

## Relationship between carbohydrate concentration and root growth potential in coniferous seedlings from three climates during cold hardening and dehardening

R. W. TINUS,<sup>1</sup> K. E. BURR,<sup>2</sup> N. ATZMON<sup>3</sup> and J. RIOV<sup>4</sup>

<sup>1</sup> Southern Research Station, USDA Forest Service, 2500 South Pine Knoll Drive, Flagstaff, AZ 86001, USA

<sup>2</sup> Coeur d'Alene Nursery, USDA Forest Service, Coeur d'Alene, ID 83814, USA

<sup>3</sup> Department of Agronomy and Natural Resources, Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel

<sup>4</sup> Faculty of Agriculture, Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

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**Summary** Greenhouse-cultured, container-grown seedlings of Aleppo pine (*Pinus halepensis* Mill.), radiata pine (*Pinus radiata* D. Don), and interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) were cold acclimated and deacclimated in growth chambers over 24 weeks. Needle and root cold hardiness and root growth potential (RGP) were measured weekly. Root, needle and stem analyses for soluble sugars and starch were performed biweekly. In all tissues, there was a close correspondence between cold hardiness and the absolute concentration of soluble sugars, as well as between the increase and decrease in concentration of soluble sugars during cold hardening and dehardening, respectively, supporting the theory that soluble sugars function as cryoprotectants in plant tissues. The magnitude of starch concentration did not parallel the magnitude of the cold hardiness attained, and changes in starch concentration were related to production and consumption factors, rather than timing of changes in cold hardiness. The rise and fall of RGP paralleled the rise and fall of total carbohydrate concentration in roots. The behavior of the three species was surprisingly similar, considering the different climates to which they are adapted.

**Keywords:** acclimation, Aleppo pine, cold hardiness, cryoprotectants, Douglas-fir, radiata pine, soluble carbohydrates.

### Introduction

Survival and field performance of nursery-grown tree seedlings depend on their ability to resist damage from environmental stresses such as cold, drought and mechanical handling, and on their ability to establish root contact with the surrounding soil quickly. The tree nursery industry has long recognized the need for rapid and accurate measurement of seedling quality, but over the last 15 years only a few tests have become operational tools, such as those that measure root growth potential (RGP) (Ritchie and Dunlap 1980) and cold hardiness (Glerum 1985). These tests are based on assump-

tions that must be kept in mind when interpreting the results, and each test gives only partial information. There remains a need to better understand the mechanisms behind these tests, as well as the limitations associated with each test's ability to measure the condition of seedlings and their readiness for lifting, storage and outplanting.

Root growth potential is greatly affected by season and the cultural practices applied during growth at the nursery, but exactly how these affect RGP is not clear (Krueger and Trappe 1967, Cannell et al. 1990). The initiation of new roots in tree seedlings appears to depend primarily on a stimulus originating in the shoot that is translocated in the phloem. In Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco), the source of stimulus for spring root growth appears to be the foliage rather than the buds or cambium, suggesting the involvement of carbohydrates (Krueger and Trappe 1967, van den Driessche 1987). New root growth, which is an energy-requiring process, uses either current photosynthate or starch reserves (Ritchie and Dunlap 1980). Elongating roots of Aleppo pine (*Pinus halepensis* Mill.) seedlings compete vigorously for soluble carbohydrates (Atzmon et al. 1994), suggesting that carbohydrate availability is an important factor in determining root growth.

However, it is probable that other endogenous factors also affect RGP (Kramer and Kozlowski 1979, Coleman et al. 1992). For example, immediately after removal of a ring of bark at the root collar, Douglas-fir root growth stops, but carbohydrate reserves in the roots remain relatively high, indicating that these reserves alone do not control root growth activity (Krueger and Trappe 1967). Rose (1992) found no relationship between percent starch in roots of loblolly pine (*Pinus taeda* L.) and RGP, although seedlings that produced new roots tended to have more starch in the whole plant than seedlings that produced no roots.

Accumulation of soluble sugars is known to serve as a cryoprotectant mechanism by which plants avoid protein denaturation and the membrane disruption caused by dehydra-

tion during ice formation (Parker 1963, Alden and Hermann 1971, Levitt 1980, Guy 1990, Lin et al. 1990). Therefore, it is not surprising that a positive relationship has been found between cold hardiness and RGP (Burr et al. 1989).

The objectives of this study were to quantify relationships among RGP, cold hardiness, and starch and soluble sugar concentrations in three conifers. A detailed knowledge of these relationships will improve our understanding of dormant-phase tree physiology and perhaps lead to new and improved diagnostic tests for determining seedling condition. This is not a new idea (Sutinen 1985), but the wide variation in cold hardiness behavior among species reported in the literature shows that there is still much to learn about the physiological processes underlying cold hardening and dehardening.

### Materials and methods

Seedlings of Aleppo pine (Mount Carmel, Israel, 32°46' N, 35°0' E, elevation 350 m), radiata pine (*Pinus radiata* D. Don) (Mendocino County, CA, 39°10' N, 123°12' W, elevation 100 m), and interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) (Blue Ridge District, Coconino National Forest, AZ, 34°37' N, 111°15' W, elevation 2134 m) were grown in a greenhouse in 400-ml Roottrainer (Spencer-Lemaire Industries, Edmonton, Canada) containers filled with a 1:1 (v/v) mix of peat and coarse grade vermiculite (Forestry Mix #1, Black Gold, Inc., Hubbard, OR). Sowing dates were staggered to achieve similar plant heights (35–45 cm) among species: Douglas-fir seedlings were grown for 9.0 months (sown April 5, 1993), Aleppo pine for 8.5 months (sown April 14, 1993), and radiata pine for 8.0 months (sown May 6, 1993). Greenhouse day/night temperatures averaged 25/19 °C ( $\pm 2$  °C), and intermittent sodium arc lighting (5–7  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the top of the plants, 24 s  $\text{min}^{-1}$ ) from 2000 to 0500 h extended the daylength to 24 h. Seedlings were watered as needed with a high-N, complete nutrient solution, as described in Table 1, for the deacclimation growth chamber stages. Seedlings were graded during the last month of growth in the greenhouse.

On December 28, 1993, seedlings of uniform shoot height and form were placed in Percival HL-60 growth chambers (Percival, Boone, IA) beginning a five-stage, 24-week cold ac-

climation and deacclimation regime (Table 1). Sodium and multivapor arc lights provided irradiance, and seedlings were watered as needed with a complete nutrient solution. A sample of 16 seedlings of each species was taken at weekly intervals to test root and needle cold hardiness and RGP. At biweekly intervals, we took root, needle and stem (plus bud for Douglas-fir only) samples for carbohydrate analyses. To permit direct comparisons among the cold hardiness test dates, each sample was randomly divided into four replications of four seedlings, and each replication was independently measured in each test.

### Freeze-induced electrolyte leakage (FIEL)

Cold hardiness of needles was measured by FIEL procedures similar to those described by Burr et al. (1990). A few needles were removed from the upper one-third of the central axis from each of the 16 trees in a sample. Needle segments, 1 cm long and cut at both ends from the middle of needles, were pooled within each replication. The segments were washed in deionized water and transferred, in random groups of 10, to 16  $\times$  125-mm culture tubes containing 0.5 ml deionized water. Six or eight tubes were prepared per replication on odd or even numbered weeks, respectively. Four tubes (one per replication) were stoppered and placed in a refrigerator set at 2 °C to serve as the undamaged controls. Treatment tubes were placed in a 64-liter Forma Scientific (Mallinckrodt, Inc., Marietta, OH) ethanol bath set at –2 °C. After 30 min, the water in the treatment tubes was nucleated with –80 °C No. 8 lead shot. The tubes were then stoppered and the ethanol bath was cooled at 5 °C  $\text{h}^{-1}$ .

Four treatment tubes (one per replication) were removed from the ethanol bath to thaw in a 2 °C water bath at each of five (odd numbered weeks) or seven (even numbered weeks) test temperatures, selected to span 20–80% indices of injury (Flint et al. 1967). After the contents of each tube thawed, 5.5 ml deionized water was added, and the tubes were placed in a 100-rpm shaker at room temperature (24–28 °C) for 18–20 h. After incubation, conductivity of the solution in each tube was measured with a YSI Model 34 conductance meter (YSI, Yellow Springs, OH), equipped with a microcel (YSI 3403) and temperature probe (YSI 3220) with automatic

Table 1. Growth chamber conditions for cold acclimation and deacclimation. Abbreviations: AP, Aleppo pine; RP, radiata pine; and DF, Douglas-fir.

Period	Duration (day no.)	Day/night temperature (°C)	Photoperiod (h)	Irradiance ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )			Nutrient regime (ppm)		
				AP	RP	DF	N	P	K
<i>Acclimation</i>									
I	0–28	20/15	8	800	800	600	20	86	151
II	29–70	10/3	8	600	600	450	20	86	151
III	71–112	3/1	8	450	450	350	20	86	151
<i>Deacclimation</i>									
IV	113–140	10/3	12	600	600	450	220	36	151
V	141–168	22/17	16	800	800	600	220	36	151

correction of conductivity for 25 °C. All tubes were then placed in a water bath (set at 92 °C) for 20 min, and after another 18–20-h incubation on the shaker, conductivity was remeasured.

The FIEL procedures for roots differed from those for needles only with respect to tissue sampling (cf. McKay 1992, McKay and White 1997). Two of the four seedlings per replication were randomly selected. Their root systems were washed free of growing medium with 20 °C tap water. From the selected seedlings, four 10-cm-long root sections were taken from the middle of each root system, excluding root tips and large taproots. These were rinsed in deionized water and combined within each of the four replications. From the 10-cm root sections, 1-cm-long root segments were cut, rinsed in deionized water, and transferred in random groups of eight to culture tubes containing 0.5 ml deionized water. Each week, six tubes were prepared per replication. The control and treatment tubes containing root segments were then handled as described for the tubes containing needle segments.

The FIEL results for both needles and roots were calculated as an index of injury based on the conductivity data taken before and after boiling (Flint et al. 1967). Each treatment tube yielded one observation for a total in each set of 20, 24 or 28 (four replications of five, six or seven test temperatures). A Weibull sigmoid model (Ratkowsky 1990) or a linear model, as appropriate, was fitted to each set of observations. The temperature causing 50% index of injury ( $T_{50}$ ), with 95% calibration (Weibull models) or confidence (linear models) limits, was estimated from each model. The model chosen to represent the data was the one with the smallest confidence interval. If the difference between them was small, and the sigmoid shape of the data was strong, the sigmoid model was chosen (Chambers et al. 1983). Differences among  $T_{50}$  estimates within species were determined by the test of non-overlapping 95% limits (Jones 1984).

#### *Root growth potential (RGP)*

The two seedlings per replication that were not used for FIEL root testing were placed in a greenhouse with their root systems suspended through the cover of an aeroponic mist chamber (Rietveld and Tinus 1987). Each chamber comprised a 0.6-m<sup>3</sup> chest freezer containing a 20-cm-deep reservoir of water. The water was heated by two 250-W aquarium heaters, and supplied intermittent mist that kept the roots moist and heated the air surrounding the roots. The combined heating and cooling capacity of each chamber enabled the root zone air temperature to be maintained at 20 °C. Three chambers were used, one per species. The chambers were set to provide conditions similar to those in the greenhouse. Mean day/night temperature was 25/19 °C ± 2 °C, with daylength extended to 24 h with intermittent sodium arc lighting from 2000 to 0500 h (5–7  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the top of the plants, 24  $\text{s min}^{-1}$ ).

Root growth potential was determined as the total number of new roots per seedling  $\geq 0.5$  cm long after 13 days in the mist chamber (Burr et al. 1989). Homogeneity of variances was rejected within each species ( $P \leq 0.005$ ) based on Bart-

lett's test (Milliken and Johnson 1984). Welch's test was used to compare all RGP means within a species. All hypotheses of equal means were rejected ( $P < 0.0001$ ). Major differences between weekly means ( $n = 8$ ) within species were expressed by the test of non-overlapping 95% confidence intervals (Jones 1984).

#### *Determination of soluble sugar and starch*

On even-numbered weeks, four of the seedlings used for FIEL root testing were sampled, one from each of the four replications, for carbohydrate analyses. After FIEL sampling, each plant was separated into remaining roots, foliage and stem, which included buds on Douglas-fir. Plant parts were placed in paper bags and oven dried for 48 h at 80 °C. Once dried, the paper bags were taped closed and sealed in airtight plastic bags.

One hundred mg of finely ground tissue was put in a small bag made of Whatman No. 1 filter paper and extracted with 12 ml of 80% ethanol for 30 min at 80 °C, with constant shaking. The supernatant was collected, and the extraction was repeated three additional times. The ethanol was evaporated from the pooled supernatants at room temperature in a fume hood. The volume of the extract was brought to approximately 10 ml with distilled water. Two hundred mg of activated charcoal (Dacro G 60, Fluka, Milwaukee, WI) (Ebell 1969) was added, and the extract was mixed gently. After 10 min with occasional mixing, the extract was centrifuged at 10,000 g for 15 min. The supernatant was collected and adjusted to 12 ml with distilled water. Soluble sugars were determined with anthrone reagent (Hassid and Neufeld 1964).

Starch was determined according to Thivent et al. (1972). The filter paper bag containing insoluble residue was dried at 60 °C to evaporate the ethanol. The residue was suspended in 6 ml of double-distilled water and boiled for 10 min. The suspension was then autoclaved for 1 h. To the cooled suspension were added 0.5 ml of 2 M sodium acetate buffer, pH 4.8, and 1 ml of double-distilled water containing 5 mg of amyloglucosidase (Sigma Chemical Co., St. Louis, MO). The volume was brought to 10 ml with double-distilled water, and the suspension was incubated for 24 h at 55 °C, with constant shaking. The suspension was filtered through Whatman No. 1 filter paper, and the residue was washed twice with 2.5 ml of double-distilled water. The volume of the solution was brought to 15 ml. The glucose released by the amyloglucosidase was measured with anthrone reagent. For each determination a mean and standard error were calculated.

## **Results**

### *Douglas-fir*

The growth chamber conditions for the five-stage cold acclimation and deacclimation regime are summarized in Table 1. The seedlings were exposed to cold hardening conditions during Periods II and III. Following Period III, which was the coldest period, the seedlings were subjected to dehardening conditions during Periods IV and V. Needles of Douglas-fir

cold hardened and dehardened in response to changes in the sequence of temperature and photoperiod regimes (Figure 1, Table 1). Douglas-fir roots hardened from  $-5$  to  $-15$  °C, which is quite substantial, but considerably less than the hardening of the shoots ( $-33$  °C; Figure 2).

The RGP of Douglas-fir declined from 38 to about seven new roots per seedling during Period I, and then increased steadily during cold acclimation, at a rate of about 0.6 roots per seedling per day ( $r^2 = 0.79$ ), to about 80 by the end of the coldest period (Period III) (Figure 3). The RGP remained high for most of Period IV, while cold hardiness decreased, but RGP plummeted to a very low value at bud break. For all three species, but especially for Douglas-fir, the major difference between changes in cold hardiness and changes in RGP was the 2–3 week lag in RGP response to the dehardening conditions of Period IV. Cold dehardening began during the second week of Period IV, whereas the decline in RGP was not evident until the fourth week of Period IV.

During Period I, soluble sugars, which made up 10% ( $100 \text{ mg g}_{\text{DW}}^{-1}$ ) of the dry weight of Douglas-fir needles and about 4% ( $40 \text{ mg g}_{\text{DW}}^{-1}$ ) of the dry weight of its stems and roots (Figure 4), did not change in any of the tissues. During Periods II and III, the concentration of sugar in needles rose sharply to  $250 \text{ mg g}_{\text{DW}}^{-1}$ , and less sharply in stems and roots to 60 and  $80 \text{ mg g}_{\text{DW}}^{-1}$ , respectively. Concurrent with cold deacclimation of the seedlings during Period IV, but before the decline in RGP, concentrations of sugars declined rapidly in all tissues, reaching values similar to those found during Period I. As bud break occurred during Period V, sugar concentrations continued to fall.

During Period I, starch concentrations did not change.

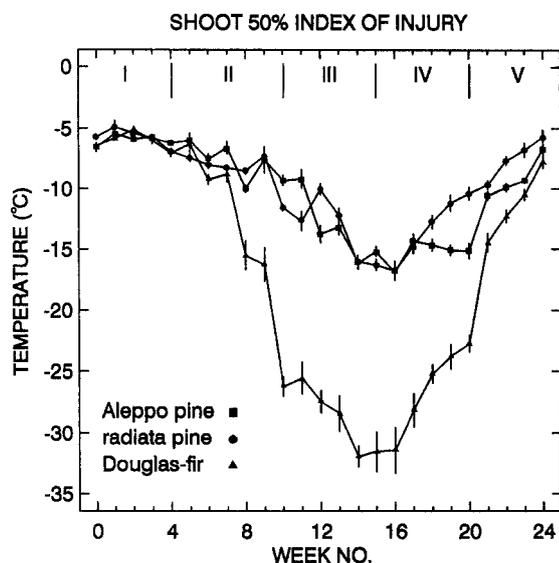


Figure 1. Cold hardiness of Aleppo pine, radiata pine, and Douglas-fir shoots measured by freeze-induced electrolyte leakage of needles during a 24-week acclimation and deacclimation regime in growth chambers. Environmental conditions for the five periods are given in Table 1. Error bars are 95% confidence limits.

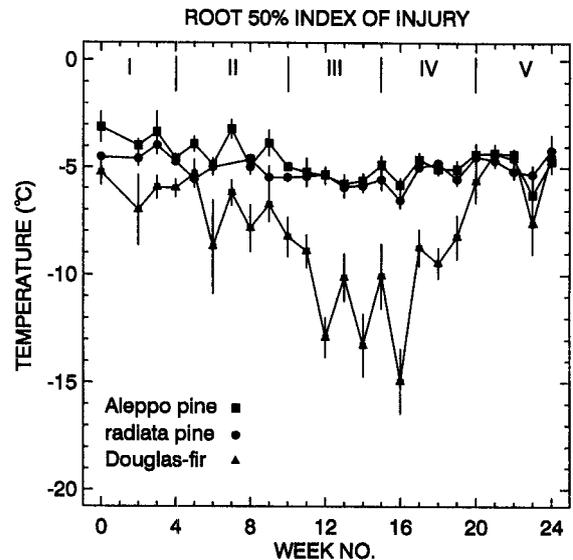


Figure 2. Cold hardiness of roots of Aleppo pine, radiata pine, and Douglas-fir measured by freeze-induced electrolyte leakage during a 24-week acclimation and deacclimation regime in growth chambers. Environmental conditions for the five periods are given in Table 1. Error bars are 95% confidence limits.

Starch concentration in needles rose from 30 to  $70 \text{ mg g}_{\text{DW}}^{-1}$  during Period II, and fell back to  $30 \text{ mg g}_{\text{DW}}^{-1}$  during the coldest period (Period III). Starch concentration in roots rose from 40 to  $110 \text{ mg g}_{\text{DW}}^{-1}$  by the end of Period II, and then declined to  $70 \text{ mg g}_{\text{DW}}^{-1}$  during Period III. There was no significant change in stem starch concentration during Periods I–III. Corresponding closely with the onset of deacclimation during Period IV, starch concentrations dramatically increased from 30 to  $150 \text{ mg g}_{\text{DW}}^{-1}$  in needles, from 40 to  $170 \text{ mg g}_{\text{DW}}^{-1}$  in stems, and from 70 to  $140 \text{ mg g}_{\text{DW}}^{-1}$  in roots. As bud break approached in the final warm period (Period V), the concentration of starch declined again in all tissues, but the proportional decline in needles was less than in stems and roots.

Total starch plus sugar concentration in Douglas-fir needles did not change during Period I, but rose rapidly in Period II and continued to rise at a slower rate through Periods III and IV, before declining during Period V. Total starch plus sugar concentration in Douglas-fir roots doubled in concentration in Period II and remained high throughout Periods III and IV, coinciding with an increase in RGP from about 8 to more than 70 roots per seedling.

#### Aleppo pine and radiata pine

Needles of Aleppo and radiata pine cold hardened very slowly at first, but the rate increased with decreasing temperatures, reaching a 50% index of injury of about  $-17$  °C by the end of Period III (Figure 1). Radiata pine began deacclimating within 1 week after the onset of Period IV, and continued to deacclimate steadily to a minimum hardiness of  $-5$  °C. In contrast, Aleppo pine retained its cold hardiness until the onset of high

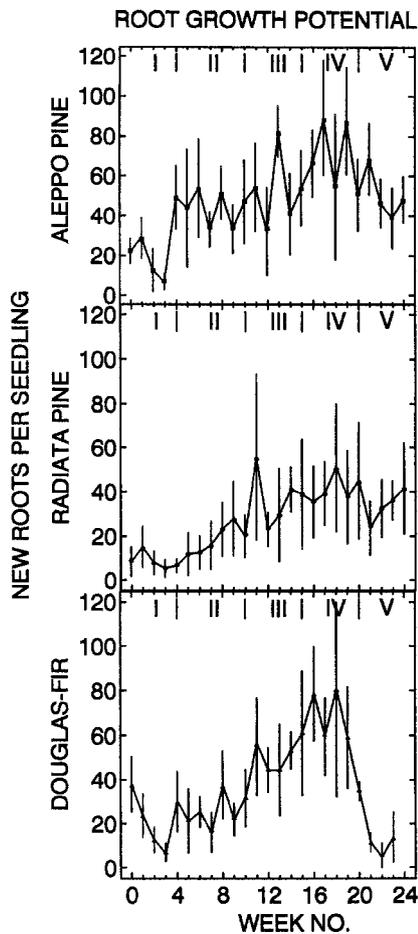


Figure 3. Root growth potential of Aleppo pine, radiata pine and Douglas-fir during a 24-week acclimation and deacclimation regime in growth chambers. Environmental conditions for the five periods are given in Table 1. Error bars are 95% confidence limits.

temperatures in Period V.

Radiata pine roots hardened by only 2 °C, and Aleppo pine roots did not harden at all. A regression analysis of cold hardiness versus time showed no significant slope or curvature for Aleppo pine hardiness and only a small slope and curvature for radiata pine.

The RGP of radiata pine, which was about eight new roots per seedling during Period I, tended to increase throughout the study period at a rate of about 0.4 new roots per seedling per day ( $r^2 = 0.89$ ) (Figure 3); however, the data were variable. Radiata pine showed no significant decline in RGP as bud break approached. In Aleppo pine, RGP declined from about 20 to about seven new roots per seedling in the first 3 weeks of Period I, increased to 30–50 new roots per seedling during Period II, peaked at around 80 new roots per seedling during Period IV, and then declined to around 45 roots per seedling as bud break approached.

In Aleppo pine needles, sugar and starch concentrations did not change during Period I (Figure 5). In Periods II and III,

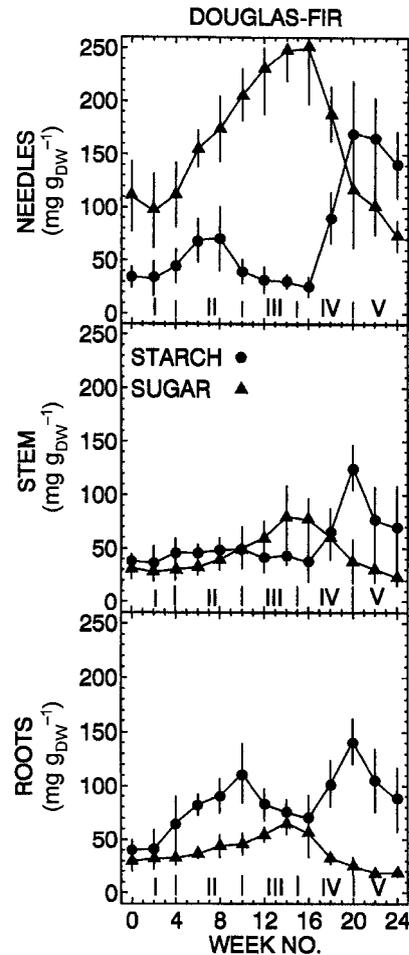


Figure 4. Starch and soluble sugar concentrations in Douglas-fir needles, stem and root tissues during a 24-week acclimation and deacclimation regime in growth chambers. Environmental conditions for the five periods are given in Table 1. Error bars are 95% confidence limits.

sugar concentration rose from 40 to 120 mg  $g_{DW}^{-1}$ , and then declined to 30 mg  $g_{DW}^{-1}$  in Periods IV and V. Soluble sugar concentration began falling early in Period IV. Starch concentration rose from 40 to 120 mg  $g_{DW}^{-1}$  in Period II, fell back to 40 mg  $g_{DW}^{-1}$  in Period III, rose again to 80 mg  $g_{DW}^{-1}$  in Period IV, and remained more or less constant in Period V. Trends in Aleppo pine stems were similar to the trends in needles, but were less pronounced. Total starch plus sugar concentration was low and did not change during Period I, rose rapidly in Period II, remained high through Periods III and IV, and then fell to low levels in Period V.

In Aleppo pine roots, sugar concentration was low but rose significantly (from 20 to 40 mg  $g_{DW}^{-1}$ ) in response to the cold treatments in Periods II and III, and then gradually declined to 20 mg  $g_{DW}^{-1}$  in Periods IV and V (Figure 5). Starch concentration was low and did not change in Period I, but increased from 30 to 80 mg  $g_{DW}^{-1}$  in Period II, remained more or less constant through Period III, rose to 140 mg  $g_{DW}^{-1}$  in Period IV, and then

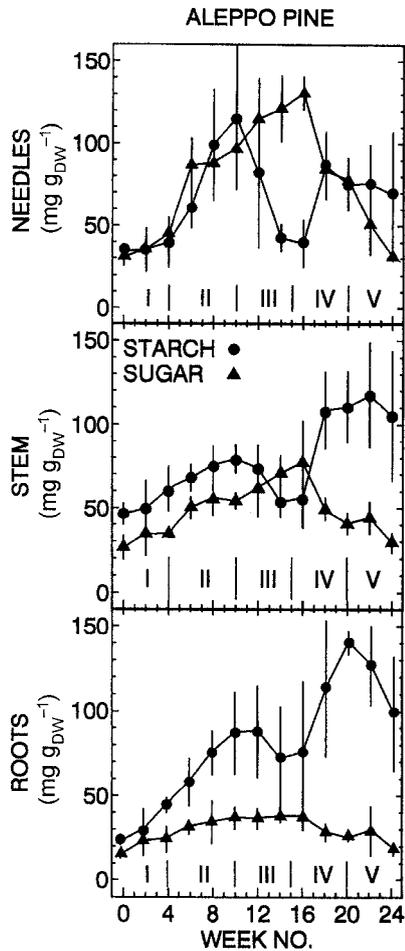


Figure 5. Starch and soluble sugar concentrations in Aleppo pine needles, stem and root tissue during a 24-week acclimation and deacclimation regime in growth chambers. Environmental conditions for the five periods are given in Table 1. Error bars are 95% confidence limits.

fell to  $100 \text{ mg g}_{\text{DW}}^{-1}$  in Period V. Total starch plus sugar concentration in roots rose steadily through Periods I, II and IV, with a pause during the coldest period (III), and began to fall in Period V. This coincided with a rise in RGP from about 16 to over 70 new roots per seedling in Week 18, followed by a decline to about 44 new roots per seedling by the end of Period V.

With some exceptions, trends in starch and sugar concentrations in radiata pine were similar to those in Aleppo pine. Radiata pine stems contained less sugar and much less starch than Aleppo pine stems (Figure 6). All three tissues of radiata pine showed a precipitous decline in starch during Period V, as was observed in Douglas-fir, whereas starch concentrations in Aleppo pine remained more or less constant during Period V.

During acclimation, there was a strong relationship ( $r^2 = 0.90$ ) between the magnitude of increase in soluble sugars and the magnitude of increase in cold hardiness in roots and needles of all three species (Table 2).

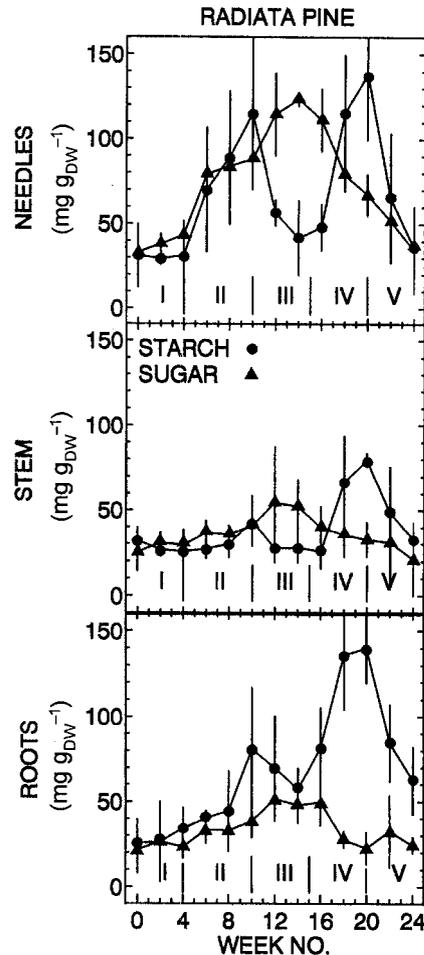


Figure 6. Starch and soluble sugar concentrations in radiata pine needles, stem and root tissues during a 24-week acclimation and deacclimation regime in growth chambers. Environmental conditions for the five periods are given in Table 1. Error bars are 95% confidence limits.

## Discussion

In Douglas-fir, patterns of RGP and cold acclimation and deacclimation were as expected for a species and ecotype native to climates with cold winters and frozen soil most of the winter. Rates of hardening and dehardening were quite linear and would lend themselves to creation of predictive models such as those developed by Tinus (1996) and Leinonen et al. (1995). The effects of photoperiod on cold acclimation and deacclimation were also quite apparent. Periods II and IV had the same day/night temperatures ( $10/3 \text{ }^\circ\text{C}$ ), but the 8-h day in Period II caused hardening, whereas the 12-h day in Period IV caused dehardening. However, the effect of photoperiod may also be influenced by where the trees are in the hardening–dehardening cycle (Leinonen et al. 1995). We found that the rate of hardening of Douglas-fir at the lowest temperature regime ( $3/1 \text{ }^\circ\text{C}$ ) was less than the rate at a day/night temperature of  $10/3 \text{ }^\circ\text{C}$ . Similar observations have been made in trees

Table 2. Relationship between increase in soluble sugar concentration and increase in cold hardiness during cold acclimation.

Tissue	Sugar gain (mg g <sub>DW</sub> <sup>-1</sup> )	Cold hardiness gain (°C)
Aleppo pine roots	24	1
Radiata pine roots	30	2
Douglas-fir roots	47	11
Radiata pine needles	91	12
Aleppo pine needles	99	12
Douglas-fir needles	154	27

approaching maximum hardiness (Leinonen et al. 1995). We have also observed spontaneous dehardening and rehardening on a week to week basis under constant conditions in trees approaching maximum hardiness (Tinus 1996), as seen here in Douglas-fir roots (Figure 2).

Radiata pine and Aleppo pine, which are both native to mild Mediterranean climates, responded similarly to the cold acclimation and deacclimation regimes. The differences observed between the two species may be associated with the fact that radiata pine is from a cool Mediterranean climate, whereas Aleppo pine is from a warm Mediterranean climate. Overall, the behavior of the three species was remarkably similar; differences were quantitative rather than qualitative. There were two main findings. First, between Periods I and III, root soluble sugar concentration doubled in all three species. Second, during Period III, the soluble sugar concentration in roots directly paralleled the magnitude of changes in root cold hardiness.

The dynamics of starch and sugar concentrations in Aleppo and radiata pines were similar to those found in Douglas-fir, except starch plus sugar concentrations in Douglas-fir were always substantially higher. This difference is presumably associated with the finding that Douglas-fir needles hardened significantly more than Aleppo pine or radiata pine needles. Patterns of sugar accumulation during acclimation, conversion to starch during early deacclimation, and the decline of both sugar and starch concentrations during late deacclimation were consistent among species and tissues. Our results are similar to those found in coastal Douglas-fir (Krueger and Trappe 1967). Although several species of tree seedlings have been shown to accumulate carbohydrates during hardening (Krueger and Trappe 1967, Leborgne et al. 1995), Cannell et al. (1990) found little change in total nonstructural carbohydrates in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) or Douglas-fir seedlings in the nursery beds from August through March. The reason for this discrepancy is not clear. It may reflect different opportunities for photosynthesis during the dormant season. Alternatively, the differences may have been masked because sugars and starch were not measured separately in the different plant organs. Ögren (1997) and Ögren et al. (1997) found a close relationship between loss of sugars and loss of cold hardiness in roots and needles of stored seedlings of Scots pine (*Pinus sylvestris* L.), lodgepole pine (*Pinus*

*contorta* Dougl.) and Norway spruce (*Picea abies* L.). Likewise, we have shown that, during acclimation, the extent of cold hardiness closely follows the increase in sugar concentration in both roots and needles of Douglas-fir, Aleppo pine and radiata pine (Table 2).

The relationship between cold hardiness and starch and sugar concentration of roots and needles may be explained by considering that the amount of starch and sugar present represents a balance between the rate of photosynthesis, consumption by respiration, and export to parts of the seedling that are growing. As buds were set and the shoot entered dormancy, the demand for photosynthate slowed; however, during Period II, temperatures were still high enough to permit photosynthesis, and so carbohydrates rapidly accumulated. As temperatures decreased during Period III, growth stopped and photosynthesis slowed. During Period V, temperature increased, growth resumed, and demand for photosynthate throughout the plant accelerated, leading to a reduction in concentrations of starch and sugars in needles. Because starch and sugar are readily interconvertible, the proportion that is present in plant tissues as sugar during acclimation and deacclimation is probably a function of the need for cryoprotection. In Douglas-fir in particular, the rapid rise in needle sugar concentration during Period II coincided with a rapid increase in needle cold hardiness. During deacclimation in Periods IV and V, the decline in sugar concentration in Douglas-fir needles coincided with loss of cold hardiness. The same was true of Douglas-fir roots, but the magnitude of the change was much less, both in cold hardiness and sugar concentrations. Abundant carbohydrate supply is a necessary but perhaps not sufficient condition for root growth. As bud break approaches, changes in source-sink relations may direct root carbohydrates to sinks other than root elongation, which may explain why RGP began to decline during Period IV, while carbohydrate concentrations were still high.

In conclusion, there was a close correspondence between concentrations of sugars and starch, RGP and cold hardiness of needles and roots in three species of conifers from different climates. Thus, with adequate calibration and field verification, measurement of starch and sugar concentrations in roots and foliage could provide another rapid test for seedling physiological condition that would aid nursery managers in determining when seedlings are ready for lifting and storage, the effect of storage, and potential performance when outplanted.

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