Frost hardening of *Pinus radiata* seedlings: effects of temperature on relative growth rate, carbon balance and carbohydrate concentration

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Summary  *Pinus radiata* (D. Don) seedlings were grown for 100 days at day/night temperatures of 10/1, 15/1, 20/1 and 25/1 °C, to determine whether temperatures above a threshold of 5 °C influence frost hardiness development. Relationships between hardening and relative growth rate, carbohydrate concentration and net carbon balance were also investigated. Seedlings hardened at a nearly constant rate in each treatment, although the rate of hardening was strongly temperature dependent. It increased as the temperature declined, but in a curvilinear fashion. Temperatures below 9.5 °C were effective in hardening the seedlings. During the daily temperature cycle, dehardening occurred at temperatures above the threshold, whereas hardening occurred at temperatures below the threshold. The net difference between the two processes determined the development of frost hardiness. The development of frost hardiness was negatively correlated with relative growth rate and positively correlated with the accumulation of starch and sugars. We conclude that frost hardening is a complex process that is causally linked to carbohydrate concentrations.

Keywords: carbohydrates, hardness, hardening rate, modeling.

Introduction

In most plant species, development of frost hardiness is environmentally controlled (Weiser 1970, Aronsson 1975, Greer and Warrington 1982, Gay and Eagles 1991) with photoperiod controlling the first phase of hardening and low temperature below some threshold controlling a second, deeper phase of hardening. Although there is much information on the effects of short photoperiods and low temperatures on frost hardening (Christersson 1978, Smit-Spinks et al. 1985, Bigras and D’Aoust 1993), the information is largely qualitative in nature. No attempt has been made to identify the underlying processes that regulate the development of frost hardiness. Knowledge of the quantitative relationships between the frost hardening process and the major environmental factors involved is also needed to develop models for simulating seasonal changes in frost hardiness (Kobayashi et al. 1983, Anişko et al. 1994, Timmis et al. 1994, Leinonen 1996).

*Pinus radiata* (D. Don) represents the main species in production forestry in New Zealand. Because many plantations are on upland areas, seedlings are often subjected to early and late-season frosts (Menzies and Chavasse 1982). Early frosts in autumn and late frosts in spring have destroyed thousands of hectares of seedlings (B. Shui, Fletcher Challenge Forests, Whakatane, New Zealand, pers. comm.) with high economic costs. For this reason, predictive models that enable assessment of frost-risk at plantation sites will improve site selection and potentially reduce the costs of plantation establishment.

Based on earlier studies of the environmental control of the frost hardening process in *Pinus radiata* seedlings, we know that an early phase of hardening in autumn is controlled by photoperiod (Greer and Stanley 1985, Greer et al. 1989). A second phase of hardening, which occurs in late autumn, is regulated by temperatures below a threshold of about 5 °C. Subsequently, the rate of hardening increases as temperatures decrease to at least to −4 °C (Greer and Warrington 1982, Greer 1983). A simple model of the relationship between temperature and rate of hardening was derived by Greer (1983). An important assumption of this model was that, at temperatures above the threshold of 5 °C, the rate of hardening was zero; however, this assumption has not been tested for *Pinus radiata*, and rarely so in other species.

Ögren et al. (1997) reported that *Picea abies* (L.) Karst. seedlings with high concentrations of soluble sugars and low rates of respiration were more frost tolerant than comparable seedlings of *Pinus sylvestris* L. and *P. contorta* Dougl. ex Loud. A general relationship between carbohydrate concentration and frost hardiness has been established in several studies (e.g., Sakai and Yoshida 1968, Aronsson et al. 1976, Chen and Li 1980, O’Neill 1983, Ögren et al. 1997). If the accumulation of carbohydrates during autumn is a prerequisite for development of frost hardiness, it should be dependent on acquisition of carbon, demand by growth and respiration and carbohydrate accumulation. However, the effects of net photosynthesis, respiration, carbohydrate concentration and growth rates on the development of frost hardiness in *Pinus radiata* have never been assessed.

The objectives of this study were to quantify the response of the hardening process in *Pinus radiata* seedlings to different
daytime temperatures and to assess relationships between frost hardening and growth, carbon gain and carbohydrate concentration during these temperature treatments.

Materials and methods

Plant material

Eight hundred 1.5-year-old Pinus radiata seedlings were grown in 1.25-liter pots containing a gravel/pumice/peat mixture (33:33:33 v/v) with added fertilizer (3–4 month Osmocote, 8–9 month Osmocote, Dolomite lime, Superphosphate with an N,P,K composition of 18,4,5,8,5 (Mintech NZ Ltd., Auckland, New Zealand) and trace elements (Micromax, Sierra Chemicals Europe, Heerlen, Netherlands). The plants were maintained in an outdoor shelter for approximately 6 months before the experiment and were watered daily.

Environmental treatment conditions

In late January, 192 actively growing seedlings were transferred to each of four controlled-environment (CE) rooms located at the HortResearch National Climate Laboratory, Palmerston North, New Zealand (Robotham et al. 1978). The CE rooms provided daytime temperatures of 25, 20, 15 or 10 ± 0.5 °C with a daytime VPD of 0.6 ± 0.05 kPa and a uniform night temperature of 1.0 ± 0.5 °C with a nighttime VPD of 0.2 ± 0.05 kPa. The thermoperiod was 9 h in the high-temperature treatment and 11 h in the low-temperature treatment and day/night changeovers were both 2 h in duration. A 9-h photoperiod was provided in each CE room by a water-screened array of four 1-kW high-pressure discharge (Metalac, Sylvania, Drummondville, Quebec, Canada) lamps and four 1-kW tungsten iodide (Halogen, Thorn, Enfield, U.K.) lamps, giving a photon flux density (PFD) of 700 µmol m⁻² s⁻¹ at the plant level. Seedlings were automatically irrigated three times daily.

To measure frost hardness of the seedlings at the start of the experiment, eight seedlings were exposed to each of three frosts at −2, −4 and −6 ± 0.5 °C. Seedlings were transferred in late afternoon to a low-temperature CE room set at 12 °C with a 9-h photoperiod at an irradiance of 8 µmol m⁻² s⁻¹. The pots were placed in polystyrene blocks on trolleys. The polystyrene blocks were 100-mm deep with circular holes cut to fit the pots closely. Each pot was covered with two semicircular sections of 10-mm polystyrene to provide additional root-zone protection. The air temperature was then reduced at midnight in the dark to the test temperature over 6 h, held at the test temperature for 6 h and then increased over 4 h back to 12 °C. Root-zone temperature was maintained and controlled at 5 °C throughout the frost treatment by means of 100-W heating element in the base of each trolley. Rates of cooling ranged between 3 and 5 °C h⁻¹.

Throughout the experiment, frost hardness evaluations were carried out at approximately 10-day intervals as described by Greer and Warrington (1982) and Greer (1983), with temperatures ranging from −3 to −16 °C. For each treatment, seven seedlings were exposed to each of three frosts, usually at 2 °C increments and at progressively lower temperatures as the experiment proceeded.

Following each frost, seedlings were placed in an outdoor shelter and visible frost damage to the needles was assessed after 4 to 6 weeks. Needle damage was measured according to the scale described by Menzies and Holden (1981): 0 = no damage, 1 = some foliage damage (< 10%), 2 = 10–30% needles damaged, 3 = 40–60% needles damaged, 4 = 70–90% needles damaged, and 5 = seedling dead. Plants were considered frost hardy to the temperature causing the equivalent of 30% foliage damage (cf. Greer and Warrington 1982).

Net photosynthesis and respiration

Daily time courses of net photosynthesis and respiration were measured on whole seedlings at approximately 20-day intervals throughout the hardening treatments. A single seedling was placed in a Plexiglas chamber (300 × 300 × 550 mm) equipped with a circulating fan and gas exchange was measured with an open gas exchange system connected to an IRGA (Binos, Leybold-Herereus, Hanau, Germany) as described by Greer (1995). All measurements were carried out at the prevailing temperature and irradiance conditions of each treatment. Following the measurements, foliage of each plant was stripped off and total leaf area measured with a leaf area meter (LI-3000, Li-Cor, Inc., Lincoln, NE). Needles and stems were then vacuum dried at 40 °C. On each occasion, two seedlings from each treatment were measured. Photosynthesis and respiration were determined on both a leaf area and shoot dry weight basis.

Net carbon balance was estimated as described by Greer and Jeffares (1998) by integrating the daily time courses for photosynthesis and respiration (dry weight basis), converting these values to grams of carbon based on the molecular fraction of C in CO₂, and then calculating the net carbon gain per day. From relative rates of shoot biomass growth and assuming about 45% (Walton and Fowke 1995) of the biomass in needles and stems was elemental carbon, the net carbon balance was determined from the difference between net carbon gain and loss as structural carbon.

Carbohydrate and growth measurements

At 20-day intervals, four plants were taken from each treatment, rapidly stripped of foliage, the stem sectioned and all stored on dry ice. These samples were then air-freighted to the laboratory at Ruakura, Hamilton and stored at −80 °C until analyzed. Each shoot was freeze-dried and weighed and a subsample taken for carbohydrate analysis. Soluble sugars, starch and sugar alcohols in each subsample were extracted with 80% ethanol and assayed by gas chromatography as described by Greer (1998). Relative growth rates of the shoots were determined by standard growth analysis procedures (Hunt 1978) from increments in dry weight for each treatment at each sample date.

Data analysis and analytical procedure

All data were subjected to analysis of variance by the general linear models in the SAS software package (1985, SAS Insti-
tute, Cary, NC). Time courses of frost hardening were analyzed by linear regression and mean rates of hardening at each temperature were determined from the regression slope. Existing data from two earlier treatments described by Greer (1983), which were similar to the experiments described here except that the day/night temperatures were 12/3 and 12/−4 °C, were included to examine the overall response of the hardening process to temperature. The combined data were used to obtain an estimate of the temperature response function for rates of hardening at the fixed 9-h photoperiod. Each treatment was described by minimum and maximum temperatures, the proportions of the day spent at each, and the proportion of time changing from one to the other temperature. We used a least-squares nonlinear regression procedure to fit a piecewise-linear model to the data to find the approximate shape of the temperature response function. A piecewise-linear model offers the flexibility of a discrete rate analysis (McNaughton et al. 1985), and at the cost of one extra parameter fits a continuous response curve instead of a histogram.

When fitting the piecewise linear model, we let the response be linear in four sections over a −4 to 25 °C temperature range, with the slope changing at 1, 8 and 15 °C.

Results

Development of frost hardiness

Before the hardening treatments, the seedlings had a mean frost hardness of −4.6 °C (Figure 1). After 40 days at 25/1 °C, frost hardness of the seedlings had increased slightly to a mean of −6 °C, but thereafter frost hardness of the seedlings in the 25/1 °C treatment remained constant. In contrast, seedlings in the 20/1, 15/1 and 10/1 °C treatments hardened to −10, −12 and −13.5 °C, respectively, after about 85 to 90 days in their respective treatments.

Table 1. Regression analyses of the time courses of development of frost hardiness in seedlings of Pinus radiata at four day/night temperature regimes.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Intercept</th>
<th>Coefficient</th>
<th>P-value</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>25/1</td>
<td>−4.9 ± 0.2</td>
<td>−0.023 ± 0.005</td>
<td>0.019</td>
<td>0.83</td>
</tr>
<tr>
<td>20/1</td>
<td>−4.6 ± 0.2</td>
<td>−0.059 ± 0.004</td>
<td>&lt; 0.001</td>
<td>0.97</td>
</tr>
<tr>
<td>15/1</td>
<td>−4.6 ± 0.4</td>
<td>−0.092 ± 0.007</td>
<td>&lt; 0.001</td>
<td>0.96</td>
</tr>
<tr>
<td>10/1</td>
<td>−4.1 ± 0.4</td>
<td>−0.113 ± 0.006</td>
<td>&lt; 0.001</td>
<td>0.97</td>
</tr>
</tbody>
</table>

For seedlings in each treatment, the shape of the hardening curve (Figure 1) was approximated by a linear increase in hardness followed by a plateau. To estimate the slope of the hardening phase, we used data to Day 40 for seedlings in the 25/1 °C treatment, data to Day 85 for seedlings in the 15/1 and 10/1 °C treatments and data to Day 100 for seedlings in the 20/1 °C treatments, respectively. Across the entire temperature range, lower daytime temperatures gave higher rates of hardening than higher daytime temperatures (Table 1).

To estimate the temperature response function for rates of hardening at a fixed 9-h photoperiod, rates of hardening at the 12/3 and 12/−4 °C temperature combinations from an earlier study (Greer 1983) were included along with the rates estimated from the four temperature combinations of the present study. The piecewise-linear temperature response function fitted to these data, along with asymptotic standard errors of the estimates at the end points and changes in slope, are shown in Figure 2. The piecewise linear temperature response showed distinct curvature. Therefore, a quadratic temperature response was fitted as shown in Figure 2. Because each treatment included a range of temperatures, we related the piece-wise linear temperature response and the quadratic temperature response to actual measurements by taking into account the relative time seedlings spent at the maximum temperature.

Figure 1. Time courses of the development of frost hardiness in seedlings of Pinus radiata grown in four temperature regimes; (○) 25/1, (■) 20/1, (▲) 15/1, (▲) 10/1. Lines are the best fit linear regressions fitted for Days 0 to 40 for seedlings in the 25/1 °C treatment, Days 0 to 92 for seedlings in the 20/1 °C treatment and Days 0 to 81 for seedlings in the 15/1 and 10/1 °C treatments. Other horizontal lines represent the mean frost hardness for the remaining period.

Figure 2. Rates of frost hardening and their asymptotic standard errors at different temperatures as calculated from a piece-wise linear model, fitted in four sections over a −4 to 25 °C temperature range, with the slope changing at 1, 8 and 15 °C. (Note these are not the treatment temperatures.) The dotted line is the quadratic model and the dashed line indicates a zero rate of hardening.
minimum, and intermediate temperatures for each treatment. A comparison of the actual and predicted rates of hardening showed larger deviation for the quadratic model (Figure 3) than for the piecewise linear model (not shown). However, the quadratic model was statistically more robust because the analysis involved fitting three parameters to six data points rather than the five parameters required for the piecewise linear model.

Independently of the fitted temperature function used, whenever the treatment temperature was above a threshold of 9.5 °C a loss of frost hardiness was indicated. This finding contrasts with the neutral effect of high temperatures on hardening that was postulated by Greer (1983). If the hardening response was neutral at temperatures above 9.5 °C, differences in the rates of hardening among the four treatments with the same temperature minimum should be small. However, a wide range in rates of hardening were observed (cf. Figure 1 and Table 1). Although seedlings in all treatments hardened to some extent, temperatures above 9.5 °C in the daily cycle reduced the hardening effect of the low temperatures.

Biomass accumulation

Shoot biomass of seedlings growing at 10/1, 15/1, 20/1 and 25/1 °C, increased 1.7-, 2.7-, 3.7- and 3.5-fold, respectively (Figure 4). Linear regressions of log dry weight with time (data not shown) were highly significant ($P = 0.001–0.006$) for seedlings in each treatment with $r^2$ values ranging between 0.32 (10/1 °C) and 0.82 (25/1 °C). The relative shoot growth rates of the seedlings showed a linear ($P = 0.04, r^2 = 0.88$) decrease between 25/1 and 10/1 °C (Table 2).

Photosynthesis and respiration

A typical example of the 24-h time course of net photosynthesis and respiration is shown in Figure 5. Photosynthetic rate was generally steady throughout the day and respiration rate responded to the changes in temperature occurring at the day/night changeovers, but otherwise was also steady. In all treatments, there were minor changes in net photosynthesis and respiration of the seedlings during the experiment (Figure 6). There were significant treatment effects on mean net photosynthetic rate, with mean rates increasing from 4.5 μmol m$^{-2}$ s$^{-1}$ in seedlings at 25/1 °C to 6.5 μmol m$^{-2}$ s$^{-1}$ in seedlings at 10/1 °C (Table 2). There were similar treatment effects on respiration rate, with the mean rate of respiration increasing from 0.66 μmol m$^{-2}$ s$^{-1}$ in seedlings at 25/1 °C to 1.5 μmol m$^{-2}$ s$^{-1}$ in seedlings at 10/1 °C.

Net carbon gain on a dry weight basis was generally similar for the seedlings in each treatment (Table 2), but because both relative shoot growth rate and rate of carbon accumulation in biomass declined with increasing temperature, net carbon balance was strongly temperature dependent. In treatments 25/1, 20/1 and 15/1 °C, the seedlings had a negative shoot carbon balance, although it was most negative at 25/1 °C and least negative at 15/1 °C. Seedlings in the 10/1 °C treatment had a positive net carbon balance.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Photosynthesis (μmol m$^{-2}$ s$^{-1}$)</th>
<th>Respiration (μmol m$^{-2}$ s$^{-1}$)</th>
<th>Carbon gain (mg C g$^{-1}$ day$^{-1}$)</th>
<th>Carbon loss (mg C g$^{-1}$ day$^{-1}$)</th>
<th>Net C gain (mg g$^{-1}$ day$^{-1}$)</th>
<th>RGR (mg gDW$^{-1}$ day$^{-1}$)</th>
<th>C skeleton cost (mg g$^{-1}$ day$^{-1}$)</th>
<th>Net C balance (mg C g$^{-1}$ day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25/1</td>
<td>4.35</td>
<td>0.61</td>
<td>4.94</td>
<td>1.21</td>
<td>3.73</td>
<td>12.6</td>
<td>5.5</td>
<td>$-1.82$</td>
</tr>
<tr>
<td>20/1</td>
<td>4.80</td>
<td>0.88</td>
<td>5.09</td>
<td>1.55</td>
<td>3.54</td>
<td>10.7</td>
<td>4.7</td>
<td>$-1.17$</td>
</tr>
<tr>
<td>15/1</td>
<td>6.53</td>
<td>1.60</td>
<td>5.79</td>
<td>2.27</td>
<td>3.53</td>
<td>9.2</td>
<td>4.1</td>
<td>$-0.52$</td>
</tr>
<tr>
<td>10/1</td>
<td>6.61</td>
<td>1.38</td>
<td>5.75</td>
<td>2.07</td>
<td>3.68</td>
<td>4.6</td>
<td>2.0</td>
<td>1.66</td>
</tr>
</tbody>
</table>

Table 2. Mean rates of photosynthesis and respiration (leaf area basis) averaged over the experiment and calculated carbon acquisition and respiratory loss, net carbon gain, accumulation of skeletal carbon, net carbon balance (dry weight basis) and relative shoot growth rate (RGR) for Pinus radiata seedlings growing in four temperature regimes.
Changes in carbohydrate status

At the beginning of the experiment, the nonstructural carbohydrates in order of decreasing concentration were: starch, glucose, fructose, sucrose, pinitol, myo-inositol and sequoyitol (Figures 7A–F). Among the nonstructural carbohydrates, the temperature treatments had the greatest effect on starch concentration (Figure 7A). In seedlings in the 10/1 °C treatment, the concentration of starch more than doubled between the start of the experiment and Day 22 and then decreased to a value below that initially present. In seedlings in the 25/1, 20/1 and 15/1 °C treatments, starch concentrations declined slowly throughout the experiment and by Day 100 the starch concentrations of seedlings in the 25/1 and 20/1 °C treatments were below those initially present. Overall, mean starch concentration declined from 67.8 ± 10 to 2.6 ± 0.6 mg g⁻¹ with the increase in daytime temperature from 10 to 25 °C.

Concentrations of total sugars changed little over time in seedlings in all treatments (Figure 7B). In response to the temperature treatments, glucose and fructose concentrations followed similar patterns and were thus combined (Figure 7C). In seedlings in the 25/1 and 20/1 °C treatments, glucose + fructose concentrations declined sharply over the first 40 days. In contrast, they declined only initially in seedlings in the 15/1 °C treatment, and no change occurred over the first 40 days in seedlings in the 10/1 °C treatment. By Day 100, however, the hexose sugar concentrations had decreased to about half the initial concentration in seedlings in all treatments. When averaged over the whole experiment, mean concentration of hexose sugars (fructose + glucose) in seedlings declined from 57.6 ± 4.4 mg g⁻¹ to 31.2 ± 3.3 mg g⁻¹ as the temperature increased from 10 to 25 °C.

Among the seedlings in the four treatments, sucrose concentrations showed generally similar changes throughout the experiment (Figure 7D). Sucrose increased from an initial concentration of 4.0 ± 0.6 mg g⁻¹ to mean concentrations (mean for Days 22 to 100) of 19.6 and 23.6 ± 3.2 mg g⁻¹ in seedlings in the 10/1 and 25/1 °C treatments, respectively. In all treated seedlings throughout the experiment, myo-inositol was present at concentrations of 4 to 5.5 mg g⁻¹ (data not shown). Changes during the experiment in the concentrations of the two main sugar alcohols, pinitol and sequoyitol are shown in Figures 7E and 7F. Concentrations of pinitol were generally similar for seedlings in all treatments, although seedlings in the 10/1 °C treatment showed a small increase in pinitol concentration from Days 40 to 100. During the experiment, sequoyitol concentrations, which were low at all times compared with concentrations of other carbohydrates, increased markedly in seedlings in the 25/1 °C treatment, less so
in seedlings in the 20/1 °C treatment and only slightly in seedlings in the 15/1 and 10/1 °C treatments. In response to an increase in treatment temperature from 10 to 25 °C, mean pinitol concentrations increased from 18.6 ± 0.8 to 19.3 ± 1.1 mg g⁻¹ and mean sequoyitol concentrations increased from 3.2 ± 0.1 to 5.5 ± 0.6 mg g⁻¹.

**Relationships between frost hardening and biomass, carbon gain and carbohydrates**

The temperature-induced differences in the time-courses of hardening were not accompanied by parallel changes in photosynthesis, respiration or any constituent carbohydrate in any of the treated seedlings. However, biomass accumulation was inversely proportional to hardening (cf. Figures 1 and 4). The rate of frost hardening was curvilinearly dependent ($P = 0.091, r^2 = 0.99$) on relative shoot growth rate (Figure 8) with higher relative shoot growth rates associated with slower rates of frost hardening, especially at shoot growth rates above about 9.0 mg g⁻¹ day⁻¹.

Mean concentrations of hexose sugars and starch, averaged over the whole experiment for seedlings across the different treatments, were both linearly correlated with the rate of hardening ($P = 0.002$ and 0.012, $r^2 = 0.99$ and 0.97, respectively) (Figure 9). The rate of hardening increased with increasing concentrations of both hexose sugars and starch in the seedling shoots.

**Discussion**

The hypothesis put forward by Greer (1983), that temperatures above a threshold of about 5 °C would not develop frost hardiness in *Pinus radiata* seedlings, could not be confirmed by the present research. On the basis of the earlier study, we had reasoned that a common low temperature, known to be effective in the development of frost hardiness, in concert with marked variation in day temperature, would have induced nearly identical rates of frost hardening in each regime, if this hypothesis were valid. As shown here, however, seedlings grown at the different temperature treatments were all capable of frost hardening, even though the extent of development of frost hardiness was strongly dependent on the actual temperature. Therefore, temperatures above the threshold have a marked effect on the development of frost hardiness in these seedlings of *Pinus radiata*. There has been an implicit assumption in many studies (Harvey 1930, Levitt 1980, Jonsson et al. 1981, Harwood 1981, Gay and Eagles 1991, Greer and Robinson 1995) that changes in frost hardiness do not occur at temperatures above the threshold temperature, but this appears to have been rarely, if ever, tested. The threshold temperature for hardening of seedlings of *Pinus radiata* was estimated to be slightly above 9 °C, and comes well within the range for other conifers (Jonsson et al. 1981), hardwoods (Harwood 1981, Greer and Robinson 1995) and grasses (Gay and Eagles 1991).

From both the earlier studies on development of frost hardness in *Pinus radiata* (Greer and Warrington 1982, Greer 1983) and the present data, it is apparent that temperature controls the rate of frost hardening. What is less clear is the form of the underlying temperature response function. Greer (1983) showed that, at temperatures between 3 and –4 °C (with a common maximum of 12 °C), rates of hardening increased linearly with decreasing temperature. The current analysis could also imply a similar linear temperature response function if the temperatures are restricted to the range below the threshold (9.5 °C). However, using the discrete-rate approach, with no a priori assumptions about the shape of the temperature response, we have demonstrated that the overall response is curvilinear and continuous over the range of temperatures tested. Furthermore, to account for the observed effects of the different day temperatures on the development of frost hardness, we must assume, and the piecewise analysis implies, that dehardening must have occurred when temperatures were above 9.5 °C and that hardening must have occurred when temperatures were below 9.5 °C. Thus, the development of frost hardness over a 24-h period depends on the net difference between the two processes of hardening and dehardening. The difference also depends on the actual temperatures and the proportions of each day that temperatures
were above or below the threshold. This concept of hardening and dehardening occurring concurrently appears to be novel in the frost hardening literature, but has been applied conceptually in dormancy breaking (Richardson et al. 1974, Shaltout and Unrath 1983) and vernalization (Chouard 1960, Weibe et al. 1992). Other frost hardening studies, e.g., Eagles and Williams (1992), have used different day/night temperature combinations but have not used discrete-rate methods of data analysis. However, Repo (1991) has shown that Scots pine (Pinus sylvestris) can reharden during dehardening if temperatures are favorable for hardening. This suggests that a continuum of response over a wide temperature range might also occur in Scots pine, at least for the dehardening phase.

Across many studies where Pinus radiata seedlings have been grown in constant temperature conditions, the time course of frost hardness development has followed an approximately linear path. Elsewhere, hardening in Lolium multiflorum over 24 days (Gay and Eagles 1991) and in Pseudotsuga menziesii (Mirb.) Franco over about 140 days (Leinonen et al. 1995) followed curvilinear kinetics, with the rate of hardening declining as the maximum capacity for frost hardiness was approached. Alnus species followed linear kinetics over 40 days (Tremblay and Lalonde 1987). Although the time course of hardening in Pinus radiata is unlikely to follow linear kinetics continuously, it provides a first approximation for model development.

In several species, initiation of the hardening process is accompanied by declining growth rates and dormancy (e.g., Valkonen et al. 1990). Pinus radiata does not form a true dormant bud and produces several growth flushes per year (Bollman and Sweet 1975, Jenkins et al. 1976), suggesting that development of frost hardness in this conifer differs from many other species where hardening is strongly associated with this stage of the annual growth cycle (Kobyashi et al. 1983). However, we found that relative shoot growth rates were negatively correlated with rates of frost hardening, indicating that reduced shoot growth in Pinus radiata seedlings is closely associated with the development of frost hardness.

Reduced growth and increased hardening of seedlings at low temperatures were associated with increased concentrations of starch and glucose + fructose. Furthermore, the mean concentrations of these carbohydrates were linearly correlated with the rate of hardening across the temperature range tested. Bigras et al. (1989) observed that, in Juniperus chinensis L. at 1 °C, sugars and starch accumulated in concert with frost hardening; however, at 8 and 15 °C, hardening was associated with little change in sugar and starch concentrations. In both Pinus sylvestris and Picea abies, carbohydrate concentrations, especially sucrose, were also highly correlated with frost hardness, particularly at low (10/5 °C) temperatures (Aronsson et al. 1976). Increases in concentrations of sucrose, fructose and glucose also paralleled increases in frost hardness in Ilex aquifolium L. (Rütten and Santarius 1988). These studies substantiate the conclusion of Ögren et al. (1997) that hardening and carbohydrate concentration are causally linked.

At the highest day temperature tested in the present study, negligible starch and low hexose sugar concentrations were symptoms of rapid, source-limited growth of the seedlings because of relatively low photosynthetic rates. Rook and Corson (1978) and Benecke (1980), reported that, in Pinus radiata, photosynthetic rates are optimal at 10–15 °C across a range of PFDs and rates are reduced by about 30% from maximum at 25 °C. Similar results were obtained in the present study. We found that seedlings in the 25/1 °C treatment had a negative carbon balance, whereas seedlings in the 10/1 °C treatment had a positive net carbon balance, showing that net carbon balance progressively increased with decreasing daytime temperatures. In parallel with the temperature-induced changes in net carbon balance, we observed progressive increases in starch and sugar concentrations and frost hardness with declining temperature.

There were no changes in the amount of pinitol in seedlings in any treatment. Sequoyitol, which was present in low concentrations, increased significantly in seedlings in the 25/1 °C treatment, less in seedlings in the 20/1 °C and no change was measured in seedlings in the 15/1 and 10/1 °C treatments. In all treatments, concentrations of myo-inositol, the precursor for both sequoyitol and pinitol, remained virtually constant in the shoots of the seedlings. Similarly, Aronsson et al. (1976) showed that, in Pinus sylvestris and Picea abies, several sugar alcohols, including pinitol and myo-inositol, were not correlated with frost hardening.

In summary, Pinus radiata seedlings developed frost hardness at rates that were dependent on growth rate and temperature. The temperature response of the hardening process was curvilinear over the range from –4 to 25 °C with hardening occurring at temperatures below a threshold value of 9.5 °C and dehardening occurring at temperatures above the threshold value. As a consequence, development of frost hardness over the daily cycle of temperature was the net balance between the underlying rates of hardening and dehardening. The development of frost hardness is associated with changes in many other plant processes, in particular the accumulation of sugars and starch; however, the mechanism underlying the development of frost hardness still remains to be elucidated.

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References


