

# **IMPROVING LODGEPOLE PINE SELECT SEED UTILIZATION THROUGH UNDERSTANDING GERMINATION BEHAVIOR**

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**March 2006**

**2005/2006 Report**

## SUMMARY AND CONCLUSIONS

Germination behavior of 18 orchard lodgepole pine (*Pinus contorta* var. *latifolia*) clones collected over two years (2001 and 2002) were assessed to evaluate the extent of variation among clones and between years. For each year, individual clone's seed were subjected to standard germination assay consisting of control (unstratified) and stratification (stratified) treatment. Three germination attributes were evaluated; including, germination capacity (GC: % of total germination), germination speed ( $R_{50}'$ : number of days required for 50% of the germinating seed to germinate; indicative of even emergence), and effectiveness of the stratification treatment on breaking dormancy differences (DI: expressed as dormancy index). The consolidated analysis (clone, treatment, year, and their interactions) highlighted the overwhelming impact of treatment (stratification) on GC and  $R_{50}'$  resulting into the production of a polarized effect that reduced the test sensitivity to evaluate either clone or year effects effectively. When the effect of treatment was removed, the impact of clone, year, and their interaction was effectively evaluated. The two independent analyses conducted on stratified and unstratified data, producing non-significant and significant results for year and clone effects, respectively, indicating that clonal (genetic background) is the main factor controlling germination behaviour, also allowing generalization of clonal performance over years. Unstratified seed produced significant GC (range; 2001: 13.3 to 89.5% and 2002: 15.8 to 89.3%) and  $R_{50}'$  (range; 2001: 8.4 to 12.3 days and 2002: 6.7 to 12.7 days) differences, interesting results from the species seed evolutionary biology point-of-view, but with no operational importance since seed production relies on pre-treated (stratified) seed. The stratified seed produced non-significant and significant for GC (range; 2001: 92.3 to 99.0% and 2002: 90.0 to 99.0%) and  $R_{50}'$  (range; 2001: 5.7 to 7.5 days and 2002: 5.5 to 6.8 days), respectively. The observed non-significant clonal effect for GC indicates that stratification was effective in eliminating the observed clonal dormancy significant differences (2001 range: 25 to 75 and 2002 range: 27 to 78), results of importance operational perspective. Additionally, the significant  $R_{50}'$  differences are indicative of the test's statistical power, but are expected to have minimal to no operational impact. The results indicate that the stratification treatment is very effective in masking clonal dormancy and speed differences and with the observed high GC, it is recommended that single seed sowing of bulked orchard 230 seedlots should be practiced. Comparisons between bulk seedlots and individual clones' performance are underway to provide empirical data supporting the bulking recommendation.

## **INTRODUCTION**

Presently, most British Columbia seed orchards seed crops are processed as bulked lots. Bulk seedlots genetic constitution and performance attributes such as storability, germination behavior, and seedling development is expected to equal the weighted average of clonal contribution. When bulk lots are processed, clonal variation are masked and difficult, if not impossible, to track. Bulking of seed orchards seed is practical and lends itself to increase efficiency by minimizing field work, reducing record keeping, seed testing and pre-treatment, and finally seedling production. However, if clonal germination attributes vary substantially, then the added costs for processing seeds by clone may offset by the added benefits in terms of maximizing seed utilization and nursery recovery. If clonal germination behavior is characterized, then the hidden variation in the bulked lots could be either overcome by the application of more effective seed pre-treatment methods or by grouping the clones in subsets of similar behaviour, thus improving performance.

The objective of this study is to assess the potential efficacy of lodgepole pine single-clone relative to bulk seedlot processing by examining differences in germination attributes and dormancy over time.

## **METHODS AND MATERIALS**

### **Seed source**

Eighteen individual lodgepole pine clonal seedlots representing two independent crop years (2001 and 2002) were used for this study. The clones are part of the B.C. Ministry of Forests and Range (MOFR) seed orchard #230 production population. Seed were used to determine clonal, annual, and clone-by-year interaction variation in germination parameters.

### **Seed germination**

Seeds were subjected to standard germination tests following International Seed Testing Association standards (ISTA, 1993) comprising four replications of 100-seed per clone-treatment-year combination. The effect of treatment (28 days stratification) was assessed and compared to a control (no stratification). Germination tests were run for 28 days; however, the data analyses were based on 21 days.

### **Germination parameters**

Three germination parameters were investigated. These are:

- 1- Germination capacity (GC), is the percentage of seed germinated by the end of the experiment, in this case, after 21 days,

- 2- Germination speed ( $R_{50}'$ ), is the number of days required for 50% of the viable (germinable) seed to germinate (Thomson and El-Kassaby 1993), and
- 3- Individual clone dormancy (DI), represented by Dormancy Index value (see below).

For each year and every individual clone, the cumulative germination data of each replication, stratified and unstratified, were expressed as a regression model using the curve-fitting option of SigmaPlot 2000 (SPSS Inc., Chicago, IL). The three-parameter Hill function's parameters were used to estimate the germination attributes listed above:

$$y = \frac{ax^b}{c^b + x^b}$$

Where:

Y = the cumulative germination count (%) at a given time,

x = time (days),

a = maximum cumulative germination (GC), set to equal the empirical value,

b = a parameter controlling the shape and steepness of the germination curve;

c = time (days) at which 50% of viable seeds have germinated ( $R_{50}'$ ).

The area in Figure 1 (shaded surface) was used as a representation of the dormancy (DI), where the Y-axis is cumulative germination capacity, and the X-axis is days of the germination test. The area between the control (unstratified) and treatment (stratified) curves is represented by:

$$Area = \int_{t_0}^{t_n} (y_{t_n} - y_{c_n}) dt$$

Where:

y = is function value from the Hill function listed above for each seedlot or treatment n or control c;

$t_0$  and  $t_n$  = beginning and end of the germination test (days), respectively.

The dormancy index (DI) was calculated as follows:

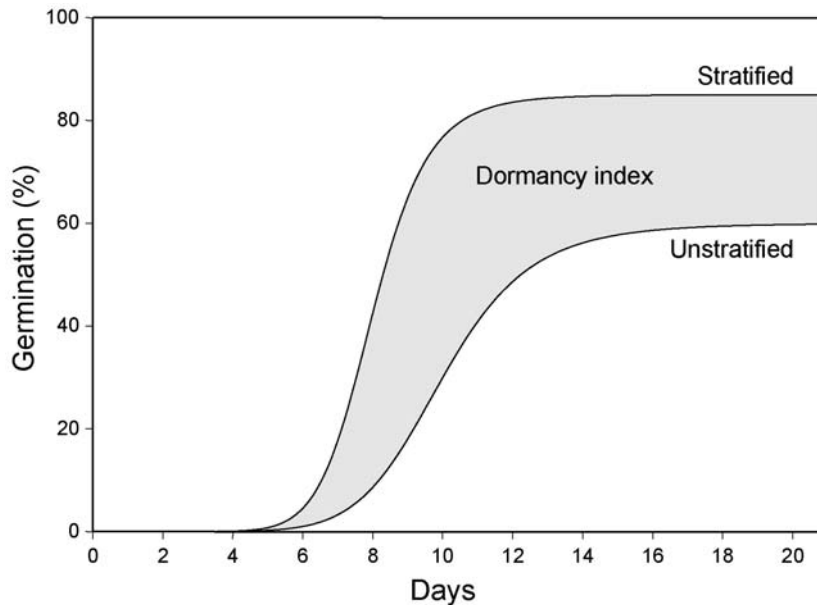
$$DI = \frac{\sum_{t_0}^{t_n} (y_{t_n} - y_{c_n}) \times 0.1}{t_n}$$

Where:

DI (no units, cf. McDonald 1993) = the surface area between the stratified and unstratified germination curves, calculated using 0.1-day intervals.

The total value of the area under the germination curve is standardized by division by the total number of test days to enable broader comparison.

Figure 1. Illustration of dormancy index concept.



## Data analysis

Germinants were counted several times a week following ISTA (1993) standards, where cumulative germination was recorded to the end of the 28 days germination test (data analyses were conducted on 21 days count and the cumulative count is equivalent to germination capacity, GC) and expressed as a percentage of the total. The cumulative germination data was then fit to the 3-parameter Hill function in SigmaStat 2000 (see above).

To estimate dormancy index (DI) for each clone-year combination, cumulative germination data for each stratified replication was randomly matched with unstratified replication to estimate the difference in area between the stratified and unstratified curves and divide the area by the duration of the germination test (number of days), resulting in the DI value (see above).

Significance of the GC,  $R_{50}'$ , and DI was tested using PROC MIXED in SAS V.9.1.3. Clones and years were treated as random effects while treatment was considered fixed. The significance of mixed model terms and their interactions was assessed using a  $\chi^2$  test with degrees of freedom accounted for by each individual model term or interaction tested (SAS Institute Inc., 2004). GC, a proportional variable, was arcsine-square root transformed to fulfill the normality requirement of ANOVA.

Data analyses were conducted on each using the following additive linear model:

$$Y_{ijkl} = \mu + C_i + T_j + Y_k + CT_{ij} + CY_{ik} + TY_{jk} + CTY_{ijk} + \varepsilon_{l(ijk)}$$

where:

- $Y_{ijkl}$  : effect of the  $l^{\text{th}}$  replication within the  $c^{\text{th}}$  seedlot,  $t^{\text{th}}$  treatment and  $y^{\text{th}}$  year,
- $\mu$  : overall mean,
- $C_i$  : effect of the  $c^{\text{th}}$  clone (random),
- $T_j$  : effect of the  $t^{\text{th}}$  treatment (stratified and un-stratified (fixed)),
- $Y_k$  : effect of the  $y^{\text{th}}$  year (random),
- $CT_{ij}$  : effect of the  $c^{\text{th}}$  clone x  $t^{\text{th}}$  treatment interaction,
- $CY_{ik}$  : effect of the  $c^{\text{th}}$  clone x  $y^{\text{th}}$  year interaction,
- $CTY_{ijk}$  : effect of the  $c^{\text{th}}$  clone x  $t^{\text{th}}$  treatment x  $y^{\text{th}}$  year interaction, and
- $\varepsilon_{l(ijk)}$  : residual term = effect of the  $l^{\text{th}}$  replication within the  $c^{\text{th}}$  clone x  $t^{\text{th}}$  treatment x  $y^{\text{th}}$  year.

When the original additive linear model (above) yielded significant treatment effect (see Table 1), the data were re-analyzed within each treatment (stratified and not-stratified) using the following reduced additive linear model (below), so the effect of clones and years as well as their interaction could be accurately discerned.

$$Y_{ijk} = \mu + S_i + Y_k + SY_{ik} + \varepsilon_{l(ij)}$$

where:

All terms similar to those of the complete model and  $\varepsilon_{l(ij)}$  is the residual term = effect of the  $l^{\text{th}}$  replication within the  $c^{\text{th}}$  clone x  $t^{\text{th}}$  treatment.

All significance tests were set to  $\alpha = 0.05$ .

## RESULTS

All individual clones' germination courses were a good fit to the Hill model ( $\text{Rad}j^2 > 0.9$ ,  $p < 0.01$ ) and the Hill function parameters (a, b and c) were generally significant in all models ( $p < 0.001$ ) (data not shown). Treatment clearly dominated the variability in the model for all parameters (Table 1), thus for a more in-depth evaluation of the allocation of variation among clones and years, the original model was reanalyzed with years, clones, and their interaction within treatments (Table 2). In this instance, all terms were random, allowing terms to be tested using PROC GLM in a random factorial design (Table 2).

Individual clones varied significantly in their dormancy and were consistent over the two study years (Table 2 and Figure 2). Visual inspection across the 18 clones confirms the statistical analyses results (Appendix 1).

Clonal germination capacity and speed produced variable results for the stratified and unstratified treatments; however, their performance was consistent over the two collection year (Figures 3 – 6; note scale differences). Clonal germination parameters averages and their standard errors are listed in Tables 3 and 4.

Table 1. *P*-values for mixed model on each germination parameters (capacity (C) and speed ( $R_{50}'$ )), and significance of treatment (fixed) LS means. Significant values ( $p \leq 0.05$ ) in **bold**. Values represent the significance of the  $\chi^2$  test of -2 log likelihood values with degrees of freedom equal to the difference in the model with and without each tested term.

Source <sup>a</sup>	Type	GC <sup>b</sup>	$R_{50}'$
Clone (C)	Random	1.0000	0.5052
Treatment (T) <sup>c</sup>	Fixed	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
Year (Y)	Random	1.0000	0.9701
C*T	Random	1.0000	1.0000
C*Y	Random	1.0000	1.0000
T*Y	Random	1.0000	1.0000
C*T*Y	Random	1.0000	1.0000
<b>Total</b>			

<sup>a</sup> df represents the maximum possible degrees of freedom for each term in the mixed model.

<sup>b</sup> All tests and estimations of LS means were performed on arcsine-square root transformed values of GC.

<sup>c</sup> Treatments with significantly different LS means are underlined.

Table 2. *P*-values for random model on dormancy index (DI) and germination parameters (capacity (C) and speed ( $R_{50}'$ )). Significant values ( $p \leq 0.05$ ) in **bold**.

Source	df	Unstratified			Stratified	
		DI	GC	$R_{50}'$	GC	$R_{50}'$
Clone (C)	1	<b>0.0003</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.4776	<b>0.0006</b>
Year (Y)	17	0.1432	0.2226	0.7759	0.9686	0.0516
C*Y	17	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
Residual	108					

Table 3. Means and standard errors of germination parameters by year.

Year	DI		Unstratified				Stratified			
	Mean	SE	GC		$R_{50}'$		GC		$R_{50}'$	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2001			53.64	2.25	9.95	0.16	96.75	0.98	6.28	0.07
2002			57.81	2.51	9.90	0.16	96.78	0.83	6.10	0.07

<sup>a</sup> All analyses were performed on arcsine-square root transformed values of a.

Table 4. Means and standard errors of parameters by family and year.

Year	Clone	Unstratified				Stratified			
		GC		R <sub>50</sub> '		GC		R <sub>50</sub> '	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
2001	3048	88.00	1.63	8.44	0.10	97.00	1.29	5.65	0.05
	3054	68.00	2.86	12.33	0.08	96.00	0.91	7.50	0.09
	3063	89.50	1.04	9.42	0.10	98.00	0.71	5.98	0.01
	3080	33.25	4.92	11.35	0.30	93.75	0.85	6.55	0.04
	3085	30.25	2.32	10.89	0.16	92.25	2.69	6.88	0.09
	3087	44.00	5.76	8.64	0.20	98.75	0.63	6.21	0.05
	3088	67.50	1.66	9.06	0.09	98.00	0.71	6.76	0.05
	3090	58.00	0.82	9.70	0.15	97.50	1.32	6.11	0.07
	3092	43.75	2.17	10.13	0.13	97.75	0.48	6.14	0.08
	3105	34.00	1.58	9.52	0.09	99.00	0.71	5.84	0.06
	3107	30.00	2.58	12.00	0.45	92.75	0.95	6.50	0.07
	3109	13.25	1.31	9.36	0.15	98.00	0.91	6.24	0.07
	3110	66.50	1.04	9.62	0.20	98.75	0.25	5.69	0.07
	3112	54.00	1.47	10.24	0.14	94.75	0.48	6.22	0.04
	3116	74.75	2.78	10.67	0.12	98.25	0.48	6.89	0.13
3134	81.00	2.45	9.49	0.17	98.25	0.85	6.25	0.06	
3169	26.25	2.50	9.17	0.19	96.25	2.78	5.73	0.07	
3179	63.50	1.66	9.06	0.06	96.50	0.65	5.87	0.07	
2002	3048	75.75	1.44	9.01	0.09	98.50	0.50	5.80	0.04
	3054	78.00	1.96	10.97	0.05	96.25	0.75	6.15	0.14
	3063	89.25	1.44	9.19	0.11	99.50	0.29	5.98	0.04
	3080	71.00	2.27	10.39	0.19	98.25	0.63	6.01	0.04
	3085	31.25	2.78	10.18	0.18	96.00	1.73	6.81	0.10
	3087	51.50	6.76	8.74	0.13	96.75	0.48	6.23	0.09
	3088	79.50	3.01	9.90	0.15	99.00	0.41	6.80	0.10
	3090	40.75	0.48	10.90	0.17	94.50	1.55	5.72	0.04
	3092	57.00	2.61	8.85	0.04	99.00	0.58	5.81	0.04
	3105	35.75	1.44	9.34	0.30	97.50	0.29	5.75	0.01
	3107	15.75	1.65	12.73	0.30	96.50	0.65	6.50	0.13
	3109	19.25	1.38	9.68	0.16	95.75	0.85	6.47	0.06
	3110	74.50	2.33	9.49	0.27	99.25	0.48	5.75	0.08
	3112	54.50	5.24	10.23	0.20	95.75	2.02	6.06	0.06
	3116	68.75	3.68	10.79	0.17	94.75	0.48	6.58	0.07
3134	77.25	2.84	10.06	0.09	90.00	1.78	6.20	0.12	
3169	38.50	1.85	8.84	0.18	97.75	0.63	5.68	0.06	
3179	82.25	2.10	8.93	0.15	97.00	0.82	5.54	0.08	

<sup>a</sup> All analyses were performed on arcsine-square root transformed values of *a*.

Figure 2. Yearly clonal dormancy index (DI) values.

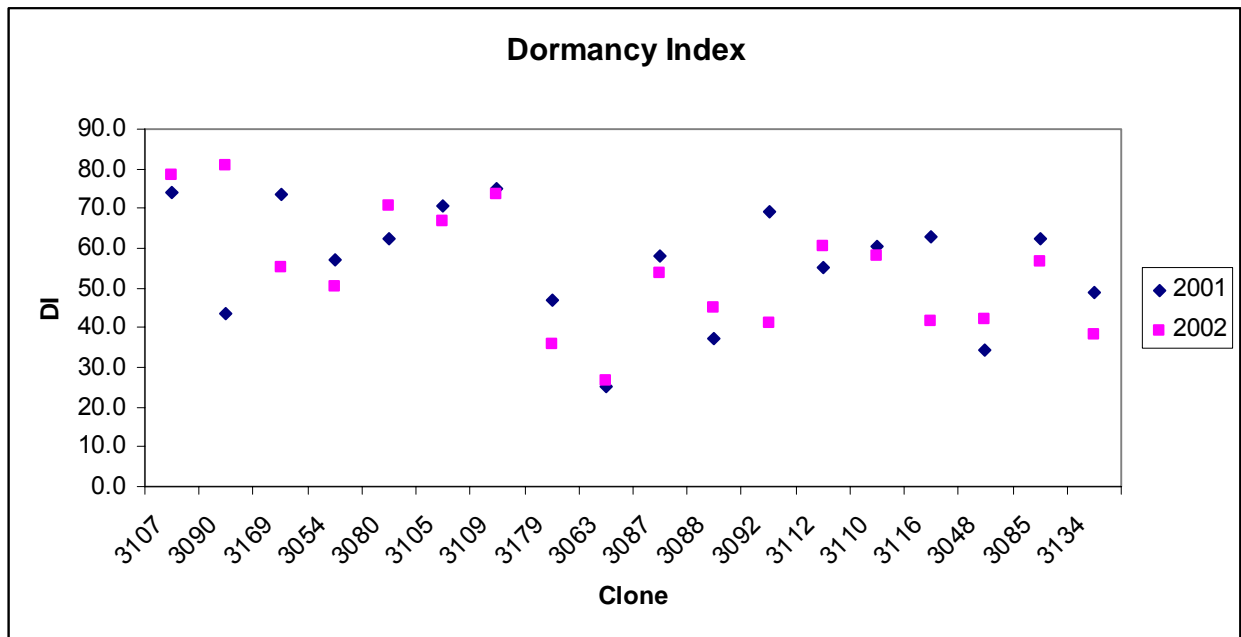


Figure 3. Yearly clonal germination capacity (GC) averages and their confidence intervals for unstratified seed.

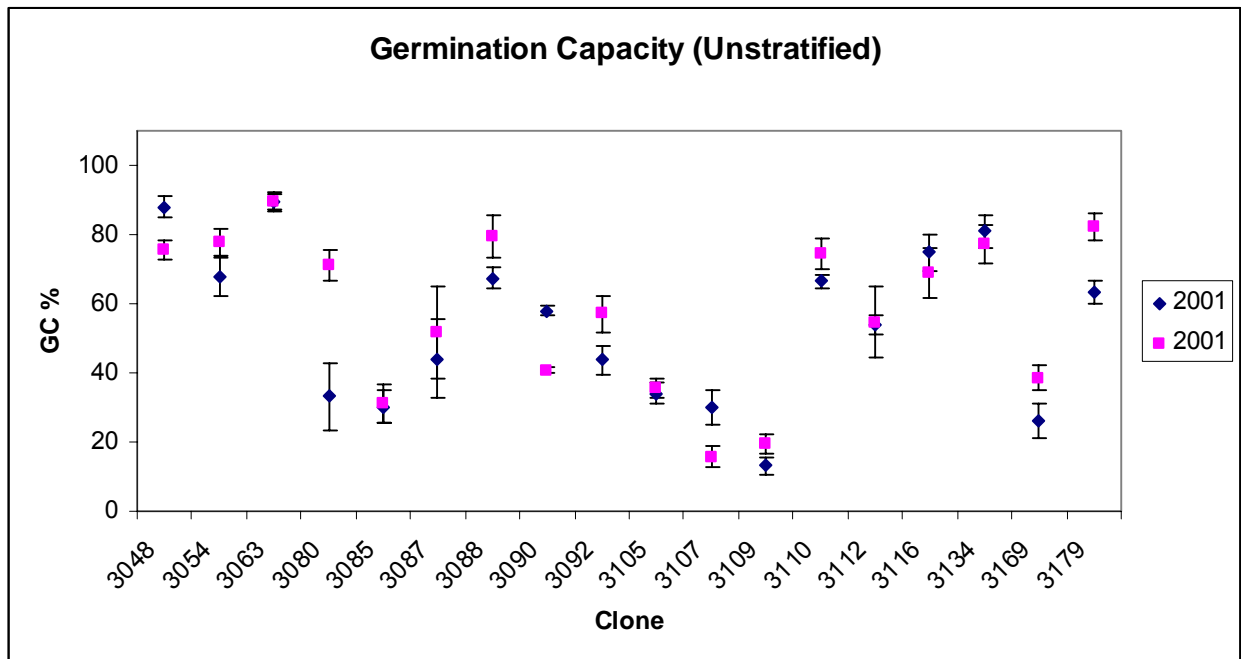


Figure 4. Yearly clonal germination capacity (GC) averages and their confidence intervals for stratified seed.

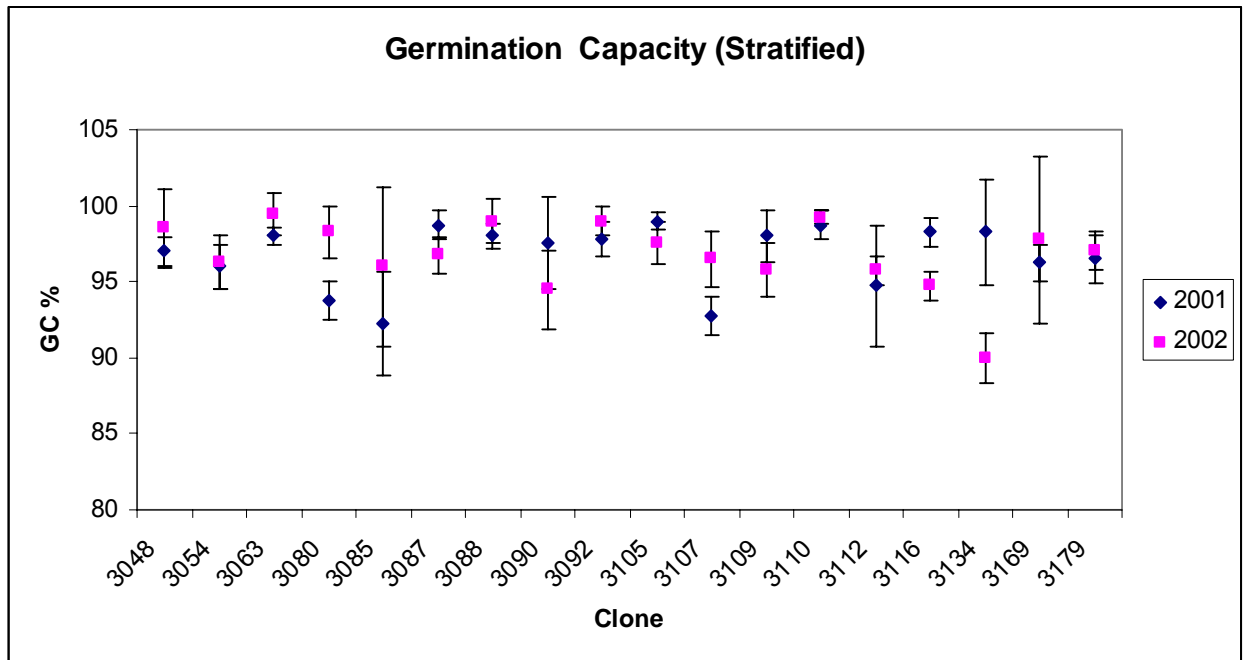


Figure 5. Yearly clonal germination speed ( $R_{50}'$ ) averages and their confidence intervals for unstratified seed.

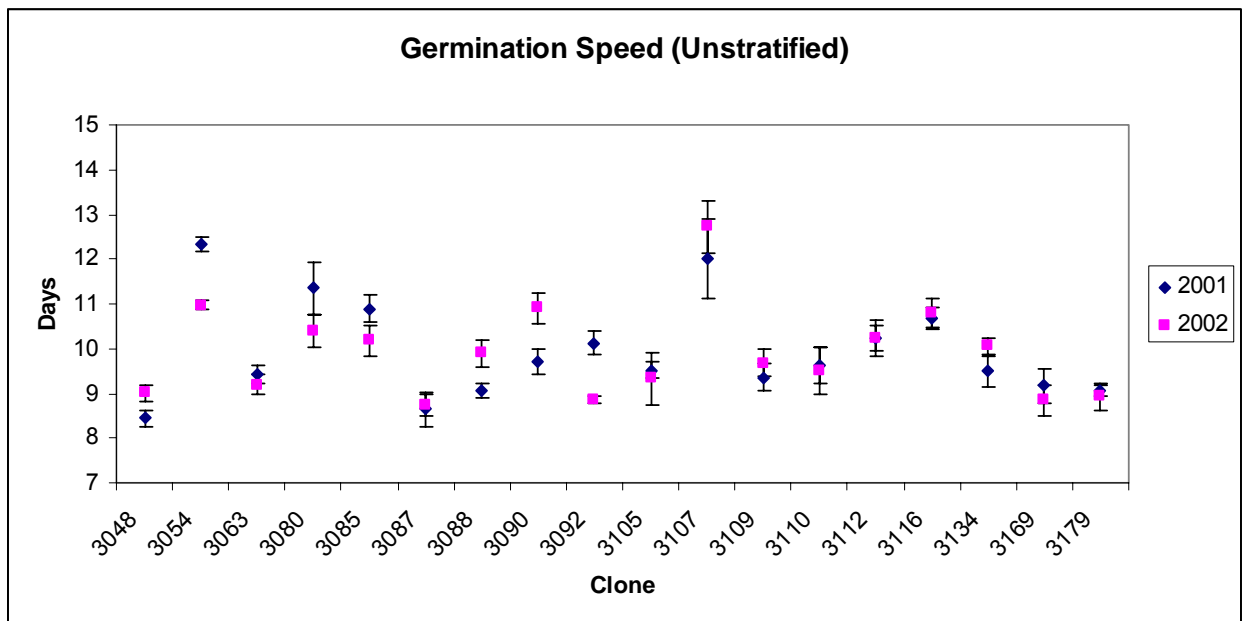
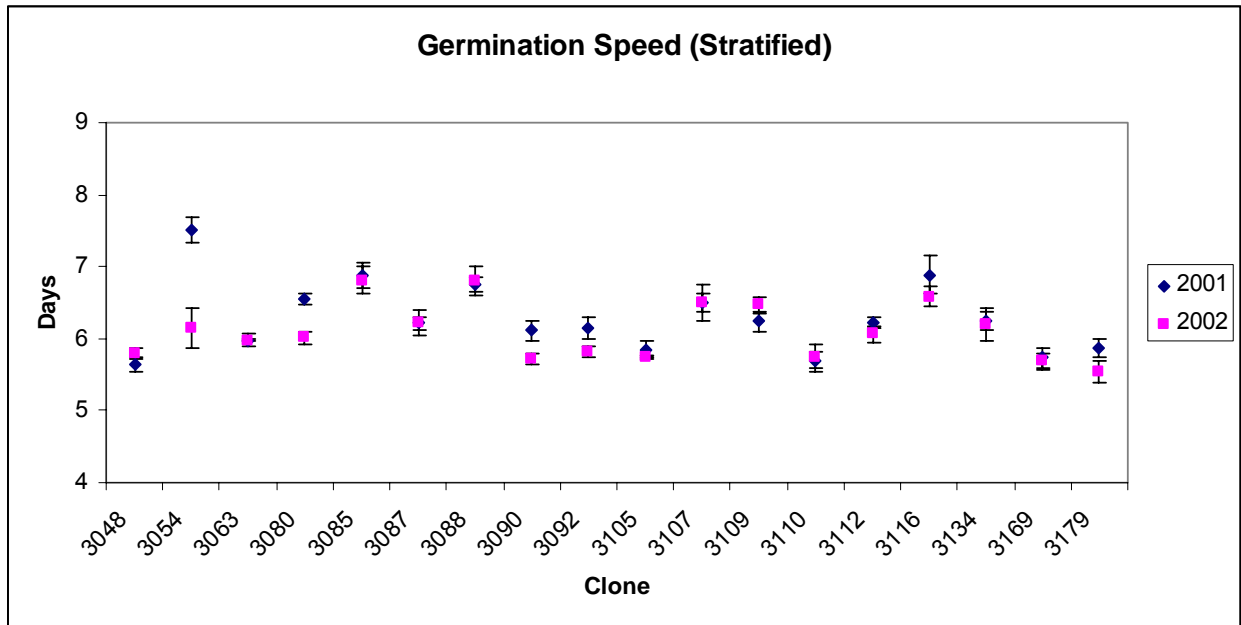


Figure 6. Yearly clonal germination speed ( $R_{50}'$ ) averages and their confidence intervals for stratified seed.



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## **APPENDIX 1.**

Germination course of stratified (upper) and unstratified (lower) of 18 lodgepole pine clones over two years (2001 and 2002).

