Relationships Among Chilling Hours, Photoperiod, Calendar Date, Cold Hardiness, Seed Source, and Storage of Douglas-Fir Seedlings

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Abstract

Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings from three nurseries in the Pacific Northwest United States were lifted on five dates from mid-October through mid-December 2006. Each nursery provided seedlings from a low- and a high-elevation seed lot. Photoperiod and accumulated chilling hours (calculated using two methods) were evaluated throughout the lifting period. Seedlings had typical patterns of fall cold hardiness development, with some indication that the high-elevation lot at each nursery was hardier than the low-elevation lot. Photosynthetic yield measured on seedlings from one of the nurseries decreased with decreasing temperatures, thereby corresponding well to levels of tissue damage at each freezing test temperature over time. Seedlings were either cold- or freezer-stored until February 2007, then tested for physiological quality and planted into a garden plot. Overall, seedlings from earlier lift dates tended to perform poorly in all attributes compared with those from later lift dates. Low-elevation seedlings tended to have lower root growth potential after storage and also reduced survival and longer bud break in the garden plot compared with high-elevation seedlings, although low-elevation seedlings tended to have more height and stem-diameter growth. Freezer-stored seedlings tended to have greater survival compared with cold-stored seedlings, although storage type did not influence growth. This study exemplifies the many influencing factors that growers must consider when determining lift dates. This paper was presented at a joint meeting of the Western Forest and Conservation Nursery Association, the Intermountain Container Seedling Growers Association, and the Intertribal Nursery Council (Boise, ID, September 9–11, 2014).

Introduction

In temperate conifer species, the growth and dormancy cycle is an adaptation to prevent shoot growth during winter, when freezing temperatures would injure such growth. These phenological patterns are influenced by species, genetics, plant vigor, and environment. As winter approaches, plants respond to cues of decreasing photoperiod (daylength) and temperature by ceasing growth, setting buds (for determinant species), and developing the ability to withstand subfreezing temperatures with little or no damage (Bigras et al. 2001, Haase 2011). This development of cold hardiness involves several physical and chemical changes within the plant tissues that enable plants to resist freezing damage (Öquist et al. 2001).

Cold hardiness is defined as a minimum temperature at which a certain percentage of a random plant population will survive or will sustain a given level of damage (Ritchie 1984a). Hardiness is most commonly quantified as LT_{50} (lethal temperature for 50 percent of a population). Seedling cold hardiness in the nursery is also an indicator of overall resistance to stresses such as those associated with lifting, packing, storing, and outplanting (Burr et al. 1990, Faulconer 1988, Ritchie 2000). Cold hardiness has also been linked to subsequent survival and growth (Simpson 1990, van den Driessche 1977) and is therefore a useful seedling-quality test (Haase 2008).

In the northern hemisphere, temperate conifer seedlings typically achieve peak dormancy in October or November. Dormancy is quantified as the length of time before plants will resume growth in the spring; it is not the same thing as cold hardiness, which commonly peaks in January (Haase 2011, Timmis et al. 1994). Seedlings require a period of chilling to complete their dormancy cycle before they will resume growth in response to longer photoperiods and favorable spring temperatures. The chilling requirement for Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) is 1,200 to 2,000

hours (Bailey and Harrington 2006, van den Driessche 1975). If not totally fulfilled by the time of lifting from the nursery, the chilling requirement may be met in cold storage (van den Driessche 1977); temperatures in freezer storage, however, are below optimum for accumulation of chilling to meet dormancy release requirements (Ritchie 1984b).

While the chilling requirement for bud break in Douglas-fir has been well documented, this information does little to assist with the more practical application of using chilling accumulation to determine the optimum timing for lifting, storing, and outplanting. For Pacific Northwest nursery applications, the typical target for Douglas-fir is a minimum of 300 to 400 chilling hours before lift and storage for optimum stress resistance. Very little research has been done, however, to verify the relationship between chilling hours and subsequent seedling quality and vigor, nor is adequate information available regarding other influencing and confounding factors. Similar questions have arisen regarding which factors are most useful for determining when to lift southern pine species (South 2013).

Various methods can be used to quantify chilling. The most common method used in forestry nurseries is the number of hours below 5 °C (41 °F). Another method, used in the fruit-tree industry, is the Richardson method (Richardson et al. 1974), which is more complex because it includes relative chilling effectiveness and variable chilling accumulation depending on temperature.

In a preliminary trial (fall 2005) to examine the relationship between shoot cold hardiness and accumulated chilling hours, Douglas-fir seedlings from six seed lots were frozen and evaluated for tissue damage and mortality every 2 weeks from mid-October to mid-December. As chilling hours accumulated from approximately 35 hours in mid-October to more than 150 hours in mid-November, the LT_{50} for all lots decreased (i.e., the seedlings became more cold hardy). When a rapid rise in chilling from 150 to more than 400 hours occurred during the 2-week period from November 17 to December 1, however, no corresponding rise in cold hardiness was observed for any of the lots. A model of the preliminary data showed that calendar date was the most significant factor related to seedling cold hardiness—more so than either chilling hours or seed lot (NTC 2006). Based on the results of that preliminary trial, this study was conducted in 2006 with the objective to further examine relationships among seed lot, chilling hours, daylength, lift date, and storage and their subsequent influence on cold hardiness, bud break, growth, and survival. Understanding these relationships can assist with nursery lifting and storage decisions to optimize seedlings' stress resistance and outplanting performance.

Materials and Methods

Seedlings, Sampling, and Storage

Three nurseries (A, B, and C) in Washington, United States, participated in the study; each chose two Douglas-fir seed lots (low and high elevations) to include in the study based on expected differences in cold hardiness (table 1). Seedlings from all nurseries were lifted every 2 weeks from mid-October through mid-December 2006 on the following five dates:

1. **October 16**
2. **October 30**
3. **November 13**
4. **November 27**
5. **December 11**

Table 1. Three nurseries participated in the study, each providing seedlings from two Douglas-fir seed lots (low- and high-elevation sources).

Seed lot	Stocktype	Seed zone[a, b] (State)	Elevation[c] (ft)
Nursery A			
Low	2+0 bareroot	042 (WA)	1,000
High		631 (WA)	3,500
Nursery B			
Low	Outside-grown container, plug-to-plug transplant, 21 in^3 (344 cm^3) plug	051 (OR)	1,000
High		452 (OR)	2,200
Nursery C			
Low	1+0 bareroot (for transplant)	262 (OR)	500
High		262 (OR)	2,000

[a] Washington seed zones from Randall and Berrang (2002). [b] Oregon seed zones from Randall (1996). [c] 1,000 ft = 305 m.

Figure 1. Seedlings were lifted from mid-October through mid-December. This photograph was taken just before lifting at Nursery A for the mid-November lift date. (Photo by Nabil Khadduri, 2006)

On each lift date at each nursery, 260 seedlings from each seed lot were lifted (figure 1). A sample of 60 seedlings was designated for cold hardiness assessment and the rest were placed in storage at Nursery A's facility. Samples of 100 seedlings of each lot were placed in cold storage (1 to 3 °C [34 to 37 °F]) and in freezer storage (-1 to 0 °C [30 to 32 °F]).

Environmental Factors

Temperature sensors were installed at each nursery to monitor soil and air temperatures until all seedlings had been lifted. Data from these sensors were used to calculate chill hours over time, using both the standard method (total hours below 5 °C [41 °F]) and the Richardson method (table 2). Photoperiod (daylength) was determined using an online calculator (http://herbert.wikispaces.com/length+of+day), using each nursery's latitude coordinates.

Table 2. Quantification of chilling hours using the Richardson method.

	°C	°F		Chill hour
1 hour at:	< 2	< 35.6	=	0.0
	2.0 to 3.0	35.6 to 37.4	=	0.5
	3.0 to 9.0	37.4 to 48.2	=	1.0
	9.0 to 12.0	48.2 to 53.6	=	0.5
	12.0 to 15.0	53.6 to 59.0	=	0.0
	15.0 to 18.0	59.0 to 64.4	=	– 0.5
	> 18.0	> 64.4	=	– 1.0

Source: Richardson et al. (1974).

Seedling Physiology at the Time of Lifting

At each lift date, cold hardiness was evaluated using the whole plant freezer test (WPFT) (Haase 2011, Tanaka et al. 1997). A sample of 60 seedlings from each nursery/seed lot was randomly divided into four groups of 15 seedlings each and randomly assigned a target freeze temperature. Four target temperatures were chosen at each lift date based on their expected ability to bracket the LT_{50}. Each group was placed into a programmable chest freezer in which the temperature was lowered from room temperature to 0 °C (32 °F) at a rate of 20 °C (36 °F) per hour, then decreased to the target temperature at a rate of 5 °C (9 °F) per hour, held at the target temperature for 2 hours, then raised back to 0 °C (32 °F) at a rate of 20 °C (36 °F) per hour (figure 2a). Due to resource limitations, each WPFT freezing

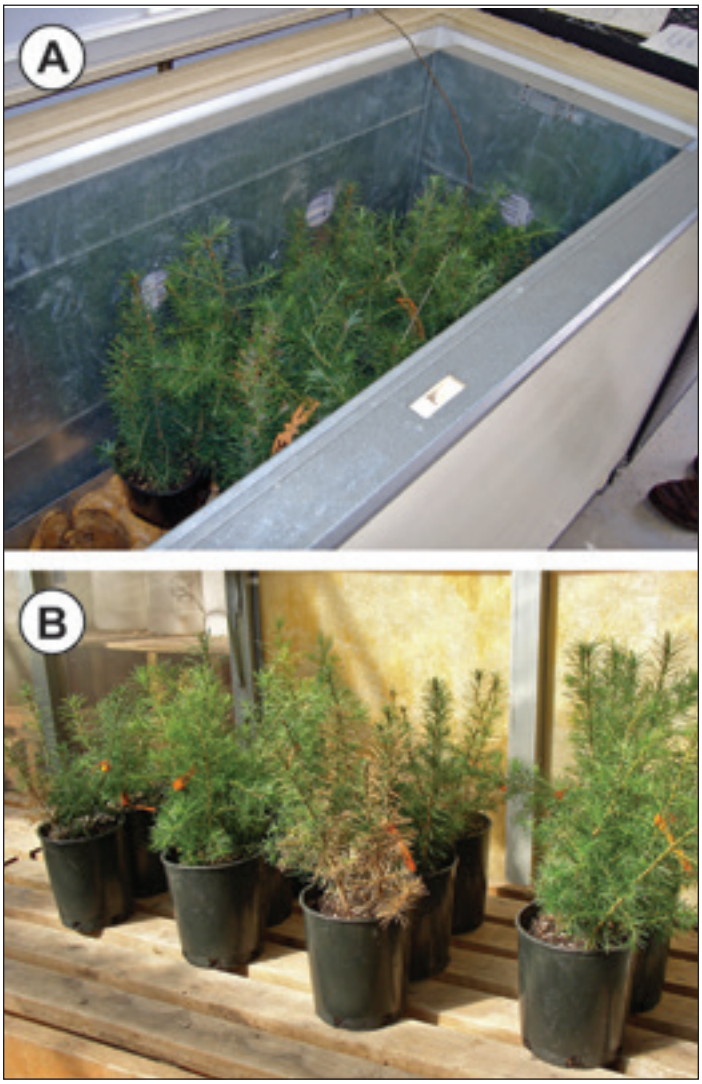

Figure 2. (a) A programmable freezer was used to subject seedlings to the whole plant freeze test. After freezing, seedlings were kept in (b) ambient conditions before assessing for freeze damage. (Photos by Diane L. Haase, 2006)

Figure 3. Six days after freezing, seedlings were evaluated for damage by examining (a) bud and (b and c) cambium tissues for browning. (Photos by Diane L. Haase, 2006)

temperature was run only once per sample date in the programmable freezer; because seedling response to freezing stress is well documented and reproducible, however, we expected that the resulting analyses would be very similar if additional freezers had been available.

After freezing, seedlings were placed into a greenhouse with adequate moisture, ambient photoperiod, and an average temperature of 20 °C (68 °F) (figure 2b). Six days after freezing, bud damage was determined by sectioning 5 to 10 randomly selected buds from throughout each seedling shoot and examining for evidence of browning (figure 3a). If more than 50 percent of the buds were damaged, then the seedling was considered nonviable. Cambial damage was evaluated by scraping the bark along the stem (figure 3b) and examining for browning (figure 3c). If the cambium was brown in the lower half of the shoot, the seedling was considered nonviable. Percent foliar damage (visual estimate) was a determining factor only when cambium or bud damage was borderline. The LT_{10} and LT_{50} for each seedling group on each date were then determined by plotting percent survival against temperature and assuming a linear relationship.

Chlorophyll fluorescence and genetic markers were also measured on seedlings from Nursery A. Due to labor-intensive sampling, only one nursery could be included for these measurements; Nursery A was chosen because the two seed sources were expected to have the greatest difference in cold hardiness. Approximately 24 hours after freezing in the WPFT procedure, chlorophyll fluorescence was measured on a single needle collected from 8 seedlings from each seed source and freezing temperature. Needles were exposed to a 3-second pulse of saturating light using a fluorometer (Model OS5-FL, Opti-Sciences, Inc.) (figure 4). The steady state (Fs) and maximal (Fms) fluorescence were determined and used to calculate photosynthetic yield (Y).

Needle and bud tissue from Nursery A seedlings (not frozen in the WPFT) were collected and processed from both seed lots on each lift date (figure 5) and sent for NSure genetic marker assessment, a molecular test for assessing cold tolerance in conifer seedlings developed at the Wageningen University and Research Centre in the Netherlands where researchers found that gene expression may be correlated with cold hardiness (Balk et al. 2007a, Joosen et al. 2006, Landis and van Wordragen 2006). The test is based on measuring the activity level of a selected set of genes.

Seedling Physiology and Performance After Storage

From late January through mid-February, seedlings were removed from storage (all seedlings from one nursery at a time). One week before removal from storage, those in freezer storage were moved to cooler storage to allow for

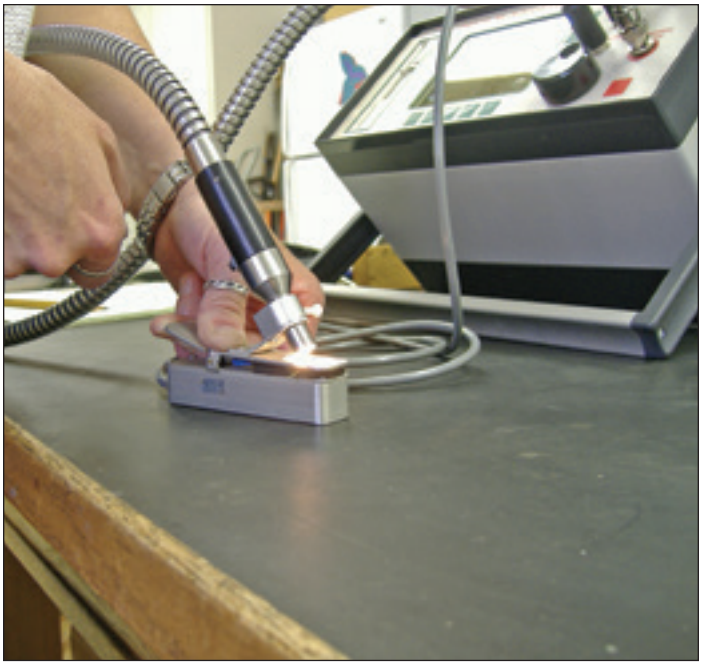

Figure 4. After freezing, chlorophyll fluorescence was measured on needles from Nursery A by exposing needles to a pulse of saturating light using a fluorometer. (Photo by Diane L. Haase, 2006)

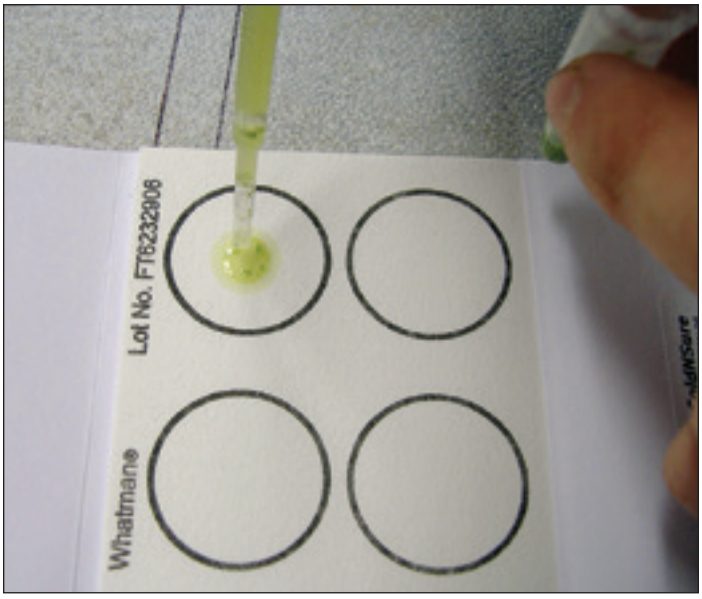

Figure 5. Needle and bud tissue from Nursery A seedlings (not frozen) were collected and processed from both seed lots on each lift date and assessed for genetic markers associated with cold hardiness. (Photo by Diane L. Haase, 2006)

thawing. A sample of 60 seedlings from each nursery/seed lot/lift date/storage treatment was immediately evaluated for cold hardiness using the WPFT. Because some groups sustained damage of more than 50 percent for all test temperatures, LT_{10} and LT_{50} could not be calculated. Thus, percent mortality at -9 °C (15.8 °F) is reported.

Root growth potential (RGP) was evaluated on a sample of 20 seedlings from each nursery/seed lot/lift date/storage treatment. Each sample was potted into 19-L (5-gal) pots (5 per pot) containing a peat-based growing medium and randomly placed in a warm greenhouse environment where they were kept well watered for 3 weeks. Seedlings were then removed from the pots and new root growth was quantified based on the following index (Burdett 1979).

RGP index	Description (1 cm = 0.4 in)
0	No new root growth
1	Some new roots but none longer than 2 cm
2	1–3 new roots longer than 1 cm
3	4–10 new roots longer than 1 cm
4	11–30 new roots longer than 1 cm
5	More than 30 new roots longer than 1 cm

The remaining 20 seedlings from each nursery/seed lot/lift date/storage treatment were randomly assigned to four replications (5 seedlings per treatment group) and planted into a garden plot at Nursery A for assessment of field vigor (figure 6). Seedling treatment groups were assessed weekly for percent bud break until late spring, when no further bud break was anticipated. In early March 2007 (before bud break), all seedlings were measured for initial height and stem diameter. In October 2007 (after bud set), seedlings were measured again for height and stem diameter and also for survival. Height and stem-diameter growth were calculated by subtracting initial values.

Statistical Analyses

Chlorophyll fluorescence yield data from Nursery A seedlings were analyzed for each sample date by analysis of variance (ANOVA) using the PROC GLM procedure of SAS software (SAS Institute Inc., Cary, NC) to determine significant differences between seed lots and among freezing temperatures.

RGP, bud break, height growth, stem-diameter growth, and survival data were all analyzed using ANOVA (PROC GLM, SAS Institute, Inc.) for a randomized complete block to determine differences among lift dates, seed lot, and storage type. Data from each nursery were analyzed separately. Fisher's Protected Least Significant Difference procedure was used to determine significant differences among treatment groups at the $\alpha \leq 0.05$ level. Tests for normality, linearity, and constant variance of the residuals were performed to ensure the validity of these assumptions on each dataset; no data transformations were deemed necessary.

In addition, probit regression was used to determine the predictive relationship of chilling hours (calculated using

Figure 6. After storage, samples of seedlings from each nursery, lift date, seed lot, and storage type were planted into a garden plot at Nursery A for evaluation of bud break, survival, and growth. (Photo by Nabil Khadduri, 2007)

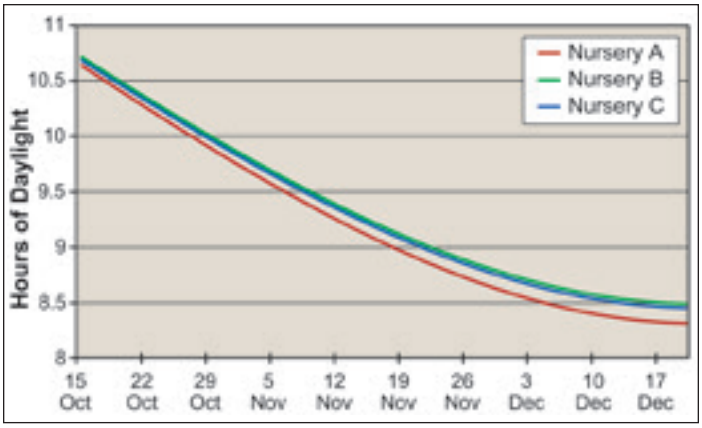

Figure 7. Photoperiod from mid-October through mid-December was similar among nursery locations.

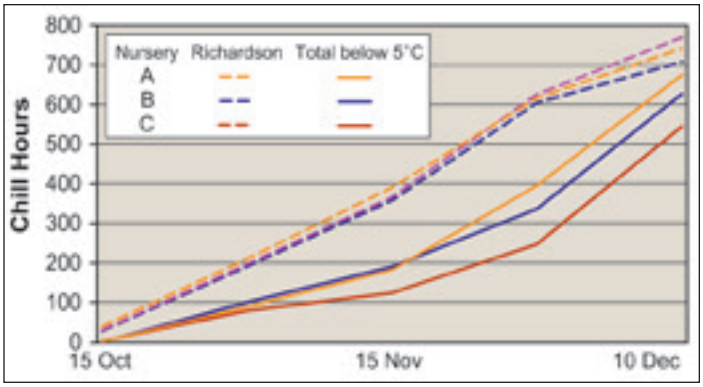

Figure 9. Chilling hours were calculated using the Richardson method or the conventional method (sum of all hours below 5 ºC [41 ºF]). The Richardson method resulted in a more rapid accumulation of chill hours from mid-October through mid-December 2006.

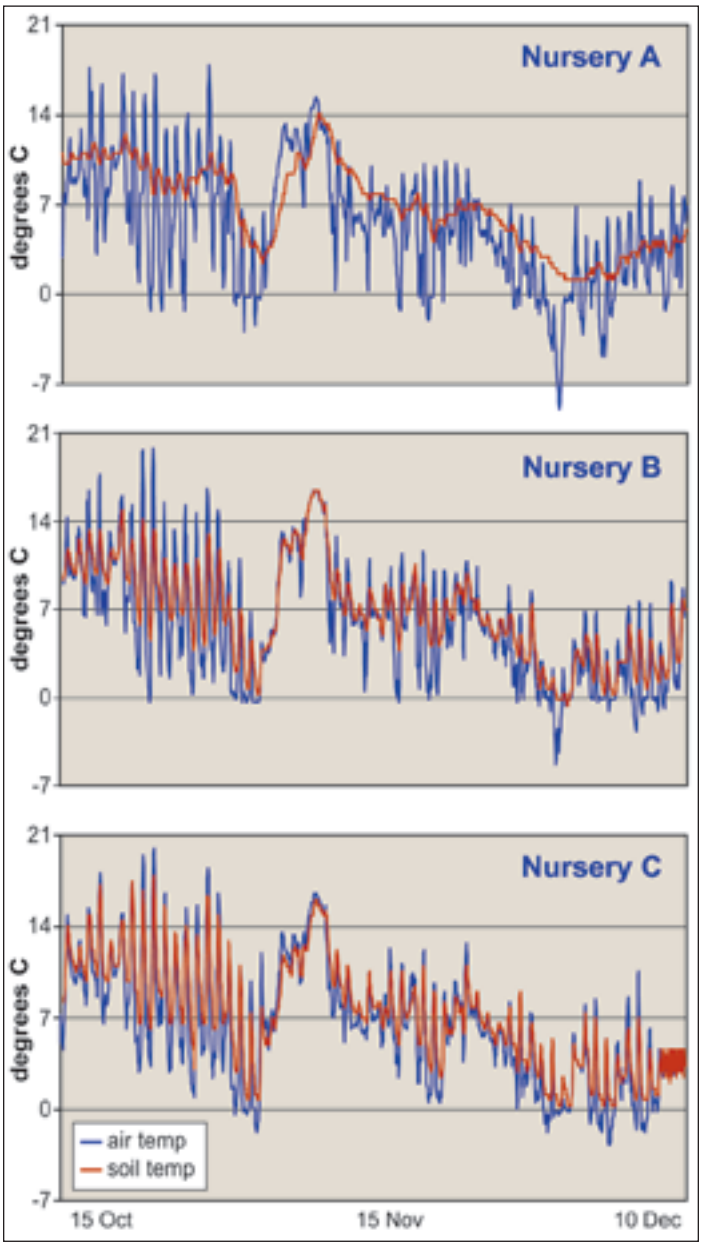

Figure 8. Air and soil temperatures were recorded during the fall 2006 lift dates at each nursery. Air temperature was used to calculate chilling hour accumulation.

either the Richardson or conventional methods) and calendar date (quantified as number of days since October 15) on seedling mortality at various freezing temperatures.

Results

Environmental Factors

Photoperiod patterns were nearly identical for Nurseries B and C, which are located at similar latitudes. Nursery A is located approximately 129 km (80 mi) north of the other two nurseries and had slightly shorter photoperiods (by approximately 3 to 9 min) from October 15 through December 20 (figure 7). Based on air temperature readings at each nursery (figure 8), calculations using the Richardson method resulted in a more rapid accumulation of chill hours as compared with the conventional method (figure 9).

Seedling Physiology at the Time of Lifting

Seedlings had typical patterns of fall cold hardiness development with some indication that the seedlings in the high-elevation lot at each nursery were hardier than those in the low-elevation lot (figure 10).

The NSure assay on needle tissue from Nursery A did not correspond to data from the WPFT test. The NSure assay on bud tissue from Nursery A, however, distinguished three stages of cold hardiness, which correlated with WPFT values as previously reported (Balk et al. 2007b) and summarized on the following page.

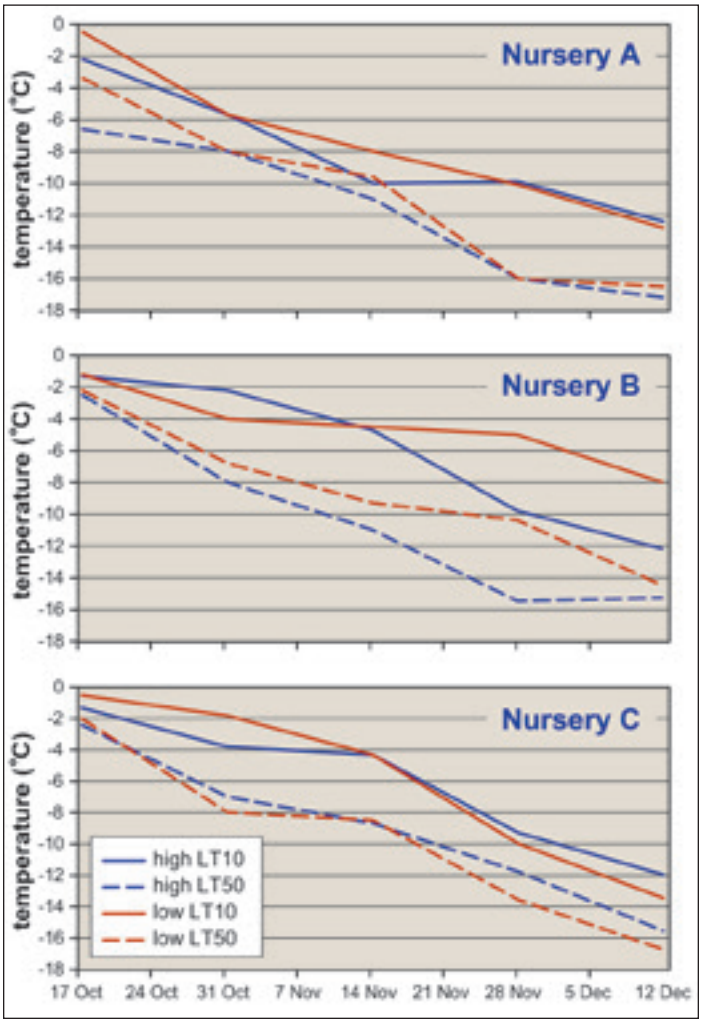

Figure 10. Cold hardiness was estimated for each seed lot from each nursery on each lift date.

NSure phase 0: No frost tolerance observed

NSure phase 1:

LT_{50} value between -5 and -10 °C (23 and 14 °F)

LT_{10} value between -1 and -5 °C (30 and 23 °F)

NSure phase 2:

LT_{50} value below -10 °C (14 °F)

LT_{10} value below -5 °C (23 °F)

Photosynthetic yield measured via chlorophyll fluorescence on Nursery A seedlings decreased with decreasing temperatures, thereby corresponding well to levels of damage from the WPFT at each freezing test temperature over time (figure 11). Seed lot affected photosynthetic yield on the October 17 sampling date (higher elevation lots had greater yield at all temperatures) and the November 28 sampling date (higher elevation lots had greater yield at all temperatures except -6.0 °C [21.2 °F]).

Seedling Physiology and Performance After Storage

Statistical analyses indicated multiple interactions among seed lot, lift date, and storage type at each nursery. In general, however, lift date had the greatest influence (based on magnitude of the F-value) on all variables for seedlings from Nursery B and Nursery C. Lift date also had the greatest influence on growth and RGP for Nursery A, but elevation had an even greater influence on survival and bud break. Overall, earlier lift dates tended to perform poorly in all attributes compared with those from later lift dates (table 3, figure 12). Seedlings

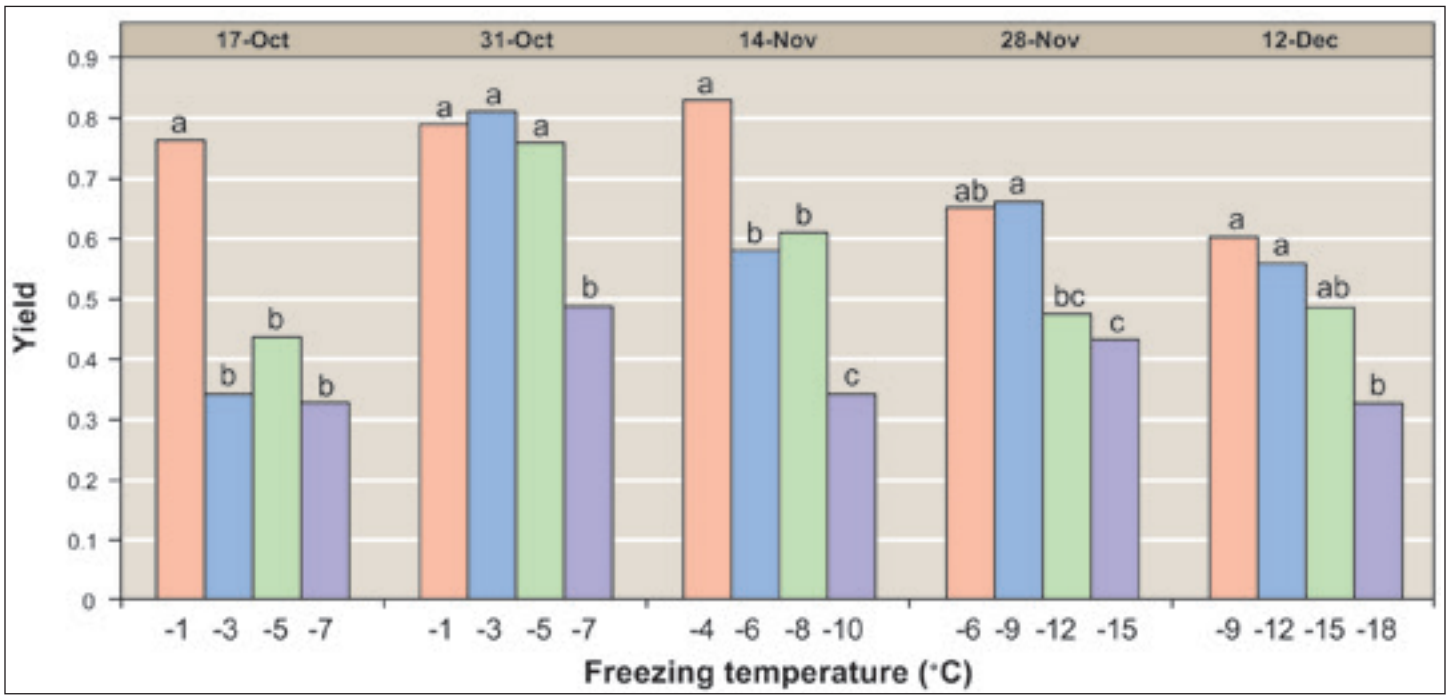

Figure 11. Yield was calculated from chlorophyll fluorescence measurements of needles from Nursery A seedlings frozen to different temperatures on each lift date. Note: A significant interaction between temperature and seed lot was observed on November 28 (needles from the high-elevation lot had greater yield than those from the low-elevation lot at all temperatures except -6.0 ºC [21.2 ºF]).

Table 3. Poststorage physiology for seedlings from each nursery group. Because, for all three nurseries, seed lot, lift date, and/or storage type significantly interacted, only means are presented here.

	Index of root growth potential				Percent mortality at –9.0 °C (15.8 °F)			
Seed lot elevation	**Low**		**High**		**Low**		**High**	
Storage Type	**Cooler**	**Freezer**	**Cooler**	**Freezer**	**Cooler**	**Freezer**	**Cooler**	**Freezer**
Lift Date	**Nursery A (2+0)**							
Oct. 16	0.65	0.60	2.75	1.45	73.3	83.3	16.7	30.0
Oct. 30	3.25	2.44	3.70	2.95	40.0	73.3	33.3	16.7
Nov. 13	3.10	2.95	3.25	2.90	13.3	63.3	20.0	20.0
Nov. 27	3.25	2.40	3.40	3.20	0.0	50.0	16.7	26.7
Dec. 11	2.83	3.00	3.55	3.10	10.0	70.0	16.7	6.7
Lift Date	**Nursery B (large plugs)**							
Oct. 16	0.05	0.20	0.20	1.21	100	96.7	100.0	93.3
Oct. 30	0.33	2.11	1.37	1.05	83.3	56.7	86.7	56.7
Nov. 13	1.42	2.45	1.50	2.65	73.3	50.0	43.3	46.7
Nov. 27	2.35	3.35	3.00	2.40	23.3	40.0	6.7	10.0
Dec. 11	3.10	3.74	3.35	3.10	16.7	3.3	20.0	20.0
Lift Date	**Nursery C (1+0)**							
Oct. 16	0	0	0	0.45	100	100	100	56.7
Oct. 30	0.25	0.17	1.15	1.85	93.3	90.0	53.3	33.3
Nov. 13	2.45	2.25	1.30	2.26	92.9	53.3	100	80.0
Nov. 27	2.60	2.56	2.95	2.95	43.3	46.7	23.3	43.3
Dec. 11	3.15	3.10	2.55	2.53	10.0	16.7	39.3	80.0

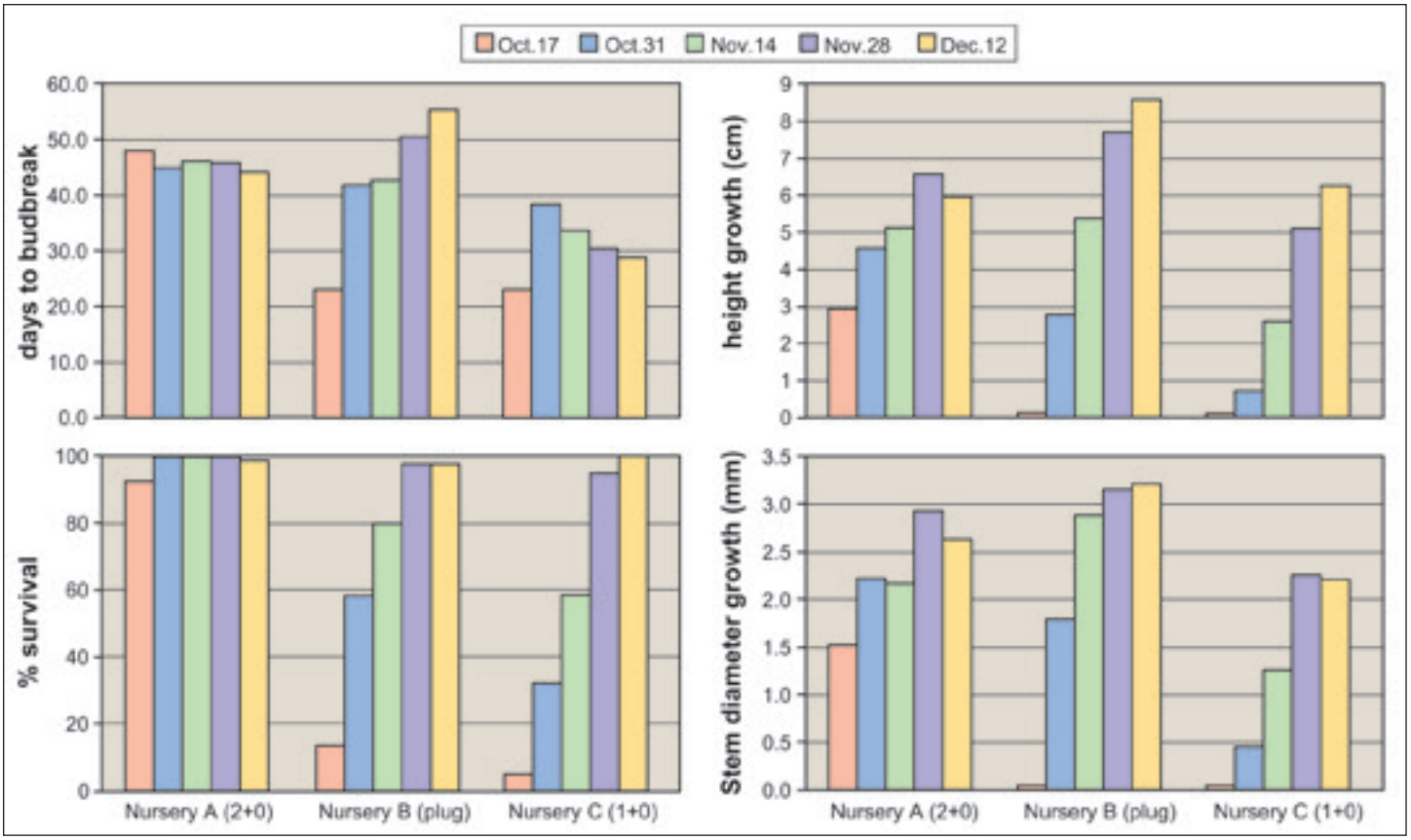

Figure 12. Lift date had a significant influence on poststorage seedling performance in the garden plot.

from the low-elevation seed lots tended to have lower RGP following storage (table 3) and lower survival and longer bud break in the garden plot compared with seedlings from the high-elevation seed lots, although seedlings from the low-elevation lots also tended to have more height and stem-diameter growth in the garden plot than those from the high-elevation lots (data not shown). Freezer-stored seedlings from Nursery B and Nursery C tended to have greater RGP compared with cold-stored seedlings, whereas the reverse was true for Nursery A seedlings (table 3). Freezer-stored seedlings also tended to have greater survival for all nurseries and seed lots in the garden plot compared with cold-stored seedlings, although storage type did not influence growth (data not shown).

Environmental Influences on Seedling Physiology

Probit analyses determined that chilling hours calculated with the Richardson method had the best fit for predicting mortality by freezing temperature (figure 13). Richardson chill hours were only a slightly better predictor of mortality than days since October 15 (data not shown); the two models did not differ significantly. Chill hours calculated with the conventional method (hours below 5 °C [41 ºF]), however, provided a significantly worse fit compared with Richardson chill hours or days since October 15.

Discussion

For the 2006–07 fall–winter season, Douglas-fir seedlings from the lots studied followed typical hardening and dehardening patterns (Haase 2011, Timmis et al. 1994). Photosynthetic yield also reflected damage levels seen in the cold hardiness test. Conifer species in northern latitudes, such as white spruce (*Picea glauca* [Moench] Voss), must achieve complete photosynthetic inactivation for protection against winter cold (Binder and Fielder 1996). Because Douglas-fir's relatively milder geographic range does not require a complete shutdown of photosynthesis, chlorophyll fluorescence is not well correlated with cold hardiness in nonfrozen seedlings (Rose and Haase 2002). Similar to results in this study, however, chlorophyll fluorescence has been shown to be well correlated with foliar damage following freeze stressing (Adams and Perkins 1993, Fisker et al. 1995), thereby serving as a quantitative and objective tool for rapid assessment of seedling vigor following freezing, although variations among tissues in freezing damage susceptibilities during the winter must be considered (Rose and Haase 2002).

Chilling hours and calendar date (days since October 15) had the strongest relationship with freeze damage at the time of lifting. Understanding the relative contributions of each factor to Douglas-fir seedling phenology, however, is nearly impossible, given that daylight, chilling hour accumulation, and calendar date are intrinsically confounded (Campbell and Sugano 1975, Fuchigami and Nee 1987). Furthermore, chilling hour accumulation varies with annual temperature patterns and by calculation method, and seedling phenology is influenced by stocktype, seed source, and nursery cultural practices. Faulconer (1988) noted several disadvantages for relying solely on chilling hour accumulation for determining seedling condition, including variations in hardiness among seedling lots, temperature changes from year to year, and uncertainties regarding the best method to quantify chilling. In an early study with several Douglas-fir seed sources, Campbell and Sugano (1975) noted that the effects of chilling, photoperiod, and temperature on subsequent bud break were highly interdependent. South (2013) also commented on confounding among multiple factors associated with chilling hours and seedling quality. While it may be possible to separate the varying factors in controlled laboratory studies, such an endeavor would not be representative of actual nursery and field conditions and would therefore be problematic to apply operationally (Haase 2014). Rather, as demonstrated by this study, it is important to consider all influences when determining lift date.

Similar to cold hardiness at lifting, poststorage RGP, cold hardiness, bud break, growth, and survival were strongly influenced by lift date (which, as described previously, is confounded with chilling hours and photoperiod). Some studies indicate that chill hours can be partially satisfied in cold storage (Carlson 1985, Ritchie 1989, van den Driessche 1977). Our study found, however, that those seedlings lifted on the earliest lift date performed poorly after outplanting. This finding indicates that seedlings require adequate time in ambient conditions to reach a certain chilling and accompanying photoperiod threshold, along with diurnal and nocturnal fluctuations, before lifting and storage, after which seedlings are less susceptible to handling stresses.

In addition to being influenced by lift date, seedling attributes were influenced by seed source. RGP after storage and also survival, bud break, height growth, and stem-diameter growth tended to differ between the low-elevation and high-elevation seedlings from each nursery. St. Clair et al. (2005) evaluated Douglas-fir seedlings from more than 1,000 locations in western Oregon and Washington and found that populations differed

considerably for adaptive traits; bud phenology, in particular, was strongly influenced by elevation and temperature. Freezer-stored seedlings tended to have greater survival compared with cold-stored seedlings. Carbohydrate reserves tend to decrease in cold storage more rapidly than in freezer storage (Ritchie 1982), which may have been a contributing factor.

In the Pacific Northwest, Douglas-fir bareroot and container seedling growers have established annual lifting and storage schedules based on factors specific to their nursery environment, weather patterns, and customer demands and also on each crop's stocktype and genetics. These decisions are based on science and experience. As temperatures increase due to

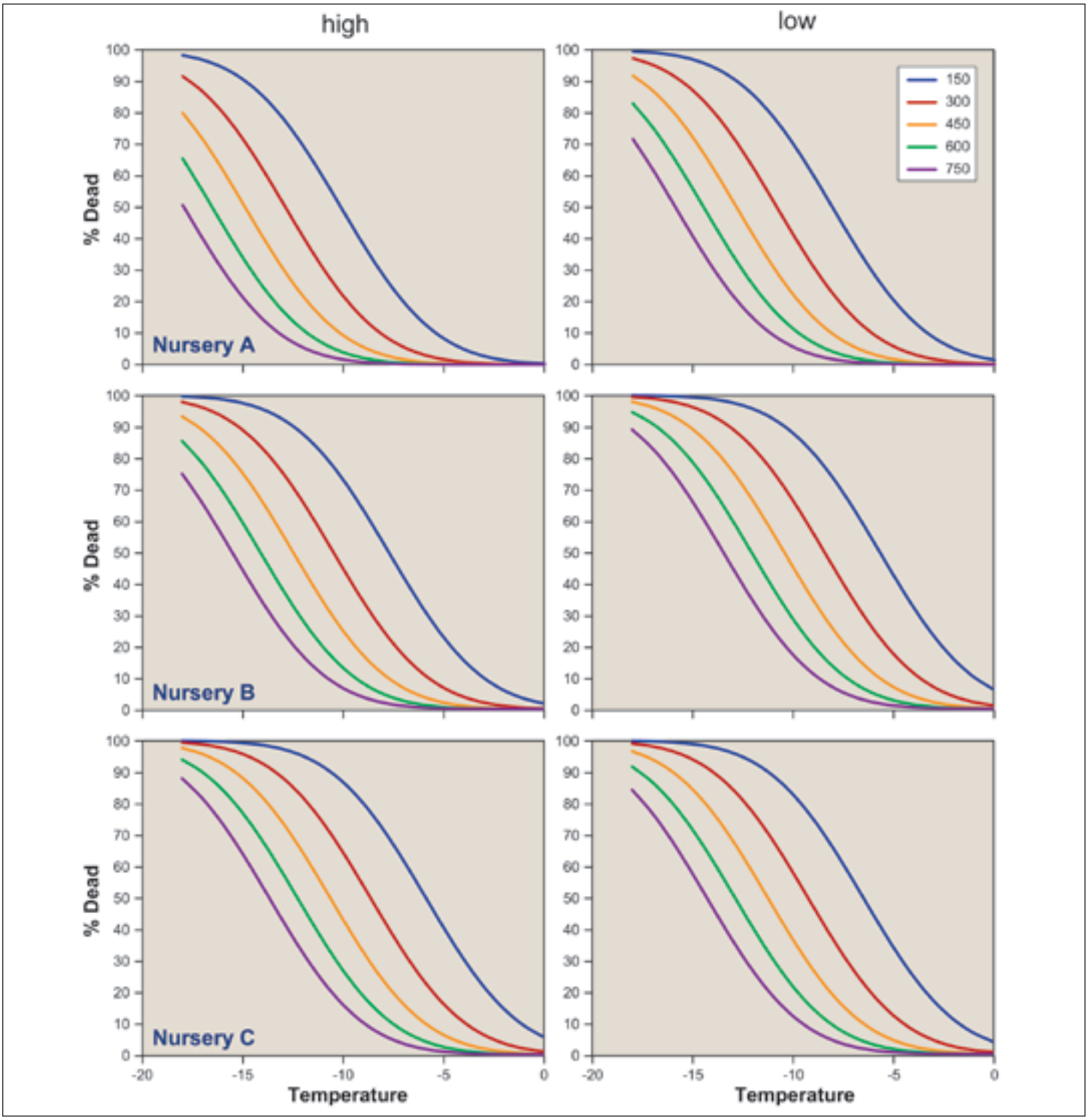

Figure 13. Probit analyses determined that chilling hours calculated with the Richardson method had the best fit for predicting mortality by freezing temperature. Days since October 15 (data not shown) also had a strong predictive fit, whereas the conventional method for calculating chill hours (hours below 5 ºC [41 ºF]) provided a significantly worse fit.

expected climate changes, however, winter temperature patterns will provide fewer annual chilling hours in temperate latitudes. This warming could affect Douglas-fir bud development and bud break. Douglas-fir seedlings grown in elevated temperature conditions had delayed cold hardening in the autumn and slowed dehardening in the spring and also had reduced maximum cold hardiness, reduced bud break, and reduced growth compared with those grown in ambient temperatures (Guak et al. 1998). In the near future, nursery managers may need to adjust their cultural practices, target species and seed sources, and lifting and storage schedules as they strive to maintain optimum seedling phenology (Tepe and Meretsky 2011, Williams and Dumroese 2014).

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REFERENCES

Adams, G.T.; Perkins, T.D. 1993. Assessing cold tolerance in Picea using chlorophyll fluorescence. Environmental and Experimental Botany. 33(3): 377–382.

Bailey, J.D.; Harrington, C.A. 2006. Temperature regulation of bud-burst phenology within and among years in a young Douglas-fir (*Pseudotsuga menziesii*) plantation in western Washington, USA. Tree Physiology. 26(4): 421–430.

Balk, P.A.; Bronnum, P.; Perks, M.; Stattin, E.; van der Geest, L.H.M.; van Wordragen, M.F. 2007a. Innovative cold tolerance test for conifer seedlings. In: Riley, L.E.; Dumroese, R.K.; Landis, T.D., tech. coords. National proceedings, forest and conservation nursery associations—2006. Proceedings RMRS-P-50. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 9–15.

Balk, P.A.; Haase, D.L.; Wordragen, M.F. 2007b. Gene activity test determines cold hardiness in Douglas-fir seedlings. In: Dumroese, R.K.; Riley, L.E., tech. coords. National proceedings, forest and conservation nursery associations—2007. Proceedings RMRS-P-57. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 140–148.

Bigras, F.J.; Ryyppo, A.; Lindstrom, A.; Stattin, E. 2001. Cold acclimation and deacclimation of shoots and roots of conifer seedlings. In: Bigras, F.J.; Colombo, S.J., eds. Conifer cold hardiness. Dordrecht, Netherlands: Kluwer Academic Publishers: 57–88.

Binder, W.D.; Fielder, P. 1996. Chlorophyll fluorescence as an indicator of frost hardiness in white spruce seedlings from different latitudes. New Forests. 11(3): 233–253.

Burdett, A.N. 1979. New methods for measuring root growth capacity: their value in assessing lodgepole pine stock quality. Canadian Journal of Forest Research. 9(1): 63–67.

Burr, K.E.; Tinus, R.W.; Wallner, S.J.; King, R.M. 1990. Comparison of three cold hardiness tests for conifer seedlings. Tree Physiology. 6(4): 351–369.

Campbell, R.K.; Sugano, A.I. 1975. Phenology of bud burst in Douglas-fir related to provenance, photoperiod, chilling, and flushing temperature. Botanical Gazette. 136(3): 290–298.

Carlson, W.C. 1985. Effects of natural chilling and cold storage on budbreak and root growth potential of loblolly pine (*Pinus taeda* L.). Canadian Journal of Forest Research. 15(4): 651–656.

Faulconer, J.R. 1988. Using frost hardiness as an indicator of seedling condition. In: Landis, T.D., tech. coord. Proceedings, combined meeting of the Western Forest Nursery Council and Intermountain Nurseryman's Association. Gen. Tech. Rep. RMRS-167. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 89–95.

Fisker, S.E.; Rose, R.; Haase, D.L. 1995. Chlorophyll fluorescence as a measure of cold hardiness and freezing stress in 1+1 Douglas-fir seedlings: response to seasonal changes in the nursery. Forest Science. 41(3): 564–575.

Fuchigami, L.H.; Nee, C. 1987. Degree growth stage model and rest-breaking mechanisms in temperate woody plants. HortScience. 22(5): 836–845.

Guak, S.; Olsyzk, D.M.; Fuchigami, L.H.; Tingey, D.T. 1998. Effects of elevated CO2 and temperature on cold hardiness and spring bud burst and growth in Douglas-fir (*Pseudotsuga menziesii*). Tree Physiology. 18(10): 671–679.

Haase, D.L. 2008. Understanding forest seedling quality: measurements and interpretation. Tree Planters' Notes. 52(2): 24–30.

Haase, D.L. 2011. Seedling phenology and cold hardiness: moving targets. In: Riley, L.E.; Haase, D.L.; Pinto, J.R., tech.

coords. National proceedings, forest and conservation nursery associations—2010. Proceedings RMRS-P-65. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 121–127.

Haase, D.L. 2014. Beyond cowboy science: simple methods for conducting credible and valid research. Tree Planters' Notes. 57(2): 32–43.

Joosen, R.V.L.; Lammers, M.; Balk, P.A.; Brønnum, P.; Konings, M.C.J.M.; Perks, M.; Stattin, E.; van Wordragen, M.F.; van der Geest, A.L.H.M. 2006. Correlating gene expression to physiological parameters and environmental conditions during cold acclimation of *Pinus sylvestris*, identification of molecular markers using cDNA microarrays. Tree Physiology. 26(10): 1297–1313.

Landis, T.D.; van Wordragen, M.F. 2006. Seedling quality testing at the gene level. R6-CP-TP-04-2006. Forest Nursery Notes (Summer). Portland, OR: U.S. Department of Agriculture, Forest Service: 4–5.

Nursery Technology Cooperative (NTC). 2006. Chilling hours and Douglas-fir seedling cold hardiness. In: NTC Annual Report 2005–06. Corvallis, OR: Oregon State University, Department of Forest Science: 24–25.

Öquist, G.; Gardeström, P.; Huner, N.P.A. 2001. Metabolic changes during cold acclimation and subsequent freezing and thawing. In: Bigras, F.J.; Colombo, S.J., eds. Conifer cold hardiness. Dordrecht, Netherlands: Kluwer Academic Publishers: 137–163.

Randall, W.K. 1996. Forest tree seed zones for western Oregon. Salem, OR: Oregon Department of Forestry. 82 p.

Randall, W.K.; Berrang, P. 2002. Washington tree seed transfer zones. Olympia, WA: Washington Department of Natural Resources. 72 p.

Richardson, E.A.; Seeley, S.D.; Walker, D.R. 1974. A model for estimating the completion of rest for Redhaven and Elberta peach trees. HortScience. 9(4): 331–332.

Ritchie, G.A. 1982. Carbohydrate reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Canadian Journal of Forest Research. 12(4): 905–912.

Ritchie, G.A. 1984a. Assessing seedling quality. In: Duryea, M.L.; Landis, T.D., eds. Forest nursery manual: production of bareroot seedlings. The Hague, Netherlands; Boston; Lancaster, United Kingdom: Martinus Nijhoff/Dr. W. Junk Publishers: 243–259. Chapter 23.

Ritchie, G.A. 1984b. Effect of freezer storage on bud dormancy release in Douglas-fir seedlings. Canadian Journal of Forest Research. 14(2): 186–190.

Ritchie, G.A. 1989. Integrated growing schedules for achieving physiological uniformity in coniferous planting stock. Forestry 62(Suppl): 213–227.

Ritchie, G.A. 2000. The informed buyer: understanding seedling quality. In: Rose, R.; Haase, D.L., eds. Advances and challenges in forest regeneration. Conference proceedings, Nursery Technology Cooperative. Corvallis, OR: Oregon State University; Western Forestry and Conservation Association: 51–56.

Rose, R.; Haase, D.L. 2002. Chlorophyll fluorescence and variations in tissue cold hardiness in response to freezing stress in Douglas-fir seedlings. New Forests. 23(2): 81–96.

Simpson, D.G. 1990. Frost hardiness, root growth capacity, and field performance relationships in interior spruce, lodgepole pine, Douglas-fir, and western hemlock seedlings. Canadian Journal of Forest Research. 20(5): 566–572.

South, D.B. 2013. Chilling hours: myths and facts. In: Haase, D.L.; Pinto, J.R.; Wilkinson, K.M., tech. coords. National proceedings, forest and conservation nursery associations—2012. Proceedings RMRS-P-69. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 3–10.

St. Clair, J.B.; Mandel, N.L.; Vance-Borland, K.W. 2005. Genecology of Douglas fir in western Oregon and Washington. Annals of Botany. 96(7): 1199–1214.

Tanaka, Y.; Brotherton, P.; Hostetter, S.; Chapman, D.; Dyce, S.; Belanger, J.; Johnson, B.; Duke, S. 1997. The operational planting stock quality testing program at Weyerhaeuser. New Forests. 13(1-3): 423–437.

Tepe, T.L.; Meretsky, V.J. 2011. Forward-looking forest restoration under climate change: Are U.S. nurseries ready? Restoration Ecology. 19(3): 295–298.

Timmis, R.; Flewelling, J.; Talbert, C. 1994. Frost injury prediction model for Douglas-fir seedlings in the Pacific Northwest. Tree Physiology. 14(7-8-9): 855–869.

van den Driessche, R. 1975. Flushing response of Douglas-fir buds to chilling and to different air temperatures after chilling. British Columbia Forest Service Research Note 71. Victoria, BC. 22 p.

van den Driessche, R. 1977. Survival of coastal and interior Douglas-fir seedlings after storage at different temperatures, and effectiveness of cold storage in satisfying chilling requirements. Canadian Journal of Forest Research. 7(1): 125–131.

Williams, M.I.; Dumroese, R.K. 2014. Assisted migration: what it means to nursery managers and tree planters. Tree Planters' Notes. 57(1): 21–26.