

## Effect of low root temperature on net photosynthesis, stomatal conductance and carbohydrate concentration in Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) seedlings

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### Summary

The effect of low root temperature on net photosynthesis, stomatal conductance and carbohydrate concentration in potted Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) seedlings was examined under controlled growth-chamber conditions. Root temperature had no effect on net photosynthesis between 10 and 20°C, however, conductance and photosynthesis declined sharply below 8°C. Net photosynthesis and stomatal conductance decreased to 50 and 34% of the initial values after 7 days at a root temperature of 0.7°C. Low root temperature also caused a decrease in photosynthetic utilization of internal CO<sub>2</sub>, carboxylation efficiency and apparent quantum yield, and it was concluded that the decrease in photosynthetic rate was caused primarily by nonstomatal limitations. Root chilling caused a small increase in starch content in needles and stems and induced the hydrolysis of starch to glucose in roots. The increase in glucose concentration may enable continued root growth at low temperatures.

### Introduction

Low soil temperature can impose a direct limitation on productivity of subalpine forested ecosystems. The seasonal increase in soil temperature under the forest canopy lags behind increases in maximum and minimum air temperature (Shanks 1956, DeLucia 1986). Consequently, low soil temperature can limit productivity long after the cessation of freezing nights.

Low soil temperature delays the onset of root and shoot growth and reduces dry matter production and height growth in conifers (Aulitzky 1961, Dougherty and Morikawa 1980, Lopushinsky and Kaufmann 1984, Tranquillini and Unterholzner 1968). Stomatal conductance and transpiration decline sharply at root temperatures below 10°C (Kramer 1940, 1942, Havranec 1972, Kaufmann 1975, Running and Reid 1980, Smith 1985). However, few investigators have examined the direct influence of low rooting zone temperatures on carbon assimilation rates. Soil temperatures below 10°C substantially reduce net photosynthesis in *Pinus radiata* D. Don. (Babalola et al. 1968), and interspecific differences in the root

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temperature-photosynthetic response are known for some herbaceous and woody species (Lawrence and Oechel 1983, McNaughton et al. 1974).

Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) is a dominant component of subalpine forests in the Rocky Mountains of western North America (Fowells 1965, Oosting and Reed 1952). At higher elevations in the central Rocky Mountains, the maximum soil temperature at 5 and 10 cm below the soil surface typically remains below 10 and 7°C, respectively (DeLucia 1986). Under field conditions, stomatal conductance in spruce is reduced by soil temperatures below 7.5°C (Kaufmann 1975, Smith 1985), suggesting that spruce may be under a root-temperature limitation for most of the growing season. In this study the response of gas exchange was examined in potted Engelmann spruce seedlings that were exposed to short-term (hours) and long-term (days) root chilling and to widely varying root temperatures. The contribution of stomatal and nonstomatal limitations to carbon assimilation at low root temperatures was determined. In addition, changes in carbohydrate content and composition as a result of root chilling were examined.

### Materials and methods

Experiments were conducted on one-year-old seedlings grown from seeds in greenhouses at Duke University. Seeds provided by the Mt. Sopris Nursery (USFS), Carbondale, Colorado, were collected from Engelmann spruce at an elevation of 3050 m in the Medicine Bow National Forest. Plants were grown in 500-ml pots in a standard greenhouse potting mix and were fertilized biweekly starting soon after emergence. Eight weeks before the start of the experiments, seedlings were moved into controlled environment growth chambers in the Duke University Phytotron (Kramer et al. 1970). Plants were maintained in a 14-h photoperiod. The day/night temperature and irradiance were 20/10°C and 350  $\mu\text{mol s}^{-1} \text{m}^{-2}$  (PPFD), respectively. Relative humidity was maintained at 70% during the day.

Root temperature was controlled by immersing sealed pots into an ethylene glycol bath. The shoots were insulated from the roots by placing loosely fitted 2-cm thick styrofoam collars around the stems at the soil surface. Each pot contained a copper-constantan thermocouple positioned at its geometric center. Several pots also contained thermocouples at different locations to determine thermal gradients. At steady state root temperature, within pot temperature differences were generally less than 0.5°C. The soil temperature in control and prechilled pots was 0.5 to 2°C warmer than the chamber air temperature when the lights were on. Soil temperature was manipulated while gas exchange measurements were being made by circulating ethylene glycol through an insulated copper coil surrounding the pot. Soil, air and bath temperatures were monitored continuously during the experiment.

Net photosynthesis was measured on whole seedlings with an open IR gas analysis system. The shoot was sealed in a well-mixed, temperature and humidity

controlled cuvette. Needle temperatures inside the cuvette were measured by looping fine-wire thermocouples around individual needles. Transpiration was measured simultaneously with dew point hygrometers placed in series before and after the cuvette. Photosynthetic measurements were made at saturating irradiance ( $1200 \mu\text{mol s}^{-1} \text{m}^{-2}$  PPFD) at an air temperature of  $20^\circ\text{C}$  and a VPD of 1 kPa. Net photosynthesis, stomatal conductance and internal  $\text{CO}_2$  concentration ( $C_i$ ) were calculated on a total leaf area basis using the equations of Von Caemmerer and Farquhar (1981).

The short-term response to root chilling was measured over a 10-h period by rapidly decreasing soil temperature while the plant was in the cuvette. Conductance and photosynthesis were at a steady state for at least one hour prior to manipulation of root temperature. As a control, diurnal gas exchange measurements were made on the same plants the day before exposure to root chilling. This procedure was replicated three times with similar results; representative data are shown.

The long-term response to root chilling was measured over several days and after a 3-day recovery period. Soil temperature during the chilling treatment was maintained at  $0.7 \pm 0.5^\circ\text{C}$ . Pots were watered to saturation before initiating the treatment and kept at field capacity throughout the experiment. The effect of root chilling on apparent quantum yield and on the relationship between carbon assimilation and  $C_i$  was measured after 1 and 5 days.

The photosynthetic and stomatal response to different root temperatures was measured by exposing plants to successively lower root temperatures from 20 to  $0.7^\circ\text{C}$  while maintaining a constant shoot temperature of  $20^\circ\text{C}$ . Plants were maintained for 4 days at each root temperature prior to measurement. Photosynthetic measurements were made between 2 and 5 h into the light period, except for the  $C_i$  and irradiance response curves which took somewhat longer.

The effect of root chilling on carbohydrate concentration was determined after 5 days at  $0.7^\circ\text{C}$ . The concentrations of sucrose, glucose and starch in root, stem and needle tissue were determined in an experimental and control group. Plants were harvested for analysis 6 hours into the light period. Xylem pressure potential was first measured with a PMS pressure chamber. The tissue was then killed in a microwave oven (30 s) and dried at  $80^\circ\text{C}$  for 14 h. Samples were ground through a 20-mesh Wiley mill, sonicated for 30 min, and then extracted with 80% (v:v) ethanol. Extracts were analyzed enzymatically for glucose and sucrose by the method of Jones et al. (1977). The starch fraction was digested to glucose for 30 min at  $55^\circ\text{C}$  with amyloglucosidase from *Aspergillus oryzae* (Sigma A-9268, grade V, dialyzed and diluted to 10 units  $\text{ml}^{-1}$  in a 50 mM Na-acetate buffer, pH 4.5) (Huber et al. 1984). The hydrolyzed samples were then analyzed for glucose (Jones et al. 1977).

## Results and discussion

The photosynthetic and stomatal responses to a rapid decrease in root temperature

are shown in Figure 1A. Gas exchange responded rapidly to a decrease in root temperature (cf. Figures 1A and B). Stomatal conductance and net photosynthesis initially increased to approximately 125% of the pretreatment values and did not return to the initial levels until 3.5 h into the chilling treatment. Both variables declined steadily to 68% (photosynthesis) and 44% (conductance) of the initial values between 3 and 10 h after the onset of root chilling.

The reason for the initial rise in photosynthesis and conductance is not clear. Stafelt (1956) observed a transient increase in stomatal conductance in *Vicia faba* L. in response to water stress. At the onset of water stress, turgor loss occurred in epidermal cells adjacent to the guard cells prior to drought-induced stomatal closure. Low epidermal turgor and high guard cell turgor permitted an increase in

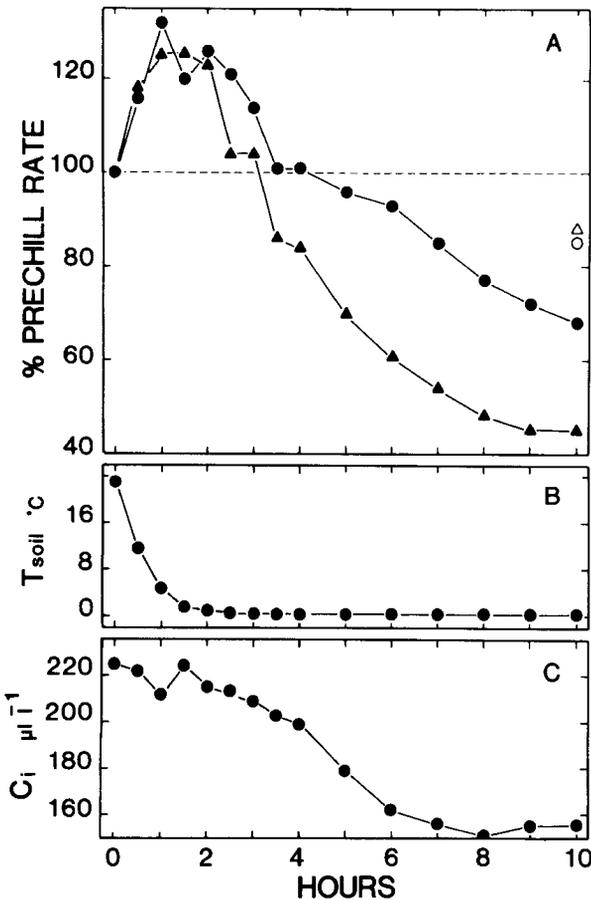


Figure 1. The short-term response of net photosynthesis (●) and stomatal conductance (▲) to root chilling in Engelmann spruce seedlings (A). Changes in soil temperature and  $C_i$  are shown in (B) and (C). The open symbols show the change in gas exchange parameters measured after 12 h in the cuvette under non-chilled conditions. The pretreatment rates of photosynthesis and conductance were  $3.12 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $44 \text{mmol m}^{-2} \text{s}^{-1}$ , respectively.

stomatal aperture and, for a short time, increased conductance. The initial increase in photosynthesis and conductance observed for spruce under root-chilling conditions may be a similar water stress response. The subsequent decrease in net photosynthesis (after 3–4 h) was a result of stomatal closure as indicated by the decrease in internal CO<sub>2</sub> (Figure 1C).

The long-term response of photosynthesis and conductance to root chilling is shown in Figure 2. The largest decrease occurred during the first 24 h of chilling, and these values decreased more slowly thereafter. After 7 days of root chilling conductance and photosynthesis were 50 and 34% of the initial values, respectively (Figure 2). The decrease in conductance was less than that observed for *Pinus contorta* Dougl. ex Loud. (Running and Reid 1980) and slightly greater than that observed for *Pseudotsuga menziesii* (Mirb.) Franco (Lopushinsky and Kaufmann 1984) measured at comparable root temperatures. *Pinus contorta* and *Pseudotsuga menziesii* are ecologically more important at lower elevations than Engelmann spruce (Marr 1961, Peet 1978) and differences in sensitivity to low root temperatures may play a role in defining the distributional patterns of these species. Interspecific differences in the effect of low soil temperature on transpiration are known for northern and southern species of *Pinus* (Kramer 1942).

The effect of a step-wise reduction in root temperature on net photosynthesis and stomatal conductance is shown in Figures 3A and B. The decrease in root temperature from 20 to 10°C had no effect on net photosynthesis. However, below 10°C both conductance and photosynthesis decreased with decreasing root temperature. An Arrhenius-type plot (Figure 4) showed a sharp decline in photo-

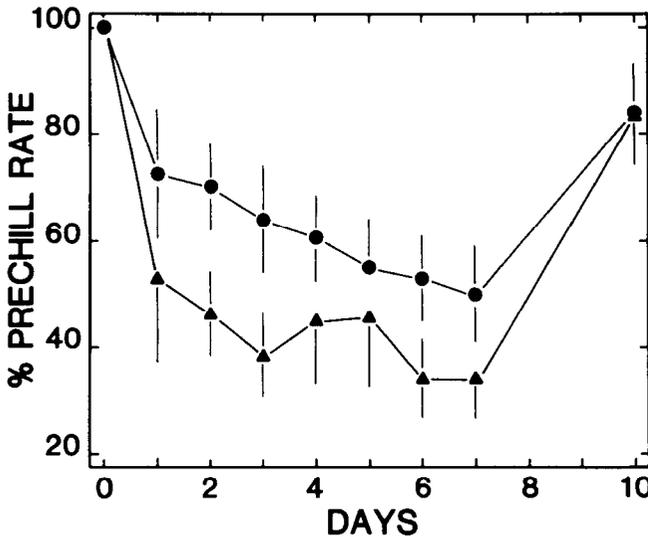


Figure 2. The long-term response of net photosynthesis (●) and stomatal conductance (▲) to root chilling (0.7°C) in Engelmann spruce seedlings. The chilling treatment was initiated on day 0 and was terminated on day 7. The pretreatment values for photosynthesis and conductance were  $2.49 \pm 0.30 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $22.5 \pm 2.7 \text{ mmol m}^{-2} \text{s}^{-1}$ , respectively. Error bars are 1 SEM,  $n = 4$ .

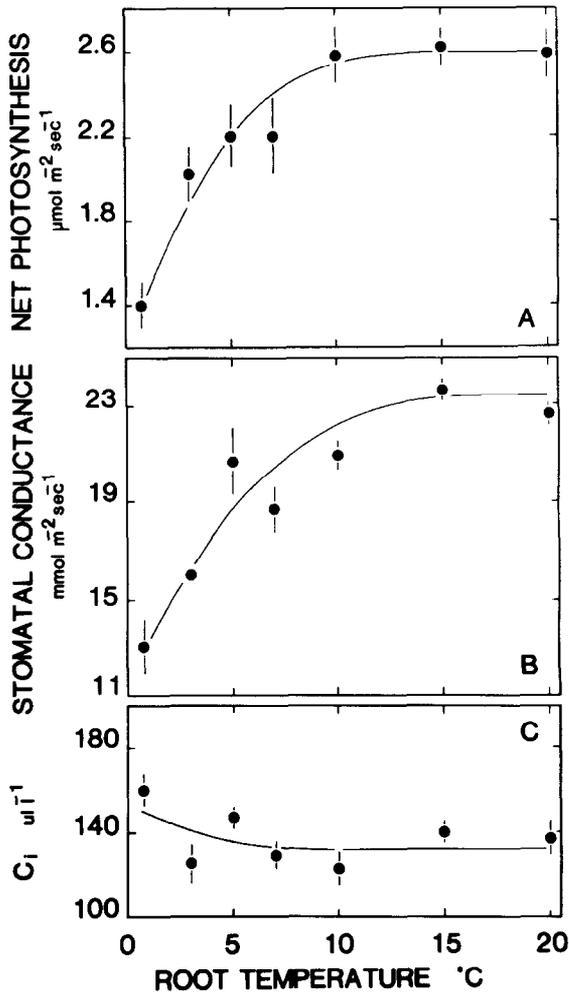


Figure 3. The effect of different root temperatures at a constant shoot temperature on net photosynthesis (A), stomatal conductance (B) and  $C_i$  (C) in Engelmann spruce seedlings. Curves were fitted by hand. Error bars are 1 SEM,  $n = 3$ .

synthetic rates below approximately 8°C. This is consistent with a decline in stomatal conductance observed for Engelmann spruce at root temperatures below 7.5°C under field conditions (Kaufmann 1975, Smith 1985). The primary cause of decreased stomatal conductance at low root temperatures is water limitation resulting from both an increase in the viscosity of water and in root resistance (Kaufmann 1975, Evans 1975).

The decrease in conductance at low root temperatures and the correlation between decreasing stomatal conductance and net photosynthesis (Figure 5) implies a causal relationship between these parameters. However, data presented indicate that primarily nonstomatal factors limited net photosynthesis.

A stomatal limitation of photosynthesis at low root temperatures should produce

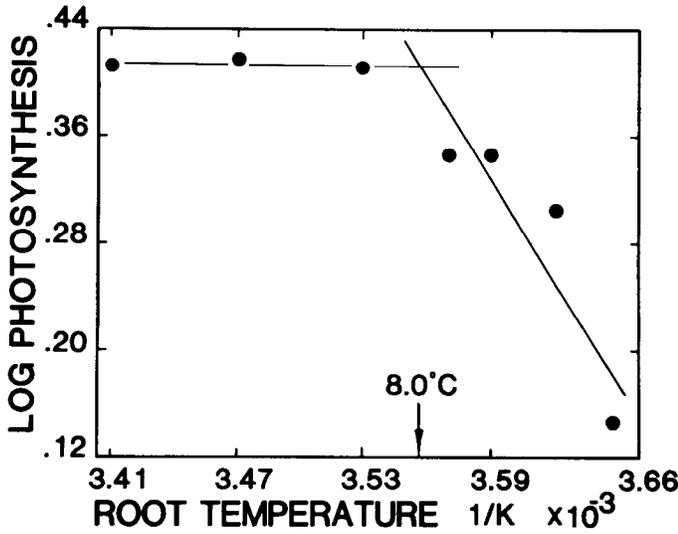


Figure 4. Arrhenius plot of the photosynthetic-root temperature response for Engelmann spruce seedlings. Log photosynthesis is the log<sub>10</sub> of the photosynthetic rate at different root temperatures (1/degrees Kelvin). Lines were fitted by least squares regression. The soil temperature at which the curve breaks is indicated by an arrow.

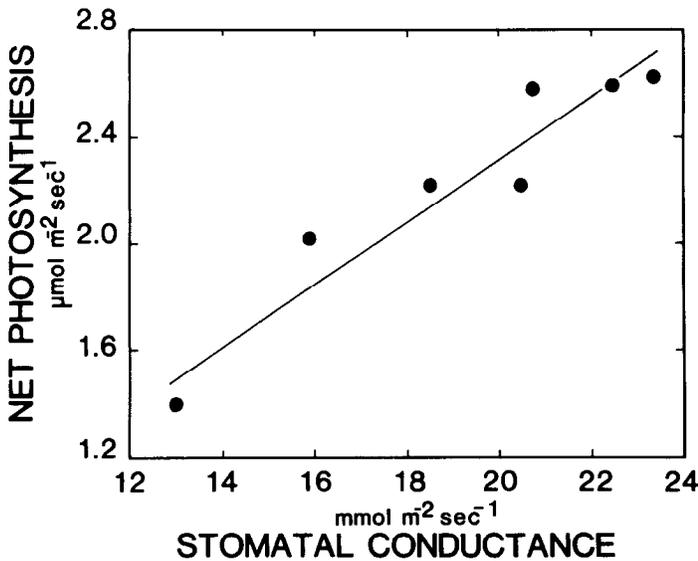


Figure 5. Net photosynthesis *versus* stomatal conductance at different root temperatures. The data are from Figure 3. The line was fitted by least squares regression, ( $r^2 = 0.90$ ).

a decrease in  $C_i$ . However,  $C_i$  remained constant between 20 and 3°C and increased slightly at 0.7°C (Figure 3C). An index of photosynthetic efficiency or photosynthetic utilization of internal CO<sub>2</sub> was derived by dividing the rate of net photosynthesis by internal CO<sub>2</sub> concentration (Sasek et al. 1985). This index of

efficiency factors out the stomatal influence on  $\text{CO}_2$  flux. At root temperatures below  $10^\circ\text{C}$  photosynthetic efficiency decreased (Figure 6) indicating a nonstomatal limitation to carbon assimilation. Moreover, adjusting  $C_i$  to the prechill level by manipulating the ambient  $\text{CO}_2$  concentration did not produce a substantial recovery of photosynthesis (Figures 7). A dramatic decrease in carboxylation efficiency also was observed 24 h after root temperature was switched from  $23$  to  $0.7^\circ\text{C}$  (Figure 7). Carboxylation efficiency, defined as the initial slope of the response of carbon assimilation to  $C_i$  (Ku and Edwards 1977), is closely correlated with the RubP saturated kinetics of RubP carboxylase (von Caemmerer and Farquhar 1981, Farquhar et al. 1980). At higher  $C_i$  net photosynthesis becomes limited by the rate of RubP regeneration, which is controlled by the production of NADPH and ATP from electron transport (Sharkey 1985). The soil temperature-induced reduction in carbon assimilation (Figure 7) and the decrease in apparent quantum yield (Figure 8) suggest that root chilling caused a reduction in electron transport capacity.

Lower rates of carbon assimilation and a decrease in quantum yield are associated with carbohydrate accumulation in several species (Azcon-Bieto 1983, DeLucia et al. 1985, Mauney et al. 1979), and it is postulated that carbohydrates can limit carbon assimilation by feedback inhibition (Herold 1980). It seems likely, therefore, that root chilling caused an increase in needle carbohydrate concentration by reducing root sink strength, ultimately limiting photosynthesis.

The starch concentration in needles increased by almost 10% after 5 days of root chilling; however, the increase was not statistically significant (Table 1). Significant differences were observed in stems and roots. The concentration of

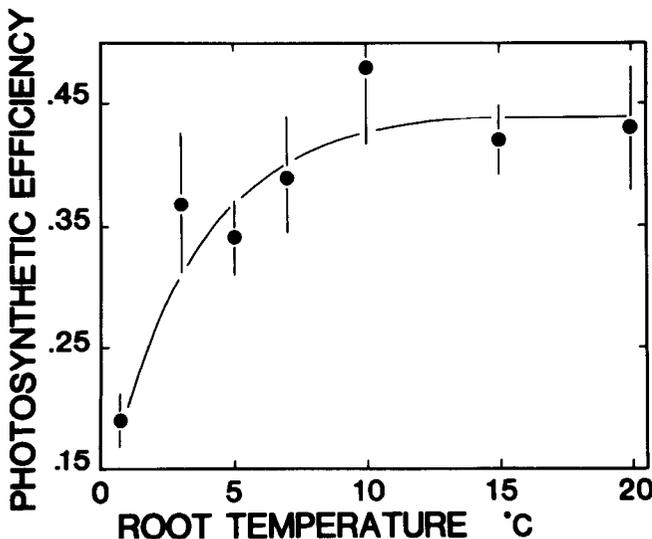


Figure 6. Photosynthetic efficiency at different root temperatures for Engelmann spruce. The unit for PE is:  $(\text{mol CO}_2 \text{ fixed m}^{-2} \text{ s}^{-1})/(\text{mol l}^{-1} \text{ intercellular CO}_2)^{-1}$ . Curve was fitted by hand. Error bars are 1 SEM,  $n = 3$ .

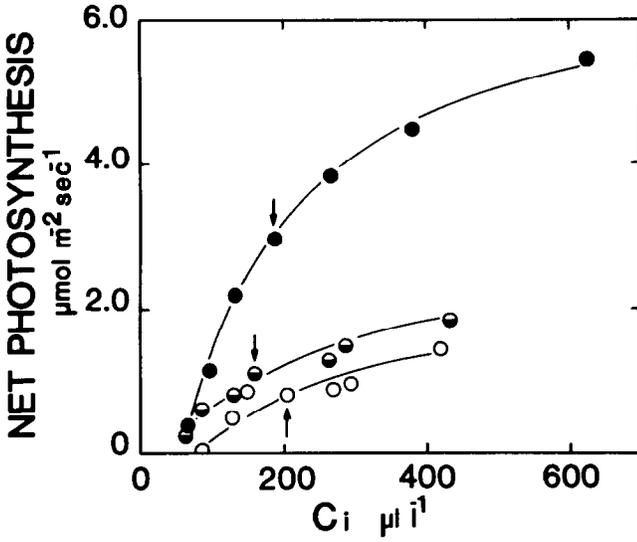


Figure 7. Net photosynthesis versus intercellular CO<sub>2</sub> concentration before root chilling (●), and after 1 (◐) and 5 (○) days of chilling (0.7°C). The C<sub>i</sub>'s at 350 µl l<sup>-1</sup> external CO<sub>2</sub> concentration are indicated with arrows. Curves were fitted by hand.

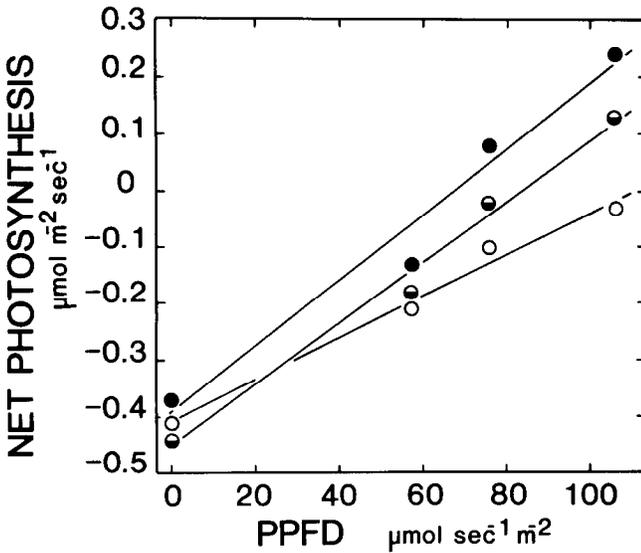


Figure 8. The response of carbon assimilation to light at low incident irradiance before (●) and after 1 (◐) and 5 (○) days of root chilling (0.7°C). The lines were fitted by least squares regression. The slope after 5 days was significantly less ( $P = 0.05$ ) than the prechilled level. Apparent quantum yields (indicated by the slope) before and after 1 and 5 days of root chilling were 0.0058, 0.0054 and 0.0037, respectively.

total soluble sugars in stems decreased and starch concentration increased after the chilling treatment (Table 1).

Table 1. The effect of root chilling on carbohydrate concentration in needles, stems and roots of Engelmann spruce seedlings. All values are mean percentages of oven dry weight  $\pm$  1 SEM. N = 8 or 9. Total carbohydrate (Tot Carb) is the sum of starch and total soluble sugars. Sol:Sta is the ratio of total soluble sugars to starch. XPP is xylem pressure potential in MPa. Means were compared with a *t*-test.

	Needle	Stem	Root	XPP
<i>Prechilled</i>				
Starch	2.52 $\pm$ 0.36	4.26 $\pm$ 0.37	4.93 $\pm$ 0.23	-0.83 $\pm$ 0.043
Glucose	2.69 $\pm$ 0.35	0.98 $\pm$ 0.11	1.48 $\pm$ 0.16	
Sucrose	3.12 $\pm$ 0.42	4.85 $\pm$ 0.32	4.12 $\pm$ 0.26	
Tot sol	5.87 $\pm$ 0.25	5.83 $\pm$ 0.30	5.59 $\pm$ 0.25	
Sol:Sta	2.73 $\pm$ 0.40	1.44 $\pm$ 0.13	1.17 $\pm$ 0.01	
Tot Carb	8.39 $\pm$ 0.45	10.09 $\pm$ 0.59	10.52 $\pm$ 0.18	
<i>Chilled</i>				
Starch	2.78 $\pm$ 0.42	5.59 $\pm$ 0.45 <sup>1</sup>	3.26 $\pm$ 0.36 <sup>1</sup>	-1.23 $\pm$ 0.111 <sup>1</sup>
Glucose	2.13 $\pm$ 0.29	0.70 $\pm$ 0.08	2.32 $\pm$ 0.16 <sup>1</sup>	
Sucrose	3.15 $\pm$ 0.50	4.36 $\pm$ 0.10 <sup>2</sup>	5.25 $\pm$ 0.25 <sup>1</sup>	
Tot sol	5.28 $\pm$ 0.38	5.06 $\pm$ 0.10 <sup>1</sup>	7.57 $\pm$ 0.27 <sup>1</sup>	
Sol:Sta	2.23 $\pm$ 0.37	0.95 $\pm$ 0.07 <sup>1</sup>	2.48 $\pm$ 0.21 <sup>1</sup>	
Tot Carb	8.02 $\pm$ 0.58	10.65 $\pm$ 0.48	10.83 $\pm$ 0.52	

<sup>1</sup> *P* < 0.05.

<sup>2</sup> *P* = 0.054.

Root tissue responded quite differently. Chilling produced a large decrease in root starch concentration and an increase in total soluble sugars, resulting in a greater than 2-fold increase in the ratio of soluble sugars to starch. The accumulation of reducing sugars, which can act as cryoprotectants, is commonly observed in the development of frost hardiness in conifers (Sakai 1966, Aronsson et al. 1976) and in potato tubers stored at low temperatures (Schwimmer et al. 1954). Low temperature treatment also causes an increase in invertase activity (Pressey and Shaw 1966) and the production of a low temperature invertase isozyme (Roberts 1979). An increase in the ratio of glucose to sucrose in roots after chilling suggests that similar changes in invertase activity occurred in spruce. The maintenance of adequate sugar supplies to the root tip is important for continued elongation. Low soil temperature caused a sharp decline in sugar concentration and root growth in maize but not in the more cold tolerant pea (Crawford and Huxter 1977). Maintenance of large pools of reducing sugars, particularly glucose, may be important to enable continued root growth in spruce at low soil temperatures.

In summary, soil temperatures below 8°C caused a decrease in carbon assimilation, stomatal conductance and xylem pressure potential (Table 1) in Engelmann spruce seedlings. The decrease in net photosynthesis during the first several hours of root chilling was caused by a decrease in conductance as indicated by a decrease in  $C_i$ . However, after 24 h, the decrease in net photosynthesis at low soil temperatures was associated with a decrease in carboxylation efficiency and apparent quantum yield and could not be explained solely by the decrease in stomatal

conductance. It is hypothesized that carbohydrate accumulation in needles at low soil temperatures caused a decrease in carbon assimilation by feedback inhibition. Starch concentration in needles increased after 5 days of chilling, however, the change was not statistically significant. Alternatively, the root temperature-induced decrease in xylem pressure potential suggests that a decrease in the turgor of mesophyll cells may be responsible for the nonstomatal reduction in photosynthetic rates. Chilling produced a decrease in starch and an increase in glucose concentration in the roots that may enable continued root growth in spruce at low soil temperatures.

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