Final Project Report – February, 2006

Title: Interrelationship of seedling dormancy status and root zone temperature in determining new root growth capacity of northern California conifer species

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Executive summary

Changes in seedling cold hardiness between January and April 2005 were assessed using the electrolyte leakage method and by determining the LT50 (lethal temperature for 50% of plant material). Seedlings were most cold-hardy in January and least cold hardy in April. Douglas-fir seedlings that received blackout treatments during nursery culture were hardier than those that did not, as reflected by the LT50 tests. Blackout treatments appear useful for increasing cold hardiness, and may improve seedling vigor under given environmental scenarios. Root zone temperature influenced the number and mass of new roots. Results are summarized in the following report, with a more in-depth examination to be published as a scientific paper. Logical future research directions, including initiation of an ongoing follow-up study, are also outlined.

Introduction

Soil temperature is known to control development of new roots in forest tree seedlings. Foresters have become increasingly interested in planting seedlings beyond the traditional spring-planting window, partly because planting seedlings in fall may theoretically allow for two seasons of root growth prior to first shoot elongation in spring. However, low soil temperatures following planting may restrict root development and limit water uptake, which may result in a condition of physiological drought leading to transplant stress and limiting planting success.

Considering the importance of soil temperature in dictating new root growth and implications that this relationship may have on regeneration success, relatively little modern research has been conducted on the subject. Ritchie (1985) suggested that soil temperature near 20ºC promotes ideal root growth of western conifer species, while temperatures below about 5ºC allow for little new root growth. Lopuschinsky and Max (1990) reported that root initiation of Douglas-fir, pacific silver fir, noble fir, lodgepole pine, and ponderosa pine occurred at 5ºC, maximum root growth was attained at 20ºC, and root growth generally ceased at 30ºC, though variation was present among species and seed sources. More information is needed specific to species and seed sources of northern California to identify optimal soil temperatures to promote new root growth for species planted in this region.

Soil temperature is not the only factor influencing new root growth, and most likely a complex interrelationship exists between this variable and seedling dormancy status. Root growth capacity appears to be strongly related to the bud dormancy cycle (Ritchie and Dunlap 1980)

(Figure 1) and date of lifting and time of storage may have a substantial impact on subsequent root growth capacity of seedlings (Ritchie 1985). Changes in seedling dormancy status may be monitored using the electrolyte leakage procedure (Tinus 1996) in which cell damage associated with freezing stress may be quantified. Examining how soil temperature and seedling dormancy status interact to influence potential for new root growth is a relatively unexplored area of research that will help nursery managers and foresters to better time lifting and planting operations to promote new root growth, limit transplant shock, and maximize regeneration success.

Objectives

This study was designed to examine how potential for new seedling root growth (i.e., extension of current roots and formation of new roots) is affected by both media temperature and changes in seedling phenology as seedlings transition through the dormancy cycle. Though it has been established that root growth of many conifer species is maximized around 20°C, few studies have examined how media temperature may interact with changes in seedling dormancy status during the period from dormancy induction in fall through dormancy release in spring. Additionally, no studies have examined either of these trends specific to seed sources in northern California. This information will be useful to help match seedling physiological status with site environmental conditions to optimize new root growth immediately following planting.

Materials and Methods

Three species were included in this experiment, each representing an important component of forest tree seedling production in northern California. The species used were Douglas-fir, consisting of a single seed source (1676 m elevation) that either did or did not receive blackout in the nursery, ponderosa pine from two seed sources (elevations of 1067 and 1524 m), and a single seed source of California red fir (1676 m elevation). Each combination of species and source or blackout will be herein referred to as seed source. Douglas-fir and ponderosa pine from the 1067 m elevation were grown in Styro-8 containers and California red fir and the ponderosa pine from the 1524 m elevation were grown in Styro-15 containers.

The experimental system was established in a controlled environment growth room with 16-hour photoperiods, a constant air temperature of 23.3°C, and light intensity of 120 μmol/m^2/sec. The study was established as a randomized complete block design with three blocks serving as replications. Within each block, four growing tanks (45.7 cm × 45.7 cm × 30.5 cm, L × W × H), with each tank corresponding to a controlled root zone temperature (10, 15, 20, and 25°C) were installed. Each tank held 10 seedlings per species source. Water in each tank is pumped through a thermoelectric chiller/heater with set points at each of the four temperatures. Pumping of water from the tank and through the chiller/heater is achieved through use of small magnetic drive centrifugal pumps connected to polyethylene tubing. Measurements conducted identified that water temperature throughout the initial four-week period was within ± 2ºC of each set point. Supplemental nutrition was not added to the water in an effort to avoid experimental confounding.

Seedling shipments were received from Cal Forest Nursery (Etna, CA) on 7 January 2005, 7 February 2005, 25 February, 23 March 2005, and 19 April 2005. On each date, seedlings of each seed source (5 treatments, 600 seedlings total) were measured for initial root volume, height, and root collar diameter. A subsample of seedlings was evaluated for numbers of new white roots. After initial measurements, seedlings were placed into a hydroponic growing system (Figure 1) to evaluate growth potential over a four-week period.

For each date, dormancy status and cold hardiness of each seed source/treatment were determined by the electrolyte leakage method. Thirty-six seedlings from each seed source, not used for the growth trial, were used for this procedure. For each treatment, approximately five needles were removed from each seedling and cut into segments 1 cm in length. These were divided into four equal groups. Segments from each group were placed in nine vials containing de-ionized water, with ten segments per vial. The nine vials corresponded to nine test temperatures [2 (control), -3, -7, -11, -15, -19, -24, -29, -34, and -40 °C]. Measurements of conductivity in each vial were made after freezing and autoclaving, allowing calculation of an index of injury at each temperature. LT50 (temperature at which mortality of 50% of plant material occurs) was also assessed.

Data were analyzed using analysis of variance (ANOVA) for a randomized complete block design to identify differences between treatments for new root growth and mass of new root growth, stem dry weight, and root dry weight. A significance level of $\alpha = 0.05$ was selected for analysis of treatment differences using Tukey's HSD. Results for treatment effects on seedling height growth, root-collar diameter growth, and root volume are not presented.

Findings

For this report, data are presented in a summative form. In the forthcoming scientific publication associated with this work, data will be presented in a manner that delves further into the results. For that reason, photosynthetic rate, changes in root volume, stem height, and root-collar diameter are not presented at this time.

Electrolyte leakage

Investigation of electrolyte leakage data clearly demonstrated an increase in cold hardiness with the use of blackout of Douglas-fir seedlings (Figures 2 and 3). Seasonal decreases in cold hardiness are mimicked by increasing electrolyte leakage values across the sampling dates. Seedlings of all species tended to have lower electrolyte leakage values early in the sampling period. Elevation did not appear to be a significant factor in determining cold hardiness of ponderosa pine seedlings (Figures 2 and 3). California red fir had lower electrolyte leakage values than other species at the first sampling date; however, by the end of the sampling period that trend dissipated.

LT50

Further quantification on the effect of cold hardiness on seedling development can be identified using a test of LT50 (Table 1). For ponderosa pine, there is little difference in cold hardiness depending on date. An examination of the LT50 data indicates that ponderosa pine was most cold hardy at the first sampling date on 7 January 2005, and decreased in ability to tolerate extreme temperatures through to the final sampling date (19 April 2005). Elevation also did not result in a difference in ponderosa pine LT50. California red fir behaved in a similar manner as ponderosa pine, with greatest cold hardiness occurring at the earliest sampling date and a progression to highest sensitivity at the last sampling date.

Douglas-fir seedlings were less sensitive to cold when seedlings were treated with blackout than those that were not. For seedlings not subjected to blackout during the growing season, the most cold hardy seedlings were sampled on 7 February 2005, with little difference between those seedlings and the 7 January and 25 February 2005 samples. By comparison, the blacked-out seedlings were able to tolerate far colder temperatures than the non-blacked-out seedlings, and also exhibited peak cold hardiness at the 7 January 2005 sampling date. This trend persisted to the 23 March 2005 sample, but by the 15 April sample both blacked-out and non-blacked-out Douglas-fir seedlings had approximately the same LT50.

New Root Development

Root zone temperature had a significant effect on root growth of Douglas-fir seedlings. The number of new roots ($p<0.0001$, Figure 4), mass of new roots ($p<0.0001$, Figure 5), and total root dry weight ($p=0.0039$) were all significantly influenced by root zone temperature. Sampling date was non-significant. Total seedling dry weight ($p<0.0001$) was significantly greater for seedlings that had not received a blackout treatment during the growing season. There was a significant interaction between blackout treatment and root zone temperature and the number of new roots ($p=0.0038$) and mass of new roots ($p=0.0461$).

As seen in Figure 4, more new roots were initiated at 15 and 20°C than at 10°C, with no difference between 25°C and other treatments although the mean value declined. New root mass followed a similar trend, though the mean value for 25°C was significantly less than that of 20°C (Figure 5). These trends indicate that regardless of blackout treatment, seedling root zone temperature is optimal for new root growth above 15°C and below 20°C.

In addition to the aforementioned findings, there were several significant interactions that need to be further investigated. Interactions between date of sampling and root zone temperature influence on the number of new roots ($p=0.012$) and new root mass ($p=0.0311$), as well as the date of sampling and whether seedlings received blackout treatment on new root mass ($p=0.0446$, Figure 6) were present. Significant interactions also existed between blackout treatment and root zone temperature and total stem dry weight ($p=0.0389$) and total root dry weight ($p=0.0249$).

As was the case with Douglas-fir, seedlings from the low-elevation ponderosa pine seed source were significantly influenced by root zone temperature. Seedlings developed more new roots ($p<0.0001$) at the 15, 20, and 25°C root zone temperatures than at 10°C. Seedlings also had greater total root mass at higher root zone temperatures than at 10°C (Table 2).

Seedlings from the high elevation ponderosa pine seed source behaved in a similar manner, with root zone temperature significantly influencing the number of new roots (p<0.0001) and new root mass (p<0.0001). Sampling date also significantly influenced the number of new roots (p=0.0353) and new root mass (p=0.0139), with seedlings removed from cold storage early growing more new roots and greater new root mass than those seedling removed from cold storage at later dates. Further explanation of this phenomenon could be found from the significant interaction between root zone temperature and sampling date (new roots, p=0.0082; new root mass, p=0.0030), which showed that seedlings removed from cold storage at the first sampling date produced more new roots at the 25°C root zone temperature than at any other temperature or sampling date combination. This may reflect an attempt by the seedlings to rapidly proliferate new roots once certain environmental conditions are met. For high elevation ponderosa pine seedlings, total stem and root dry weight were not influenced by root zone temperature.

California red fir seedling root development was also significantly influenced by root zone temperature. The number of new roots (p=0.0029) was highest at 20°C and lowest at 10 and 25°C. New root mass did not differ significantly across root zone temperatures (p = 0.0707); however, trends were similar to those of the number of new roots (Table 3). Total stem and root mass were not influenced by root zone temperature.

Conclusions and future directions

Nursery blackout plays a significant role in Douglas-fir cold hardiness and tolerance of lower root zone temperatures. California red fir and Douglas-fir that did not receive a blackout treatment showed a sharp seasonal decrease in cold hardiness. The effect of this was mitigated with blackout treatment. There was little difference in EL data and LT50 for ponderosa pine seedlings of different elevations.

Given the apparent significance of blackout in affecting Douglas-fir seedling performance identified in this study, future studies should be designed to more closely examine the influence of blackout timing and duration on Douglas-fir seedling physiology and transplant performance. In cooperation with Cal Forest Nursery, a subsequent study is presently underway to examine physiological changes of Douglas-fir seedlings throughout the growing season for four different seed zones in northern California. In 2005, seedlings were subjected to two different three-week blackout treatments (early or late) and a control (no blackout). These results will be forthcoming and should provide a supplement to the data from this study report. Research is also needed to help identify trends in root growth at different temperatures throughout the growing season, as Iivonen et al. (2001), for example, found that Scots pine seedlings response to different root zone temperatures was dynamic across the growing season.

The results of the current study will be analyzed further and presented as a scientific paper. This more in-depth examination of the data, coupled with knowledge gathered from our ongoing study, will help improve our basic and applied understanding of the effect of blackout on seedling performance as well as changes in seedling development across root-zone temperatures.

Literature Cited

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Table 1. LT50 data for each species and each harvest date.

	Douglas-fir		Ponderosa pine		California red fir
Sampling date	No blackout	Blackout	Low elevation	High elevation	
7 January 2005	-24.43	-46.50	-27.75	-26.04	-41.70
7 February 2005	-26.76	-39.04	-24.96	-23.65	-38.28
25 February 2005	-22.97	-37.32	-19.86	-20.60	-29.53
23 March 2005	-16.99	-28.82	-14.58	-15.78	-26.03
19 April 2005	-16.93	-17.91	-16.93	-16.93	-21.12

Table 2. Number and mass of new roots of ponderosa pine seedlings grown from low and high elevation seed sources. Numbers represent means, letters represent significant within-column differences using Tukey's HSD with α=0.05.

	New roots (#)		New root mass (mg)	
Temperature	Low elevation	High elevation	Low elevation	High elevation
10°C	6.00 A	3.96 a	4.5 A	2.79 a
15°C	20.21 B	13.83 b	16.29 B	10.17 a
20°C	22.63 B	28.38 c	20.86 B	30.33 b
25°C	25.75 B	31.42 c	25.54 B	38.08 b

Table 3. Number and mass of new roots of California red fir seedlings. Numbers represent means, letters represent significant within-column differences using Tukey's HSD with α=0.05.

Temperature	New roots (#)	New root mass (mg)
10°C	2.33 A	0.83 a
15°C	7.21 AB	3.86 a
20°C	7.71 B	3.50 a
25°C	2.33 A	1.17 a

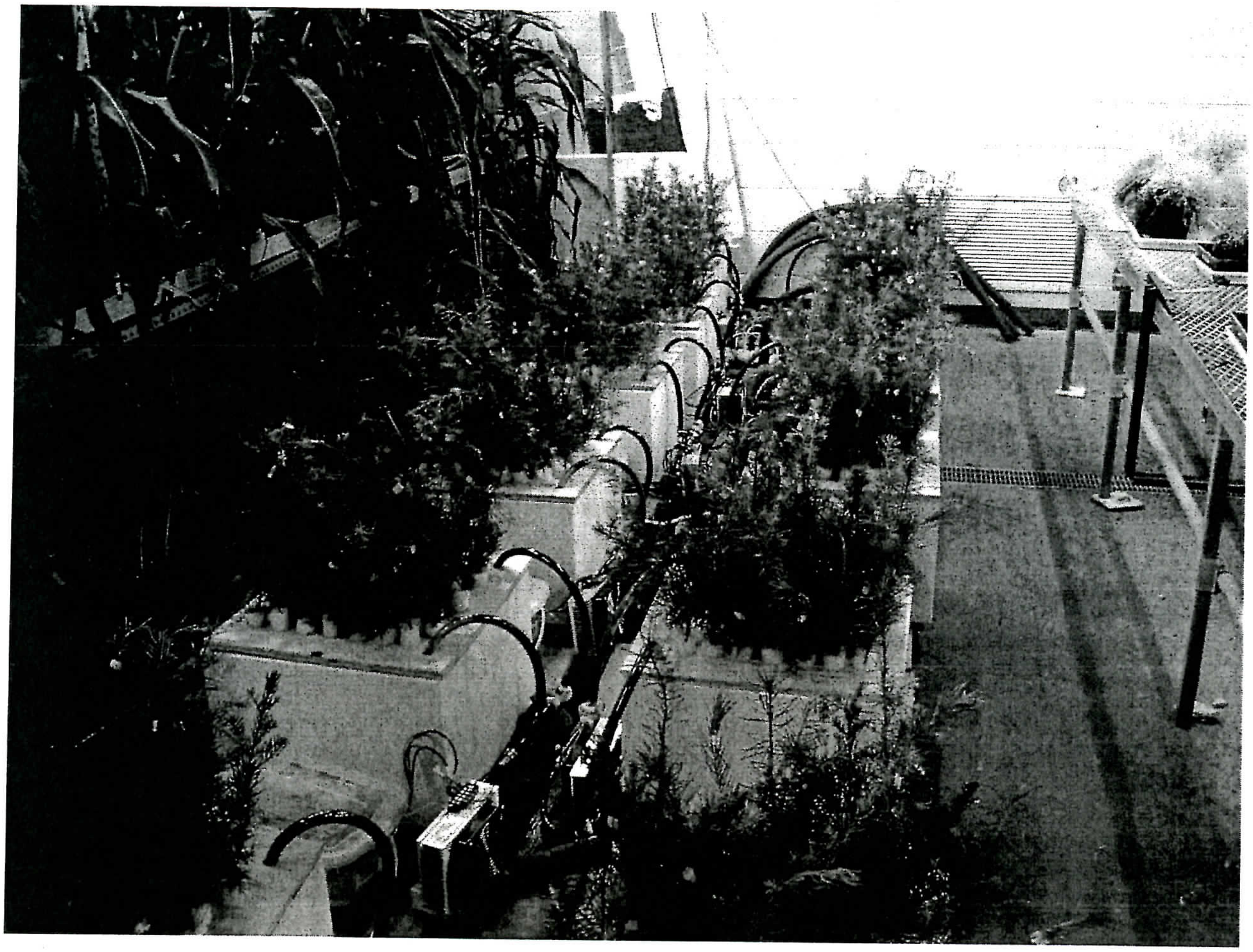

Figure 1. Experimental setup of conifer seedlings. Water in each tank is held at 10, 15, 20, or 25°C, and each treatment is replicated three times.

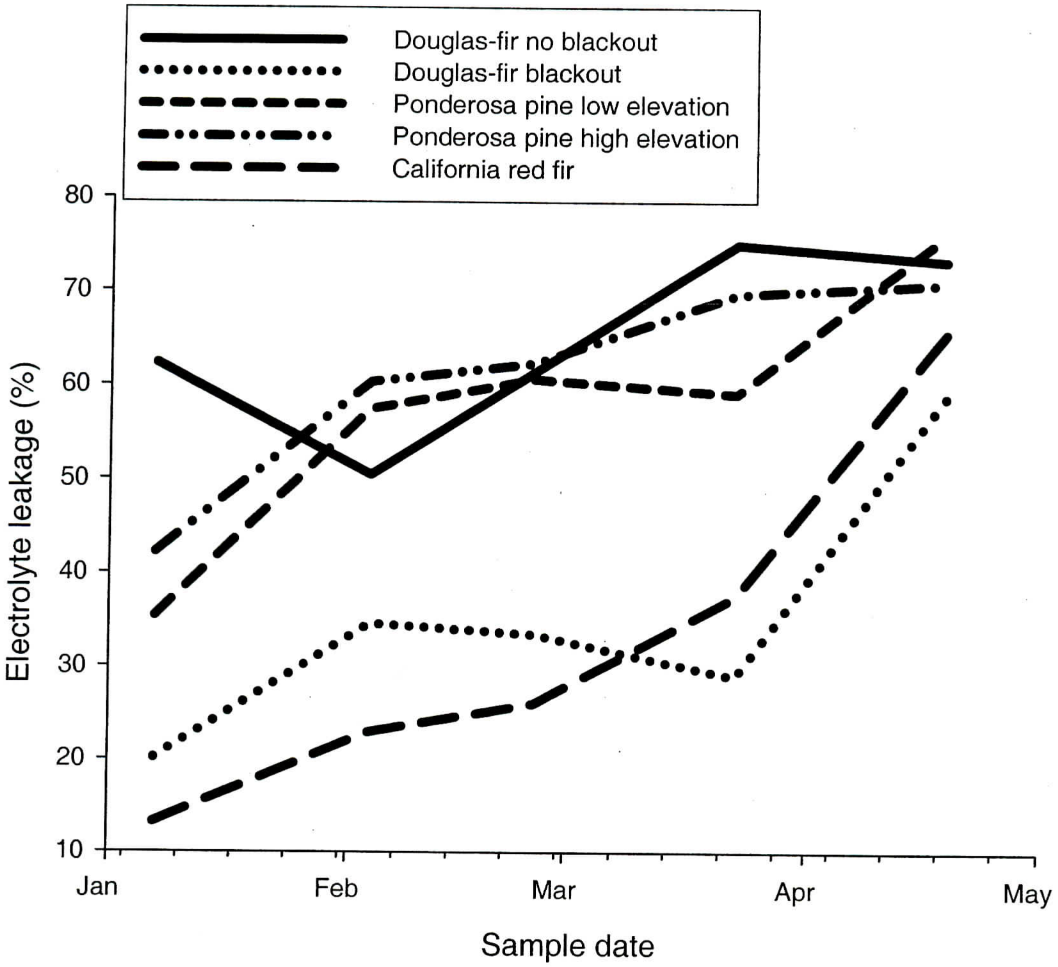

Figure 2. Variation in electrolyte leakage at -28°C by species/seed source across sampling dates 7 January 2005, 7 February 2005, 25 February, 23 March 2005, and 19 April 2005.

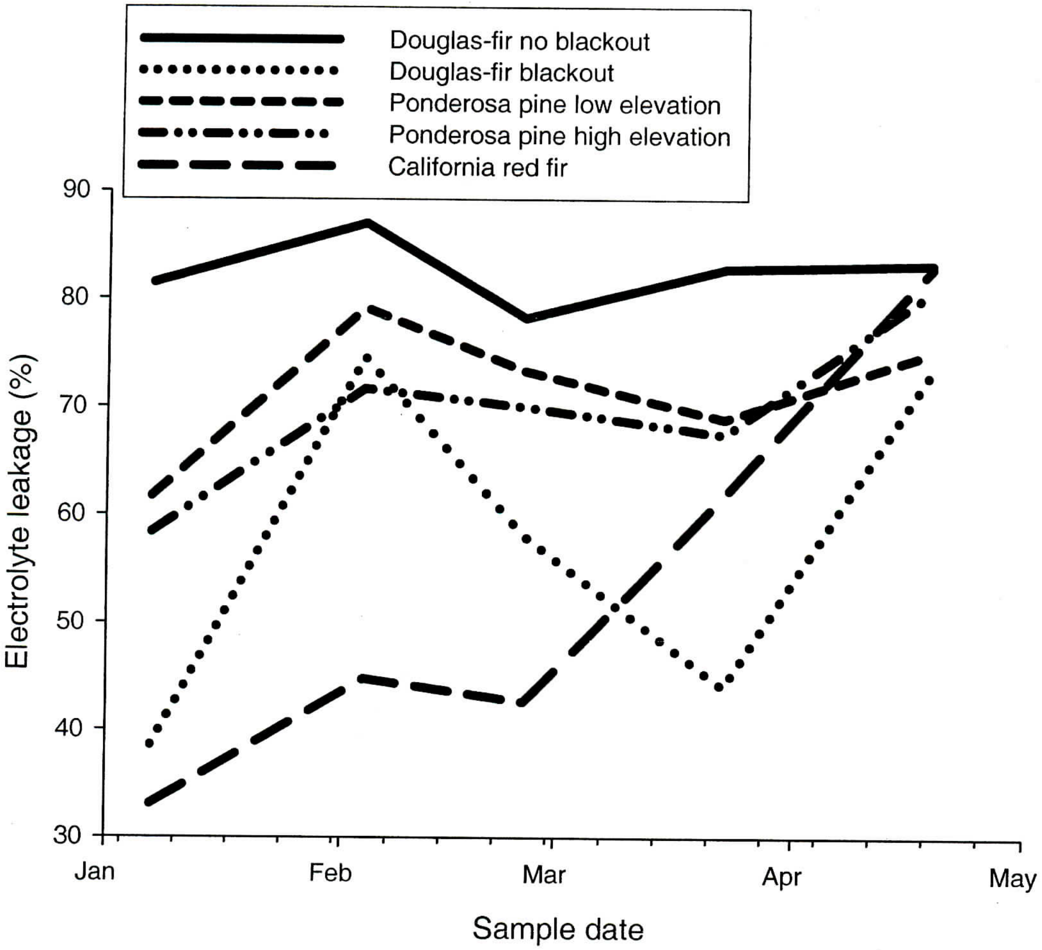

Figure 3. Variation in electrolyte leakage at -40°C by species/seed source across sampling dates 7 January 2005, 7 February 2005, 25 February, 23 March 2005, and 19 April 2005.

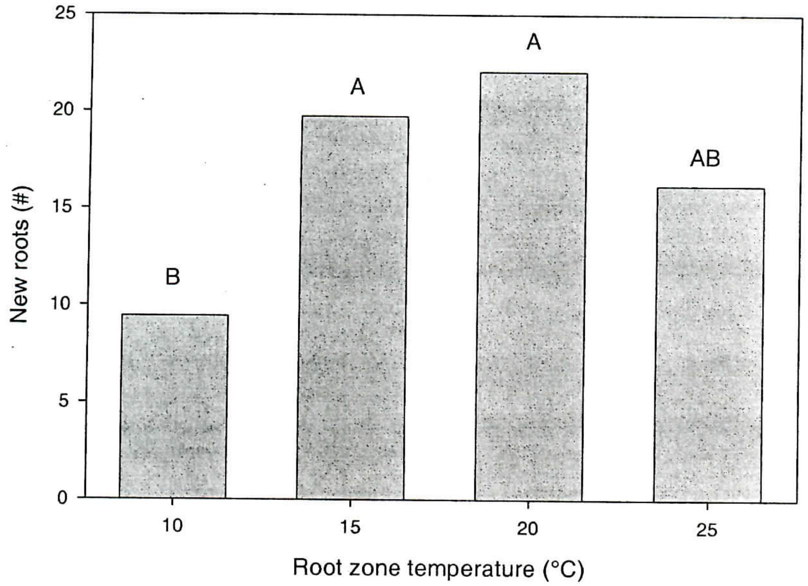

Figure 4. Number of new roots of Douglas-fir seedlings grown at different root zone temperatures. Bars represent means and letters represent significant differences within each graph using Tukey's HSD at $\alpha=0.05$.

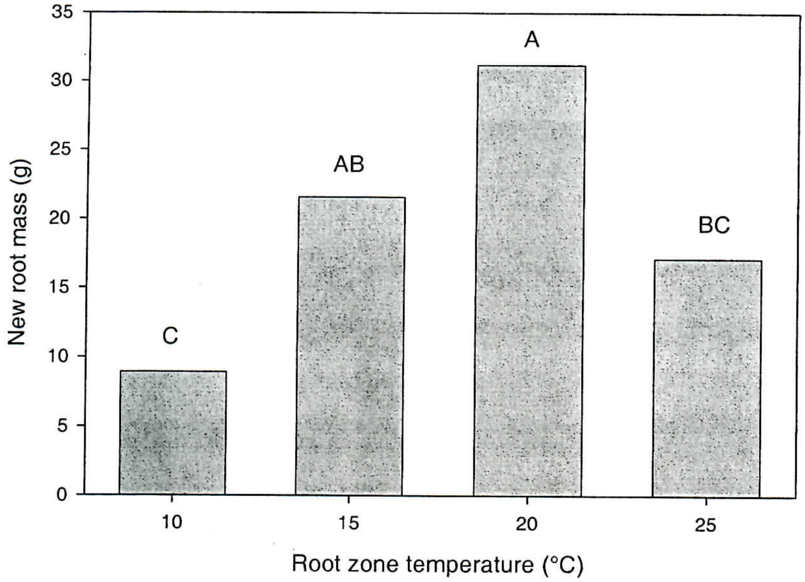

Figure 5. New root mass of Douglas-fir seedlings grown at different root zone temperatures. Bars represent means and letters represent significant differences within each graph using Tukey's HSD at α=0.05.

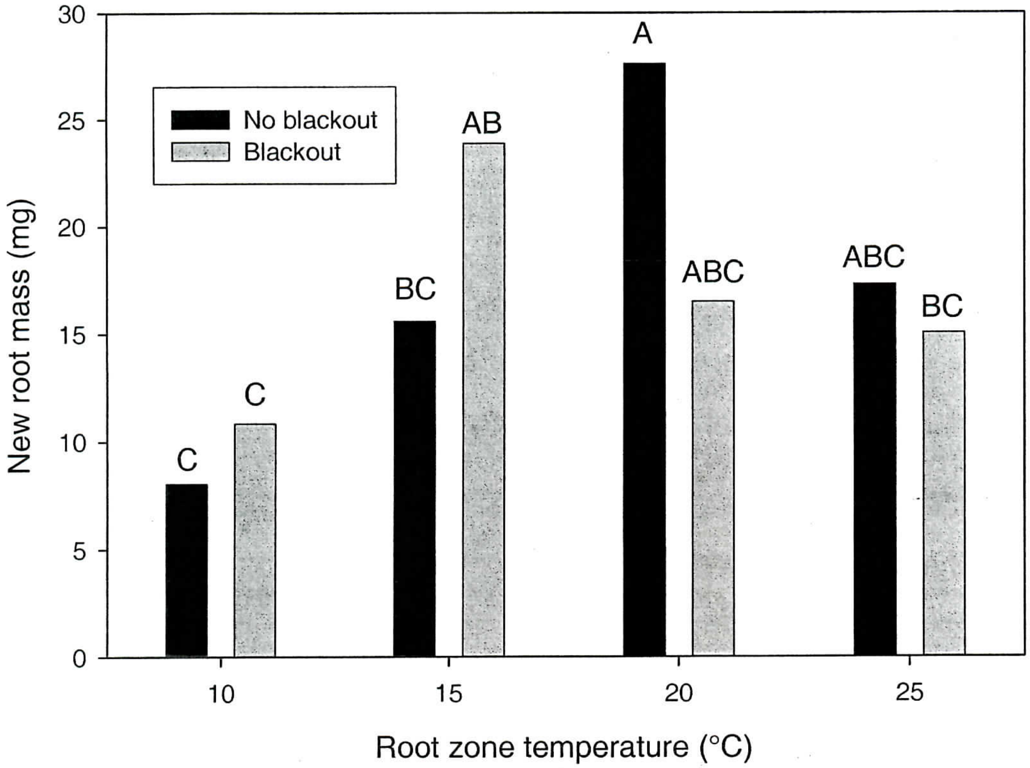

Figure 6. Investigation into the effect of interaction between blackout treatment and root zone temperature on new root mass of Douglas-fir seedlings. Bars represent means and letters represent significant differences within each graph using Tukey's HSD at α=0.05.