

# Short-day treatment alters Douglas-fir seedling dehardening and transplant root proliferation at varying rhizosphere temperatures

Douglass F. Jacobs, Anthony S. Davis, Barrett C. Wilson, R. Kasten Dumroese, Rosa C. Goodman, and K. Francis Salifu

**Abstract:** We tested effects of shortened day length during nursery culture on Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) seedling development at dormancy release. Seedlings from a 42°N source were grown either under ambient photoperiods (long-day (LD)) or with a 28 day period of 9 h light : 15 h dark photoperiods (short-day (SD)). Seedlings were periodically removed from freezer storage from January to May. Sensitivity of plant tissues to cold temperatures was investigated via electrolyte leakage at nine temperatures ranging from 2 to -40 °C. New root growth was assessed with rhizosphere temperatures of 10, 15, 20, and 25 °C. From 2 to -13 °C, there was no difference between treatments in cold hardiness. However, at or below -18 °C, LD seedlings exhibited higher indices of damage than SD seedlings. The LT<sub>50</sub> (temperature at which 50% cell electrolyte leakage occurred) was consistently lower for SD than LD seedlings. Rhizosphere temperature differentially influenced new root proliferation: LD seedlings had greater new root production than SD seedlings at 20 °C, whereas the opposite response was detected at 10 °C. Our results confirm photoperiod sensitivity of Douglas-fir sources from relatively low (i.e., <45°N) latitudes. Increased spring cold hardiness and greater rooting at lower rhizosphere temperatures may improve field performance potential of SD-treated seedlings.

**Résumé :** L'effet d'une exposition à des jours courts sur le développement de semis de douglas vert (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) lors de la levée de la dormance a été évalué pendant la culture en pépinière. Les semis provenant d'une source à basse latitude (42°N) ont été exposés à un traitement de photopériode ambiante (jours longs (JL)) ou à une photopériode de 9 h de lumière : 15 h d'obscurité (jours courts (JC)) pendant 28 jours. Les semis ont été périodiquement enlevés de l'entreposage au froid entre janvier et mai. La sensibilité des tissus végétaux aux températures froides a été évaluée par la méthode de fuite des électrolytes à neuf températures différentes variant entre 2 et -40 °C. La croissance de nouvelles racines a été mesurée à des températures de rhizosphère de 10, 15, 20 et 25 °C. Entre 2 et -13 °C, aucune différence n'a été décelée entre les traitements au niveau de la résistance au froid. À des températures entre -18 et -40 °C, les semis exposés à des JL ont subi plus de dommages que les semis exposés à des JC. La température causant une augmentation de 50 % de la fuite des électrolytes (LT<sub>50</sub>) était continuellement plus basse pour les semis exposés à des JC que pour les semis soumis à des JL. Les semis exposés à des JL ont développé plus de nouvelles racines que les semis soumis à des JC à une température de rhizosphère de 20 °C, alors que le contraire a été décelé à 10 °C. Les résultats confirment une plus grande sensibilité à la photopériode de semis de douglas vert provenant d'une source à basse altitude (<45°N). L'augmentation de la résistance au froid au printemps ainsi qu'une plus grande production de nouvelles racines à des températures de rhizosphère plus basses pourrait améliorer la performance des semis exposés à des JC après la mise en terre.

[Traduit par la Rédaction]

## Introduction

Rapid initiation of new roots after seedling planting is critical to regeneration success (Grossnickle 2005), because new roots lacking an endodermis or cork layer have more efficient water uptake (i.e., lower resistance to water flow) than older roots (Sands et al. 1982; Grossnickle 1988). New root growth can help alleviate transplanting stress (Ritchie and Dunlap 1980; Nambiar and Sands 1993), which is largely a function of water limitations (Burdett 1990; Haase

and Rose 1993), and improve survival in a wide variety of conifer species (Grossnickle 2005). Without new root growth and water uptake shortly after transplanting, photosynthesis is limited, which further reduces the potential for new root growth (Burdett 1990; Grossnickle 2000) because new root growth after planting in conifers is largely dependent on current photosynthate (van den Driessche 1987). The need for new root growth to ensure seedling establishment depends on limiting environmental conditions on the outplanting site (Grossnickle 2005); the relative importance

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increases under harsh conditions (Simpson and Ritchie 1997).

Low soil temperature inhibits root growth of newly planted conifer seedlings (Lopushinsky and Kaufmann 1984; Grossnickle 2005), and much research has examined effects of low root zone temperatures on seedling growth and physiology. Soil temperature near 20 °C is optimal for root growth of newly planted conifers, whereas temperatures below about 5 °C allow for little root growth (Ritchie 1985; Lopushinsky and Max 1990). Soil temperatures on the outplanting site are frequently not optimum for root growth. For example, low soil temperatures (e.g.,  $\leq 10$  °C) persist during spring, the standard planting time in many regions. Low soil temperatures can limit seedling physiological function (i.e., gas exchange and root respiration) and water uptake because of decreased root permeability and increased water viscosity (Kaufmann 1975; Day et al. 1990); stresses may be expressed rapidly (Grossnickle 1988).

Avoidance of transplanting stresses, such as low soil temperature, is partly associated with seedling condition at time of planting (Burdett 1990), which may be mediated through nursery cultural treatments. For example, the shoot/root mass ratio is an indicator of the balance between seedling water loss through transpiration and water uptake capability by root systems (Burdett 1990; Grossnickle 2000). Additionally, adequate stress resistance is needed for response to drought and low temperature events (Ritchie 1984a; Burdett 1990). Cold hardiness, a surrogate measure of dormancy status (Ritchie 1984a; Fuchigami and Nee 1987), provides an effective predictor of stress resistance during lifting, storage, handling, and outplanting (McKay 1997).

In many temperate species, cold hardiness development is initiated by photoperiod reduction and coincides with growth cessation (Campbell and Sugano 1975). Early seedling dormancy induced via photoperiod manipulation during propagation (i.e., short-day treatments or blackout) is an option practiced in conifer seedling production (Hawkins et al. 1996; Turner and Mitchell 2003). This technique was adopted to arrest growth in conifer seedlings that would otherwise exhibit lammass growth and was based on the premise that reduced photoperiods induce seedlings to initiate bud dormancy (Wareing 1956), thereby conditioning stock for lifting. Short-day (SD) treatments during nursery culture may affect development of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedling cold hardiness (Turner and Mitchell 2003; MacDonald and Owens 2006). Additionally, because root growth capacity is strongly related to the bud dormancy cycle (Ritchie and Dunlap 1980; Ritchie 1985) and to shifts in shoot–root carbohydrate balance (Burdett 1990), SD treatments during nursery culture could affect timing and vigor of subsequent root growth.

Ability to effectively anticipate potential for seedling root proliferation following transplanting could improve coupling of seedling growth capacity to outplanting conditions. Despite investigations of responses of SD-treated conifer seedlings for cold hardiness and timing of bud break, little published research has examined transplant rooting responses to varying root temperatures. With increasing interest in planting forest tree seedlings beyond the traditional spring planting period (Adams et al. 1991; Seifert et al. 2006), it is important to study the interrelationship between nursery

stock quality and responses at varying root temperatures. Additionally, most studies related to SD treatment during nursery culture examined patterns of hardening in fall (e.g., Coursolle et al. 1998; Fløistad 2002), but fewer have explored cold hardiness during spring dormancy release (Coursolle et al. 1997), which is the critical period when seedlings must resist stresses of cold storage, transport to the outplanting site, and planting. Furthermore, interactions between SD periods and latitude of seed lot origin may affect dormancy development and cold hardiness (Coursolle et al. 1997; Partanen 2004). Although SD treatments have effectively modified plant physiological status at higher latitudes (i.e.,  $\geq 50^\circ\text{N}$ , Krasowski and Owens 1991; Oleksyn et al. 1992; Hawkins and Shewan 2000; Fløistad 2002; Partanen 2004; Rostad et al. 2006;  $45\text{--}50^\circ\text{N}$ , Grossnickle et al. 1991a; Bigras and D'Aoust 1992; Coursolle et al. 1997; Coursolle et al. 1998; Turner and Mitchell 2003; MacDonald and Owens 2006), little research has examined effects of SD treatments for midlatitude sources (i.e.,  $<45^\circ\text{N}$ ). We applied SD versus long-day (LD) treatments during nursery culture of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) seedlings from a  $42^\circ\text{N}$  seed source and examined dehardening at periodic intervals from January to May along with whole-plant and rooting responses at varying rhizosphere temperatures.

## Materials and methods

### Plant material and photoperiod treatments

Douglas-fir seeds were collected from a coastal provenance in southern Oregon, USA (State of Oregon Seed Zone 081;  $42^\circ 37'\text{N}$ ,  $123^\circ 30'\text{W}$ , elevation 1067 m), and sown into Styroblock™ 415C containers (ninety-one 130 mL cavities per container; Beaver Plastics, Edmonton, Alta.) with a 85:15 peat : fine sawdust (by volume) medium in mid-March 2004. All seedlings were grown under equivalent operational practices at Cal-Forest Nursery near Etna, California, USA ( $41^\circ 28'\text{N}$ ,  $122^\circ 49'\text{W}$ ), except that one-half of the experimental seedlings received SD treatment, whereas the remaining seedlings were grown in an adjacent greenhouse under ambient photoperiods (LD treatment). The SD treatment was accomplished using a blackout curtain system (Cravo Equipment, Ltd., Brantford, Ont.) suspended at a height of 1.7 m above the seedling containers. The SD treatment was initiated on 12 July and consisted of restricting seedlings to 9 h light: 15 h dark photoperiods (i.e., 09:00–18:00, solar time) for 28 days. Overhead sprinkler irrigation was applied to all seedlings until media saturation on the day of sowing, reduced to maintain moist media until germination, and thereafter applied to saturation after the medium dried to approximately 50% soil water content as determined by block masses. All seedlings received fertigation (100 ppm N, 10 ppm P, 100 ppm K with minor nutrients; Romeo Packing Co., Half Moon Bay, Calif.) at each irrigation. This equates to total delivery of approximately 100 mg N, 10 mg P, and 100 mg K per plant. The pH of irrigation water was adjusted to 5.5 using sulfuric acid injection. Seedlings were lifted from containers on 15 December and couriered to Purdue University, West Lafayette, Indiana, USA, where they were freezer stored ( $-2^\circ\text{C} \pm 2^\circ\text{C}$ ).

### Cold hardiness assessment

Seedlings were removed from freezer storage on six dates in 2005 (7 January, 7 February, 25 February, 23 March, 19 April, and 9 May). At each removal date, cold hardiness of LD and SD seedlings was determined via freeze-induced electrolyte leakage (FIEL; Flint et al. 1967). Thirty-six seedlings from each nursery photoperiod treatment were randomly selected and destructively sampled for this procedure. For each treatment, approximately five needles were removed from each seedling, cut into 1 cm segments, and divided into four replicates. Ten segments from each replicate were placed into each of nine 20 mL copolymer polypropylene vials (RPI Corp., Mount Prospect, Ill.) and filled with 15 mL deionized water. The nine vials corresponded to nine test temperatures (2 [control], -3, -8, -13, -18, -23, -28, -34, and -40 °C). The control treatment was placed into a refrigerator (2 °C), and remaining treatments were placed into a programmable freezer (So-Low, Inc., Cincinnati, Ohio). Beginning with an initial temperature of 0 °C, the temperature was decreased at 0.25 °C·min<sup>-1</sup>. Each test temperature was maintained for a period of 20 min, after which time the vials designated for that test temperature were removed, and the temperature then continued to decrease to the next test temperature. Vials were thawed at 2 °C for approximately 24 h and then moved to ambient conditions to complete thawing. After thawing, FIEL of the excised stem portions was measured with an HI 9813 portable conductivity meter (Hanna Instruments, Inc., Woonsocket, R.I.). Maximum conductivity was determined by placing vials in an autoclave (Gettlinge USA, Inc., Rochester, N.Y.) at 110 °C for 20 min. Electrolyte leakage values were then expressed as a percentage of conductivity at each test temperature compared with that at maximum conductivity.

### Rhizosphere temperature treatments

At each removal date from storage, 120 additional seedlings of each of the SD and LD treatments were randomly selected, and roots were washed free of medium in preparation for transfer to hydroponic tanks. Seedlings were then measured for initial shoot height (cotyledon scar to base of terminal bud) and stem diameter (1 cm above cotyledon scar) and transplanted into a hydroponic growing system for 14 days to evaluate root system proliferation under varying rhizosphere temperatures. The experimental system was established in a controlled environment growth room at the Purdue University Horticulture and Landscape Architecture Plant Growth Facility (40°25'N, 86°55'W) with 16 h light: 8 h dark photoperiods, day:night air temperature of 24:18 °C (±0.5 °C), relative humidity of 65% (±25%), and photosynthetic photon flux density (PPFD) at seedling top height of 120 µmol·m<sup>-2</sup>·s<sup>-1</sup>. Light was provided by a combination of 400 W metal halide and high-pressure sodium lamps. The experimental design was a randomized complete block design with three blocks serving as replications. Within each block, four hydroponic growing tanks (45.7 cm × 45.7 cm × 30.5 cm, L × W × H), with each tank corresponding to one of four controlled root zone temperatures (10, 15, 20, and 25 °C), were installed. Each tank held 10 seedlings of each of the LD and SD nursery photoperiod treatments.

Each growing tank was filled with water, and temperature was controlled with a computer (model TC-24-25; TE Technology, Inc., Traverse City, Mich.) programmed thermo-electric chiller (model LC-061; TE Technology, Inc.), with constant water flow through the unit maintained at 680 L·h<sup>-1</sup> by a magnetic drive centrifugal pump (model 1A-MD-1, March Manufacturing, Inc., Glenview, Ill.) connected to polyethylene tubing. Adequate aeration was provided to seedling roots through continuous air flow pumped into the tank at 200 L·h<sup>-1</sup> (Aquarium Pharmaceuticals, Inc., Chalfont, Pa.). Periodic measurements indicated that water temperature throughout each 14 day period was within ± 2 °C of each set point. No supplemental nutrition was provided to the seedlings.

Following 14 days in hydroponic tanks, all seedlings were harvested, and new roots ≥ 1 cm in length were counted and excised for dry mass determination following drying at 70 °C for 48 h. Seedlings were also reassessed for shoot height, stem diameter, and shoot and root dry mass.

### Data analysis

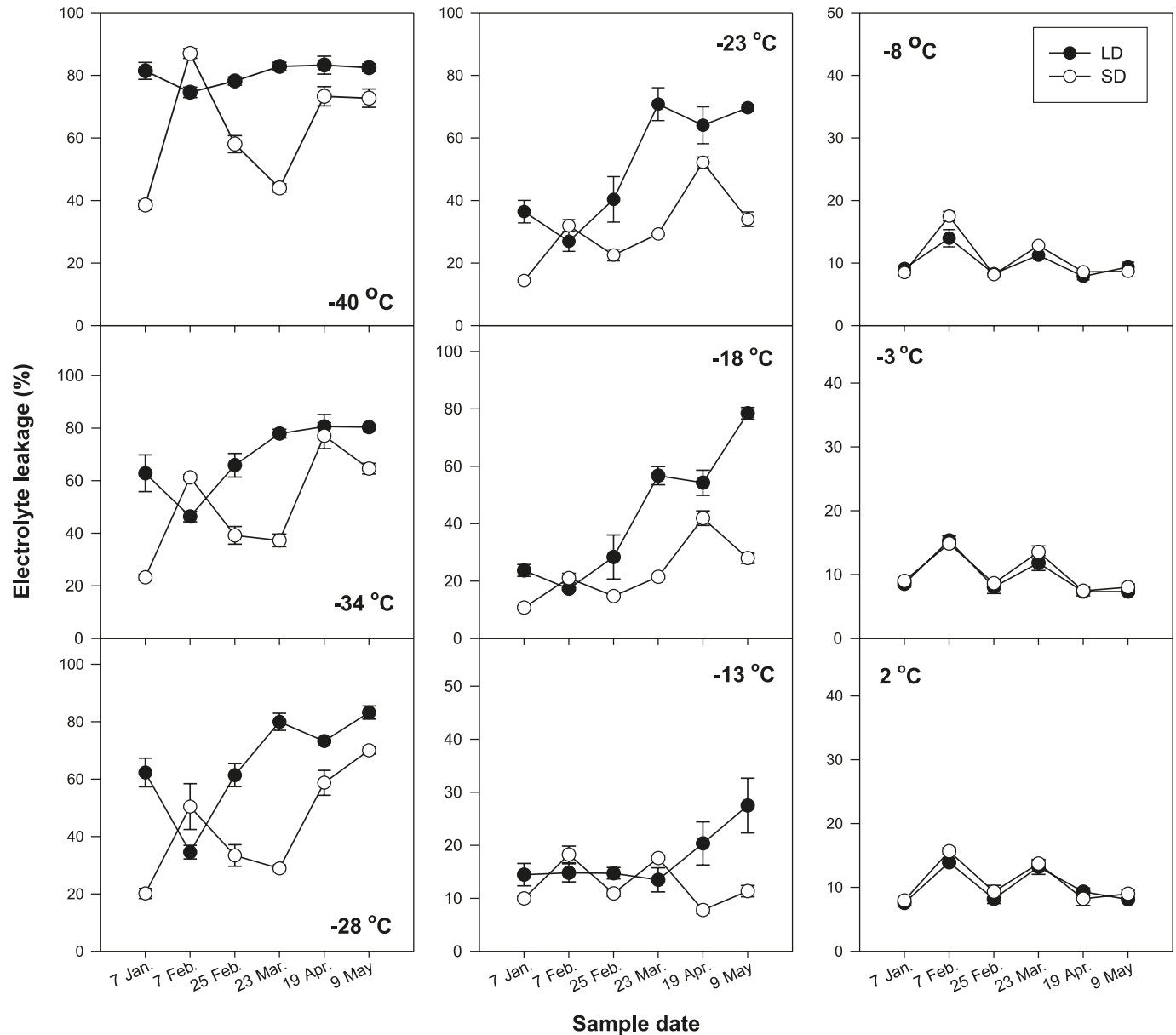
Tests for normality of residuals and constant variance were performed to confirm that assumptions of analysis of variance (ANOVA) were met, and no transformations were required. Subsequently, three-way ANOVA was conducted to evaluate effects of root zone temperature at four levels, photoperiod at two levels, and sample date at six levels. Another three-way ANOVA was used to test sensitivity of plant tissues to cold temperature at nine levels, photoperiod at two levels, and sample date at six levels. Replicate means comprised the experimental units and ANOVA effects were considered significant at  $P \leq 0.05$ . Cold-hardiness data was expressed as an LT<sub>50</sub> (i.e., interpolated temperature at which 50% cell electrolyte leakage occurs, relative to 100% leakage in heat-killed samples), described extensively for tree seedling applications in Burr et al. (1990). Each LT<sub>50</sub> was calculated by fitting simple quadratic regressions ( $P \leq 0.05$  in all cases) for LD and SD seedlings based on electrolyte leakage values at each of the nine test temperatures. Where sample date was nonsignificant, data were pooled across dates and analyzed, which only occurred for component dry mass, new root numbers, and new root dry mass. Significant treatment mean differences were ranked according to Duncan's multiple range test ( $\alpha = 0.05$ ). Treatment interaction means were separated by orthogonal contrasts (Nogueira 2004), using Tukey's honest significant difference (HSD) test ( $\alpha = 0.05$ ). These contrasts, for example, evaluated LD versus SD within each rhizosphere temperature regime. SAS, version 9.1 (SAS Institute Inc., Cary, N.C.), was used for all statistical data analysis.

## Results

### Cold hardiness as affected by photoperiod and removal date

The FIEL was significantly ( $P < 0.0001$ ) affected by removal date, photoperiod, test temperature, and all associated interactions. Values for FIEL generally increased from January (24.9%) to May (41.8%), reflecting spring deacclimation. Test temperatures below -8 °C caused progressively increased cell leakage and temperatures below -23 °C

**Fig. 1.** Mean ( $\pm$ SE) electrolyte leakage values for each freezing test temperature across sampling dates for either ambient (long-day (LD)) or midsummer short-day (SD) photoperiod treatments.



resulted in >50% FIEL. Averaged across removal dates and test temperatures, SD-treated seedlings had lower FIEL values (27.6%) than seedlings exposed to ambient photoperiods (LD, 39.3%). Figure 1 illustrates the significant removal date  $\times$  photoperiod  $\times$  test temperature treatment interaction. Relatively few differences between photoperiod treatments were detected at temperatures above  $-9^{\circ}\text{C}$ . At the remaining test temperatures, SD seedlings had consistently lower FIEL values from 25 February through 9 May.

Values for  $LT_{50}$  were significantly ( $P < 0.0001$ ) affected by removal date, photoperiod, and removal date  $\times$  photoperiod. Results of orthogonal contrasts for LD versus SD seedlings for each sampling date are given in Table 1. For both photoperiod treatments,  $LT_{50}$  values became less negative during the course of the sampling period. SD seedlings had significantly lower  $LT_{50}$  values than LD seedlings for all but

**Table 1.** Mean values ( $\pm$ SE) of  $LT_{50}$  (i.e., interpolated temperature at which 50% cell electrolyte leakage occurred) for seedlings grown under either ambient (long-day (LD)) or midsummer short-day (SD) photoperiods within each sample date evaluated using orthogonal contrasts.

Sample date	$LT_{50}$ ( $^{\circ}\text{C}$ )	
	LD	SD
7 January	-28.7 (0.8)a	-48.3 (0.8)b
7 February	-33.6 (0.7)b	-29.6 (0.5)a
25 February	-27.7 (1.5)a	-37.2 (0.5)b
23 March	-19.8 (1.0)a	-43.9 (0.3)b
19 April	-20.3 (0.6)a	-25.6 (0.6)b
9 May	-15.6 (0.5)a	-28.3 (0.2)b

**Note:** For each sample date, values in same row with different letters differ statistically according to Tukey's HSD test at  $\alpha = 0.05$ .

**Table 2.** ANOVA summary of *P* values for morphological parameters.

Source	Shoot height		Stem diameter		New root		Dry mass			
	Initial	Final	Initial	Final	No.	Dry mass	Root	Shoot	Plant	Shoot/root
Date (D)	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	0.6094	0.0856	0.1609	0.4595	0.2777	0.4044	0.0848
Photoperiod manipulation (PM)	<b>0.0001</b>	<b>0.0001</b>	0.7165	0.2576	0.3540	0.5038	0.1785	<b>0.0001</b>	<b>0.0006</b>	<b>0.0001</b>
D × PM	<b>0.0001</b>	<b>0.0001</b>	<b>0.0065</b>	<b>0.0467</b>	0.5127	0.5146	0.7720	0.4935	0.5607	0.3067
Rooting zone temperature (RZT)	0.6319	0.5608	0.2158	<b>0.0153</b>	<b>0.0003</b>	<b>0.0003</b>	<b>0.0037</b>	0.1014	<b>0.0230</b>	0.1109
D × RZT	0.3892	0.1057	0.4570	0.3881	0.0600	0.3973	<b>0.0400</b>	0.6151	0.3139	0.1801
PM × RZT	0.6453	0.3432	0.3944	0.6433	<b>0.0016</b>	<b>0.0382</b>	<b>0.0262</b>	<b>0.0367</b>	<b>0.0172</b>	0.7398
D × PM × RZT	0.4092	0.4441	0.1448	0.5865	0.9792	0.9319	0.1013	0.8274	0.5784	0.4419

Note: Significant ( $P < 0.05$ ) treatment effects are given in boldface.

**Table 3.** Main effects of photoperiod manipulation (PM) for seedlings grown under either ambient (long-day (LD)) or midsummer short-day (SD) photoperiods and of root zone temperatures (RZT) on seedling morphology after removal from freezer storage (initial) and following transplant into varying rhizosphere temperatures for 14 days (final).

Treatment	Shoot height (cm)		Stem diameter (mm)		Shoot/root dry mass
	Initial	Final	Initial	Final	
PM					
LD	37.2a	37.7a	5.1a	5.4a	4.1a
SD	29.2b	29.9b	5.1a	5.3a	2.5b
RZT					
10 °C	32.8a	33.4a	5.0a	5.1b	3.7a
15 °C	33.2a	33.8a	5.1a	5.3ab	3.0a
20 °C	33.3a	33.9a	5.2a	5.4ab	2.9a
25 °C	33.4a	34.2a	5.2a	5.6a	3.5a

Note: Values with different letters in same column and within each treatment differ statistically according to Duncan's multiple range test at  $\alpha = 0.05$ .

one date (7 February). Differences in  $LT_{50}$  values for LD versus SD seedlings were pronounced for several dates (19 °C on 7 January, 24 °C on 23 March, and 12 °C on 9 May) (Table 1).

### Responses to varying rhizosphere temperatures

At the time of transplanting to the hydroponic tanks, shoot height differed significantly by photoperiod treatments, with LD seedlings having greater mean height than SD seedlings (Tables 2 and 3). Removal date was significant in the ANOVA (Table 2), but no trend was apparent, and this effect was attributed to sampling variance. Stem diameter did not differ by nursery photoperiod treatments (Tables 2 and 3).

Number of new roots and new root dry mass were significantly affected by photoperiod × rhizosphere temperature, whereas associated interactions with removal date were not significant (Table 2). For LD seedlings, both mean number and dry mass of new roots increased with increasing rhizosphere temperature up to 20 °C, but declined at 25 °C (Fig. 2). Mean number and dry mass of new roots increased for SD seedlings from 10 to 15 °C, but mean values subsequently declined at 20 °C and continued to decrease at 25 °C (Fig. 2). Results of orthogonal contrasts for LD versus SD seedlings for each rhizosphere temperature showed significantly greater number and dry mass of new roots for LD versus SD seedlings at 20 °C (Fig. 2). However, at 10 °C, mean number of new roots was significantly greater for SD versus LD seedlings (Fig. 2A).

Root, shoot, and total plant biomasses at harvest were also significantly affected by photoperiod × rhizosphere temperature (Table 2). Shoot dry mass was greater for LD versus SD seedlings for all but the 15 °C rhizosphere temperature (Fig. 3A). Root dry mass was significantly greater for SD versus LD seedlings at the 15 °C rhizosphere temperature (Fig. 3B). Total plant dry mass was significantly greater for LD versus SD seedlings only at the 20 °C rhizosphere temperature (Fig. 3C). Shoot/root dry mass varied significantly between photoperiod treatments (Table 2), with LD seedlings having a mean value of 4.09 compared with 2.45 for SD seedlings (Table 3).

## Discussion

### Nursery photoperiod effects on cold hardiness and morphology

Increased cold hardiness of SD-treated seedlings in fall has been frequently reported and associated with reduced physiological activity and dormancy induction (McCreary et al. 1978). Increased fall cold hardiness of SD-treated seedlings reduces risk of cold damage to seedlings during fall hardening (Bigras and D'Aoust 1993; Rostad et al. 2006). Greater seedling cold hardiness could also facilitate fall outplanting, which is of current interest in some areas where spring outplanting predominates (Adams et al. 1991; Seifert et al. 2006). We observed generally greater cold hardiness of SD versus LD seedlings throughout the spring deacclimation period (Fig. 1; Table 1), which suggests that increased fall

cold hardiness associated with SD treatment may be maintained under freezer storage conditions through spring dormancy release. Bigras and D'Aoust (1992) found that black spruce (*Picea mariana* (Mill.) BSP) and white spruce (*Picea glauca* (Mill.) B.S.P.) seedlings that received SD treatments exhibited more rapid hardening but lower levels of cold tolerance during dehardening after transplant to a growth chamber (21 days at 10 °C with 14 h light: 10 h dark photoperiods). It is likely that this dehardening response contrasted with our results because we sampled FIEL immediately after removal from storage compared with following exposure to favorable environmental conditions. Cannell et al. (1990) reported that dehardening of cold-stored (0.5 °C) Douglas-fir seedlings was slowed compared with seedlings exposed to ambient environmental conditions; dormancy release is delayed further under subzero storage conditions (Ritchie 1984b). Increased cold hardiness is correlated to higher stress resistance (Fuchigami and Nee 1987), which reduces susceptibility of spring-planted stock to stresses associated with lifting, handling, and storage.

Cooling rates used in this study and elsewhere (e.g., Burr et al. 1990) for controlled laboratory testing of seedling cold hardiness are more rapid than ambient conditions. Increasing cooling rate from 1.0 to 2.9 °C·h<sup>-1</sup> can mean the difference between cell survival and cell death in herbaceous plants (Steffen et al. 1989; Palta and Weiss 1993). This may limit capacity to draw direct inferences to nursery or field conditions. Rapid cooling may also affect the ability to detect treatment effects, because the absence of differences may be solely associated with the high cooling rate. This suggests that methodology for future cold hardiness testing may need to be refined to ensure the ability to accurately test for treatment effects and to confirm operational relevance.

Seedlings exposed to SD treatment had 22% shorter shoot height (measured after removal from storage), although stem diameter did not differ (Table 3). Arrested height associated with early budset has long been reported in relation to photoperiod manipulation of conifers, in particular Douglas-fir (McCreary et al. 1978; Turner and Mitchell 2003), western red-cedar (*Thuja plicata* Donn ex D. Don; Krasowski and Owens 1991), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.; Grossnickle et al. 1991a), and Sitka spruce (*Picea sitchensis* (Bong.) Carr.; Hawkins et al. 1996). Shoot/root dry mass sampled at the end of the 14 day rhizosphere testing period was reduced by 39% in SD versus LD seedlings (Table 3). Shoot/root ratio also decreased in Sitka spruce (Hawkins et al. 1996) and western hemlock (Grossnickle et al. 1991a) following photoperiod manipulation, although this effect was not previously observed for Douglas-fir (Burdett and Yamamoto 1986). Reduced shoot/root ratio improves the ability of seedlings to resist transplanting stresses through regulating water supply and the balance between transpiration and absorption (Ritchie 1984a; Burdett 1990) and, likely, confers an advantage to SD-treated seedlings on drought-prone sites.

Variation by seed source in response to photoperiod manipulation has been documented in conifers (Coursolle et al. 1998; Hawkins and Shewan 2000). Partanen (2004) found that Norway spruce (*Picea abies* (L.) Karst.) and European white birch (*Betula pendula* Roth) seedlings from

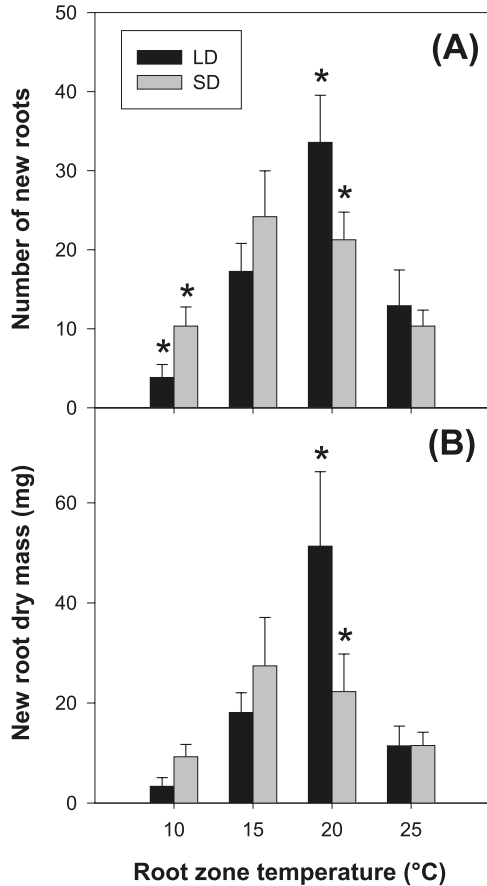
northern origins generally ceased growth earlier than those from southern origins under similar photoperiodic durations. Oleksyn et al. (1992) tested 24 European populations of Scots pine (*Pinus sylvestris* L.) using 50°N or 60°N photoperiods (simulating natural day-length changes from 1 May to 1 September) and reported that northern (56–61°N) plants responded to a combination of increasing night length and accumulated degree-days. However, central and southern populations (40–55°N) did not respond to increased night length: they all ceased growth earlier in the 60°N than the 50°N photoperiod. Oleksyn et al. (1992) suggested that central and southern populations are either photoperiod insensitive or respond to short nights or accumulated degree-days at their native latitudes. Despite the relatively low latitude of the seed source used in our study (42°N), photoperiod manipulation affected seedling physiological and morphological status. We are not aware of any other published research that reports effects of photoperiod manipulation on conifer seed sources <45°N latitude. Although variation in responses is likely to occur both among and within species, our results lend support to effective use of this practice for the culture of more southerly seed sources of coastal Douglas-fir than are traditionally used.

#### Nursery photoperiod effects under varying rhizosphere temperatures

Rhizosphere temperature affected seedling morphological development following transplanting to hydroponic tanks, particularly in relation to new root development (Figs. 2 and 3; Table 3). Similar to our results, low root temperature reduced new root growth of several forest tree species (Lopushinsky and Max 1990; Apostol et al. 2007). Low root temperature inhibits root hydraulic conductivity and metabolic activity, which may be linked to limitations in new root growth (Bowen 1991). Reduced root proliferation at low root temperatures results in less functional roots available for water transport, which may deter successful seedling establishment. Restricted water uptake in cold soils may occur even with adequate soil water availability because of resistance in water flow (Kaufmann 1975; Day et al. 1990).

We observed significant photoperiod × rhizosphere temperature interactions for new root production, with greater mean new root numbers (34 vs. 21) and more mean new root biomass (51 vs. 22 mg) for LD versus SD seedlings at 20 °C and generally the opposite trend (4 vs. 10 for new root numbers and 3.3 vs. 9.3 mg for new root biomass, although the difference for new root biomass was not statistically significant) at 10 °C (Fig. 2). Relatively little research has examined spring root development in SD-cultured nursery seedlings. Turner and Mitchell (2003) found that root growth capacity (i.e., number of new roots) was reduced in Douglas-fir seedlings with earlier versus later blackout initiation date, although there was no LD treatment and soil temperature was not documented. MacDonald and Owens (2006) reported that SD treatment did not affect Douglas-fir seedling root growth capacity after transplanting of seedlings into pots for 15 days in a controlled-environment chamber (constant air temperature of 20 °C, soil temperature not reported). Hawkins and Shewan (2000) examined new root development of interior spruce

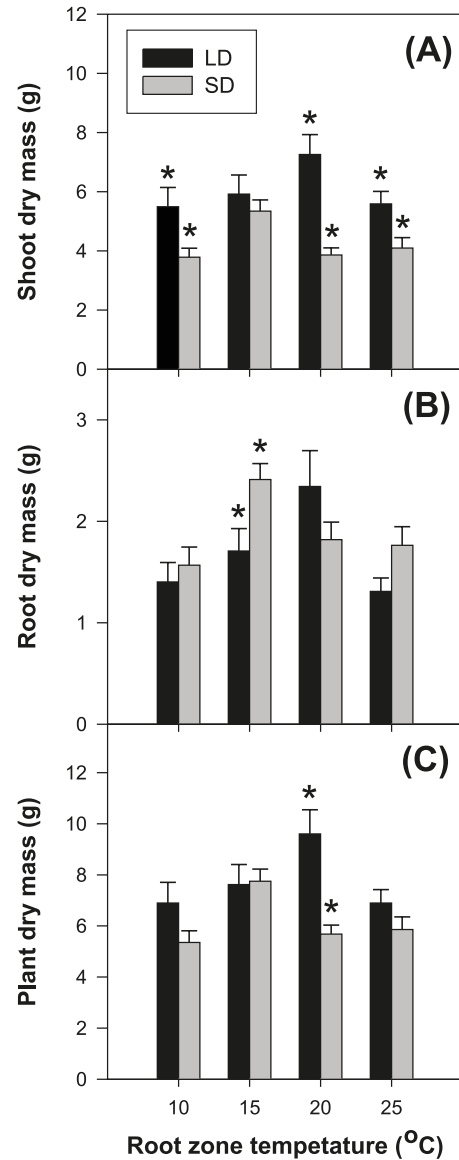
**Fig. 2.** Mean ( $\pm$ SE) values for (A) new root numbers and (B) new root biomass according to rhizosphere temperature for either ambient (long-day (LD)) or midsummer short-day (SD) photoperiod treatments. At a given rhizosphere temperature, asterisks indicates a significant difference between photoperiod treatments according to orthogonal contrasts using Tukey's HSD test at  $\alpha = 0.05$ .



(*Picea glauca*, *Picea engelmannii* Parry ex Englem., and their naturally occurring hybrids) following SD treatment by transplanting into hydroponic culture at 20 °C for 7 days. Similar to our results at this rhizosphere temperature, they reported that SD seedlings produced fewer new roots than LD seedlings (mean values of 0.5 and 2.2, respectively).

Grossnickle et al. (1991a) is the only other known published report to examine transplant rooting response of SD-cultured conifer seedlings under more than one root temperature regime. They examined responses of SD- and LD-cultured western hemlock seedlings in combination with relatively wet or dry watering regimes on transplant rooting responses in hydroponics at 5 and 22 °C root zone temperatures for 14 days. Similar to our results, well-watered SD seedlings produced more new roots than well-watered LD seedlings at 5 °C root temperature and the opposite trend occurred at 22 °C. In a complementary field trial, Grossnickle et al. (1991b) found greater root proliferation of SD-treated seedlings 1 month (30 March) following planting and attributed this to better adaptability of SD seedlings to low (3–10 °C) soil temperatures that occurred up to that point. Grossnickle et al. (1991a) noted that the lower number of new roots produced by SD seedlings at 22 °C

**Fig. 3.** Mean ( $\pm$ SE) values for (A) shoot dry mass, (B) root dry mass, and (C) total plant dry mass according to rhizosphere temperature for either ambient (long-day (LD)) or midsummer short-day (SD) photoperiod treatments. At a given rhizosphere temperature, asterisks indicates a significant difference between photoperiod treatments according to orthogonal contrasts using Tukey's HSD test at  $\alpha = 0.05$ .



may have little biological consequence, because all seedlings produced >35 new roots, which is indicative of good potential for survival following transplant. Similarly, although LD seedlings produced significantly more roots than SD seedlings at 20 °C in our study, a mean value of 21 new roots for SD seedlings suggests high survival potential (Grossnickle 2005). Additionally, PPFD was relatively low following transplanting in our study ( $120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), because light compensation and saturation values of approximately 15 and  $438 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively, at 20 °C air temperature have been reported for coastal Douglas-fir seedlings (Krueger and Ferrell 1965). This suggests that root proliferation may have been increased under higher PPFD conditions.

Grossnickle et al. (1991a) hypothesized that differences in new root production among photoperiod treatments at the 5 °C root temperature was a function of higher photosynthetic rates recorded for SD seedlings at this temperature, because new root growth in conifers is largely associated with current photosynthate (van den Driessche 1987). However, they also reported no significant differences in gas exchange at the 22 °C root temperature. Interestingly, on a field planting site, LD seedlings had higher gas exchange rates than SD-treated seedlings in early spring under low (3–10 °C) soil temperatures (Grossnickle et al. 1991b). These contrasting results suggest that other specific physiological mechanisms may be involved in increased root proliferation of SD-treated seedlings at low soil temperatures (e.g., variation in phenological status or shifts in root–shoot carbohydrate balance).

Grossnickle et al. (1991a) also found that SD-treated western hemlock seedlings had significantly less resistance to water movement at low (5 °C) temperatures and suggested that this could confer greater water flow efficiency in SD seedlings immediately after early spring planting. Root elongation often begins early in the growing season, while soil temperature is still low (Oliver and Larson 1996), and precedes prioritization of carbohydrate transfer to shoot growth following bud break (Burdett 1990). Thus, treatments that facilitate root development under colder soil temperatures, such as the photoperiod treatment employed in this study, may help minimize transplanting stress and benefit initial plantation establishment success.

The short test cycle duration (14 days) in our study precludes our ability to evaluate longer term growth of SD-treated seedlings following planting. Similar to new root development, we observed significant photoperiod × rhizosphere temperature effects for shoot, root, and total plant dry mass (Fig. 3). We attributed the 88% larger shoot dry mass in LD versus SD seedlings (Fig. 3A) to pretransplant differences as reflected in the significantly different initial shoot height (Table 3). Interestingly, shoot dry mass did not differ significantly at 15 °C, the same temperature at which root dry mass was significantly greater for SD seedlings (Fig. 3B). This suggests that greater mean new root development of SD-treated seedlings at this temperature (Fig. 2) may have benefited aboveground growth. Similarly, greater new root biomass in LD seedlings at 20 °C (Fig. 2B) probably stimulated the significantly greater whole-plant dry mass at this temperature (Fig. 3C). Previous studies of photoperiod manipulation during nursery culture have reported positive (Hawkins et al. 1996), detrimental (Grossnickle et al. 1991b; O'Reilly et al. 1994), and no (MacDonald and Owens 2006) effects associated with early dormancy induction in terms of subsequent season height growth.

## Conclusions

We observed that SD treatment applied during nursery culture of Douglas-fir seedlings increased cold hardiness upon removal from freezer storage throughout the dormancy release period. This suggests that increased cold hardiness of SD-treated seedlings in fall observed in other studies may be maintained in freezer storage through spring. Our results indicate that coastal Douglas-fir sources from lower (i.e., <45°N) latitudes may exhibit strong morphological

and physiological adaptations to photoperiod. This refutes suggestions of Oleksyn et al. (1992) that southerly sources may be photoperiod insensitive and indicates that SD treatments may potentially serve as an effective cultural tool for a wide range of Douglas-fir seed sources.

Seedlings treated with abbreviated photoperiods (SD) had increased new root proliferation at low soil temperature (10 °C) compared with LD seedlings, whereas the opposite response occurred at 20 °C. Our results support recommendations of Grossnickle et al. (1991b) in regard to the importance of matching stock conditions manipulated by SD treatments to environmental conditions on the planting site. Under low soil temperature conditions, SD-treated seedlings may be at a competitive advantage associated with their demonstrated greater rooting capacity. Additionally, SD seedlings may better resist drought on dry sites because of lower resistance to root water flow (Grossnickle et al. 1991a) and reduced shoot:root. On sites where early season low soil temperatures or drought conditions are not expected, LD seedlings may be better adapted.

Future studies should more closely examine transplant rooting responses associated with timing of SD treatments in relation to natural growth rhythm: this was shown to affect cold hardiness of Norway spruce in the subsequent growing season (Fløistad 2002). Although we did not identify variation in rooting response according to transplant date, further research should work to identify trends in root growth at different temperatures throughout the growing season; for example, Iivonen et al. (2001) found that Scots pine seedling response to different root zone temperatures was dynamic across the growing season. Although our investigations were limited to the dormancy release period during spring, transplant rooting responses of seedlings exposed to photoperiod manipulation should also be examined during fall to aid in coupling seedling physiological status to site conditions likely to be encountered during fall planting.

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