Effect of Nursery Photoperiod Manipulation on Coastal Douglas-fir Seedling Development: Early Results

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Abstract

Photoperiod manipulation by artificial short-day treatment (blackout) is increasingly used as a tool to induce dormancy in nursery-grown seedlings. This article summarizes preliminary results from a project to evaluate optimum blackout protocols for Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) seedlings. We subjected seedlings to three blackout intensities (mild, moderate, and long) and compared morphological and physiological responses at the time of lifting and during the next growing season with seedlings in a control treatment (ambient day length) and a progressive blackout treatment involving a gradual reduction of light during the hardening phase. We additionally characterized morphology, bud break, and root growth in response to varying rhizosphere temperatures. Preliminary results indicate that seedlings subjected to blackout treatments had earlier bud break in both a controlled hydroponic culture and a field plot. Seedlings from the mild, moderate, and long blackout treatments had less root growth in the hydroponic trial but greater early spring shoot and root biomass in the field plot. By the end of the growing season, however, biomass in the field plot did not differ among treatments. Cold hardiness was unaffected by treatments. Additional results from this trial and another trial to examine blackout effects on varying seed sources will be published at a later date. This paper was presented at the annual meeting of the Western Forest and Conservation Nursery Association (Eugene, OR, October 26–27, 2015).

Introduction

Dormancy induction of nursery-grown seedlings in early autumn is important to prevent frost damage, promote better shoot-to-root ratios, and minimize stress during lifting and storage. Dormancy can be induced by reducing fertilization or irrigation or by physical manipulation (Grossnickle and South 2014). It can be difficult, however, to successfully induce dormancy. For example, container-grown Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings did not move into an endodormant state when exposed to water stress in combination with reduced fertility in early fall (Macdonald and Owens 2006). These techniques also have the potential to reduce plant vigor and thus decrease subsequent survival and performance after outplanting (Villar-Salvador et al. 2015). For example, Douglas-fir seedlings with low internal nitrogen (N) levels were less cold hardy than seedlings with higher N levels (Timmis 1974).

Because of fewer negative effects on plant quality compared with conventional techniques, forest tree nurseries increasingly are using photoperiod manipulation during propagation (i.e., short-day treatments or blackout) to slow growth and induce dormancy in conifer species from northern latitudes (Hawkins et al. 1996). In general, the greater the reduction in photoperiod and the earlier the initiation of light deprivation, the more rapidly dormancy is induced. Intense light deprivation, however, can reduce plant reserves and hence reduce growth in the next year (Hawkins et al. 1996). Research indicates that blackout can be used to induce dormancy in species such as Douglas-fir (Jacobs et al. 2008, Turner and Mitchell 2003). To define the optimum blackout protocol for this species, it is necessary to examine the effects of different blackout treatments on seedling dormancy, morphology, and performance after planting.

Root growth and stress resistance also influence seedling survival and performance after outplanting (Grossnickle and South 2014, Villar-Salvador et al. 2015). Vigorous root development following field planting is necessary to minimize potential for seedling physiological drought and ensure survival (Grossnickle 2005). Soil temperature in most temperate outplantings during winter or early spring is usually relatively cold (i.e., <10 ºC [50 °F]) and can limit root growth and establishment of planted seedlings (Jacobs et al. 2008, Villar-Salvador et al. 2015). Adequate cold hardiness and stress resistance are needed during drought and low-temperature events (Grossnickle and South 2014, Villar-Salvador et al. 2015). Frost resistance is especially important in both fall and early spring for seedling performance and survival. Photoperiod has an important role in frost tolerance, because it affects both bud set and dormancy release. Seedlings additionally must resist stresses of transport to the outplanting site and, following planting, seedlings usually must deal with late spring frosts. Thus, cold resistance in late spring is of particular interest, but it has been little studied in combination with blackout treatments.

This article summarizes preliminary results from an ongoing study to test blackout treatments and rhizosphere temperature effects on Douglas-fir phenology, quality, and vigor, with the objectives of determining optimal blackout protocols and coming to a better understanding of seedling performance after outplanting.

Materials and Methods

Seedlings

Douglas-fir seeds were collected from the Washington State Department of Natural Resources Meridian Seed Orchard (Olympia, WA), representing a composite of collections harvested from 2001 through 2010 from the South Sound (0 to 610 m [0 to 2,000 ft] elevation) breeding block.

Seeds were sown on February 18, 2014, into 170 mL (10 in^3), 77-cell Styroblock™ containers (Beaver Plastics Ltd., Acheson, AL, Canada). Soil media consisted of an 80:20 peat:perlite medium with 60 g seedling^{-1} of Nutricote Total 18-6-8 Type 180 controlled-release fertilizer preincorporated into the medium. All seedlings were grown under equivalent operational practices at the L.T. Mike Webster Forest Nursery near Olympia, WA (46°57’00” N. 122°57’36” W.). Seedlings were grown under greenhouse cover until June 3, 2014, when they were moved outdoors.

Blackout Treatments

We tested five treatments with varying periods of light deprivation. Treatments were initiated on July 14, 2014, as follows:

1. Control—ambient photoperiod.
2. Mild—7 days blackout, 7 days ambient light, 7 days blackout.
3. Moderate—14 days blackout, 7 days ambient light, 7 days blackout.
4. Long—21 days blackout, 7 days ambient light, 7 days blackout.
5. Progressive—continuous (initiated August 1).

It is conventional to apply blackout treatments in a static manner at the same time each day for the treatment period, as in our mild, moderate, and long blackout treatments that received 9 hours of light and 15 hours of darkness during blackout. The conifer phytochrome system, however, which, in part, is responsible for stimulating bud set during shortening photoperiods, likely responds to environmental inputs other than the single mechanism of absolute day length. For example, the phytochrome system may respond to increasing or decreasing day lengths (Greer et al. 1989). Thus, we included the progressive treatment that consisted of a progressive reduction of light based on the natural photoperiod of 1 month ahead and gradually (weekly) decreasing to meet the natural photoperiod at the end of October. We started with 120 min light reduction on August 1 (to simulate light levels on September 1) and finished with 10 min of light reduction on October 30. A total of 616 seedlings were assigned to each treatment.

All treatments received equal amounts of water stress by gradually lowering gravimetric block weights to 55 to 60 percent from late June through late September. Seedlings were lifted from containers on December 3, 2014, and cooler stored at 2±1°C (35±1°F).

Morphology Characterization

Forty-eight seedlings per treatment were destructively sampled after lifting. Shoots were cut at the point of insertion of the cotyledon and separated into shoots and roots. Root plugs were carefully washed to remove growing medium. Then all seedling fractions were gently washed with tap water, rinsed in deionized water, oven dried for 72 h at 60 °C (140° F), weighed, and ground. Shoot-to-root ratio was estimated by dividing shoot mass by root mass.

Cold Hardiness Assessment

Cold hardiness was determined on 48 seedlings per treatment in mid-April 2015. Five subsamples of 100 mg of leaves and five subsamples of 100 mg of roots from each seedling were placed into 20 ml (0.67 oz) vials and filled with 10 ml (0.34 oz) of deionized water. The five subsamples per organ corresponded to five test temperatures: 2 (control), -10, -20, -30 and -40 ºC (35.6, 14, -4, -22, and -40 ºF). The control treatment was placed into a refrigerator (2 ºC [35.6 ºF), and the four remaining vials per organ were placed into a programmable freezer (So-Low Environmental Equipment Co., Inc., Cincinnati, OH). Beginning with an initial temperature of 0 ºC (32 ºF), the temperature was decreased by 0.3 ºC min-1 (0.5 ºF min^{-1}). Each test temperature was maintained for a period of 30 min, after which time the vials designated for that test temperature were removed, and the temperature continued to decrease to the next temperature. Vials were thawed at 2 ºC (35.6 ºF) for approximately 24 h and then moved to ambient conditions to complete thawing. After thawing, electrolyte leakage of leaves and roots was measured with an HI 9813 portable conductivity meter (Hanna Instruments, Inc., Woonsocket, RI). Maximum conductivity was determined by placing vials in an autoclave (Getinge USA, Inc., Rochester, NY) at 120 ºC (248 ºF) for 20 min. Electrolyte leakage liberation (ELL) values were then calculated as a percentage of conductivity at each test temperature compared with that at maximum conductivity, and finally were expressed as the difference of ELL between control temperature and frost temperatures (ELLc).

Root Temperature Treatments

Seedling growth under different rhizosphere temperatures was assessed by growing seedlings in hydroponic tanks. On May 4, 2015, seedlings were removed from cold storage and the root plug was cleaned of substrate. Then, seedlings were transplanted into hydroponic tanks in a greenhouse with root zone temperatures of 5, 10, or 20 ºC (41, 50, or 68 °F), corresponding to low, normal, and optimum soil temperatures in the spring (figure 1). We used three to four tanks per temperature with eight seedlings per treatment in each tank. The number of new roots was recorded after 4 weeks. In addition, budbreak day of each seedling in the different tanks was recorded.

Field Trial

A garden plot was established at the Webster Nursery to evaluate field response following blackout treatments. Approximately 300 seedlings per treatment were planted on February 26, 2015, in a research block at the nursery. The soil is a Cagey loamy sand. In the first year of field growth, we recorded the budbreak date for each seedling. In addition, 40 seedlings per treatment were randomly chosen and excavated on May 11 and again on August 16 of the first year (second-year data will be obtained in 2016). Excavated seedlings were divided into new shoots, old shoots, old roots (plug roots), and new fine roots and were measured for dry mass.

Study Design and Data Analyses

The effect of blackout treatment on shoot and root mass at lifting was analyzed with one-way analysis of variance (ANOVA). For cold hardiness, temperature

Figure 1. Douglas-fir seedlings from different blackout treatments were grown in a hydroponic system for 28 days under controlled root zone temperatures. (Photo by Mercedes Uscola, 2015)

and blackout treatments were considered as independent variables in a two-way ANOVA for each organ separately. For budbreak date in the field trial and hydroponic culture, we ran an event history analysis. The analysis function used is the Cox mixed-effects proportional-hazards model with days to bud break as the dependent variable and blackout and temperature (in the hydroponic trial) as the independent variable(s). For the number of new roots in the hydroponic culture experiment, tank was considered as the random effect in a generalized linear model, and temperature and blackout treatments were the independent variables. In the field trial, shoot and root mass growth was obtained by subtracting average initial biomass at lifting from biomass of each seedling at the time of harvest. Then, mass growth was analyzed independently for each sampling date by a one-way ANOVA with blackout treatment as the independent variable. When significant factor effects were detected, Tukey's Honestly Significant Difference test was used to identify differences between treatment means at α = 0.05. Statistical analyses were conducted with R version 3.1.0 (Spring Dance, release 2014-04-10).

Preliminary Results and Discussion

Blackout Treatment Effects on Morphology

At lifting, the progressive and mild treatments were too mild to stop shoot mass growth compared with the control seedlings (P = 0.0065; figures 2 and 3). The other blackout treatments, however, effectively reduced shoot mass growth compared with control seedlings. It is interesting to note that differences in morphology also occurred below ground (P = 0.017). The mild treatment produced seedlings with the highest root mass, the control and progressive treatments resulted in the lowest root mass, and the long and moderate treatments had intermediate root mass.

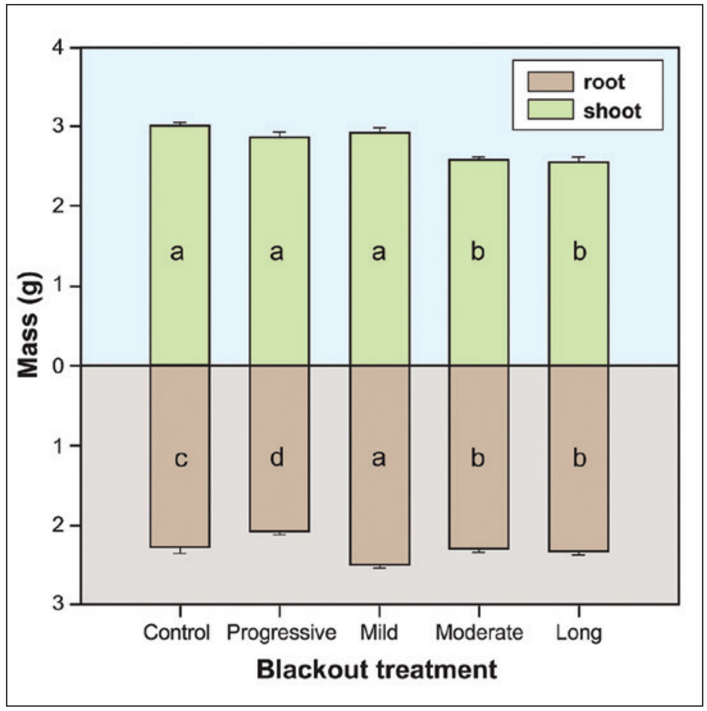

Figure 3. Shoot and root mass of Douglas-fir seedlings subjected to five different blackout treatments to induce bud set. Different letters denote significant differences in root or shoot mass at α = 0.05.

These differences in shoot and root mass promoted lower shoot-to-root ratios in blackout-treated seedlings, especially in mild and moderate seedlings (data not shown). A well-balanced shoot-to-root system, with a sturdy stem and a large fibrous root system, provides the best chance for seedling survival, especially in droughty sites (Grossnickle 2012).

Cold Hardiness

Jacobs et al. (2008) found that greater cold hardiness of blackout-treated seedlings compared with seedlings under normal day-length conditions was maintained throughout the spring deacclimation period. They suggest increased fall cold hardiness associated with blackout treatment may be maintained under

Figure 2. Douglas-fir seedlings subjected to five different blackout treatments varied in morphology at the time of lifting. (Photo by Nabil Khadduri, 2014)

freezer-storage conditions through spring dormancy release. In this study, however, we found similar cold resistance in late spring for both blackout-treated seedlings and control seedlings at all temperatures for both leaves and roots (figure 4).

Root Temperature Effects

Rhizosphere temperature ($P < 0.001$) had an effect on bud break, which was independent of blackout treatment (interaction $P = 0.67$). By the end of the experiment (28 days), 90.6 percent of seedlings in the 20 ºC (68 ºF) treatment broke bud, but only 60.3 and 30.5 percent of seedlings exhibited bud break in the 10 and 5 ºC (50 and 41 °F) treatments, respectively. Higher rhizosphere temperature also significantly increased the number of new roots, with maximum growth at 20 ºC (68 ºF), independent of the blackout treatment (figure 5a). Limitations to new growth at low temperatures can be explained, at least in part, because of inhibitions to root hydraulic conductivity and metabolic activity (Bowen 1991).

The number of new root tips was greatest in progressive and control seedlings (figure 5b). By contrast, Jacobs et al. (2008) showed that blackout treatment increased Douglas-fir root growth compared

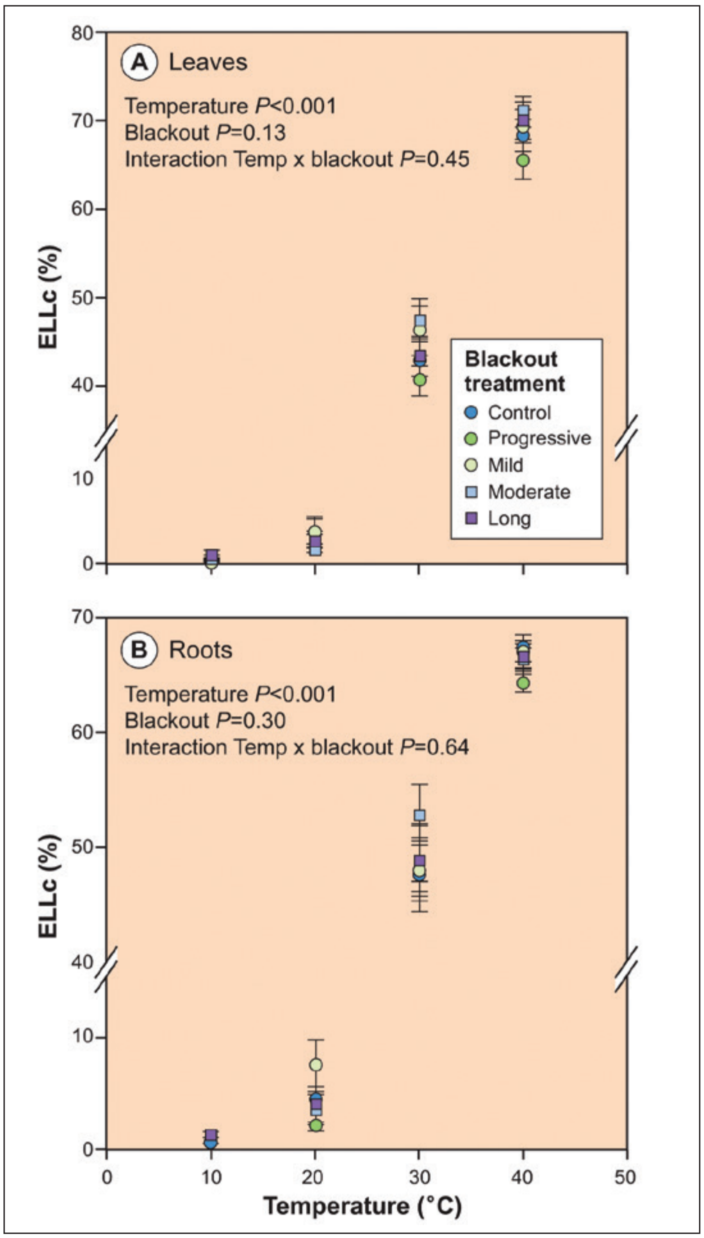

Figure 4. Electrolyte leakage liberation (ELL) following freezing of (a) leaves and (b) roots of Douglas-fir seedlings subjected to five different blackout treatments. Values at each temperature were corrected by subtracting the ELL at control temperature (2°C [35.6 ºF]).

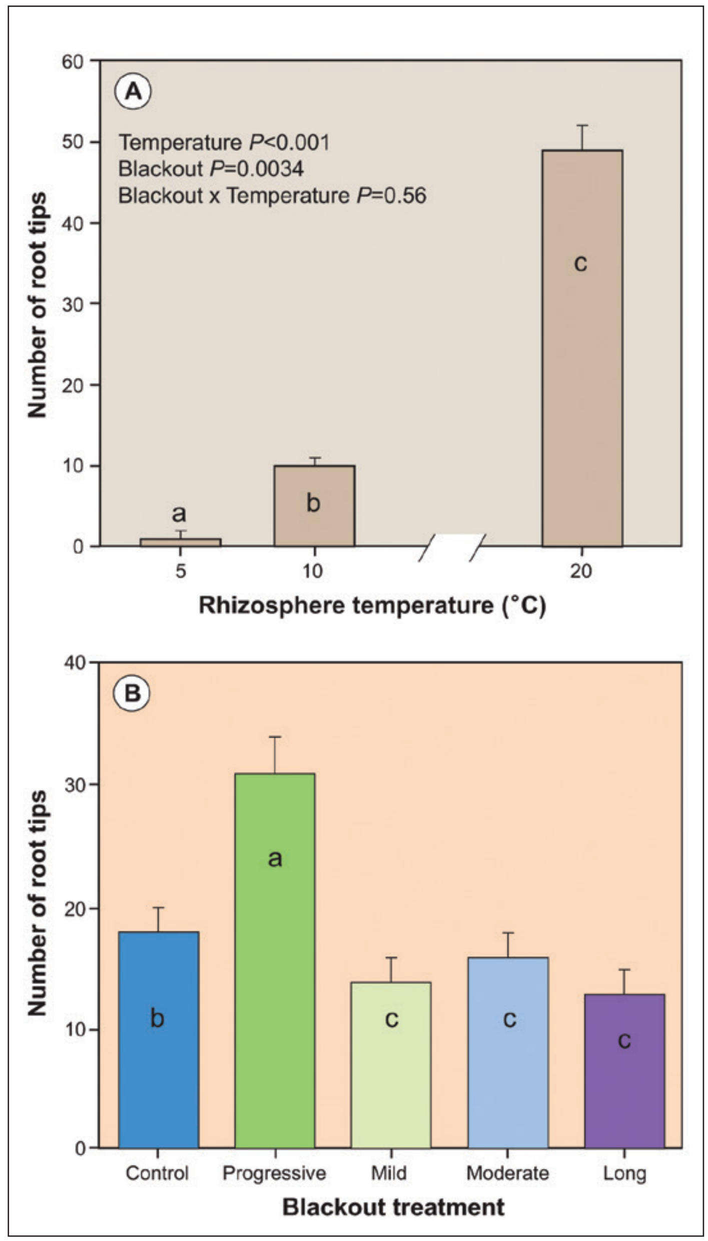

Figure 5. Effect of (a) rhizosphere temperature and (b) blackout treatment on the number of new root tips of Douglas-fir seedlings after 28 days in hydroponic tanks.

with controls at low soil temperatures. In this experiment, root growth of blackout-treated seedlings was less than controls, especially in the most intensely blackout-treated seedlings. This finding could indicate that the most intense blackout treatments reached a limit in which negative effects appeared, perhaps due to reduction of carbohydrates (Carpenter et al. 1983). Blackout treatment ($P < 0.001$) also had an effect on bud break in the hydroponic experiment. By the end of the experiment (28 days), mild-, moderate-, and long-treated seedlings had 62 to 67 percent bud break, whereas the control and progressive-treated seedlings had only 50 percent bud break.

Field Performance and Phenology

The effect of blackout treatment on bud break occurred in a similar manner under field conditions, with the control and progressive-treated seedlings breaking bud later than the other treatments ($P < 0.001$; figure 6). The number of days needed for 50 percent of the seedlings to break bud was less than 20 days in the mild, moderate, and long blackout-treated seedlings, but the control and progressive-treated seedlings needed 27 and 30 days, respectively. Reduction in photoperiod has been shown to decrease days to bud break in several temperate conifer species (Brigas and D´Aoust 1993, Hawkins et al. 1996). In Douglas-fir, photoperiod effect on bud break can be strongly dependent on provenance (Campbell and Sugano 1975). Spring phenology of temperate forest trees is optimized to maximize the length of the growing season while minimizing the risk of freezing damage. Earlier bud break can be advantageous for seedlings to be more competitive with vegetation, but it could also increase the risk of frost damage in early spring. In early spring, shoot and root biomass of excavated seedlings differed significantly among treatments ($P < 0.001$ for both roots and shoots; figure 7a). In general, control and progressive seedlings had lower biomass growth than seedlings from the mild, moderate, and long blackout-treated seedlings. These differences may be explained, in part, by bud-break differences. By the end of the first growing season, however, dry mass of shoots and roots did not differ among treatments ($P = 0.71$ and $P = 0.70$ for roots and

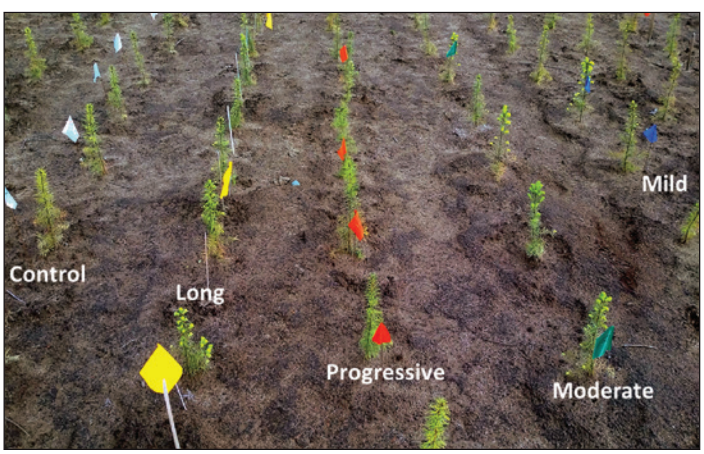

Figure 6. Spring bud break varied among Douglas-fir seedlings subjected to different blackout treatments. Note that the progressive-treated and control seedlings have later bud break. (Photo by Nabil Khadduri, 2015)

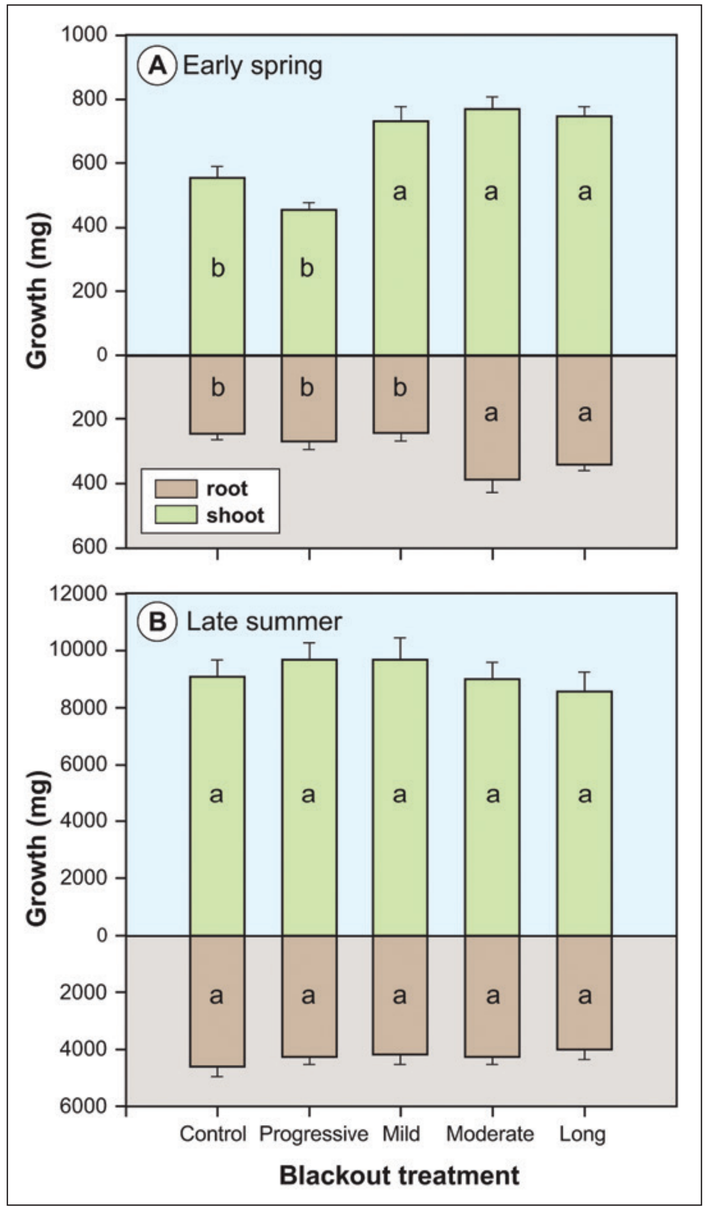

Figure 7. Shoot and root growth (a) in early spring and (b) at the end of the first growing season in the field for Douglas-fir seedlings previously subjected to five different blackout treatments.

shoots, respectively; figure 7b). Nonetheless, due to initial mass differences among treatments, total mass differences persisted after 1 year of growth in the field (data not shown).

Future Directions

Whether the observed effects of blackout will persist under field conditions after a second year is unclear. Thus, we are continuing to evaluate field performance over a second growing season. Interactions between blackout treatments and latitude of seed lot origin may also affect dormancy development and cold hardiness (Coursolle et al. 1997). To address these factors, we are also conducting a new trial looking at the effect of blackout on seed sources from southern Oregon, Washington, and British Columbia. Results will be published in a forestry journal in the next couple of years.

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REFERENCES

Bowen, G.D. 1991. Soil temperature, root growth and plant function. In: Waisel, Y.; Dekker, M., eds. Plant roots: the hidden half. New York: Science: 309–330.

Brigas, F.J.; D´Aoust, A.L. 1993. Influence of photoperiod on shoot and root frost tolerance and bud phenology of white spruce seedlings (*Picea glauca*). Canadian Journal of Forest Research. 23(2): 219–228.

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.

Carpenter, A.S.B.; Immel, M.J.; Smith, N.D. 1983. Effect of photoperiod on the growth and photosynthetic capacity of Paulownia seedlings. Castanea. 48(1): 13–18.

Coursolle, C.; Bigras, F.J.; Margolis, H.A.; Hébert, C. 1997. Dehardening and second-year growth of white spruce provenances in response to duration of long-night treatments. Canadian Journal of Forest Research. 27(8): 1168–1175.

Greer, D.H.; Stanley, C.J.; Warrington, I.J. 1989. Photoperiod control of the initial phase of frost hardiness development in *Pinus radiata*. Plant Cell and Environment. 12(6): 661–668.

Grossnickle, S.C. 2005. Importance of root growth in overcoming planting stress. New Forest. 30(2): 273–294.

Grossnickle, S.C. 2012. Why seedlings survive: influence of plant attributes. New Forest. 43(5): 711–738.

Grossnickle, S.C.; South, D.B. 2014. Fall acclimation and the lift/store pathway: effect on reforestation. The Open Forest Science Journal. 7: 1–20.

Hawkins, C.; Eastham, A.; Story, T.; Eng, R.Y.N.; Draper, D.A. 1996. The effect of nursery blackout application on Sitka spruce seedlings. Canadian Journal of Forest Research. 26(12): 2201–2213.

Jacobs, D.F.; Davis, A.S.; Wilson, B.C.; Dumroese, R.K.; Goodman, R.C.; Salifu, K.F. 2008. Short-day treatment alters Douglas-fir seedling dehardening and transplant root proliferation at varying rhizosphere temperatures. Canadian Journal of Forest Research. 38(6): 1526–1535.

Macdonald, J.E.; Owens, J.N. 2006. Morphology, physiology, survival, and field performance of containerized coastal Douglas fir seedlings given different dormancy-induction regimes. HortScience. 41(6): 1416–1420.

Timmis, R. 1974. Effect of nutrient stress on growth, bud set, and hardiness in Douglas-fir seedlings. In: Tinus, R.W.; Stein, W.I.; Balmer, W.E., eds. Proceedings, North American containerized forest tree seedling symposium. Pub. No. 68. Denver: Great Plains Agricultural Council: 187–193.

Turner, J.; Mitchell, S.J. 2003. The effect of short day treatments on containerized Douglas-fir morphology, physiology and phenology. New Forest. 26(3): 279–295.

Villar-Salvador, P.; Uscola, M.; Jacobs, D.F. 2015. The role of stored carbohydrates and nitrogen in the growth and stress tolerance of planted forest trees. New Forest. 46(5): 813–839.