified germination tests of each replication, thus no treatment (stratification) effect (table 3).

To test the effect of stratification duration on germination parameters including dormancy, two bulk seedlots (created after mixing equal proportions of the sampled 18 families) were used for each collection year (2001 and 2002). The stratification duration (Time) varied between 1 and 5 weeks (i.e., 5 stratification treatments, starting with 1 week and ending with 5 weeks) using the following additive linear model:

$$Y_{ijk} = \mu + L_i + T_j + LT_{ij} + \epsilon_{(ij)k} \quad [\text{Eq. 3}]$$

where:

Y_{ijk} is the observation within the k^{th} replication, i^{th} seedlot and j^{th} stratification duration,

μ is the overall mean,

L_i is the effect of the i^{th} seedlot (random, $i = 2$),

T_j is the effect of the j^{th} stratification duration (fixed, $j = 5$),

LT_{ij} is the effect of the i^{th} seedlot \times j^{th} stratification duration interaction, and

$\epsilon_{(ij)k}$ = the residual term ($k = 4$).

Expected mean squares for Eq. 3 were calculated and components of variance were estimated and percentage contributions of the experimental variables to the total variation were determined (table 4).

Table 1. Germination parameters ANOVA for the 18 lodgepole pine families' seedlots over seed pre-treatments and collection years. (source of variation (SOV), degrees of freedom (d.f.), expected mean squares (E.M.S.) and percent of total variance attributed to each random source of variation).

S.O.V.	d.f.	MS	E.M.S.	GC ^{1,2}		R ₅₀ ³	Lag	D _{lag-50}
				Variance component %				
Family (F)	17	MS _F	$\sigma^2_e + 8\sigma^2_{fy} + 16\sigma^2_f$	22.88**	34.28**	10.50**		17.32**
	1	MS _S	$\sigma^2_e + 4\sigma^2_{fsy} + 72\sigma^2_{sy} + 8\sigma^2_{fs} + 144\sigma^2_s$	--.3**	--**	--**		--**
	1	MS _Y	$\sigma^2_e + 8\sigma^2_{fy} + 144\sigma^2_y$	0.00 ^{ns,4}	0.00 ^{ns}	15.78 ^{ns}		0.00 ^{ns}
	17	MS _{FS}	$\sigma^2_e + 4\sigma^2_{fsy} + 8\sigma^2_{fs}$	36.95**	28.30**	0.00 ^{ns}		14.13*
F × Y	17	MS _{FY}	$\sigma^2_e + 8\sigma^2_{fy}$	7.90**	10.90**	3.30*		5.25**
S × Y	1	MS _{SY}	$\sigma^2_e + 4\sigma^2_{fsy} + 72\sigma^2_{sy}$	17.36**	10.28 ^{ns}	20.56 ^{ns}		35.65 ^{ns}
F × S × Y	17	MS _{FSY}	$\sigma^2_e + 4\sigma^2_{fsy}$	6.29**	7.81**	10.47**		11.10**
Residual	216	MS _e	σ^2_e	8.62	8.44	39.39		16.56

¹See text for parameters description; ²Germination % (GC) is arcsine transformed; ³Variance component percentages were not determined for treatment (fixed effect); ⁴Negative or very small (<0.0001) variance component; ^{ns}not significant; *significant at P<0.05; **significant at P<0.01

Table 2. Germination parameters ANOVA for the 18 lodgepole pine families' seedlots after the removal of the seed pre-treatments.

S.O.V.	d.f.	E.M.S.	Unstratified			Stratified				
			GC ^{1,2}	R ₅₀ [']	lag	D _{lag-50}	GC	R ₅₀ [']	lag	D _{lag-50}
			Variance component %							
Family (F)	17	$\sigma_e^2 + 4\sigma_{fy}^2 + 8\sigma_f^2$	77.92**	68.39**	17.37 ^{ns}	48.05**	0.00 ^{ns,3}	59.03**	17.36 ^{ns}	38.15*
Year (Y)	1	$\sigma_e^2 + 4\sigma_y^2 + 72\sigma_y^2$	0.87 ^{ns}	0.00 ^{ns,3}	2.12 ^{ns}	0.45 ^{ns}	0.00 ^{ns,3}	5.82 ^{ns}	0.00 ^{ns,3}	6.22 ^{ns}
F x Y	17	$\sigma_e^2 + 4\sigma_{fy}^2$	14.75**	19.62**	11.69 ^{ns}	18.95**	36.82**	25.29**	29.66**	29.33**
Residual	108	σ_e^2	6.46	11.99	68.81	32.55	63.18	29.03	52.99	26.30

¹See text for parameters description; ²Germination % (GC) is arcsine transformed; ³Negative or very small (<0.0001) variance component; ^{ns}not significant; *significant at P<0.05; **significant at P<0.01

Table 3. Dormancy index ANOVA for seedlots from 18 lodgepole pine families for the 2001 and 2001 collection years.

S.O.V.	d.f.	E.M.S.	2001	2002
			Variance component %	
Family	17	$\sigma_e^2 + 4\sigma_f^2$	93.35**	92.89**
Residual	54	σ_e^2	6.65	7.11

**significant at $P < 0.01$

Results and discussion

The combined ANOVA mixed model [Eq.1] indicated that seed pre-treatment effect (i.e., stratification) was highly significant ($P > 0.01$) across all germination parameters (% germination (GC), germination speed (R_{50}'), time at germination onset (Lag), and the duration between the time at germination onset and that at 50% germination (D_{lag-50})) (table 1). The use of the mixed-model, negated any quantification of the seed pre-treatment effect's variance component (i.e., quantifying fixed effects violates ANOVA assumptions); however, it allows only for assessment of its significance. Of interest is the drastic difference between the remaining main effects, where family (i.e., genetics effect) was highly significant for all germination parameters (range: 10.5 and 34.3% for Lag and R_{50}' , respectively) while year was non-significant (environmental effect) (table 1). The complex nature of this combined analysis and the fact that 4 out of the 16, 1st and 2nd order interactions were either significant or highly significant, necessitating the use of the abbreviated liner model [Eq. 2] to allow isolating the effect of each source of variation (table 2).

When seed pre-treatment effect was removed, the role of family (genetics) and year (environment) effects emerged and their interplay with the stratification treatment resulted in revealing some interesting observations:

1- Under no seed pre-treatment (i.e., unstratified/control):

a. Family effect was highly significant ($P > 0.01$) for:

- i. Germination percent, GC , accounting for the majority of variation (77.9%), producing overall yearly means of 52.9 (range: 12.6 and 88.6%) and 57.5% (range: 16.3 and 88.2%) for 2001 and 2002, respectively. The observed drastic GC mean difference among families, determined by the range values, is indicative of their dormancy variability (figures 1 and 2),
- ii. Germination speed, R_{50}' , amounting to 68.4% of the total variation (table 2) with overall yearly means of 9.8 (range: 8.4 and 12.2) and 9.9 days (range: 8.7 and 12.8) for 2001 and 2002, respectively, and again confirming the presence of greater family variability in germination speed (figures 1 and 2), and
- iii. Time between germination onset and R_{50}' , D_{lag-50} , harbouring 48.2% of the total variation (table 2), with yearly average of 4.2 (range: 3.0-5.7) and 4.4 days (range: 3.0-7.3) for 2001 and 2002, respectively, reflecting the significant variation observed for R_{50}' (figures 1 and 2).

Table 4. ANOVA for germination parameters (stratified treatment only) and dormancy index (*DI*) for 2 bulk lodgepole pine seedlots (representing 2001 and 2002 collection years) subjected to 5 stratification durations (time: 1 – 5wks).

S.O.V.	d.f.	E.M.S.	<i>GC</i> ^{1,2}	<i>R</i> ₅₀ '	<i>lag</i>	<i>D</i> _{lag-50}	<i>DI</i>
			Variance component %				
Seedlot (L)	1	$\sigma_e^2 + 40\sigma_l^2$	0.00 ^{ns,3}	3.22 ^{ns}	25.00**	1.36 ^{ns}	0.00 ^{ns,3}
Time (T)	4	$\sigma_e^2 + 8\sigma_{lt}^2 + 8\theta_t$	--*	--**	--ns	--**	--**
L x T	4	$\sigma_e^2 + 8\sigma_{lt}^2$	8.02 ^{ns}	0.00 ^{ns,3}	19.05*	0.67 ^{ns}	0.00 ^{ns,3}
Residual	30	σ_e^2	91.98	96.78	56.00	97.97	100.00

¹See text for parameters description; ²Germination % (*GC*) is arcsine transformed; ³Negative or very small (<0.0001) variance component; ^{ns}not significant; *significant at $P < 0.05$; **significant at $P < 0.01$

- iv. Unlike the three significant germination parameters listed above, time of germination onset (*Lag*) produced non-significant family effect indicating that germination of unstratified seed, while somewhat variable (17.4%), harboured extensive variation among each family's replications resulting into greater overlap among families (see 95% CI overlap; figures 1 and 2) with yearly mean of 5.65 (range: 4.8-6.7) and 5.45 days (range: 4.7-6.3) for 2001 and 2002, respectively. This observation is supported by the large variation residing within the residual term (68.8%) (table 3).
 - b. Year (environment) effect was striking, yielding no or very little variation (table 2), indicating that families had consistent performance over the two studied years and generalization could be made. Families tended to perform similarly over the two years and the results of their correlations were highly significant ($r = 0.83, 0.77, 0.69$, and 0.71 for *GC*, *R*₅₀', *Lag*, and *D*_{lag-50}, respectively).
- 2- Generally, the stratification treatment produced similar results to that observed under no stratification (above):
- a. Family effect was:
 - i. Highly significant ($P > 0.1$) for *R*₅₀' accounting for 59.03% of the total variation with overall means of 6.26 (range: 5.7-7.5) and 6.08 days (range: 5.5-6.8) for 2001 and 2002, respectively (figures 1 and 2).
 - ii. Significant ($P > 0.5$) for *D*_{lag-50} accounting for 38.15% of the variation with yearly mean of 2.28 (1.54-3.57) and 2.07 (1.41-2.77) for 2001 and 2002, respectively (figures 1 and 2).
 - iii. Surprisingly non-significant for both *GC* and *Lag*, indicating that the stratification treatment was extremely effective in producing high germination, thus harmonizing differences among families (table 2). Visual examination of figures 1 and 2 indicated that all families' germination percent were high and associated with very small 95%CI. Yearly mean *GC* was 97.01 (range: 92.8-99.1) and 96.72% (89.89-99.40) for 2001 and 2002, respectively, highlighting the effectiveness of the stratification treatment on removing existing family

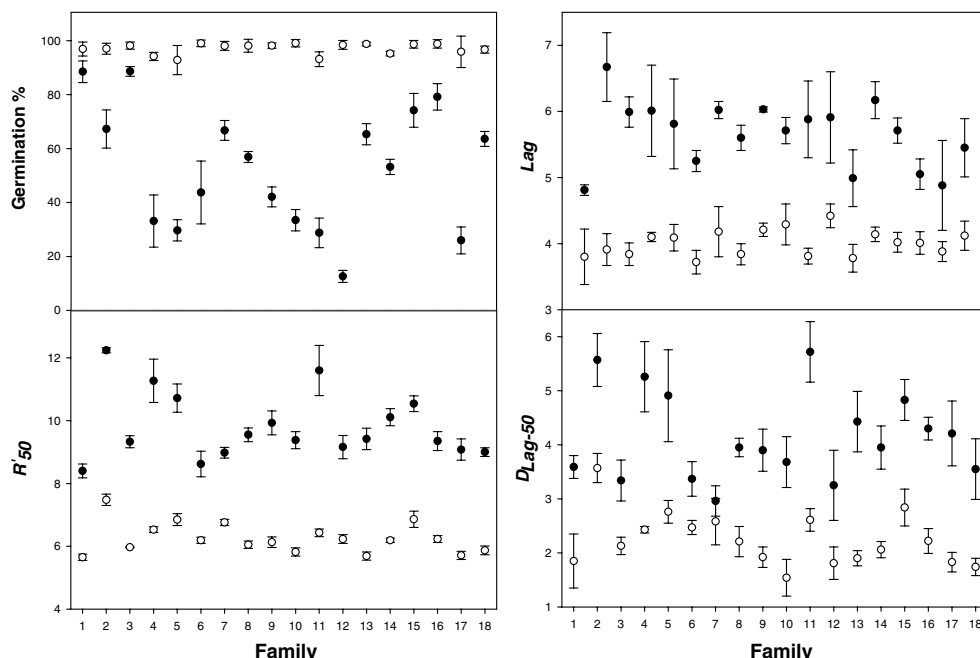


Figure 1. Germination parameters for 2001, 18 stratified (O) and unstratified (●) lodgepole pine families (see text for parameters explanation). Vertical lines represent 95% confidence intervals.

differences, thus resulting into the observed lack of family differences (table 2). Similarly, Lag produced the same trend and both yearly means were 4 days with ranges of (3.7-4.4) and (3.4-4.3) for 2001 and 2002, respectively (figures 1 and 2).

- b. Year effect again was either non-existent (GC and Lag) or accounted for very little variation (R'_{50} : 5.82 and D_{lag-50} : 6.22%) (table 3), indicating minor environmental (year) effect and consistent family performance over years.

Dormancy index significantly varied among families and accounted for 93% of the total variation for both years, indicating the dominant role family exerts on this evolutionary important attribute (table 3). Broad-sense heritability estimate (repeatability) of 0.93, for both years, is indicative of the strong genetic control over this trait. Families' yearly dormancy indices were consistent over the two studied years ($r = 0.84$; $P > 0.01$), supporting the estimated high heritability values. The observed large family differences in dormancy were neutralized by the stratification treatment, resulting in uniform performance for all germination parameters.

Three important findings, namely; 1) the major and highly significant effect of seed pre-treatment (i.e., stratification), 2) the significant role of family (genetics) as unravelled after the removal of the treatment effect, and 3) the minor influence of year (environment)

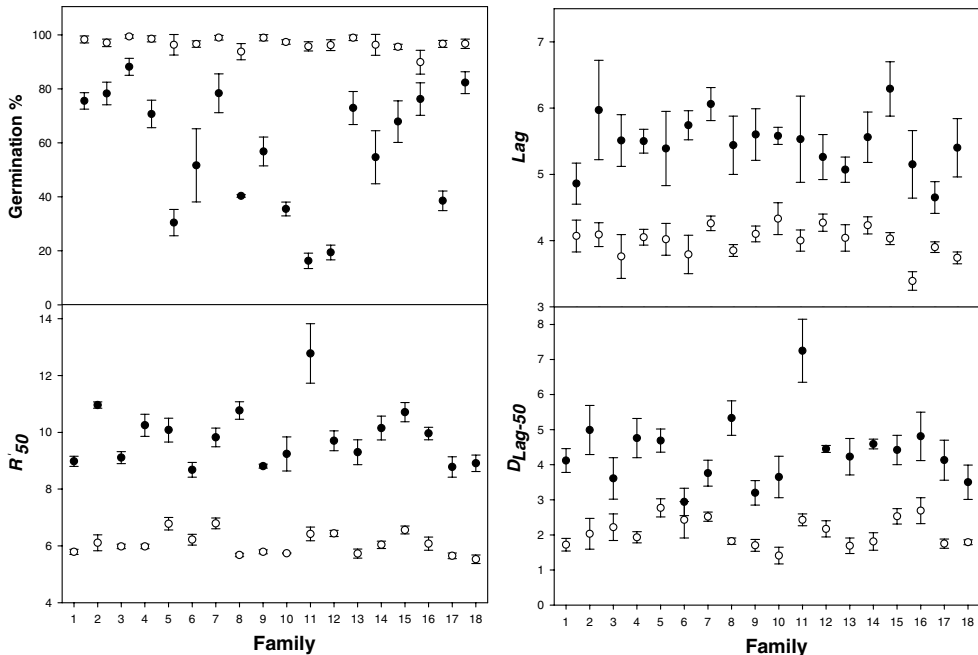


Figure 2. Germination parameters for 2002, 18 stratified (○) and unstratified (●) lodgepole pine families (see text for parameters explanation). Vertical lines represent 95% confidence intervals.

on the germination behaviour of lodgepole pine were revealed in the present study. While the difference between family and year roles is of biological interest, operationally the impact of seed pre-treatment is important in many ways. Stratification improved GC by 44 (2001) and 39% (2002) and reduced its range from 76 to 6 (2001) and from 72 to 10% (2002), increased R_{50} (i.e., shortening the time) by 4.6 (2001) and 3.8 days (2002) and minimized its variability from 3.8 to 1.8 (2001) and from 4.1 to 1.3 days (2002), prompting Lag by 1.7 (2001) and 1.4 days (2002) while compacting its heterogeneity from 1.9 to 0.7 (2001) and from 1.6 to 0.9 days (2002), reduced D_{lag-50} by 1.9 (2001) and 2.3 days (2002) with associated reduction of variation from 2.8 to 2.0 (2001) and from 4.3 to 1.4 days (2002). All these factors act in concert to speed and increase germination, increase seedling crop uniformity, and collectively eliminating any unintentional bias towards the selection of a whole or part of specific families during thinning of excess germinants and/or culling of sub-standard seedlings during crop grading (El-Kassaby and Thomson 1995; El-Kassaby 2000) if multiple sowing is practiced and potentially reducing heating costs associated with early seed germination and germinant development (i.e., reducing the number of days of heating required).

The role of stratification appeared to be instrumental in the overall improvement of lodgepole pine seed germination; however, it should be stated that the 4-week stratification treatment applied in the present study is one week longer than that currently recommended by ISTA (note: starting from 2005, ISTA has reduced the stratification treatment duration

for this species from 4 to 3 weeks). Thus at this stage, it was not clear that increasing stratification period by one week was adequate to attain the maximum improvement potential, accordingly the impact of stratification duration was tested on two bulk seedlots representing the two collection years (2001 and 2002) after mixing equal proportions of seed from the sampled 18 families. This test mirrored the method used by Edwards and El-Kassaby (1995) to test the efficacy of stratification on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seed. We intentionally applied a range of stratification durations to flank the ISTA's new recommendation with two treatments shorter (1 and 2 weeks) and two longer (4 and 5 weeks) in addition to the control (i.e., no stratification).

Germination parameters were assessed using new mixed ANOVA models [Eq. 3]. In general, the two seedlots' germination courses produced a steady improvement in all germination parameters with increased stratification period, resulting into either the expected steady increase for GC or decrease for R_{50}' , Lag and D_{lag-50} (figure 3). Results from the mixed model were not surprising producing highly significant ($P>0.01$) stratification duration effect (table 4). The studied seedlots did not differ in the majority of germination parameters including dormancy index (table 4). It is noteworthy to mention that the 3-week stratification treatment, recommended by ISTA, produced germination % lower than that of the 5-week or was either slightly higher or lower than that for the 4-week, indicating that neither the recommended 3-week and/or the applied 4-week were adequate to produce the attained maximum germination potential (figure 4).

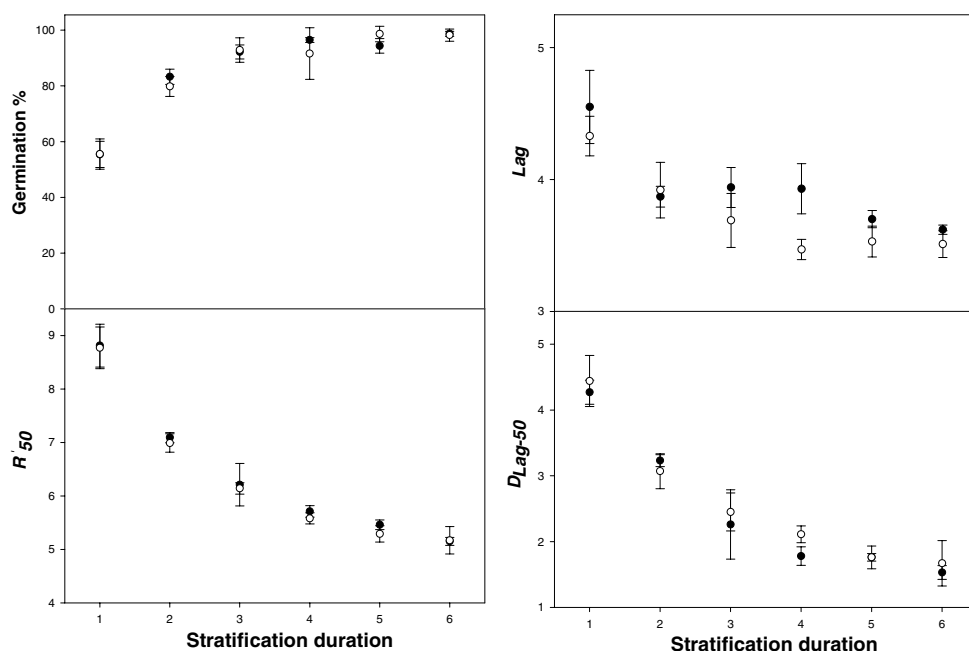


Figure 3. Germination parameters as affected by stratification duration (control (1) and 1- to 5-week (2-6)) on two lodgepole pine seedlots (2001 and 2002 are represented by open and closed circles, respectively). Vertical lines represent 95% confidence intervals.

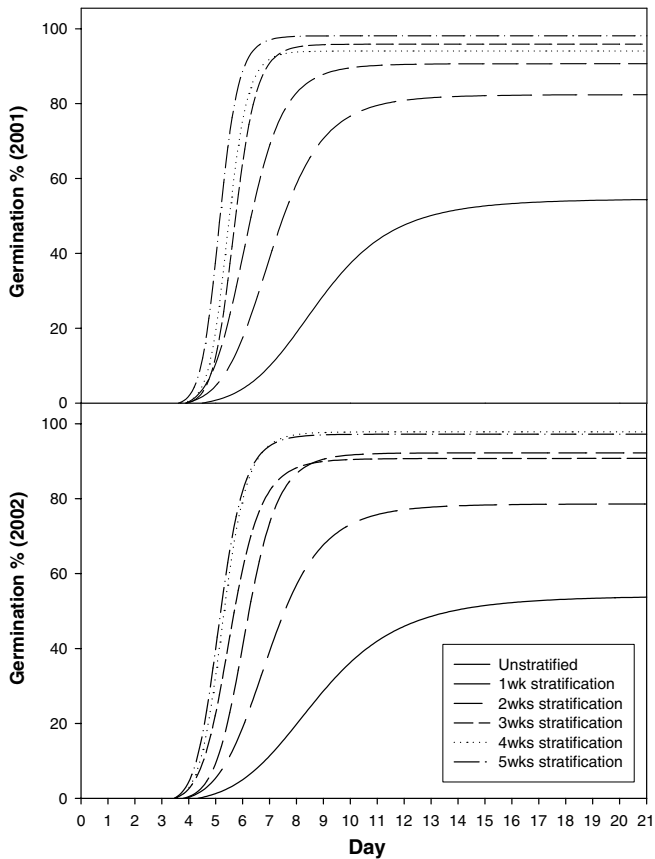


Figure 4. Effect of stratification duration (control and 1- to 5-week) on two lodgepole pine seedlots' germination for composite samples representing 2001 and 2002 bulk seedlots.

Since stratification is applied, to overcome dormancy, before nursery sowing to improve germination and attain emergence and seedling development uniformity, the complementary analysis [Eq. 3] was conducted on stratified seed to assess any germination capacity gain/loss associated with modifying the ISTA recommendation. Increasing stratification time beyond the ISTA recommended 3 weeks resulted into increased average germination capacity by 2.5 and 4.4% for 4 to 5 weeks, respectively. Additionally, the difference between the 4 weeks, currently practiced in British Columbia, and the 5 weeks resulted into 2% increase. To assess the impact of this seemingly small 2% increase in germination capacity, we factored this slight improvement into the 2006 Province of British Columbia's 1,002 lodgepole pine sowing requests (information accessed through the Seed Planning and Registry Application (British Columbia Ministry of Forests and Range, 2008 by D.K.) as follows:

- 1- 2% increase in germination capacity up to a maximum of 100% was added to all seedlots,

- 2- Potential seedlings production was estimated as = (# of grams of seed) (# seeds/gram) / # of seed required producing a seedling,
- 3- The total number of potential seedling was estimated by summing up the product of (2) over the 1,002 seedlots = 126.5 million seedlings,
- 4- Comparing the results of (3) to that estimated before factoring in the 2% germination increase (115.4 million seedlings) was approximately equalled 11 million seedlings,
- 5- Estimating the number of additional hectares that could be planted with this increase assuming planting density of 1,600/ha was approximately equal to 6,900 ha
- 6- The increase of seed utilization = $(11/115.4) * 100 = 9.6\%$

The above scenario demonstrated the potential of increasing seedling production if seed pre-treatment is modified; however it should be emphasized that the realization of this improvement (i.e., higher germination and its associated estimated increase in seedling production) can only be accomplished through the implementation of changes/adjustments to nurseries sowing factors (Vyse and Rudd 1974; British Columbia Ministry of Forests and Range. 2008) reflecting the amount of germination improvement. In other words, if germination improvement results in increasing germinant count/production without adjusting sowing factors (i.e., multiple sowing is practiced), then the impact of the observed improvement will be nullified by the unnecessary seed over use. In most cases, balance between economic gains based on excess seed use and progressive adjustments to sowing factors for better seed utilization should be reached to allow the maximum utilization of available seed.

In conclusion, seed utilization should not be viewed as an independent nursery issue; it encompasses the full aspects of its delivery system starting from seed collection and/or production from natural stands or seed orchards, to seed extraction, storage, and seed pre-treatment to nursery use. To maximize seed efficiency, a comprehensive well integrated seed utilization policy and practices are required. This study demonstrated the possibility of increasing seed germination through slight modification to recommended seed pre-treatment prescriptions and highlighted the need to translate the attained improvement into increasing in seedling production through continuous adjustments throughout the whole seed utilization delivery system.

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