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The role of moist-chilling and thermo-priming on the germination characteristics of white spruce (*Picea glauca*) seed

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Summary

The individual and combined effect of moist-chilling and thermo-priming on germination parameters (capacity, speed, lag and dormancy index) were evaluated using seed lots representing British Columbia's five white spruce (*Picea glauca*) seed planning zones. The combined effect of moist-chilling followed by a 3-day priming at 15 or 20°C, stimulated seed germination across all seed lots. These combined treatments resulted in substantial improvement of all germination parameters including reducing the dormancy index (14 vs. 25). Generally, three days of priming at 20°C yielded a higher percent germination than priming for the same period at 15°C, irrespective of whether or not seeds had received a previous moist-chilling treatment. Thermo-priming alone led to some improvement in germination characteristics such as reducing the time to germination onset (lag) and increasing germination speed. These results indicate that the combined application of moist-chilling and thermo-priming could improve container nursery practices for commercial seedling production of white spruce. The positive combined effect of moist-chilling and thermo-priming on seed germination offer great potential for generalised application to other conifers; however, species-specific treatment adjustment is required.

Introduction

Prompt and uniform germination can ameliorate under-stocked seedbeds, reduce vulnerability to soil-borne diseases and maximise nutrient use efficiency (Cantliffe, 2003; O'Reilly and Doody, 2005). Crucially, synchronisation of germination within the population and rapid seedling emergence are of great commercial significance. For example, tree nurseries in northern climates must maintain greenhouse temperatures between 22 and 25°C for an extended period. This is essential to favour adequate germination and seedling establishment, and to improve growth rates during early seedling development. More than 100 million white spruce seedlings are produced yearly for regeneration across Canada (Bousquet *et al.*, 2007) and the cost for heating greenhouses is a major expenditure (Wood, 1995). Rapid, synchronous and full-capacity germination within a seed population are thus pivotal components of conifer seedling production.

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From the commercial point of view, efficient seedling production in forest nurseries requires the use of treatments that will effectively break seed dormancy. From a biological point of view, seed dormancy is an adaptive trait as it safeguards against seed germination when conditions are unfavourable (Baskin and Baskin, 1998). The dormancy of gymnosperm (*Coniferales*, *Gnetales*) seed is mainly caused by physiological dormancy (Baskin and Baskin, 2004), and is largely coat-enhanced (Bewley *et al*., 2012). Seeds of gymnosperms *in situ* will often gradually break dormancy as the temperature increases, a process that generates a series of temperature-driven events. Changes in photoperiod are also likely to contribute to dormancy release. Under controlled laboratory conditions, exposure of seeds to moist-chilling prior to germination conditions has proven very effective in relieving the dormancy of most conifer species' seeds (Wang and Berjak, 2000). Indeed this treatment has long been recognised as an effective means of overcoming the physiological dormancy of seeds (Barton, 1930). Standard procedures for moist-chilling are now in place to improve dormancy-breakage and germination performance of seeds of most temperate conifers (Edwards, 1986; Barnett, 1989). The beneficial effects of moist-chilling might be attributed to the stimulation of appropriate enzyme activity and initiation of inherent repair mechanisms (Villiers, 1971, 1972; Ward and Powell, 1983). Changes in hormone flux in both the megagametophyte and embryo contribute to the positive effects of moist-chilling on the dormancy breakage of conifer seeds, especially a higher relative rate of catabolism of the germination inhibitor abscisic acid (ABA) (Schmitz *et al.*, 2002; Zeng *et al.*, 2003; Feurtado *et al.*, 2004; Kermode, 2005; Feurtado *et al.*, 2007; Pieruzzi *et al.*, 2011). The bi-directional movement of solutes from embryo to the environment is also proposed to underlie the efficacy of this practical method (Taylor *et al.*, 1998; Ma *et al.*, 2003). The waxy seed coat and mechanical restraint imposed by the megagametophyte are significant factors that participate in conifer seed dormancy mechanisms (e.g., through restriction of water uptake and as a barrier to radicle emergence, respectively) (Downie and Bewley, 1996; Ren and Kermode, 1999, 2000). Generally, a prolonged soak at the beginning of the dormancy-breaking regime is necessary to ensure that adequate moisture is absorbed by the embryo early in the process (Tillman-Sutela and Kauppi, 2000). Even for conifer species such as white spruce that have relatively shallow dormancy, moist-chilling appears to stimulate physiological changes yielding rapid and/or uniform germination (Downie *et al.*, 1998).

Seed priming can also enhance germination and it can provide the environmental conditions necessary to trigger the initiation of germination (e.g., moisture, temperature and light) (Taylor *et al*., 1998; Holdsworth *et al*., 2008). Essentially seeds are brought to the 'brink' of germination, such that when seeds are transferred to germination conditions they very rapidly complete germination (De Atrip *et al*., 2007). Priming can involve different matrices and other conditions that regulate seed moisture content; this can be achieved through the use of various aqueous solutions, solid particulate systems and a spectrum of controlled moisture contents (Taylor *et al*., 1998). The associated methods are referred to as hydro-priming (exposing seeds to a controlled amount of water at a specific temperature), osmo-priming (soaking seeds in inorganic salts, PEG, mannitol or glycerol), solid matrix priming (pre-sowing seeds in a solid matrix to attain a threshold moisture content), drum-priming (controlled vapour-generated hydration) and bio-priming

(coating seeds with bacterial agents and imbibing in warm water) (Wartidiningsih *et al*., 1994; Warren and Bennett, 1997; Wu *et al*., 2001; Masuda *et al*., 2005; O'Reilly and Doody, 2005; Moeinzadeh *et al*., 2010). Thermo-priming is another seed pretreatment that involves exposing bulk seeds to specific moisture (usually a full soak) and specific temperature conditions in darkness.

The present study is a comparative analysis of the germination performance of five seed lots of white spruce (*Picea glauca* [Moench] Voss). Pre-treatments were designed to achieve rapid and synchronous germination and we used a parameters extraction protocol, namely; the four-parameter Hill function (El-Kassaby *et al*., 2008) to measure germination parameters and to accurately assess their individual and combined effects. Standard seed pre-treatments (moist-chilling and thermo-priming) were imposed using bulk seed lots obtained from natural stands representing five white spruce seed planning zones within British Columbia, Canada.

Materials and methods

Seed material

Five seed lots of white spruce (*Picea glauca*) representing five different seed planning zones (SPZ) in British Columbia, Canada were used and all exhibited similar average standard germination percentages (table 1). Seed lots from natural stands are required to pass two tests for registration and reforestation use on Crown land. These are: purity (> 97%) and moisture content on fresh weight basis (4.9 to 9.9%) (Kolotelo *et al*., 2001). Seeds were maintained at -18°C (moisture content between 4.9 and 9.9%). Just prior to use, the seeds were transferred to vials (75 per vial) and stored at 4°C.

Table 1. Characteristics of the white spruce seed lots used in the present study.

Seed lot Number	Seed Planning Zones[1]	Elevation (m a.s.l.)	Year Collected	Mean Annual Temperature[2] °C	Annual Heat Moisture Index[2]	Germination[3] %	Moisture Content %
33356	WK (50°15'N 118°10'W)	1190	1991	3.0	13.6	96	7.0
35707	MIC (51°02'N 118°48'W)	1200	1991	3.4	9.3	97	7.0
37842	MGR (54°26'N 121°44'W)	850	1992	4.5	12.1	95	8.0
39450	CP (55°03'N 125°02'W)	875	1994	2.5	20.1	95	7.6
45353	SM (54°39'N 128°45'W)	800	1996	1.3	10.8	97	7.0

[1] The Seed Planning Zones in BC are abbreviated WK, MIC, MGR, CP, and SM; these denote West Kootenay, Mica, McGregor, Central Plateau, and Submaritime zones, respectively.

[2] Based on ClimateBC (http://www.genetics.forestry.ubc.ca/cfcg/ClimateBC40/Default.aspx).

[3] As carried out by the Tree Seed Centre (Tree Improvement Branch, Ministry of Forests, Lands and Natural Resource Operations) according to International Seed Testing Association (ISTA) rules.

Seed treatments and germination conditions

The experimental design was a completely randomised design implemented in a three-way factorial ($5 \times 2 \times 3$ levels) with five white spruce seed lots (random effect), with or without moist-chilling treatment (fixed effect), and without priming or with thermo-priming at 15 or 20°C (fixed effect). Moist-chilling was conducted as per ISTA (1999) rules; for white spruce seeds, this involves soaking the seeds for 24 hours at room temperature, surface-drying the seeds and then conducting 21 days of moist-chilling at 2°C. The control was comprised of non-moist-chilled seeds. Thermo-priming treatments were conducted at 15 or 20°C for three days in the dark; the control comprised non-thermo-primed seeds. A final control comprised seeds that were neither subjected to moist-chilling nor thermo-priming. Each treatment combination consisted of four replications of a 75-seed sample. Treatments were conducted in a manner that allowed for germination assays to begin on the same day.

Seeds were imbibed for 24 hours in distilled water prior to moist-chilling. The soaked seeds were then placed in clear plastic germination boxes (4.5 L × 4.5 W × 1.5 D cm) lined with moistened cellulose wadding (Kimpack®) and filter paper, and were chilled for 21 days at 2°C under high humidity conditions and without supplemental light (Bonner and Karrfalt, 2008). After moist-chilling, replicates destined for thermo-priming were transferred to a light-proof and temperature-set chamber at 15 or 20°C for three days. For the thermopriming (alone) treatment seeds were placed in clear plastic germination boxes ($4.5 \times 4.5 \times 1.5$ cm) lined with moistened cellulose wadding (Kimpack®) and filter paper, and were transferred to the temperature-set chamber at 15 or 20°C for three days.

Germination was conducted in an incubator with alternating temperatures of 20/30°C (dark/light) with eight hours of fluorescent illumination (approximately 13.5 μmol m^{-2} s^{-1}) per day. Germination was conducted over a 28-day period following standard ISTA conditions (ISTA, 1999). Germinants were counted daily throughout the germination test. Seeds were counted as germinated if the radicle emerged to four times the seed length (approximately 4 mm for white spruce seeds) and showed gravitropic curvature, as this is the standard in the forest industry (Kolotelo *et al.*, 2001). The numbers of empty and diseased seeds were determined at the end of the test by cutting open non-germinated seeds.

Germination parameters

The four-parameter Hill function (4-PHF) (El-Kassaby *et al*., 2008) was used to quantify the germination of each seed lot (5) - treatment (6) - replication (4) combination (N = 120). Parameters estimated were: *a*, the maximum cumulative germination percentage equivalent to germination capacity (GC); *b*, a mathematical parameter controlling the shape and steepness of the curve (the larger the *b* value, the steeper the rise toward *a*); *c*, the time required to achieve 50% germination, equivalent to R_{50} [germination speed (Thomson and El-Kassaby, 1993)]; TMGR, the time of maximum germination rate; lag, the time of germination onset; $D_{lag\text{-}50}$, the duration between *lag* and *c*; and *DI*, dormancy index, the area between the germination curves of control and any pre-treatment.

Data analyses

The effect of seed lot, moist-chilling, thermo-priming and their interactions on 4-PHF

parameters was investigated using the GLM procedure in SAS® vers. 9.1.3 (SAS Institute, 1999). The germination parameters were subjected to analyses of variance using the following additive linear model as follows:

$$Y_{ijkl} = \mu + S_j + P_k + C_l + SP_{jk} + SC_{jl} + PC_{kl} + SPC_{jlk} + \varepsilon_{i(jkl)} \quad [1]$$

where, μ is the overall mean, S_j is the effect of the j^{th} seed moist-chilling treatment (j = 1 to 2, fixed effect), P_k is the effect of the k^{th} thermo-priming treatment (k = 1 to 3, fixed effect), C_l is the effect of the l^{th} seed lot (l = 1 to 5, random effect), SP_{jk} is the interaction between seed j^{th} moist-chilling and k^{th} thermo-priming treatment combination, SC_{jl} is the interaction between j^{th} moist-chilling and l^{th} seed lot, PC_{kl} is the interaction between k^{th} thermo-priming and l^{th} seed lot, SPC_{jkl} is the three-way interaction among j^{th} moist-chilling, k^{th} priming and l^{th} seed lot; and $\varepsilon_{i(jkl)}$ is the residual term (i = 1 to 4) (see table 2 for sources of variation (SOV), degrees-of-freedom (df) and component of variance (EMS).

In addition, we used a reduced model that represents a subset of the above-mentioned full model. This was needed to conduct a simplified analysis that isolated the moist-chilled treatment. The threshold for statistical significance was always set at $P < 0.05$.

Results

We first examined the effects of the various treatments on the germination characteristics of the five different seed lots (figure 1). The best treatment for seed lot 37842 was the 20°C-thermo-priming combined with moist-chilling. This treatment was substantially superior to that of the standard treatment (i.e., moist-chilling alone) (figure 1; seed lot 37842). In the absence of moist chilling, a 20°C thermo-priming treatment stimulated relatively prompt germination of non-moist-chilled seeds, noticeably affecting both the 'lag' parameter and the total germination (compared with the no-thermo-priming control). Likewise, the other seed lots showed a significant benefit of the thermo-priming following moist-chilling (figure 1), and again the thermo-priming at 20°C yielded better germination than the thermo-priming at 15°C.

Without exception, the five seed lots yielded higher estimates of DI following the combined treatment of moist-chilling and three days of 20°C thermo-priming compared with standard moist-chilling alone (note: a high DI value means greater differences between treatments, indicating an improved germination pattern) (figure 2).

Seven out of the 12 first-order interactions involving moist-chilling (moist-chilling × priming and moist-chilling × seed lots) as a source of variation were significant, indicating that perhaps the inclusion of the control treatment (no moist-chilling and no priming) in the analyses may have polarised the results of the germination parameter analyses (table 2). This in turn may have obscured the true effects of the treatments. To overcome this, we repeated the previous analyses for the moist-chilled and non-moist-chilled seeds separately (table 3). Unlike the 3-way analyses, the differences between the two priming treatments were consistently significant across all germination parameters (table 3B).

Table 2. ANOVA for the germination full model. (A) Expected Mean Squares (EMS) for the ANOVA model used to analyse the germination parameters using five interior spruce seed lots and (B) Mean square (MS), *F*- and *P*-values for the various sources of variation across the studied germination parameters of five interior spruce seed lots (see text for germination parameters explanation).

(A)

SOV	df	EMS
Moist-chilling (M)	1	$60\phi_m + 12\sigma_{ms}^2 + \sigma_e^2$
Priming (P)	2	$40\phi_p + 8\sigma_{ps}^2 + \sigma_e^2$
Seed lot (S)	4	$24\sigma_s^2 + \sigma_e^2$
M × P	2	$20\sigma_{mp}^2 + 4\sigma_{mps}^2 + \sigma_e^2$
M × S	4	$12\sigma_{ms}^2 + \sigma_e^2$
P × S	8	$8\sigma_{ps}^2 + \sigma_e^2$
M × P × S	8	$4\sigma_{mps}^2 + \sigma_e^2$
Residual	90	σ_e^2

(B)

SOV	***a***			***b***			***c***		
	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Moist-chilling (M)	2014	9.94	0.0344	225.8	94.03	0.0006	162.8	230.20	0.0002
Priming (P)	5.8	0.53	0.6103	142.4	46.12	0.0001	41.1	222.58	0.0001
Seed lot (S)	592.0	48.5	0.0001	33.5	16.57	0.0001	11.9	161.98	0.0001
M × P	4.3	0.21	0.8123	5.3	1.60	0.2612	2.5	9.54	0.0076
M × S	202.6	16.60	0.0001	2.4	1.19	0.3220	0.7	9.61	0.0001
P × S	11.1	0.91	0.5148	3.1	1.53	0.1592	0.2	2.51	0.0164
M × P × S	20.0	1.64	0.1257	3.3	1.65	0.1217	0.3	3.51	0.0014
Residual	12.2			2.0			0.1		

SOV	***TMGR***			***Lag***			$D_{lag\text{-}50}$		
	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Moist-chilling (M)	139.6	162.86	0.0001	25.5	217.53	0.0001	59.4	71.73	0.0011
Priming (P)	46.1	220.84	0.0001	47.4	135.54	0.0001	0.3	0.72	0.5136
Seed lot (S)	12.4	175.81	0.0001	4.4	22.89	0.0001	3.1	15.43	0.0001
M × P	1.8	6.62	0.0201	0.4	1.99	0.1991	3.3	10.56	0.0057
M × S	0.9	12.15	0.0001	0.1	0.62	0.6527	0.8	4.16	0.0039
P × S	0.2	2.96	0.0055	0.3	1.83	0.0807	0.5	2.38	0.0225
M × P × S	0.3	3.83	0.0006	0.2	1.13	0.3504	0.3	1.59	0.1401
Residual	0.1			0.2			0.2		

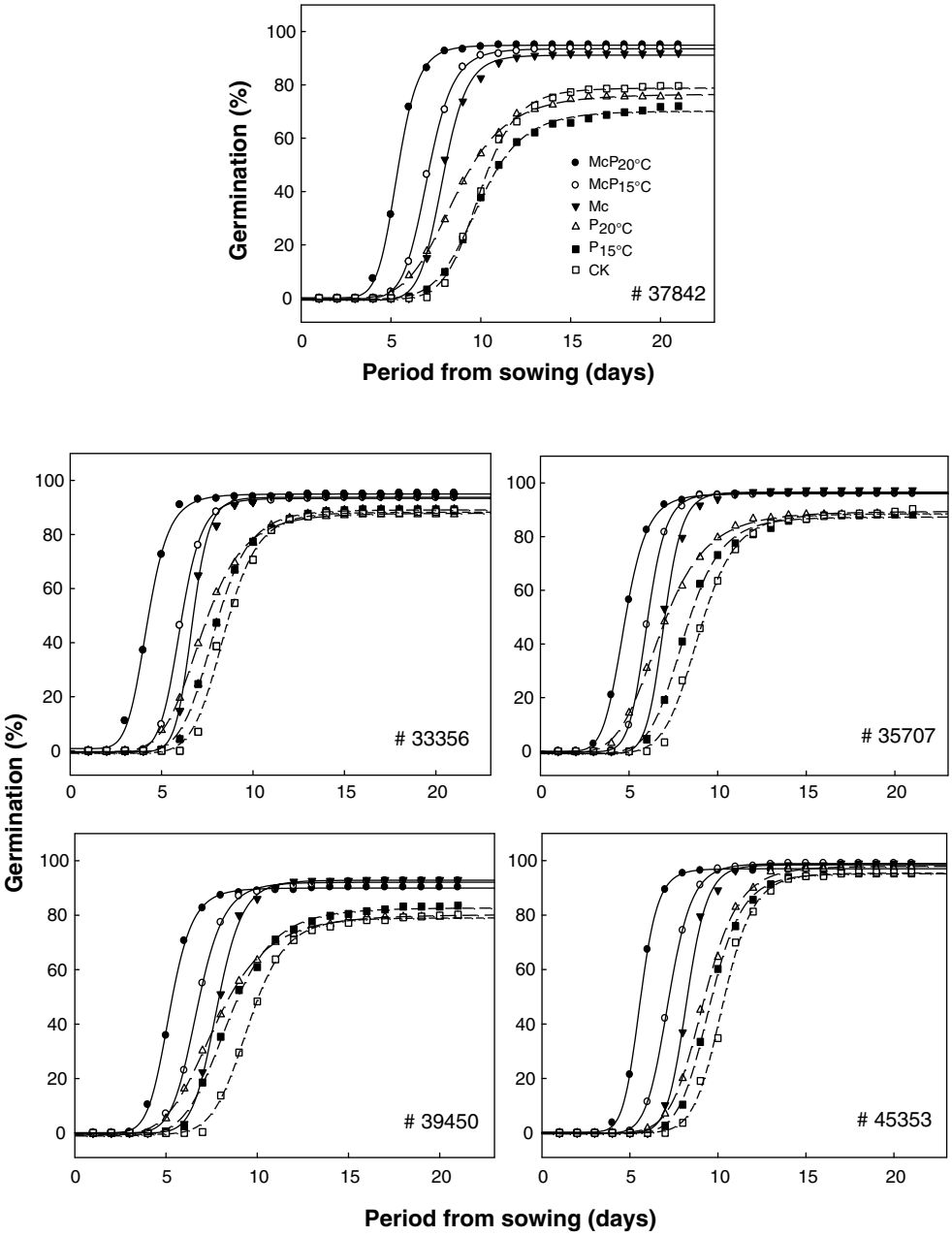

Figure 1. Effects of moist-chilling and priming on germination. (Mc: moist-chilling; $P_{20°C}$: priming at 20°C for three days; $McP_{20°C}$: moist-chilling followed by 20°C priming for three days; $P_{15°C}$: priming at 15°C for three days; $McP_{15°C}$: moist-chilling followed by 15°C priming for 3 days; CK: control (no treatment). Seed lot numbers are indicated on the bottom right-hand side of each graph.

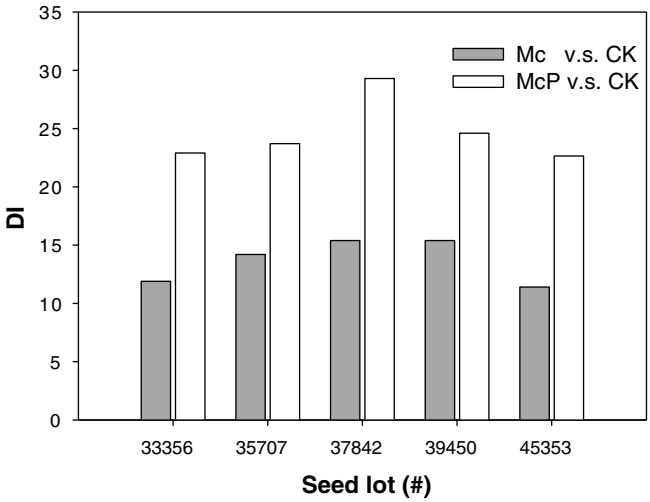

Figure 2. Dormancy index (DI) of the five white spruce seed lots. (Mc: moist-chilling; McP: moist-chilling followed by 20°C priming for three days; CK: control (no treatment). The data for DI calculation are based on the average of four replicates of each treatment.

Thus priming at 20°C (compared with 15°C) produced a similar germination capacity (*a*), but led to a steeper rise toward maximum germination (*b*), faster germination speed (*c*), shorter time to reach the maximum germination rate (*TMGR*), shorter germination lag (*lag*) and a shorter duration between the time at germination onset and time to reach 50% germination ($D_{lag\text{-}50}$) (table 4B). The results also confirmed the added benefits of the combined moist-chilling-priming treatment. Additionally, among the five seed lots studied, there were significant differences for four out of the six germination parameters (table 3B). The first-order interaction between priming and seed lot was not significant for four out of six germination parameters, thus justifying the abbreviated analysis (table 3). Additionally, the two significant first-order interactions between priming and seed lots were related to promptness and evenness of germination (*lag* and $D_{lag\text{-}50}$) rather than to the germination rate (table 3B), indicating that priming was effective in optimising maximum germination rates.

The summary statistics of germination parameters provided a generalised depiction of the various treatments on germination behaviour (table 4). While results from the control treatment (no moist-chilling and no priming) represent the baseline performance of the seed lots, they could not be used as a benchmark for comparison because the standard seed pre-treatment commonly applied prior to seedling production required moist-chilling for 21 days at 2°C. Thus we use this treatment as the benchmark for improved germination patterns for seedling production. Generally, all germination parameters of both the combined treatments of priming and moist-chilling were better than that of moist-chilling alone. Furthermore, 3-days priming at 20°C was consistently better than priming at 15°C for the same duration (table 4). Additionally, the same trend is observed when priming alone (15 or 20°C) was compared with the control treatment.

Table 3. Reduced ANOVA model after the removal of the control treatment (no moist-chilling-no priming). (A) Expected Mean Squares (EMS) for the ANOVA model used to analyse the germination parameters using five interior spruce seed lots and (B) Mean square (MS), *F*- and *P*-values for the various sources of variation across the studied germination parameters of five interior spruce seed lots (see text for germination parameters explanation).

(A)

SOV	df	EMS
Priming (P)	2	$20\phi_p + 4\sigma_{ps}^2 + \sigma_e^2$
Seed lot (S)	4	$12\sigma_s^2 + \sigma_e^2$
P × S	8	$4\sigma_{ps}^2 + \sigma_e^2$
Residual	45	σ_e^2

(B)

SOV	***a***			***b***			***c***		
	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Priming (P)	3.8	0.67	0.5396	99.4	17.45	0.0012	31.8	469.55	0.0001
Seed lot (S)	78.9	14.42	0.0001	11.9	3.77	0.0099	4.1	102.61	0.0001
P × S	5.6	1.03	0.4275	5.7	1.80	0.1021	0.1	1.71	0.1224
Residual	5.5			3.2			0.04		

SOV	***TMGR***			***Lag***			$D_{lag\text{-}50}$		
	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Priming (P)	32.9	583.28	0.0001	23.7	58.92	0.0001	1.4	2.76	0.1228
Seed lot (S)	4.1	101.7	0.0001	1.9	12.6	0.0001	1.5	10.16	0.0001
P × S	0.1	1.41	0.2204	0.4	2.59	0.0204	0.5	3.34	0.0044
Residual	0.04			0.2			0.2		

Discussion

Moist-chilling is the most widely used method to break dormancy and stimulate prompt and even germination for seeds of most conifers and other boreal and north-temperate tree species (Smith, 1973; Adkins *et al*., 1984; Wang and Berjak, 2000; Krakowski and El-Kassaby, 2005; Rawat *et al*., 2008). Notwithstanding the effectiveness of moist-chilling on germination behaviour, within-species and seed lot variation is common and has a genetic and/or environmental basis (Stoehr and Farmer, 1986; Chaisurisri *et al*., 1992; El-Kassaby *et al*., 1992; Farmer, 1997). Stoehr and El-Kassaby (2011) implicated seed dormancy as the main cause for the non-uniform germination patterns frequently observed in container nurseries. Unintentional genetic selection can occur in container nurseries during the thinning of excess germinants when multiple sowing is practiced (El-Kassaby

Table 4. Average and range of the germination parameters across treatments for five white spruce seed lots. Treatments: none, as control (no moist-chilling, no priming); Mc, moist-chilling; $P_{20°C}$, priming at 20°C for three days; and $P_{15°C}$, priming at 15°C for three days.

Treatment	Parameters in average (range)		
	a	*b*	*c*
none	87.3 (76.8-98.3)	9.8 (8.1-13.8)	9.4 (8.3-10.4)
M.c.	95.2 (91.1-99.9)	13.4 (10.3-18.6)	7.5 (6.6-8.5)
M.c. + $P_{20ºC}$	94.5 (85.7-99.4)	9.0 (6.0-13.4)	5.0 (4.0-5.7)
M.c. + $P_{15ºC}$	95.2 (88.9-99.9)	10.7 (8.2-13.4)	6.6 (5.9-7.6)
$P_{20ºC}$	86.7 (71.1-97.9)	6.7 (4.6-10.9)	7.9 (6.5-9.4)
$P_{15ºC}$	86.2 (66.2-97.7)	8.3 (5.9-11.6)	8.8 (7.7-10.5)

Treatment	Parameters in average (range)		
	TMGR	*Lag*	$D_{Lag\text{-}50}$
none	9.2 (8.1-10.3)	5.6 (5.1-6.7)	3.9 (2.9-4.9)
M.c.	7.4 (6.5-8.3)	4.8 (3.6-5.7)	2.7 (1.9-4.2)
M.c. + $P_{20ºC}$	4.9 (3.8-5.6)	2.6 (1.6-3.6)	2.4 (1.9-3.3)
M.c. + $P_{15ºC}$	6.5 (5.8-7.5)	3.7 (2.5-4.7)	2.9 (2.0-3.9)
$P_{20ºC}$	7.5 (5.9-9.0)	3.4 (2.4-5.2)	4.5 (3.6-5.9)
$P_{15ºC}$	8.5 (7.4-10.1)	4.8 (3.9-6.0)	4.0 (3.1-5.5)

and Thomson, 1996; El-Kassaby, 2000). Even in species with shallow dormancy such as white spruce, dormancy-caused germination differences can occur amongst seeds of individual trees, seeds derived from different collection years or from different populations, or even seeds produced from the same tree (Caron *et al*., 1993; Downie *et al*., 1998). Decidedly, it is of great significance to improve germination uniformity and promptness to preserve genetic diversity during seedling production.

Priming is a seed pre-treatment often applied to initiate germination processes that occur prior to radicle emergence. In general, successful priming is dependent on adjusting moisture equilibria by exposing seeds to osmotic treatments such as PEG, KNO_3 or KH_2PO_4. Alternatively pre-treatments can also involve direct application of plant growth regulators such as gibberellic acid or ethephon to promote germination (Cantliffe *et al*., 1984; Taylor *et al*., 1998; Mortensen and Eriksen, 2004; Feurtado and Kermode, 2011). Irrespective of the method used to invigorate seed germination, the effects of priming are often determined empirically and there is no simple recipe or method available to guarantee optimal results. Some of the treatments for increasing germination rate and uniformity are intended to advance seed maturity, repair cellular damage or increase cell elongation and division activities when seeds are subsequently placed in germination

conditions (Welbaum *et al*., 1998; Dadlani *et al*., 2010). Several reports on priming under laboratory-scale conditions validate the treatment; only a small proportion of this work has been tested on a larger-scale due to treatment costs and/or the need to implement engineering tools (Berlage and Brandenburg, 1984; Taylor and Harman, 1990; Taylor *et al*., 1998; Hill, 1999; Black and Bewley, 2000). Thermo-priming enhances the germination rate of moist-chilled seeds of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), noble fir (*Abies procera* Rehd.), Columbine (*Aquilegia caerulea* James, *A. canadensis* L., and *A. hinckleyana* Munz.), cowpea (*Vigna sinensis* L.) and other species (Finnerty *et al*., 1992; O'Reilly and Doody, 2005; Farahani *et al*., 2011).

The two basic and seemingly most important factors for consideration in thermo-priming are treatment time and temperature; most studies indicate that adjustment of both priming time and temperature can significantly affect the germination rates (Hardegree, 1994; Meyer *et al*., 2000; Hardegree *et al*., 2002; O'Reilly *et al*., 2007). However, nursery practitioners are more accepting of the traditional treatments such as moist-chilling and indeed this treatment does yield vigorous seedling growth, which may be superior to other less tested methods involving various reagents. Since moisture content tends to be a critical factor, it may be that alternatives to moist-chilling are more difficult to control in this regard (Feurtado and Kermode, 2011). Further, seed technologists with expertise in engineering may help to implement seed enhancement techniques in the practical setting by modifying existing equipment or developing new equipment. In this context, the cumulative germination for the combined treatments of moist-chilling followed by three days of 20°C priming ($McP_{20°C}$) resulted in the highest improvement of germination parameters across seed lots. Under these conditions, the germination across seed lots ranged from 86 to 100%, compared with 77 to 98% for seed lots that were not subjected to any treatment (table 4).The difference of the cumulative germination between these two treatments was as high as 22% for seed lot 37842, and as little as 1% for seed lot 45353 (figure 1). These differences in the germination responses of the seed lots likely reflect differences in the 'depth' of dormancy, which are in turn associated with the different origins of the seed lots. Interestingly, these two seed lots (i.e., 37842 and 45353) originate from populations from similar geographical variables (i.e. similar elevation, longitude and latitude) but with very different climate variables (table 1). This reinforces the conclusion that the environment during seed maturation, especially temperature and moisture, plays a critical role in determining the level or depth of seed dormancy (Walter and Breckle, 2002; Walck *et al.*, 2011).

Moist-chilling, thermo-priming and seed lot had significant effects on the 4-PHF parameters with few exceptions (table 2). Interestingly there were significant differences among the five seed lots, suggesting that seeds from different seed planning zones reacted differently to the treatments. These differences are expected as the seed lots represent different ecological niches (seed planning zones; SPZ); hence there exist different dormancy-breaking requirements for different seed lots. In addition, thermo-priming can ameliorate germination performance, indicating that in the case of an emergency at a nursery (e.g., when sowing windows have passed due to unforeseen circumstances), a quick 3-day priming treatment could be used to overcome any negative consequences on germination (table 4).

In contrast to the $McP_{20°C}$ treatment, $P_{20°C}Mc$ (i.e., 20°C priming prior to moist-chilling) was also performed but was inferior (data not shown). The germination of seeds in response to $P_{20°C}Mc$ was initiated very early (on the first or second day of germination conditions) but levelled off for a relatively long duration and then slowly started to increase again. Obviously there was an adverse effect of $P_{20°C}Mc$, which may be attributed to the fact that some of the superficially-dormant seed could be directly stimulated to germinate by priming while the more dormant seed benefited little from priming without being subjected to moist-chilling first to relieve dormancy. An extra lag time was needed before germination processes were re-initiated. Consequently, priming prior to moist-chilling is not justifiable. The contrasting differences between the $McP_{20°C}$ and $P_{20°C}Mc$ treatments intimate that storing seeds in high temperature and high humidity conditions would adversely impact seed germination even if moist-chilling was implemented. Finally, it should be noted that 15 or 20°C is lower than most temperatures maintained by nursery greenhouses (i.e. 22 to 25°C). However, it should be emphasised that thermo-priming should be conducted immediately following moist-chilling at 2°C and under a complete hydration matrix (i.e. when seeds are fully imbibed).

This research was conducted to investigate the combined effects of moist-chilling and thermo-priming on dormancy-breakage of white spruce seeds. The combined treatment elicited a conspicuous improvement in seed germination performance for many of the seed lots. The priming treatment improves germination performance without the need for chemicals, which is advantageous for its practical implementation. Use of a moist-chilling-thermo-priming protocol has the potential to deliver real gains in the efficiency of forest nursery operations, which depend on swift, synchronous and full-capacity germination. To follow up on this work, we are in the process of tracking the molecular mechanisms that underlie the effectiveness of moist-chilling and thermo-priming in triggering dormancy release.

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