

The Effect of Interim Cold Storage on Root Growth Potential of Hot-Lifted Western Redcedar and Coastal Douglas-Fir Seedlings

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Abstract

Seedlings planted in the summer and early fall are still physiologically active and thus require special handling requirements to maintain quality. The effect of cold storage duration on potential seedling establishment for hot-lifted seedlings in western Canada and the Pacific Northwest is unclear. This study tested the effect of interim storage duration on hot-lifted, fall planted western redcedar (*Thuja plicata* Donn ex D. Don) and coastal Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *menziesii*) seedling quality. Root growth potential (RGP) and cold hardiness (via chlorophyll fluorescence) were used to evaluate seedling quality. Results showed that coastal Douglas-fir and western redcedar seedling quality were not compromised during 2 weeks of interim cold storage in closed boxes at 4 °C (39 °F). After 3 or 4 weeks of storage, RGP declined for both species, although the coastal Douglas-fir would still have been acceptable to plant according to current British Columbia Ministry of Forests criteria. This paper was presented at the Joint Annual Meeting of the Western Forest and Conservation Nursery Association and the Forest Nursery Association of British Columbia (Portland, OR, September 19–21, 2023).

Introduction

Approximately 80 percent of container forest seedlings in western Canada and the Pacific Northwest are harvested and packaged when dormant in the late fall and early winter and stored frozen for up to 6 months until planting the following spring. The other 20 percent of container seedlings are harvested in the summer or fall and outplanted shortly thereafter. As foresters look for strategies to cope with changing climates and labor

shortages, alternative planting dates may become more common. Compared with seedlings destined for frozen storage, seedlings harvested for immediate planting are still physiologically active and are thus referred to as “hot lifted” and “hot planted.” To maintain the quality of hot-lifted seedlings, specific requirements regarding temperature during handling must be met because the seedlings generate heat via maintenance respiration. In response to these requirements, restrictive stock handling stipulations have been put in place to ensure seedling quality is not compromised. However, the underlying scientific basis for some of these guidelines is unclear.

The general handling guidelines to care for seedlings during hot planting is to keep the seedlings cool and plant them as soon as possible (Dunsworth 1997, Kiiskila 1999, Landis et al. 2010, Paterson et al. 2001, Stjernberg 1997). A 10 °C (18 °F) increase in temperature approximately doubles seedling respiration rate (Kramer and Kozlowski 1979). Thus, the temperature inside a closed box of seedlings can quickly increase (Binder and Fielder 1995), resulting in a loss of stored carbohydrates and vigor (Landis et al. 2010). Therefore, the recommendation is to keep hot-lifted seedlings refrigerated at 2 to 10 °C (35 to 50 °F) once packaged, with 2 °C (36 °F) being the ideal interim storage temperature (Grossnickle et al. 2020). Since most commercial refrigeration units on trailers and cold storage units can fluctuate up and down by 2 °C (4 °F), the lowest temperature setting used to prevent inadvertent freezing of hot-lifted stock is usually 4 °C (39 °F).

Seedlings picked up from the nursery in the early morning or evening and planted shortly thereafter are

often transported without refrigeration and stored onsite in a field cache under shade. To compensate for the lack of refrigeration, hot-lifted seedlings are typically packaged upright without bags in waxed seedling boxes so that the boxes can be opened to allow for heat dissipation and irrigation (Kiiskila 1999, Landis et al. 2010). At remote planting sites, it may be a week from the time seedlings are packaged at the nursery until they are planted. Under these conditions, the use of refrigerated storage at the nursery, during transportation, and in the field cache is important to maintain seedling quality. Because temperature interacts with the length of time from nursery harvest to planting, some reforestation contracts have time stipulations specifying that the seedling boxes be opened within 5 days of being closed at the nursery (Anonymous 2021).

Root growth after planting is critical for seedling survival and establishment (Grossnickle 2005, Grossnickle and Ivetić 2022). Thus, the seedling's ability to grow roots under optimum conditions is commonly assessed (i.e., root growth potential [RGP]) prior to planting (Haase 2008, Nelson 2019). RGP tests are usually not performed, however, on hot-lifted seedlings as is done for dormant frozen- or cold-stored seedlings prior to planting (Moeller 2022). Nonetheless, root growth after planting is still considered very important to the successful establishment of hot-planted seedlings (Grossnickle and MacDonald 2021). Sufficient cold hardiness of the shoots to withstand potential low temperatures is another trait required for successful hot planting in the fall (Grossnickle and MacDonald 2021). In British Columbia (BC), cold hardiness is routinely measured each fall via chlorophyll fluorescence to determine when seedlings are ready to be harvested for frozen storage (Moeller 2018). Measurement of the optimal quantum yield (i.e., maximum fluorescence/variable fluorescence from photosystem II) provides a direct estimate of the overall photosynthetic efficiency (Mohammed et al. 1995) and thus can be used to detect cold damage to the photosynthetic system (Ritchie 2005, Rose and Haase 2002).

While there are various recommendations and contract stipulations as to interim storage temperature and duration for hot-lifted seedlings in western Canada and the Pacific Northwest, the effect of storage duration under ideal temperatures on potential seedling establishment success is not known. In Finland,

even a few days in dark, closed boxes reduced root growth and cold hardiness after planting for hot-lifted, fall-planted seedlings, although the boxes were not stored under refrigerated conditions (Luoranen et al. 2019). Most stipulations regarding how soon seedlings must be planted after nursery harvest do not specify an interim storage temperature. The ability to safely increase interim storage duration would increase logistical flexibility during the hectic hot-lift/hot-plant season. Thus, the objective of this trial was to examine the effect of interim storage duration on hot-lifted, fall planted western redcedar (*Thuja plicata* Donn ex D. Don) and coastal Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *menziesii*) seedling quality.

Methods

Seedlings

Coastal Douglas-fir and western redcedar seedlings from a large order of hot-lifted seedlings grown commercially at Arbutus Grove Nursery in North Saanich, BC were used for this study. After grading, the coastal Douglas-fir and western redcedar average shoot height for the entire crop was 22.9 ± 2.8 cm (9.0 ± 1.1 in) and 26.3 ± 4.0 cm (10.3 ± 1.6 in), respectively, and the average stem diameter was 3.4 ± 0.4 mm (0.13 ± 0.01 in) and 2.8 ± 0.4 mm (0.11 ± 0.01 in), respectively. Coastal Douglas-fir seedlings were grown in 412A/10S Styroblocks™ (42-mm [1.62-in] diameter with 116-mm [4.58-in] depth; Beaver Plastics, Alberta, Canada), and the western redcedar were grown in 412B Styroblocks™ (36-mm [1.42-in] diameter with 116-mm [4.58-in] depth). The seedlings were grown under standard commercial growing regimes like those described in Wenny and Dumroese (1990, 1992) and Landis et al. (1989). For the coastal Douglas-fir, this regime entailed sowing the seeds into a double-poly greenhouse at the end of March and growing them under cover until mid-June at which time the poly was removed and the seedlings were exposed to full sunlight. In early July, the coastal Douglas-fir received 4 weeks of 14-hr blackout (i.e., short-day) treatment to induce budset, which was carried out with increasing levels of drought stress prior to each irrigation. The western redcedar seedlings were sown in early February in a double-poly greenhouse

and grown until mid-April when they were moved outside and grown under a low fertilizer regime to reduce shoot growth.

Ten 10-seedling bundles of each species were randomly selected during hot-lift operations on September 12, 2022. Eight bundles of each species were placed upright in a poly bag inside one waxed seedling box. Both the bag and box were closed. The box was then stored on a wooden pallet in an operational cold storage unit at Arbutus Grove Nursery at $4\text{ }^{\circ}\text{C}$ ($39\text{ }^{\circ}\text{F}$) $\pm 2\text{ }^{\circ}\text{C}$ ($4\text{ }^{\circ}\text{F}$) (figure 1). Two bundles of each species were packaged into a cardboard box with a frozen ice pack and shipped to the University of Northern British Columbia (UNBC) I.K. Barber Enhanced Forestry Laboratory in Prince George, BC. Two bundles of each species were sent each week for 5 consecutive weeks. Seedlings in the first shipment (week 0) did not have any time in cold storage before being transported to UNBC. Each subsequent shipment underwent an additional week in cold storage. The fifth shipment (week 4) spent an extra day in cold storage as the regular shipment date was a national holiday. It took 1 to 2 days from the time seedlings left the nursery until temporary placement in a walk-in cooler at $4\text{ }^{\circ}\text{C}$ ($39\text{ }^{\circ}\text{F}$) at UNBC.

Root Growth Capacity

Within a half day of arrival, seedlings sent to UNBC were taken from the cooler into a lab at room temperature ($20\text{ }^{\circ}\text{C}$ [$68\text{ }^{\circ}\text{F}$]) where root collar diameter (RCD) and height were measured. Seedlings were labelled and potted individually by hand into 3.8-L (1-gal) pots filled with moistened ProMoss (Premier Tech Horticulture, Rivière-du-Loup, Québec) 100 percent *Sphagnum* peat moss. Each week, the 40 seedlings were placed into a Conviron PGR15 (Convion Canada, Winnipeg, Manitoba) growth chamber (unit A) in a 10 by 4 rectangle of alternating coastal Douglas-fir and western redcedar (figure 2). The pots were then watered to field capacity. The growth chamber was set to 14-hr days at $460\text{ }\mu\text{mol/s2/m2}$ and 10-hr nights. The temperature was set to $22\text{ }^{\circ}\text{C}$ ($72\text{ }^{\circ}\text{F}$) during the day and $16\text{ }^{\circ}\text{C}$ ($61\text{ }^{\circ}\text{F}$) at night, and the relative humidity was 50 percent both day and night. These settings were chosen to mimic typical conditions seedlings may experience when planted on Vancouver Island in the early fall. After 7 days, all seedlings in growth chamber unit A were moved to a similar Conviron PGR15 growth chamber (unit B) set to the same light, temperature, and



Figure 1. Trial seedlings were stored in a waxed seedling box on a wooden pallet in a commercial cold storage cooler. (Photo by Steven B. Kiiskila, 2022)



Figure 2. Western redcedar and coastal Douglas-fir seedlings were placed in growth chambers to determine root growth potential following varying cold-storage durations. (Photo by Jennifer Baker, 2022)

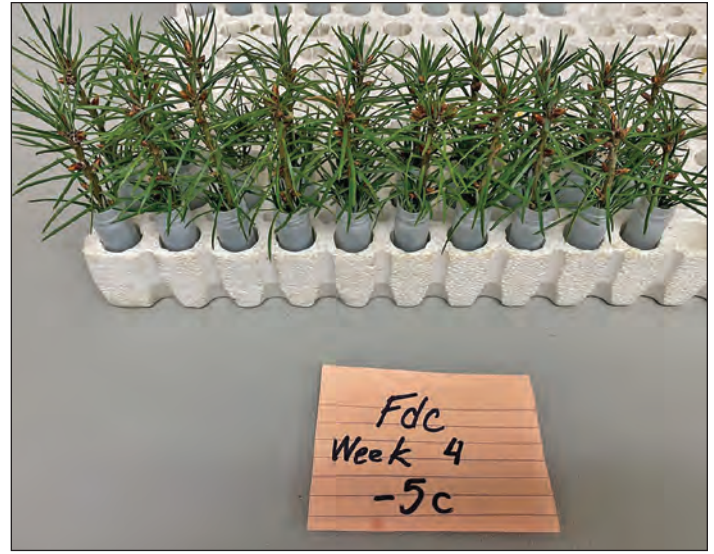


Figure 3. Seedling shoot tips were placed in vials prior to freeze treatment and chlorophyll fluorescence measurement. This photo shows Coastal Douglas-fir before the $-5\text{ }^{\circ}\text{C}$ ($23\text{ }^{\circ}\text{F}$) treatment following 4 weeks of storage and 2 weeks in the growth chambers. (Photo by Jennifer Baker, 2022)

relative humidity settings and once again watered to field capacity. Seedlings were moved to the second growth chamber after 1 week to account for potential differences in growth chamber conditions.

After 2 weeks in the growth chambers, seedlings were unpotted and the peat moss was gently removed from the roots. RGP of each seedling was then classified using the following modified Burdett (1979) scale:

- 0 – no roots
- 1 – some new roots $< 10\text{ mm}$ (0.39 in)
- 2 – 1 to 9 new roots $\geq 10\text{ mm}$ (0.39 in)
- 3 – 10 to 19 new roots $\geq 10\text{ mm}$ (0.39 in)
- 4 – 20 or more new roots $> 10\text{ mm}$ (0.39 in)

The seedling shoots were also assessed as either dead, inactive, swollen, or flushed and any signs of foliar disease or necrosis were noted.

Shoot Cold Hardiness

After the RGP assessment, shoot cold hardiness was assessed by following current British Columbia Government procedures (Moeller 2022). The top 10 cm (3.9 in) of each shoot was severed and placed in a vial filled with water (figure 3). The vials were then

placed in a chest freezer at $4\text{ }^{\circ}\text{C}$ ($39\text{ }^{\circ}\text{F}$) in the dark, after which the temperature was ramped down to $-5\text{ }^{\circ}\text{C}$ ($23\text{ }^{\circ}\text{F}$) over 1 hour, kept at $-5\text{ }^{\circ}\text{C}$ ($23\text{ }^{\circ}\text{F}$) for 1 hour, and then ramped back up to $4\text{ }^{\circ}\text{C}$ ($39\text{ }^{\circ}\text{F}$) over 1 hour. The vials were then placed in a growth chamber at $6\text{ }^{\circ}\text{C}$ ($43\text{ }^{\circ}\text{F}$) to thaw in the dark. After 8 hours, the growth chamber lights were turned on and the temperature was increased to $24\text{ }^{\circ}\text{C}$ ($75\text{ }^{\circ}\text{F}$) for another 8 hours. Finally, the lights were turned off again and seedlings were kept in the dark for a minimum of 20 min to ensure they were in a fully dark-adapted state.

The dark-adapted maximum quantum yield of PSII (F_v/F_m) was then measured using a pulse modulated chlorophyll fluorometer Opti-Sciences OS1p (Opti-Sciences, Inc., Hudson NH). The F_v/F_m reading represents an index of cold injury following freezing, with values greater than or less than 0.65 classified as either alive or dead, respectively. These steps were then repeated at $-12\text{ }^{\circ}\text{C}$ ($10\text{ }^{\circ}\text{F}$) on the same samples for week 0 (no storage) seedlings. Because all seedlings failed the freezing test at $-12\text{ }^{\circ}\text{C}$ ($10\text{ }^{\circ}\text{F}$) in week 0, it was decided to test cold hardiness at $-5\text{ }^{\circ}\text{C}$ ($23\text{ }^{\circ}\text{F}$) and $-8\text{ }^{\circ}\text{C}$ ($18\text{ }^{\circ}\text{F}$) for the remaining 4 weeks. Results from the colder exposure of $-8\text{ }^{\circ}\text{C}$ ($18\text{ }^{\circ}\text{F}$) should be interpreted with caution, as the same sample was used to test both $5\text{ }^{\circ}\text{C}$ ($23\text{ }^{\circ}\text{F}$) and $-8\text{ }^{\circ}\text{C}$ ($18\text{ }^{\circ}\text{F}$), rather than testing new samples as is typically done.

Data Analysis

An analysis of covariance (ANCOVA) was used to analyze the statistical significance of RGP and quantum yield differences between weeks for each species using RStudio (version 2022.12.0+353, Posit, Boston, MA). Seedling height and RCD were set as covariates to account for possible interactions due to differences in height and RCD between weekly measurements. To determine if there was a relationship between seedling height and RCD, the measurements were plotted against one another for each species. No discernible relationship for either species occurred, thus height and RCD were plotted separately, which showed the data was variable enough to exclude an interaction between the two covariates.

Species were analyzed separately, and Tukey's Honest Significant Difference test was used to determine the significance of differences in weekly measured parameters. Differences with a p-value ≤ 0.05 were considered significant. Analyses were performed on RGP and dark-adapted maximum quantum yield after exposure to $-5\text{ }^{\circ}\text{C}$ ($23\text{ }^{\circ}\text{F}$) and $-8\text{ }^{\circ}\text{C}$ ($18\text{ }^{\circ}\text{F}$).

Results

Root Growth Potential

There was a statistically significant ($p = 0.05$) decline in the number of new roots greater than 1 cm (0.39 in) with increasing cold-storage duration for both species (figure 4). Variability in new root growth among seedlings also increased with increasing cold storage, especially in the western redcedar (figures 5 and 6), which had one dead seedling with no new root growth after 3 weeks of storage and two dead seedlings after 4 weeks of cold storage.

Seedling Shoot Condition

After 2 weeks in the growth chamber, 5 and 10 percent of western redcedar seedlings that had received 1 or 2 weeks of cold storage, respectively, grew new foliage. Swollen buds were observed on 20 percent of coastal Douglas-fir seedlings that were not cold stored after 2 weeks in the growth chamber, compared with 10 percent of those that were cold stored for 1 week.

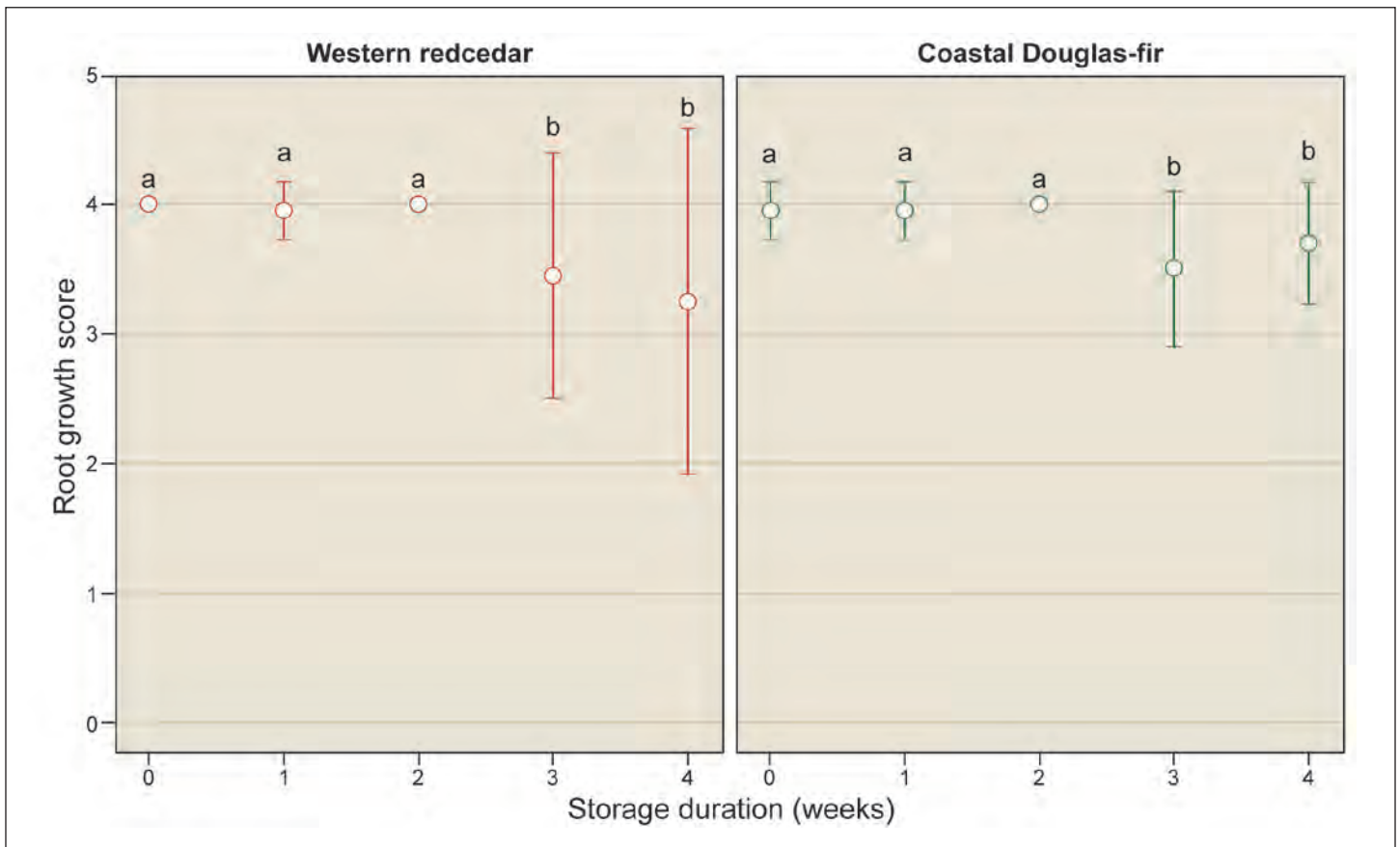


Figure 4. Mean RGP values varied for western redcedar and coastal Douglas-fir seedlings stored in a closed seedling box in cold storage at $4\text{ }^{\circ}\text{C}$ ($39\text{ }^{\circ}\text{F}$) for 0 to 4 weeks. Vertical bars are standard deviations of the mean. Weeks for each species with the same letter are not significantly different ($p = 0.05$)

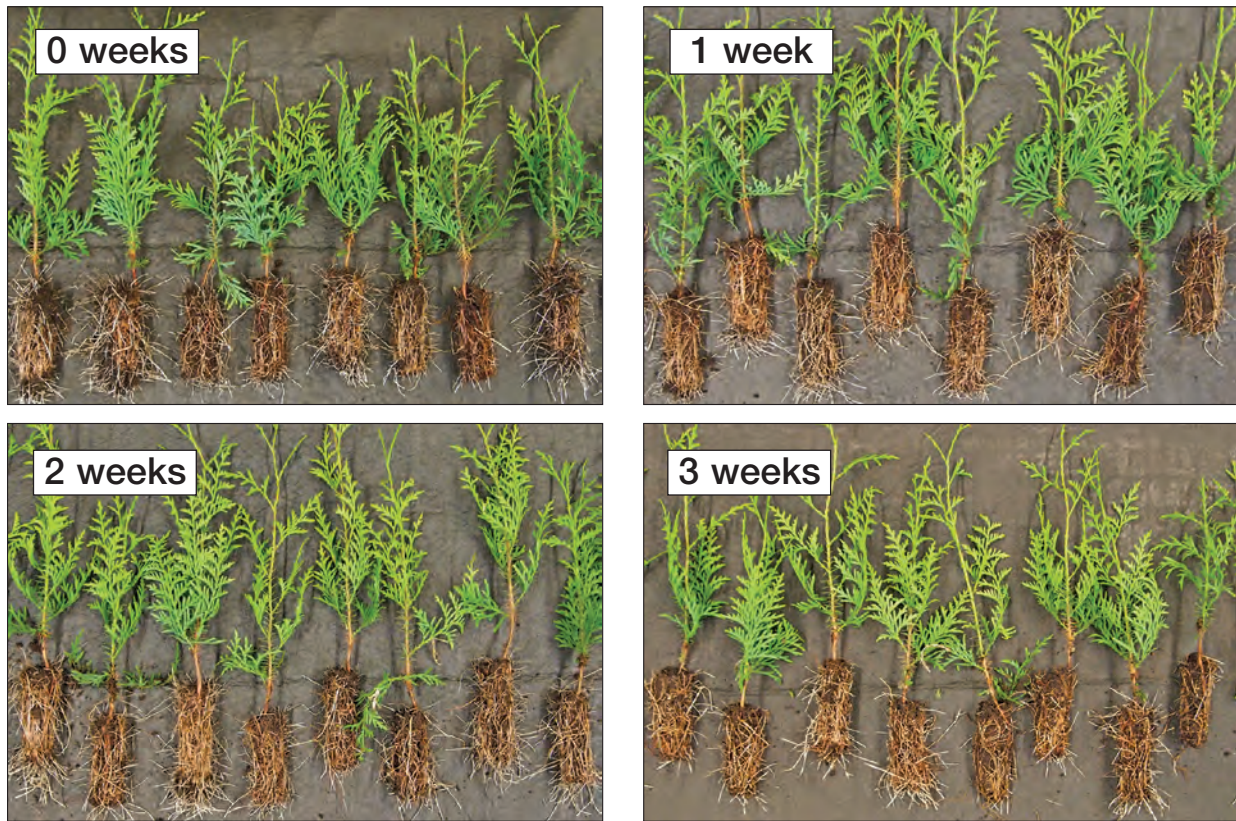


Figure 5. Western redcedar seedlings previously stored in a closed seedling box in cold storage at 4 °C (39 °F) for 0 to 3 weeks, and then grown for 2 weeks in a growth chamber exhibited varying levels of root growth. The RGP of seedlings stored for 4 weeks did not differ significantly than those stored for 3 weeks and are thus not shown for brevity. (Photos by Jenifer Turner, 2022)

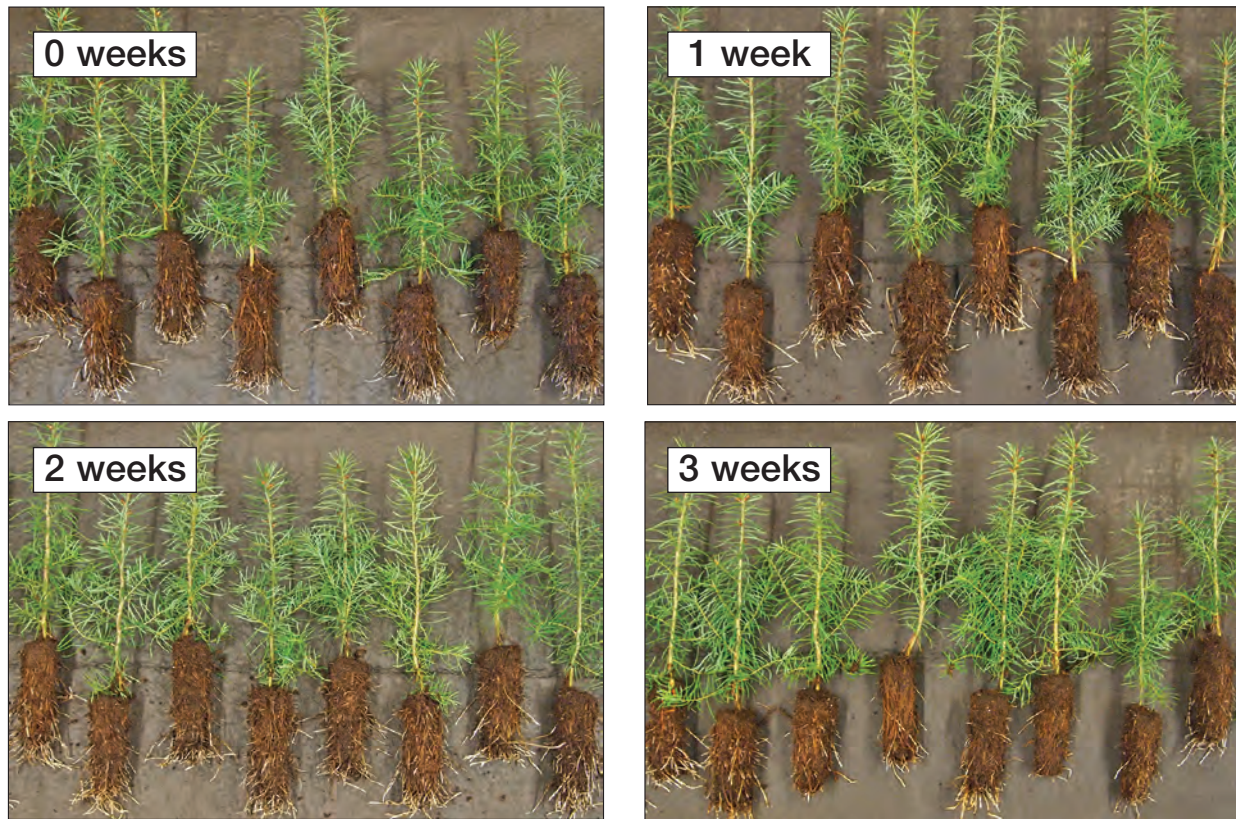


Figure 6. Coastal Douglas-fir seedlings previously stored in a closed seedling box in cold storage at 4 °C (39 °F) for 0 to 3 weeks, and then grown for 2 weeks in a growth chamber exhibited varying levels of root growth. The RGP of seedlings stored for 4 weeks did not differ significantly than those stored for 3 weeks and are thus not shown for brevity. (Photos by Jenifer Turner, 2022)

Coastal Douglas-fir seedlings in the longer storage treatments did not have bud swelling, although one of the terminal buds of a seedling stored for 2 weeks began to flush. Three western redcedar seedlings (one from the 3-week and two from the 4-week cold storage treatment) were assessed as dead during the RGP assessment. Foliar necrosis was also observed on four of the western redcedar seedlings cold stored for 3 weeks and on one stored for 4 weeks (figure 7). There was no seedling mortality or foliar damage observed in the coastal Douglas-fir.

Shoot Cold Hardiness

Maximum quantum yield among seedlings after exposure to -5 °C (23 °F) and -8 °C (18 °F) varied considerably. After the -5 °C (23 °F) exposure, the maximum quantum yield values for both species and all storage durations were deemed high at ≥ 65 . Statistically significant differences occurred among storage durations for both species, but there was no clear cold hardiness trend (figure 8a). The maximum quantum yield after the -5 °C (23 °F) exposure was similar between species.

Overall, maximum quantum yield means for seedlings cold stored 1 to 4 weeks after exposure to -8 °C (18 °F) was lower and more variable than exposure at -5 °C (23 °F) for both the western redcedar and coastal Douglas-fir. Maximum quantum yield from all test dates for both species was ≤ 65 , the value required to pass the BC Ministry of Forests cold

hardiness test for storability. Although statistically significant differences in quantum yield means were found among storage durations in both species, there was no clear cold hardiness trend (figure 8b). The maximum quantum yield after the -8 °C (18 °F) exposure was more variable and slightly lower in western redcedar compared with coastal Douglas-fir.

Discussion

Results from this trial suggest that hot-lifted, fall-planted western redcedar and coastal Douglas-fir can be kept in closed boxes at 4 °C (39 °F) for at least twice the current BC Government stock handling guideline of 5 days (Anonymous 2021). Summer- and fall-planted southern pines (*Pinus* spp.) have been safely stored at 2 °C (35 °F) for 4 to 6 weeks (Grossnickle and South 2014, Jackson et al. 2012). Thus, longer storage durations have been successful. In contrast, no more than 1 week of storage in closed boxes is recommended for Norway spruce (*Picea abies* [L.] Karst.) and Scots pine (*Pinus sylvestris* L.) in Finland (Luoranen et al. 2019). Most stock handling recommendations do not specify an interim storage temperature at the nursery or field cache, or simply recommend that seedlings be kept below 10 °C (50 °F). Seedlings in this trial were stored under cool conditions, thereby slowing respiration and conserving carbohydrates. While there was a significant decline in RGP after 3 and 4 weeks of cold storage, the RGP results would still deem coastal Douglas-fir acceptable to plant according to current BC Ministry of Forests RGP evaluation criteria. Current RGP criteria used by the Ministry require a value of 3.0 on the Burdett RGP index (Burdett 1979) after 1 week in the greenhouse or growth chamber, which is only four new roots longer than 10 mm (0.39 in). A score of zero (no new roots) would negate the sample and require a retest, thus the western redcedar stored for 3 and 4 weeks in this trial would technically not be approved for planting without further investigation as there was some mortality.

The correlation between RGP and outplanting performance is weak at best (Simpson and Ritchie 1996). Nonetheless, RGP tests can provide valuable information regarding seedling quality. Knowing that the seedlings to be planted have the potential to grow a certain number of roots under ideal conditions assures the nursery and land managers that the seedlings are



Figure 7. Foliar necrosis occurred on a western redcedar seedling after 3 weeks of cold storage and 2 weeks in a growth chamber. (Photo by Jennifer Baker 2022)

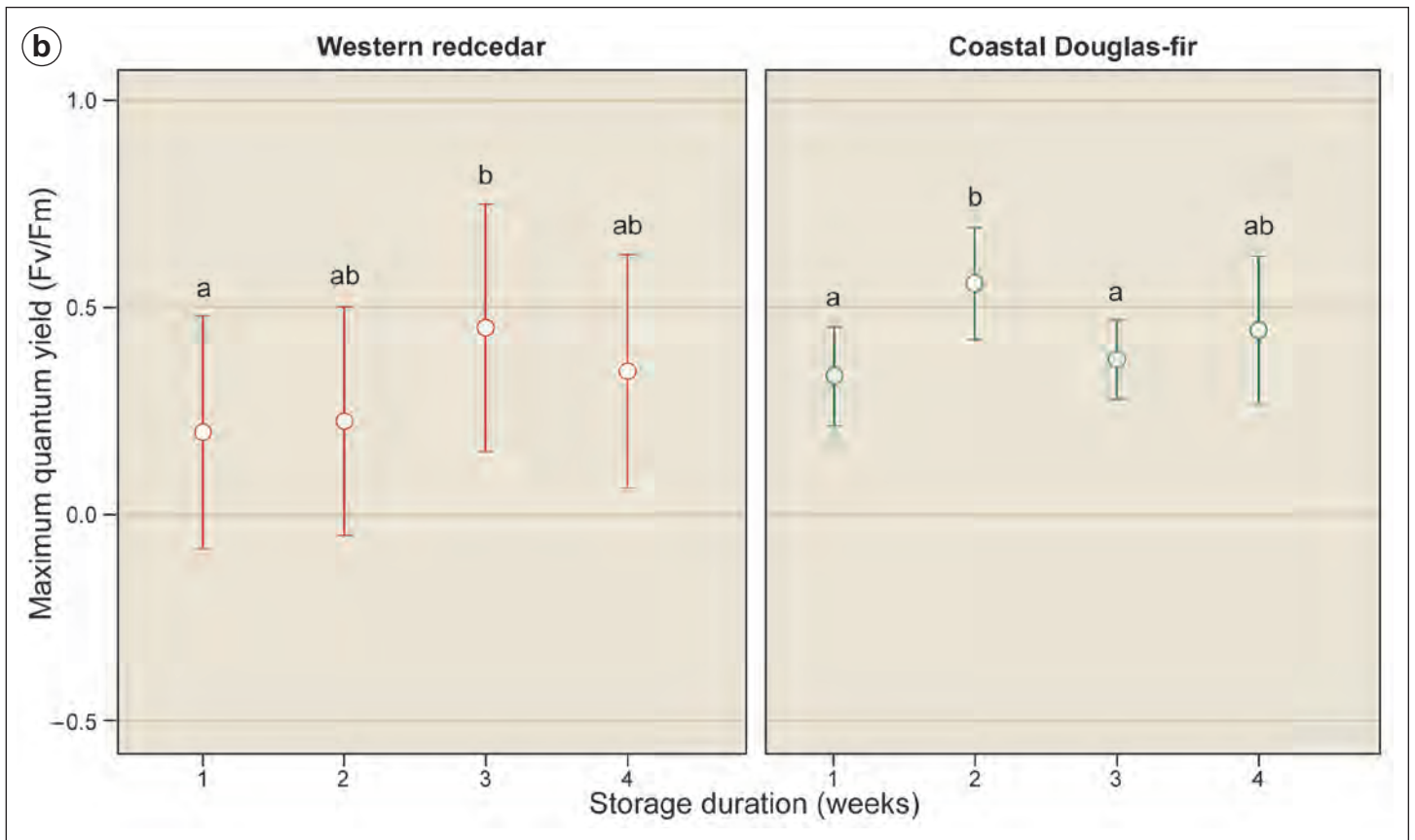
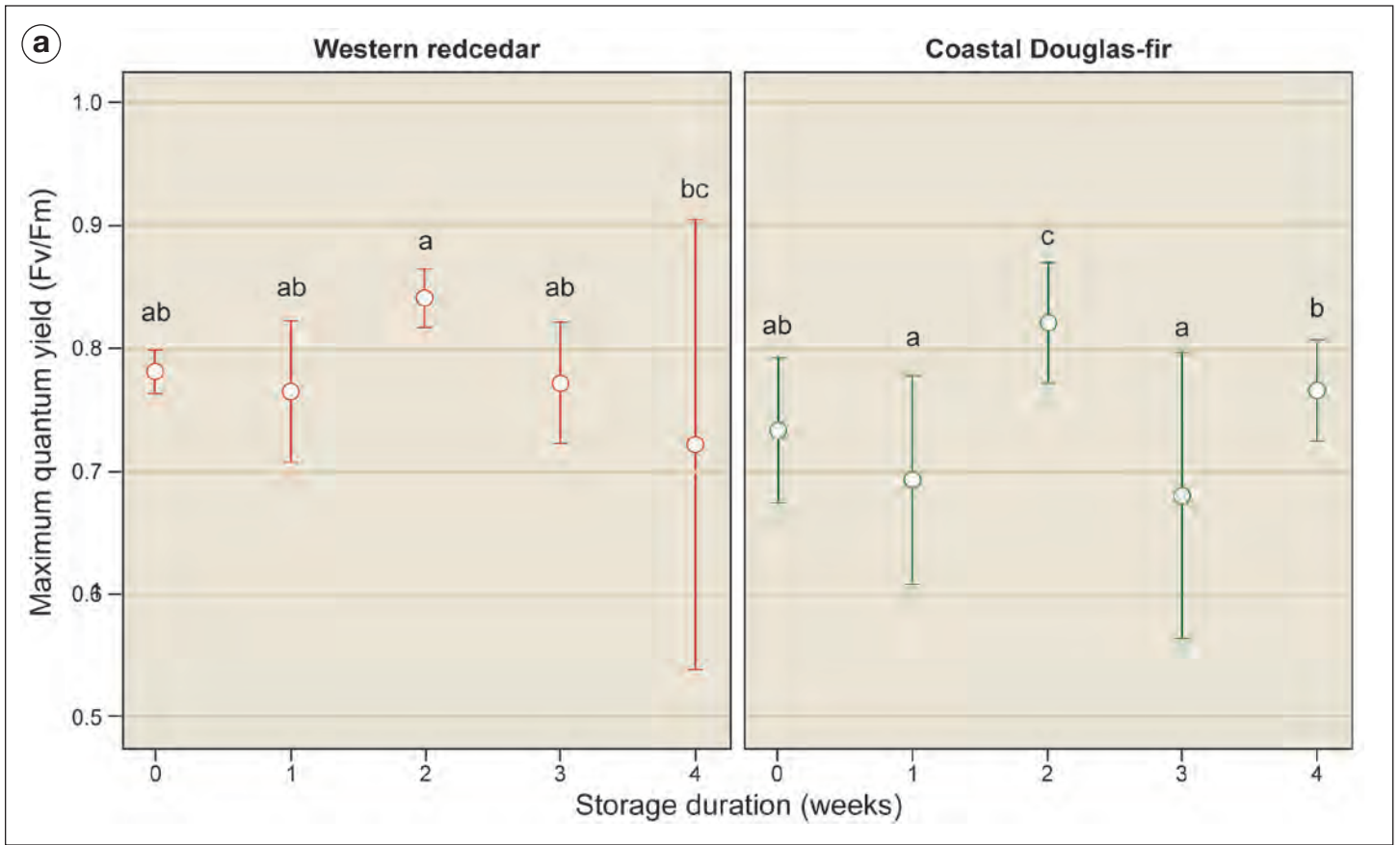


Figure 8. Mean dark-adapted maximum quantum yield values of western redcedar and coastal Douglas-fir seedlings varied by storage duration after exposure to (a) -5 °C (23 °F) or (b) -8 °C (18 °F). Vertical bars are standard deviations of the mean. Weeks for each species with the same letter are not significantly different ($p = 0.05$).

not compromised. The interpretation of RGP test results is affected by variations in test environment, test duration, species, season, and rating criteria. Various iterations of the Burdett (1979) RGP index are in use, such as the four-point scale used for commercial RGP screening at the I.K. Barber Enhanced Forestry Lab at UNBC, to which an additional classification was added to this trial for increased precision. Some RGP testing laboratories count all the new roots larger than a certain size (e.g., 10 mm [0.39 in]), although a threshold RGP value may exist after which more new roots do not result in increased aboveground growth (Nelson 2019).

RGP test results on hot-lifted seedlings should be interpreted with caution. Until a standardized RGP test procedure is developed, the test environment should be considered when interpreting RGP values. For example, because conifers primarily use current photosynthate for root growth (van den Driessche 1987, Villar-Salvador et al. 2015), root growth is expected to be greater under higher light intensity and duration. Healthy seedlings may not grow roots even under ideal environmental conditions due to seasonal periodicity in root growth, which may further be influenced by nursery cultural practices such as blackout or short-day treatment (Grossnickle and Ivetić 2022). Understanding all factors will help the nursery and forester to make changes to the nursery culture, planting date, or both to optimize seedling quality.

Hot-lifted seedling boxes are sometimes opened upon arrival at the planting site if they will not be planted in a day or two due to concerns that dark conditions reduce seedling quality (Lavender 1989). Some recommendations even suggest seedlings be removed from refrigerated storage into a warmer ambient environment so that the boxes can be opened (Anonymous 2021). In this trial, seedlings kept in the dark did not have adverse effects on RGP after 2 weeks. The closed seedling box, however, can be conducive for seedling disease (Camm et al. 1994). While seedlings were not cold hardy enough to tolerate -8 °C (18 °F), they were sufficiently hardy to -5 °C (23 °F). Thus, it is unlikely the western redcedar mortality and foliar damage was caused by the storage at 4 °C (23 °F). Seedling cold hardiness was also not impacted by the duration of cold storage. This agrees with previous studies on

Douglas-fir (Ritchie 1982) and Sitka spruce (Cannell et al. 1990) where freezing tolerance was found to be maintained throughout cooler storage.

Hot-lifted seedlings planted in the summer or fall are typically stood upright in waxed seedling boxes without a bag so that stock can be cooled if the boxes are opened and so any irrigation water will drain. On the other hand, fall/winter lift stock to be freezer stored are packaged inside a poly or poly-paper bag in a waxed seedling box to prevent moisture loss and desiccation. In this trial, the seedlings were stood upright inside a closed poly bag to remove the potential variable of moisture loss. While properly hardened seedlings have developed a high level of drought tolerance by harvest, the potential moisture loss from hot-lifted seedlings packaged and stored for a couple of weeks in boxes without bags should be investigated because desiccation of root systems prior to out-planting can negatively impact seedling establishment (Genere and Garriou 1999). Preliminary, nonreplicated weight measurements of a hot-lifted box of Douglas-fir stored at 4 °C (23 °F) without a bag declined in total weight by 1, 3, 5 and 8 percent after 1, 2, 3, and 4 weeks, respectively.

Conclusions

Interim storage is an operational handling step that is required in the process of moving hot-lifted seedlings from the nursery to the planting site. Increasing the interim storage duration of hot-lifted seedlings would allow for more flexibility in the reforestation pipeline. Results from this trial show that coastal Douglas-fir and western redcedar seedling quality were not compromised during 2 weeks of interim cold storage in closed boxes. Future studies could examine storage effects on different species during the summer hot-lift period. It is recommended that RGP test conditions be standardized and a more detailed root classification system be used to aid in evaluation of the results.

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Acknowledgments

The paper is based on an April 2023 undergraduate thesis written by Jennifer Baker while at the University of Northern British Columbia. The authors also thank Arbutus Grove Nursery for supplying the seedlings, the University of Northern British Columbia I.K. Barber Enhanced Forestry Lab for supplying the growth chambers and conducting the RGP testing, and Ché Elkin (University of Northern British Columbia) for initial planning and overseeing Jennifer Baker's thesis.

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