Effect of the Timing of Cold Storage on Cold Hardiness and Root Growth Potential of Douglas-fir'

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Abstract.--Container-grown Douglas-fir seedlings were cold acclimated in growth chambers over 20 weeks. At weekly intervals, cold hardiness and root growth potential (RGP) were measured, and additional seedlings were placed in 1°C storage for 4 weeks. Cold hardiness and RGP were reassessed following storage. Cold hardening continued in storage regardless of when during acclimation seedlings were stored. However, the rate of cold acclimation increased or decreased during storage depending on the level of cold hardiness at the start of the storage period. RGP generally declined during storage, though occasionally remained the same or increased without apparent relation to level of cold hardiness.

INTRODUCTION

The recent focus of tree seedling quality research has been on seedling attributes that indicate physiological condition and stress resistance, with the recognition that both morphological and physiological measures of quality are necessary (Duryea 1985, Ritchie 1984, Sutton 1979). Such physiological attributes include cold hardiness, root growth potential (RGP), and bud dormancy. There is much to be gained by understanding the interrelationships of these attributes, not only as it contributes to basic science through defining the annual physiological cycle of nursery-grown seedlings, but also from a practical perspective. Quickly measured information on cold hardiness could be used as a rapid estimator of RGP and bud dormancy, attributes more time consuming to measure, if a consistent relationship between the three could be established.

Toward this end, the U.S. Forest Service Rocky Mountain Forest and Range Experiment Station stress physiology project at Flagstaff, Arizona has been examining the interrelationships between cold hardiness, RGP, and bud dormancy in southwestern conifers. The initial approach was to simulate that portion of the annual cycle from bud set to bud break under controlled growth chamber conditions, and to measure all three attributes concurrently at frequent intervals (Tinus et al. 1986). In this way, relationships between the attributes were established. However, these relationships were observed only under a single set of temperature and photoperiod conditions. Recently completed experiments examined the cold acclimation and deacclimation of interior Douglas-fir and the associated changes in RGP and bud dormancy using several different sets of temperature conditions. Additionally, the effects of transferring seedlings to optimum growing conditions or to cold storage at intervals throughout acclimation and deacclimation were measured. This paper presents the results from one of those cold acclimation regimes and the effects on cold hardiness and RGP when seedlings were transferred to cold storage at intervals throughout acclimation.

MATERIALS AND METHODS

Seedlings of interior Douglas-fir (Pseudotsuga menziesii var. glauca) of the same seed source as in our previous experiments (Tinus et al. 1986) were greenhouse-grown in 240-ml Rootrainer[3] book containers in a peat-vermiculite mix for 8.5 months (Mar. 17 - Dec. 1, 1987). Greenhouse temperatures averaged 25°C daily and 22°C at night. Daylength was extended to 22 hours

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[3]Trade names are used for brevity and specificity, and do not imply endorsement by the USDA to the exclusion of other equally suitable products.

with fluorescent light (7.5 umol s^{-1} M^{-2}). Watering was as needed, with a high-nitrogen complete nutrient solution (223 ppm N, 36 ppm P, 151 ppm K). Other cultural conditions were as recommended by Tinus and McDonald (1979). Actively growing seedlings were then placed in Percival HL-60 growth chambers for a 2-stage, 20-week cold acclimation regime. Day/night temperatures were 20°/15°C during the first 4-week stage, and 10°/3°C during the second 16-week stage. Daylength was 10 hours throughout the 20 weeks (518 umol s^{-1} m^{-2}). Watering was as needed, with a low-nitrogen complete nutrient solution (20 ppm N, 86 ppm P, 151 ppm K).

A random sample of 24 seedlings was taken from the growth chamber population at weekly intervals. Needle tissue was removed from each seedling for a freeze-induced electrolyte leakage (FIEL) test of cold hardiness. The seedlings were then randomly divided into two equal groups, with one group used in an aeroponic RGP test, and the other placed in 1°C storage for 4 weeks. Cold hardiness of the stored seedlings was measured with the FIEL test weekly, or after 1, 2, and 4 weeks, during the 4-week storage period. RGP of the stored seedlings was measured at the end of the storage period.

Cold Hardiness Test

The FIEL test procedures to measure needle tissue cold hardiness were similar to those previously described (Burr et al. 1986). Needles were removed from the second to the last flush along the central axis of each seedling in the sample to be tested. An equal number of needle segments, 1 cm long, cut at both ends, were prepared from each seedling. Segments were pooled into four equal groups such that the trees represented by each group were the same every time testing was conducted. The segments were washed in distilled water and transferred to culture tubes containing 0.5 ml distilled water. Each group of segments was used to fill **six** tubes, six needles per tube.

The 24 tubes were then divided into sets of 4 such that each set included 1 tube from each of the 4 groups of segments. In this way, four groups of seedlings within each sample were independently monitored. One set of tubes was stoppered and placed in a refrigerated water bath at 1°C as a control. The other 5 sets of treatment tubes were placed in a Forma Scientific ethanol bath at -2°C. After 0.5 hour, the water in the treatment tubes was nucleated and the tubes were stoppered. The ethanol bath was then cooled at the rate of 5°C per hour.

At each of 5 test temperatures, selected to span 20 to 80% injury, 1 set of treatment tubes was removed to thaw in the 1°C water bath. After all tubes were removed from the ethanol bath and thawed, 5.5 ml of distilled water were added to each of the 24 tubes, and all were stoppered and placed in a 100-rpm shaker at 24°C for 20 hours incubation. Conductivity of the solution in each tube was measured after incubation with a YSI conductance meter and microcell, and the tubes were then placed in a boiling water bath for 15 minutes to induce complete tissue injury. Conductivity was remeasured after an additional 20 hours incubation in the shaker.

Test results, which were available in 2 days, were measured as percent index of injury according to Flint et al. (1967). A modified Gauss sigmoid model (Grosenbaugh 1965) was fitted to each data set, and the temperature at 50% index of injury (LT50) was estimated by inverting each model.

Root Growth Potential (RGP) Test

RGP was measured using an aeroponic system similar to that described by Burr et al. (1987). The mist chamber measured 1.0 m wide x 2.4 m long x 0.6 m high, was constructed of 5-cm-thick rigid urethane foam, and was fitted with a copper tubing, 3-nozzle mist system 25 cm above the floor of the chamber. Conditions within the chamber were maintained at 100% relative humidity and 27°C by a warm-water intermittent mist and a 10-cm layer of vermiculite in the bottom of the chamber. Rootballs, with potting mix intact, were suspended within the chamber using foam-lined redwood seedling clamps which formed the top of the chamber. RGP tests were conducted in a greenhouse with day/night temperatures averaging 21°/18°C and a 22-hour photoperiod extended with fluorescent light (7.5 umol s^{1} M^{-2}).

RGP was quantified as the total number of new roots per seedling =0.5 cm in length after 14 days in the mist chamber. Means and standard errors were calculated for each sample of 12 seedlings.

Storage Treatment

The 1°C storage treatment was maintained in a 1.5 m x 0.7 m x 1.3 m cooler. Stored seedlings were kept in darkness except when removed from the cooler for weekly sampling of tissue for the FIEL test and for watering, which was as needed with the low-nitrogen nutrient solution. Seedlings, in the book containers, were placed upright in the cooler without wrapping or packaging.

RESULTS Cold Hardiness

Douglas-fir cold hardiness increased from -5°C to -32°C during the 2-stage, 20-week growth chamber cold acclimation regime that excluded exposure to freezing temperatures (fig. 1). Cold acclimation proceeded slowly during the first 4-week stage when day/night temperatures were 20°/15°C. When day/night temperatures were lowered to 10°/3°C in the second stage, cold acclimation proceeded rapidly, reaching a maximum rate of approximately 1°C per day during the ninth week. Maximum cold hardiness, under these conditions, was reached in 14 weeks. No further cold acclimation occurred between week 14 and week

20, though cold hardiness may have oscillated somewhat. Cold hardiness as a function of time was highly predictable under these conditions. The LT50's for the entire 20-week period were regressed using a weighted least squares nonlinear regression assigning higher weight to early weeks (R^2= .983).

Cold acclimation continued during the 4-week, 1°C storage period regardless of when seedlings were stored during the 20-week acclimation regime (fig. 2). This change in seedling environment, from growth chamber to storage conditions, was accompanied by an increase or decrease in the rate of cold acclimation, depending on the level of cold hardiness at the start of the storage period. Seedlings stored in weeks 0 through 2, with cold hardiness between -5° and -6°C, acclimated more rapidly in storage than seedlings remaining under growth chamber conditions. Seedlings stored in weeks 3 through 10, which included the period of most rapid cold acclimation in the growth chambers, acclimated more slowly in storage than seedlings remaining under growth chamber

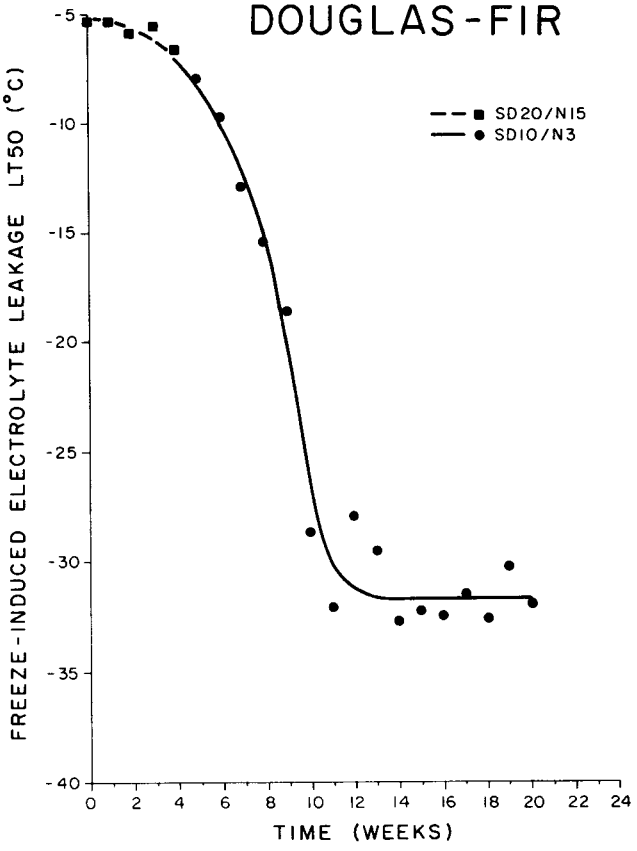

Figure 1.--Temperatures resulting in 50% index of injury to needle tissue in the FIEL test (LT50), as a function of time under growth chamber conditions. R^2= .983 LT50 = -31.83 + 26.27e exp(-0.000031$W^{4.706}$) where W= week. Dashed line = short day 20°C, night 15°C. Solid line = short day 10°C, night 3°C.

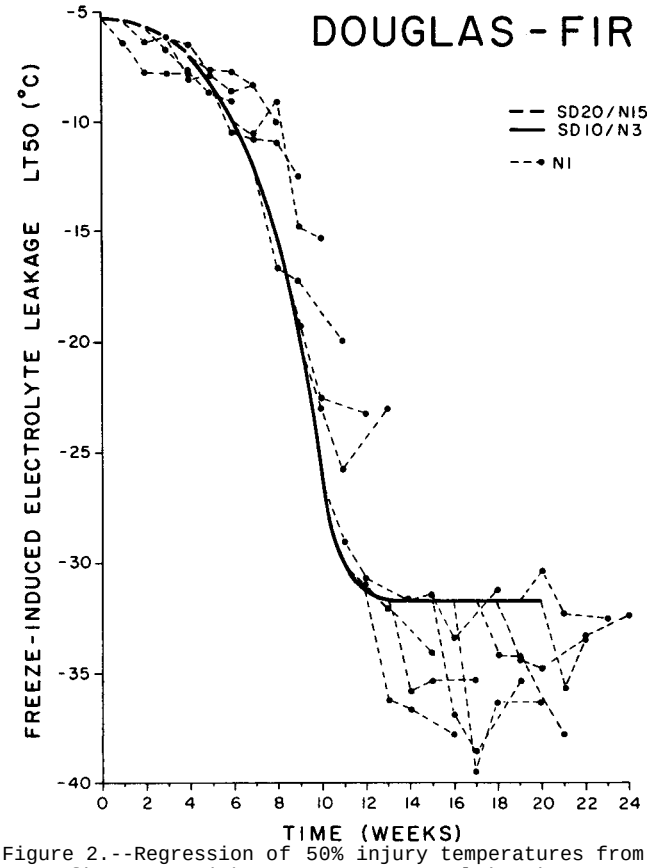

Figure 2.--Regression of 50% injury temperatures from figure 1, with temperatures resulting in 50% index of injury to needle tissue when seedlings were transferred at weekly intervals to 1°C storage (N1) for 4 weeks.

conditions. Seedlings stored in weeks 11 through 20 continued to cold acclimate appreciably in storage, while seedlings remaining under growth chamber conditions reached maximum cold hardiness and stopped acclimating.

Root Growth-Potential (RGP)

Douglas-fir RGP, measured as total number of new roots per seedling at 14 days, increased from 55 to 145 during the 20-week growth chamber cold acclimation regime (fig. 3). The initial rapid rise in RGP from 55 to 125 new roots per seedling coincided with the period of rapid cold hardening during the first 12 weeks. When seedling cold hardiness was at or near maximum under the growth chamber conditions, weeks 12 through 20, RGP remained high but fluctuated widely.

RGP generally declined during the 4-week, 1°C storage period, though occasionally remained the same or increased without apparent relation to the level of cold hardiness at the start of the storage period (fig. 4). However, RGP after 4 weeks storage increased from 25 new roots per seedling placed in storage week 0 to almost 130 new roots per seedling placed in storage week 20.

DISCUSSION

Cold Hardiness

The pattern of cold acclimation (fig. 1) was similar to that measured in the previous growth chamber experiment with this seed source (Burr et al. 1986, Tinus et al. 1986). However, the maximum rate of cold acclimation was twice as fast as previously measured. Seedlings in the current experiment reached an LT50 7°C lower than seedlings in the previous experiment did in the same length of time. Changes in greenhouse cultural conditions during seedling production to prevent bud set and promote active growth until the start of the current experiment, such as a reduction in moisture stress through more frequent watering, may have predisposed the current crop of seedlings to cold acclimate faster. Timmis and Tanaka (1976) observed that severe moisture stress under long days reduced the rate of Douglas-fir cold acclimation, while mild stress under long days enhanced cold acclimation when both treatments were followed by short days at low temperatures. Cold hardening to -20°C without exposure to freezing temperatures has been previously observed in other conifers (Glerum 1973, Cannell and Sheppard 1982) but hardening to -30°C and below

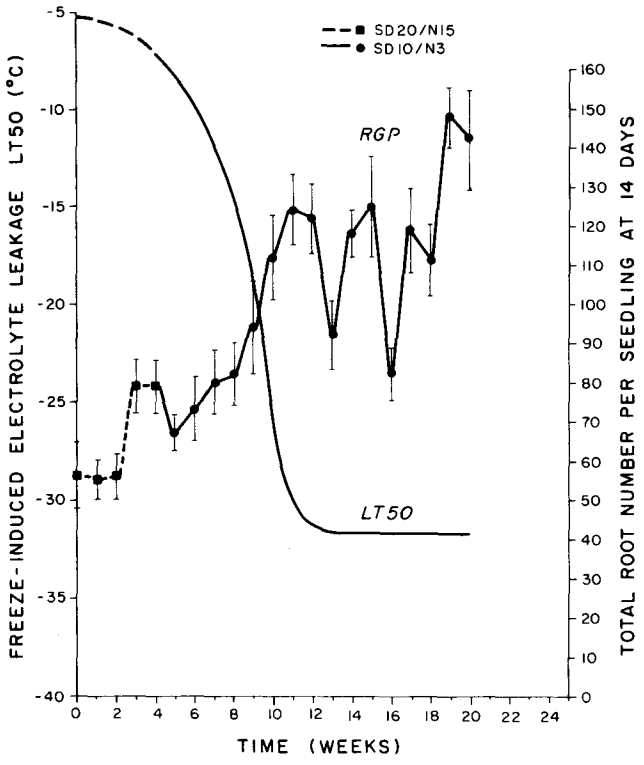

Figure 3.--Total number of new roots per seedling at 14 days (RGP) as a function of time under growth chamber conditions, with 'regression of 50% injury temperatures from figure 1 (LT50). Vertical bars are ±1 standard error. Standard errors ranged from 5 to 15, with a median of 8.

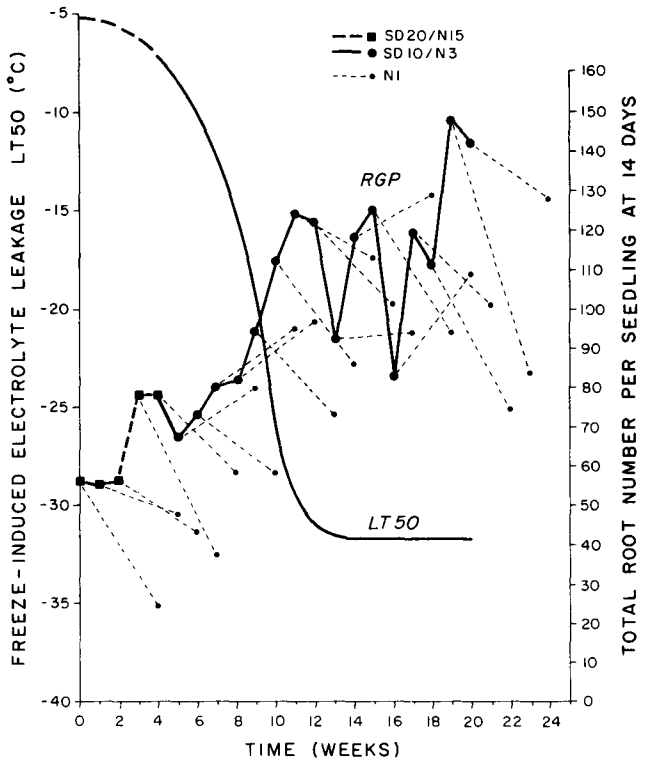

Figure 4.--Total number of new roots per seedling at 14 days (RGP) before (as in fig. 3) and after transfer at weekly intervals to 1°C storage (N1) for 4 weeks, with regression of 50% injury temperatures from figure 1 (LT50).

was of interest, especially since this FIEL tissue test was found to be approximately 10°C more conservative than a whole-plant freeze test LT50 estimate in this hardiness range (Burr et al. 1986).

The continuation of cold hardening in storage, regardless of when during acclimation seedlings were stored, and the restarting of the hardening process in storage after cold hardiness had stabilized under growth chamber conditions, suggest the importance of temperature, in the absence of photoperiod, for regulation of seedling physiology (fig. 2). Changes in the rate of cold acclimation were not random, but occurred in a readily identifiable pattern when seedlings were transferred from the growth chamber conditions into storage. It is anticipated that the extension of this pattern of rate changes over longer storage periods will result in a stabilizing of seedling cold hardiness throughout a wide range of LT50's such that the greater the level of cold hardiness at the start of storage, the lower the final LT50 attained. The lowest attainable LT50 for a sample of seedlings stored at any point along the acclimation curve would then be a function of genotype, production history, acclimation history, and storage conditions.

This seed source may or may not respond similarly to other handling and storage conditions. It should be noted that the container seedlings in this experiment were stored upright, with undisturbed root systems, good air circulation, and regular watering. Thus, many of the stresses associated with standard lifting, packaging, and storing procedures were minimized. This may have been critically important for the continuation of the cold hardening process in storage. Faulconer (1989) reported bareroot coastal Douglas-fir seedlings, lifted and packaged using operational. procedures, deacclimated in storage unless stored when cold hardy to at least -15°C. A comparison of the two studies suggests that the stresses associated with the lift and pack process may disrupt physiological mechanisms permiting continued cold hardening in storage (Faulconer 1988).

Root Growth Potential

The RGP pattern (fig. 3) was similar to that previously measured for this seed source under cold acclimating growth chamber conditions (Tinus et al. 1986). RGP of the seedlings in both experiments was low when cold hardiness was minimum, increased rapidly during the period of rapid cold acclimation, and remained high but fluctuating when cold hardiness was maximum. The resolution of the pattern has been improved in the current experiment by more frequent measurement and the use of larger sample sizes. There are many reports of conifer seedling RGP increasing under natural conditions during the cold acclimation period (DeWald and Feret 1987, Jenkinson 1980, Ritchie and Dunlap 1980) and the pattern presented here was comparable. The relationship between the rapid increase in cold hardiness and the rapid rise in RGP, consistent in both this and the previous experiment, further supported the hypothesis that such relationships exist.

Storage was generally detrimental to seedling RGP throughout the cold acclimation period (fig. 4). There was no indication of an increase in seedling capacity to maintain RGP in storage as cold acclimation progressed. There are, however, many reports that early-lifted bareroot seedlings store poorly, losing RGP in storage, while fully hardy seedlings store without loss of RGP, and often with increased RGP, under storage temperatures and durations similar to those used in this experiment (DeWald and Feret 1988, Ritchie and Dunlap 1980). An explanation for this difference is not readily available. Until it becomes possible to identify and monitor the physiological changes occurring during storage that are manifested as a change in RGP, it will remain difficult to explain why RGP increases, decreases, or remains unchanged during any given period of time.

Though there was a general decline in RGP during storage throughout the acclimation period, RGP levels following storage increased with time as cold hardiness at the onset of storage increased. Seedlings stored after cold acclimating to at least -15° to -20°C maintained RGP levels during the 4-week storage period at least as high as unstored seedlings at the start of the acclimation period. Thus it was still possible to increase RGP over the initial level of 55 new roots per seedling, with a 4-week storage period, by placing seedlings in storage after a moderate level of cold hardiness was attained.

In summary, this was the second experiment in which Douglas-fir seedlings of a single seed source were cold acclimated in growth chambers at the U.S. Forest Service Flagstaff facility. The patterns of cold acclimation and RGP, as well as the relationship between the two patterns, were consistent in both experiments. At weekly intervals throughout the acclimation period, seedlings were placed in 1°C storage for 4 weeks. Cold acclimation continued in storage, though the rate of acclimation was altered in a predictable manner. RGP generally declined in storage, though RGP levels after storage increased with greater cold hardiness at the onset of storage. Effects of longer storage periods on cold hardiness and RGP remain to be determined.

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