Seed germination: Mathematical representation and parameters extraction

Article *in* Forest Science · April 2008

Seed Germination: Mathematical Representation and Parameters Extraction

Yousry A. El-Kassaby, Ian Moss, Dave Kolotelo, and Michael Stoehr

Abstract: A method for mathematically describing cumulative seed germination using the four-parameter Hill function is described. The function's four parameters allowed both direct or indirect biological interpretation of germination behavior and the impact of seed pretreatments on germination improvement. Three parameters, *a*, *b*, and *c*, allowed direct assessment of germination capacity (%), the shape and steepness of the germination course, and germination speed (time to reach 50% germination). The fourth parameter y_0 permitted estimation of the lag time (germination onset). The mathematical expression of cumulative seed germination of unstratified and stratified seed made it possible to quantitatively estimate seedlot dormancy and the amount of germination improvement caused by a specific seed pretreatment. This was accomplished by estimating the area between the two cumulative germination curves through integration. The utility of the proposed approach was demonstrated using germination data from wind-pollinated, individual genotypes (half-sib families) of lodgepole pine (*Pinus contorta* var. *latifolia*) and bulk seedlots of lodgepole pine and white spruce (*Picea glauca*). FOR. SCI. 54(2): 220–227.

Keywords: seed germination, germination parameters, dormancy, four-parameter Hill function, curve-fitting

THE GERMINATION PERFORMANCE of a seedlot can be characterized by three parameters; time of germination onset (lag), germination speed (rate), and extent or capacity (cumulative germination percentage at the end of the testing period). The genetic makeup of a seedlot varies from a bulk collection (e.g., originating from an unknown number of parents) to a full-sib family (derived from controlled pollination) and strongly affects the variability of its germination parameters (El-Kassaby et al. 2002). Germination parameters are useful for estimating the conversion of seeds to seedlings and, thus, the suitability of a seedlot for commercial seedling production. Germination parameters are also useful in determining the type of seed pretreatment as well as nursery management practices needed to attain a high level of germination (Kolotelo et al. 2001).

In most cases, germination capacity (% germination) is the most important parameter in determining the suitability of a seedlot for commercial use, but germination rate influences the uniformity of emergence in nurseries (Ching 1959; Thomson and El-Kassaby 1993; El-Kassaby 2000). Several attempts have been made to simplify the characterization of seed germination performance by distilling the various germination parameters into a single index or value (e.g., Czabator 1962). However, reducing multiple germination parameters into one index provides an incomplete picture of germination behavior.

When germination is properly fitted to a mathematical function, the parameters of that function can be used to better understand germination behavior and the effect of seed pretreatments on germination enhancement. Many curve-fitting routines have been used and their suitabilities for characterizing and managing seedlots have been critically assessed (Tipton 1984; Brown and Mayer 1988a, 1988b).

Curve-fitting methods using the Weibull, Gompertz, and probit functions have been used to characterize the germination of conifer seeds, compare seed pretreatments, and measure seedlot differences (Bonner and Dell 1976; Rink et al. 1979; Campbell and Sorensen 1979; Bramlett et al. 1983; Dell et al. 1983; Leadem 1986, Stoehr et al. 1998). However, the parameters in these functions do not lend themselves to simple biological interpretation. The use of curve-fitting to interpret and understand germination patterns has been reviewed and endorsed by Scott et al. (1984).

In this article, we introduce the four-parameter Hill function (4-PHF) as a curve-fitting method for describing coniferous seed germination. We also show that this function is amenable to biological interpretation, thereby enhancing our understanding of germination differences among genotypes, seedlots, and seed pretreatments.

The 4-PHF and Germination Parameters

The cumulative germination count of a seedlot was modeled to fit the 4-PHF (Equation 1) using the curve-fitting routine in MATLAB (The MathWorks, Inc. 2005), with the

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Acknowledgments: We extend our thanks to M. El-Sharkawi (University of Washington, Seattle, WA), M. Fayed and M. Yasein (University of Victoria, Victoria, BC, Canada), and M. Senousy (University of British Columbia, Vancouver, BC, Canada) for assisting with the model development and introduction to MATLAB. This work was funded, in part, by the Johnson's Family Forest Biotechnology Fund, British Columbia Forest Genetics Council's Applied Forest Genetics and Biotechnology Grant, and The Natural Sciences and Engineering Research Council of Canada-Industry Research Chair Funds to Y.E.K.

Manuscript received March 14, 2007, accepted September 21, 2007

duration of the germination test and cumulative germination percentage representing the x- and y-variables, respectively:

$$y = y_0 + \frac{ax^b}{c^b + x^b}, \tag{1}$$

where y is the cumulative germination percentage at time x, y_0 is the intercept on the y axis (≤ 0), a is the asymptote, or maximum cumulative germination percentage, which is equivalent to germination capacity, b is a mathematical parameter controlling the shape and steepness of the germination curve (the larger the b parameter, the steeper the rise toward the asymptote a, and the shorter the time between germination onset and maximum germination), and c is the "half-maximal activation level" measured in days (Keshet 2006) and represents the time required for 50% of viable seeds to germinate (c is equivalent to the germination speed [R_{50}'] parameter of Thomson and El-Kassaby [1993]), and x is time in days. The time at germination onset (lag) is computed by solving Equation 1 after setting y to 0 as follows:

$$\text{lag} = b\sqrt{\frac{-y_0 c^b}{a + y_0}}, \tag{2}$$

where y_0 is the intercept on the y axis.

Another way to characterize the speed of germination is to consider the duration ($D_{\text{lag-50}}$) between the time at germination onset (lag) and that at 50% germination (c). The shorter the $D_{\text{lag-50}}$, the more prompt and uniform the germination and the steeper the germination curve (i.e., higher b). Thus, $D_{\text{lag-50}}$ and b should be negatively correlated, and $D_{\text{lag-50}}$ is a good indictor of germination speed, uniformity, and vigor. Collectively, the $D_{\text{lag-50}}$, c, and lag parameters provide a better indicator of germination speed than R_{50}' alone. This is because R_{50}' is a function of time only and could be the same for two seedlots with different germination behaviors (i.e., similar R_{50}' but different germination onset times and/or different germination capacities).

The instantaneous rate of germination was also estimated from the partial derivative of Equation 1 and can be used to measure the sensitivity of any specific variable as follows:

$$s = \frac{\partial y}{\partial x} = \frac{abc^b x^{b-1}}{(c^b + x^b)^2}, \tag{3}$$

where s is the daily rate of germination. This function was plotted against time, and the time required to reach maximum germination rate (time at maximum germination rate [TMGR]) was determined as

$$\text{TMGR} = b\sqrt{\frac{c^b(b-1)}{b+1}}, \tag{4}$$

The location of TMGR on the germination curve is the point at which the instantaneous slope is at maximum (Figure 1). This point is different from Czabator's "peak value (PV)" which is "the maximum quotient derived from all of the cumulative full-seed germination percent on any day divided by the number of days to reach this percent" (Czabator 1962). The difference between PV and TMGR is similar to the difference between mean germination increment and periodic germination increment. The former represents the maximum total amount of germination divided by the total time elapsed, whereas the latter are maximum rates of germination at a point or small interval in time that may be alternatively referred to as instantaneous rates of germination. By definition the maximum instantaneous rate must exceed the maximum average rate of germination and so too, the maximum instantaneous rate must occur in advance of the maximum average rate of germination (Table 1). Both TMGR and PV are important to the production of seedlings. However, TMGR defines the inflection point of the cumulative germination curve or the point in time when the instantaneous rate of germination starts to decline. It also defines the scale of the cumulative germination curve as this is proportional to the maximum instantaneous rate of germination. In contrast, PV is no more important than any other point along the line in terms of uniquely defining the shape and scale of a sigmoid curve. Thus, TMGR is biologically more meaningful. The shorter the TMGR, the more vigorous is the seedlot and the time to reach R_{50}' is shorter. Therefore, the 4-PHF is suitable for extracting meaningful germination parameters and characterizing the behavior of germination at different times during the germination test. Furthermore, if germination is expressed by a mathematical function, then this function could be used to assess the effectiveness of seed pretreatments using the integration method of Richter and Switzer (1982). Using this method, dormancy is defined as the amount of germination improvement caused by a specific dormancy-breaking treatment. The improvement is quantitatively determined by the difference between the areas under the germination curves before and after seed pretreatment (Figure 2) and is defined as the dormancy index (DI), which is calculated as

$$\text{DI} = \int_{t_0}^{t_n} (y_1 - y_2)^{\mathrm{d}t}, \tag{5}$$

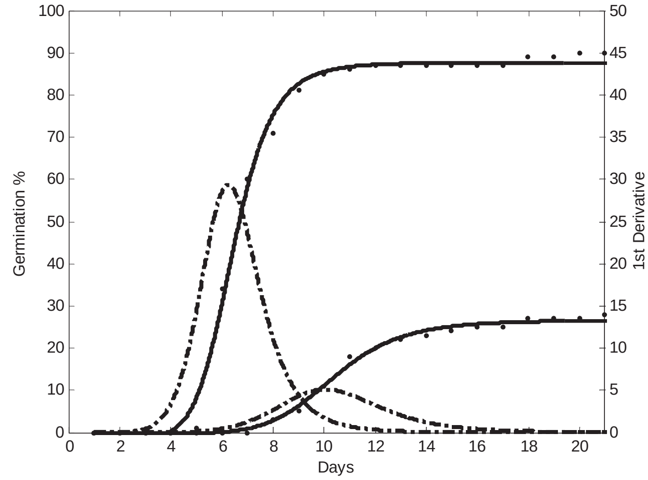

Figure 1. The 4-PHF for stratified (solid line: upper curve) and unstratified (solid line: lower curve) seedlot of lodgepole pine (single family) and the instantaneous rate of germination for the same stratified (dashed line: upper curve) and unstratified (dashed solid line: lower curve) seedlot. TMGR is the peak point for lower curves. The dots represent the cumulative germination over time, whereas the solid line represents the fitted curves.

Table 1. Comparison between Czabator's peak values (PV) determined from cumulative germination over time and the Time at Maximum Germination Rate (TMGR) determined from the instantaneous rate of germination values for the unstratified and stratified lodgepole pine seedlots presented in Figure 1

	Unstratified			Stratified		
Day	Cumulative Germination	PV	TMGR	Cumulative Germination	PV	TMGR
1	0	0.00	0.00	0	0.00	0.00
2	0	0.00	0.00	0	0.00	0.12
3	0	0.00	0.01	0	0.00	1.24
4	0	0.00	0.04	0	0.00	5.97
5	0	0.00	0.13	2	0.40	16.57
6	0	0.00	0.36	42	7.00	**26.08**
7	0	0.00	0.84	61	8.71	21.14
8	0	0.00	1.67	70	8.75	12.30
9	3	0.33	2.84	81	**9.00**	6.18
10	5	0.50	4.07	84	8.40	3.03
11	15	1.36	4.87	92	8.36	1.52
12	18	1.50	**4.89**	92	7.67	0.80
13	21	1.62	4.23	93	7.15	0.44
14	25	1.79	3.29	93	6.64	0.25
15	27	1.80	2.38	93	6.20	0.15
16	30	**1.88**	1.66	93	5.81	0.09
17	30	1.76	1.14	93	5.47	0.06
18	31	1.72	0.78	93	5.17	0.04
19	33	1.74	0.53	93	4.89	0.02
20	34	1.70	0.37	94	4.70	0.02
21	36	1.71	0.26	94	4.48	0.01

PV and TMGR values and their respective times are presented in bold.

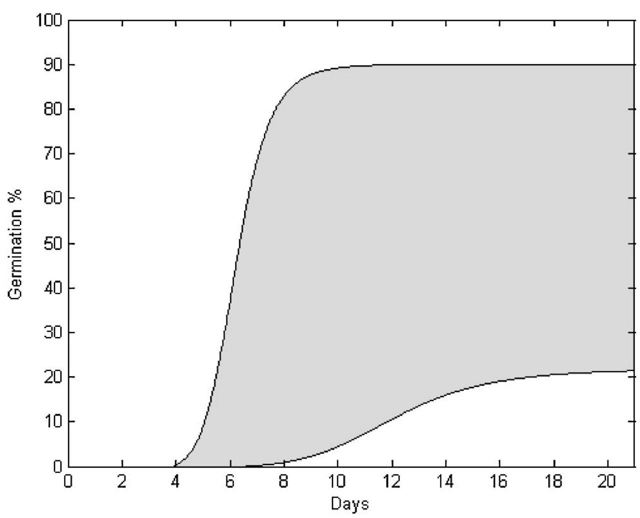

Figure 2. DI is represented by the area between the germination curves of stratified (upper curve) and unstratified (lower curve) for a lodgepole pine seedlot (single family).

where DI is the dormancy index (surface area), y_1 and y_2 are the 4-PHF for the seedlot after and before the dormancy breaking treatment, respectively, and t_0 and t_n are the time in days at the beginning and end of the germination test, respectively. Thus, zero and negative values imply that the treatment either has no effect on improving seedlot performance, or is detrimental (see below).

Parameters Demonstration

Germination parameters for 18 wind-pollinated seedlots (families) from individual lodgepole pine (*Pinus contorta* var. latifolia) orchard parents were determined from standard germination protocols (International Seed Testing Agency 2006). The seed pretreatment consisted of a simple stratification treatment (soaked in water for 1 day) followed by surface drying and storage at 4°C for 4 weeks. The seeds were germinated at alternating temperatures between 30°C for an 8-hour day and 20°C for a 16-hour night. Light, at about 13.5 μmol m^{-2} s^{-1} was provided during the day by means of cool-white fluorescent tubes. Additionally, DI was evaluated on bulk lodgepole pine and white spruce (*Picea glauca*) seedlots under five stratification durations, ranging from 1 to 5 weeks. Germination tests for individual family and bulk seedlots were conducted on four replications of 100 seeds each. Germinant counts were conducted daily, and the cumulative germination for each replication was determined. 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.

Statistical Analyses

Germination parameters (a, b, c, TMGR, and lag) for each replication within each family were generated by fitting the 4-PHF curve and then subjecting the parameters to analyses of variance using the additive linear model,

$$Y_{ijk} = \mu + T_i + F_j + TF_{ij} + \varepsilon_{(ij)k}, \qquad (6)$$

where μ is the overall mean, T_i is the effect of the ith seed pretreatment (stratification) (i = 1 to 2, fixed effect), F_j is the effect of the jth family (i = 1 to 18, random effect), TF_{ij} is the effect of the interaction between ith family and seed ith pretreatment; and $\varepsilon_{(ij)k}$ is the residual error (k = 1 to 4). With the exception of germination capacity (which was

Table 2. Person product-moment correlation coefficients among germination parameters for unstratified and stratified seedlots for 18 lodgepole pine families (critical $r = 0.232$ and 0.302 for p at 5 and 1%, respectively; $n = 18$)

	b*	$c = R_{50}$	TMGR	Lag	$D_{\text{lag-50}}$
Unstratified					
a	0.41	−0.25	−0.22	−0.02	−0.24
b		−0.75	−0.70	0.19	−0.82
$c = R_{50}$			1.00	0.18	0.91
TMGR				0.22	0.89
Lag					−0.24
Stratified					
a	0.18	−0.21	−0.21	−0.13	−0.18
b		−0.43	−0.37	0.27	−0.71
$c = R_{50}$			1.00	0.63	0.89
TMGR				0.67	0.86
Lag					0.20

*See text for parameters description.

arcsine transformed), all parameters were analyzed using their original values. Expected mean squares were calculated, and components of variance were estimated and their percentage contributions to the total variation were determined.

DI was analyzed after removing treatment effects from the additive linear model given above (note that DI for each replication was estimated using both stratified and unstratified germination tests, i.e., no treatment effect). Differences among germination parameters for the stratification treatments of the bulk lodgepole pine and white spruce seedlots were presented graphically.

Results and Discussion

The reliance on a single germination index such as Czabator's germination value (Czabator 1962) or even any of its individual components (i.e., mean daily germination or PV) is inadequate. The reason for the inadequacy of these measures is their lack of association with time over the germination course, even though time is used to estimate each of the index components. This caveat was recognized by seed technologists, and PV values are commonly presented with their respective time references (The British Columbia Ministry of Forests and Range's Tree Seed Centre, pers, comm.). Comparison of Czabator's PV and TMGR for the stratified and unstratified seedlots of lodgepole pine indicated that germination behavior was not adequately described by either parameter alone (Table 1 and Figure 1). PV is almost meaningless unless it is reported with the corresponding germination percentage. Similarly, TMGR characterizes maximum daily germination but does not provide information on the germination value. Although both values showed consistent trends for stratified and unstratified seed (i.e., higher PV value and lower TMGR, respectively), their use alone does not allow one to draw any informative conclusions about the germination behavior of a seedlot. The peak values of 2 and 9 obtained for the unstratified and stratified germination courses; respectively, are meaningless if they are not reported with the reference times (i.e., 16 and 9 days) and the germination percentages (i.e., 30 and 81%) (Table 1). Similarly, TMGR values of 12 and 6 days are meaningless if they are not reported with the incremental increase in daily germination (Table 1). These examples highlight the drawbacks of using a single "index" or parameter.

Germination parameters derived by fitting the 4-PHF for the 18 unstratified and stratified lodgepole pine families produced several significant correlations (Table 2). For example, there was a perfect correlation between TMGR and R_{50}'. Careful examination of TMGR and R_{50}' values revealed that they were almost equal, but R_{50}' was consistently slightly lower than TMGR. This indicates that TMGR could be used as a reasonable proxy for R_{50}' and vice versa. This perfect correlation is not surprising. If c in Equation 1 is set to be equal to x, then

$$y = y_0 + \frac{ac^b}{c^b + c^b} = y_0 + a/2, \tag{7}$$

Table 3. ANOVA for the germination parameters of seedlots from 18 lodgepole pine families subjected to seed pretreatment (4 weeks stratification)

SOV	df	EMS	a	b	R_{50}	TMGR	Lag	$D_{\text{lag-50}}$	DI
			%						
Treatment (T)*	1	$\sigma_e^2 + 4\sigma_{tf}^2 + 72\theta_t$	–**	–**	–**	–**	–**	–**	NA†
Family (F)	17	$\sigma_e^2 + 8\sigma_f^2$	36.4	37.6	58.9	57.0	22.9	43.9	93.4**
$T \times F$	17	$\sigma_e^2 + 4\sigma_{tf}^2$	52.0**	40.7**	32.3**	34.4**	18.8**	26.6**	NA
Residual	108	σ_e^2	11.6	21.7	8.8	8.6	58.3	30.5	6.6

SOV, source of variation; df, degrees of freedom; EMS, expected mean squares. See text for parameters description.

* Although no variance components or percent of total variation were estimated for the fixed effect (treatment), the treatment effect was highly significant for all tested parameters.

† DI was tested using a reduced additive linear model (i.e., no T and $T \times F$ effects) with residual's df = 54.

** Significant at $P < 0.01$.

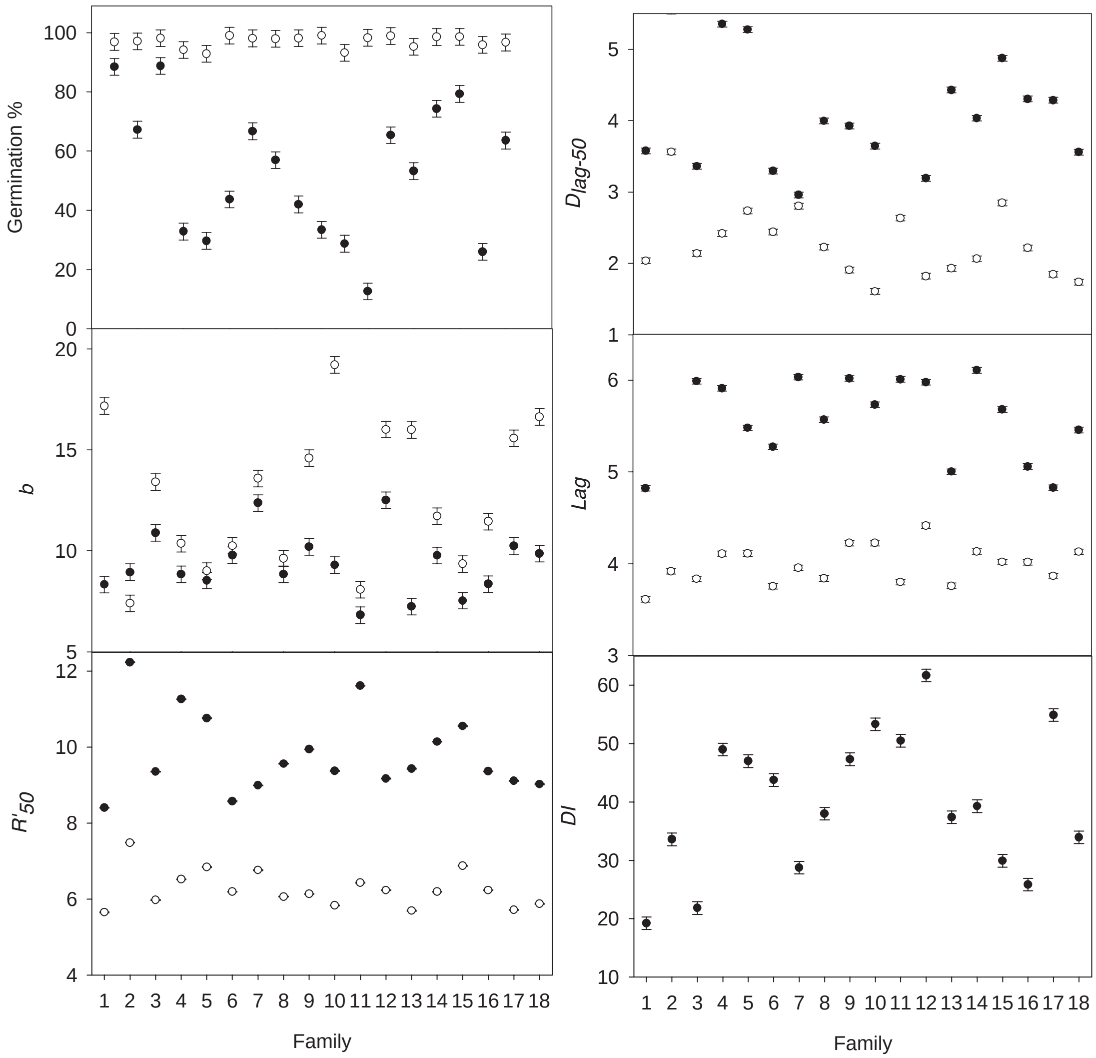

Figure 3. Germination parameters for 18 stratified (○) and unstratified (●) lodgepole pine seedlots (families) (see text for parameters explanation). Vertical lines represent 95% confidence intervals.

which is precisely why the parameter c is referred to as thehalf-maximum rate. Insofar as c decreases, so too will the time to the point of inflection because the inflection point will generally be somewhere in the vicinity of the 50%

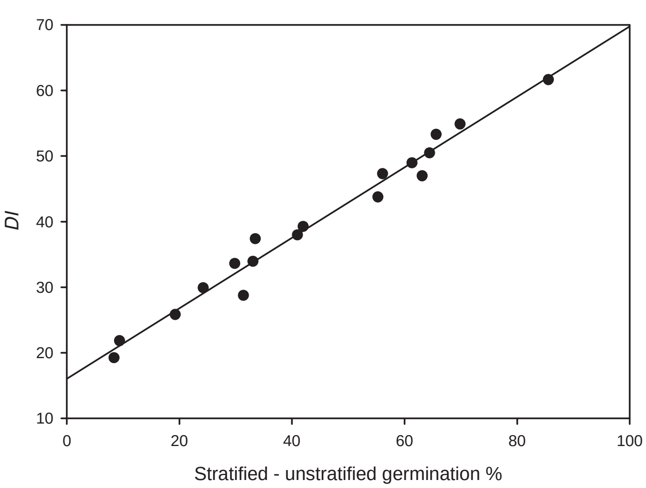

Figure 4. Correlation between the difference between stratified and unstratified germination percentages versus DI as determined by the Hill function for 18 lodgepole pine seedlots (families).

level of germination. Another correlation of interest was the negative correlation between b and $D_{\text{lag-50}}$ (−0.71 and −0.82 for the stratified and unstratified seedlots, respectively). This correlation confirms the role of germination onset (promptness) and speed on compacting the germination duration, which in turn affects the curve shape and steepness (Table 2). As expected, the faster the germination, the earlier R_{50}' is reached, resulting in a shorter $D_{\text{lag-50}}$ period. Thus, correlations between the time of germination onset (lag) and both TMGR and R_{50}' are expected to produce contrasting results for stratified (TMGR = 0.63, R_{50}' = 0.67) and unstratified (TMGR = 0.18, R_{50}' = 0.22) seed. These correlations indicate that the stratification treatment was effective in speeding the onset of germination as well as the time needed to reach 50% germination (Table 2). This result is further confirmed by the high correlation between TMGR and $D_{\text{lag-50}}$ for both stratified (0.86) and unstratified (0.89) seed (Table 2). The parameter b (shape and steepness) of the stratified seed curve did not correlate with its germination capacity (a), indicating that the steepness of the germination curve is not associated with higher germination. Finally as expected, b produced significant negative

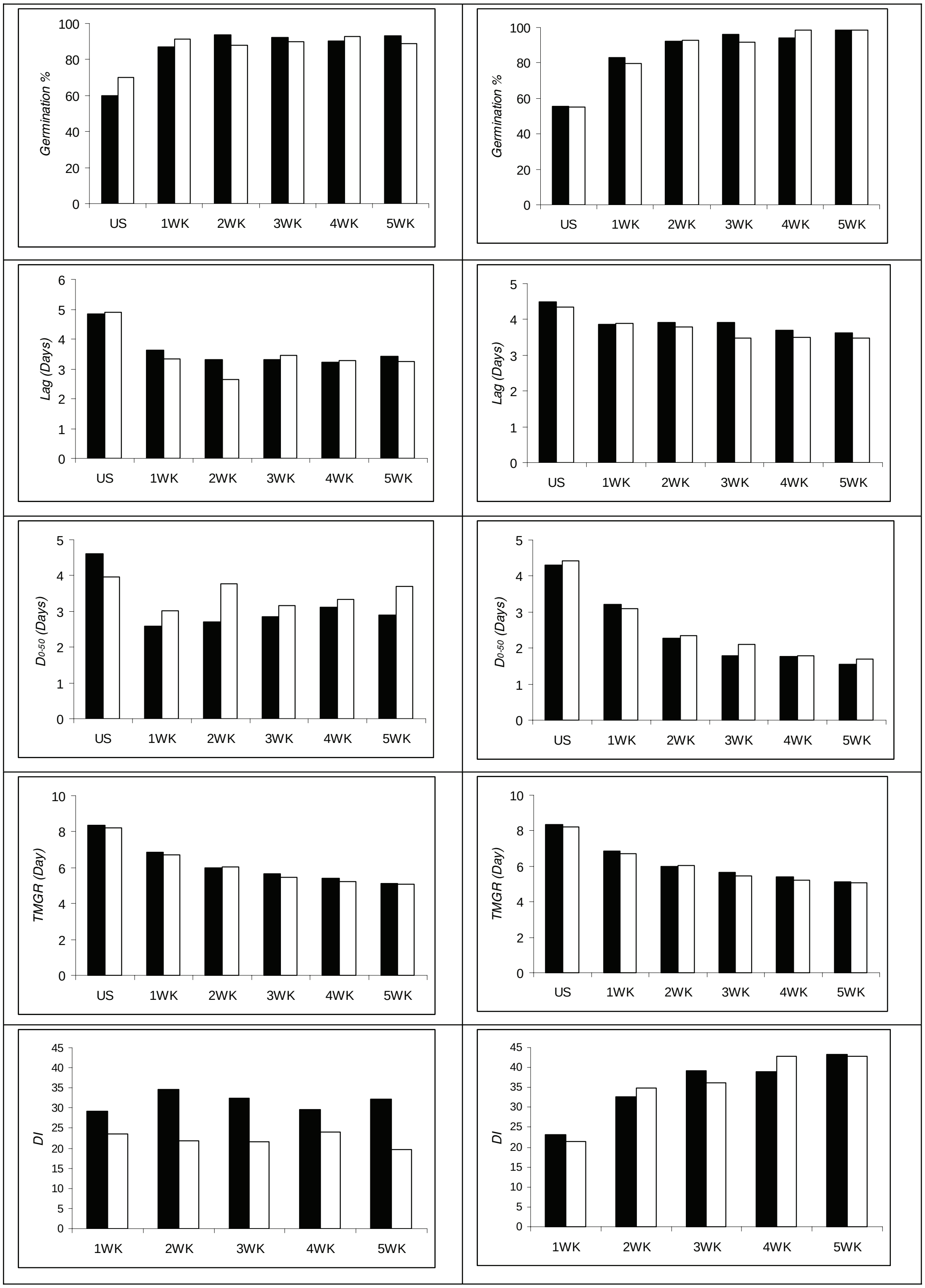

Figure 5. Interior spruce (left) and lodgepole pine (right) germination parameters as affected by stratification duration. Black and white columns represent interior spruce seedlots 60751 and 60425 and lodgepole pine 2001 and 2002 seed orchard lots, respectively. US, unstratified.

correlations with early germination parameters such as TMGR and $D_{\text{lag-50}}$ (Table 2).

Variation in germination parameters was highly significant for the treatment × family interaction (Table 3). Thus, graphical representations are best for interpreting treatment and family effects (Figure 3). Seed pretreatment was highly significant for all germination parameters; however, the use of the mixed model did not allow us to estimate treatment differences (i.e., quantifying fixed effects violates analysis of variance assumptions). The family effect and the seed pretreatment × family interaction accounted for 23–59% and 19–52% of the variation, respectively (Table 3). These results are in line with previously published estimates for the same species (Krakowski and El-Kassaby 2005). However, the germination parameters tested in the present study are the product of curve fitting using the 4-PHF. Graphical representation of each germination parameter allowed clear assessment of either the seed pretreatment and family effects on the observed variation (Figure 3). Seed pretreatment had a dominant effect on improving percent germination (parameter *a*), lag, R_{50}', and $D_{lag\text{-}50}$, resulting in smaller differences among families after stratification (Figure 3). Parameter *b* confirmed the observation from the correlation analyses (above) and produced mixed results for the stratified and unstratified seed. This supports the hypothesis that the shape and steepness of the germination curves are independent of germination performance (Table 2 and Figure 3). Family differences were large for all germination parameters, particularly for unstratified seed; therefore, the stratification treatment was successful in reducing these differences and harmonizing germination among the 18 families (Figure 3).

DI varied significantly among families, with families accounting for 93% of the total variation. This finding indicates that dormancy level is a family-specific attribute and is under strong genetic control (broad-sense heritability/repeatability = 0.93) (Table 3 and Figure 3). Although family dormancy differences were large, they were substantially lessened by the stratification treatment, producing uniform germination parameters (Figure 3). Kolotelo (2006) advocated the development of a simple and quick method for estimating dormancy so it could be used operationally. Therefore, we determined the differences between the stratified and unstratified germination percentages for the 18 lodgepole pine families and correlated these differences with the actual DI for each family. Surprisingly, a high and significant correlation ($r = 0.99$) was obtained ($R^2 = 0.978$). This indicates that 98% of the variation in DI could be explained by differences in percent germination (Figure 4). Thus, we propose this approach for estimating dormancy.

The lodgepole pine and white spruce bulk seedlots responded differently to the stratification treatments (Figure 5). Lodgepole pine bulk seedlots produced steady improvement in percent germination with increasing stratification time. This improvement was also associated with a steady decline in the other parameters (lag, $D_{lag\text{-}50}$, TMGR, and R_{50}') (Figure 5). DI, estimated as the difference between the areas under the various stratification treatments and the control (unstratified), also showed a steady increase with increasing stratification. An increase in DI indicates that the difference between the stratified and unstratified germination curves is increasing and that stratification improves germination. The trend observed for the white spruce bulk seedlots mirrored that of the lodgepole pine seedlots (i.e., improved germination with stratification), but the two spruce seedlots differed in their response (Figure 5). These differences were most notable for $D_{lag\text{-}50}$ and DI, indicating that extended stratification could have a detrimental effect on white spruce. This finding indicates that generalizations across the two species could be inappropriate. White spruce seedlots responded similarly after 1 week of stratification, and responses were inconsistent over the different stratification times (Figure 5), suggesting that this species has shallow dormancy. These results are consistent with earlier work on a larger sample of 26 seedlots, including natural stand and seed orchard collections (Kolotelo 1994).

When Hill (1910, 1913) first derived his sigmoidal function using three parameters, he based it on a mechanistic model for the binding of oxygen to the enzyme hemoglobin (Christopoulos and Lew 2000). The Hill equation was first thought to be mechanistic because the resulting parameters provided actual information about the underlying properties of the interaction. Subsequent experiments revealed that it was inadequate for this purpose, although it continues to be used as a mechanistic model (i.e., when its validity is confirmed) and as an empirical model when the shape of its curve approximates that of experimental data.

The main reason for choosing the 4-PHF versus alternatives (e.g., four-parameter Weibull, Gompertz, Chapman, and logistic functions) was the ease with which the various parameters could be interpreted. Equation 1 was also found to fit the data with a reasonable level of precision, such that reliable comparisons could be made between stratified and unstratified seedlots. The program used to fit the equations converged quickly onto each solution without the need to arbitrarily constrain any of the parameters. For broad application, however, it may be desirable to constrain the asymptote to values $\leq$100% germination. Most of the fitted equations were statistically significant, and most of the R^2 values were $\geq$0.9. A few seedlots with poor germination rates (e.g., <30% at the end of 21 days) produced lower *F* test and R^2 values than those described above. In any event, such seedlots would not be operationally deployed for seedling production, particularly if they did not demonstrate substantial improvement in germination after stratification treatment.

No adjustments were made in the curve-fitting procedure to deal with the effects of autocorrelation and heteroscedasticity. Although TMGR is constrained to be less than *c*, this is justified because TMGR tends to overestimate rather than underestimate the inflection point. TMGR provides a better estimate of the inflection point because it is based on all of the data points. Finally, although it may have been possible to obtain better fits using a different form of the sigmoidal equation and better accounting of the error structure, this would not have made any substantive difference to the results or conclusions derived from this study.

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