

# The effect of freezing nights on photosynthesis, stomatal conductance, and internal CO<sub>2</sub> concentration in seedlings of Engelmann spruce (*Picea engelmannii* Parry)

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**Abstract.** The effect of freezing night temperatures on net photosynthesis, stomatal conductance, and internal CO<sub>2</sub> concentration was investigated in unhardened seedlings of Engelmann spruce. Exposure to  $-2.5^{\circ}\text{C}$  in the dark for 10 h caused a slight and reversible reduction in gas-exchange parameters on the following days. Substantial and irreversible inhibition of photosynthesis occurred after exposure to  $-4^{\circ}\text{C}$  or  $-5^{\circ}\text{C}$ . Despite a parallel decline in stomatal conductance and net photosynthesis, exposure to a hard freeze caused a decrease in the stomatal limitation to gas exchange. Hard-freeze conditions (less than  $-4^{\circ}\text{C}$ ) also caused a decrease in carboxylation efficiency and apparent quantum yield, indicating a freeze-induced failure of the dark reactions and electron transport. There was no significant difference in the photosynthetic response to freezing temperatures in different elevational populations of spruce, although acclimatory adjustments were observed. Gas exchange in seedlings grown under cool conditions ( $14^{\circ}\text{C}$  day/ $9^{\circ}\text{C}$  night) was less affected and recovered more rapidly after exposure to a hard freeze than in seedlings grown under warm conditions ( $24^{\circ}\text{C}$  day/ $19^{\circ}\text{C}$  night).

**Key-words:** Engelmann spruce; freezing; photosynthesis; *Picea engelmannii*; stomatal conductance.

## Introduction

Engelmann spruce is a dominant component of high elevation forests in the Rocky Mountains of North America (Fowells, 1965). Freezing night temperatures occur with a high frequency through June, and begin again in September throughout the elevational distribution of spruce, although freezing nights can occur at any time of year (Barry, 1973). In a previous study the importance of low night temperature as a potential limitation to photosynthesis in Engelmann spruce was assessed in the field (DeLucia & Smith, 1987). Shoots exposed to a light freeze ( $-2.5^{\circ}\text{C}$ ) did not show a significant inhibition of photosynthesis subsequent to the treatment. Exposure to a hard

freeze ( $-5.0^{\circ}\text{C}$ ), however, caused a substantial and irreversible reduction in several gas-exchange parameters, including net photosynthesis, stomatal conductance, apparent quantum yield, and the ability to assimilate internal CO<sub>2</sub>. As a result of cold-air drainage, the frequency and severity of freezing nights during the summer is greater at low elevations (Kaufmann, 1984) suggesting that intermittent summer freezes pose a potentially greater limitation to photosynthesis at low rather than high elevations.

In this study the effect of freezing nights on photosynthesis and other gas exchange parameters in seedlings of Engelmann spruce was investigated. As a consequence of its extensive elevational distribution, different populations of spruce are subject to different temperature regimes and freezing frequencies. These differences can provide a selective force creating genetic differentiation in sympatric populations. Ecotypic differentiation of the peroxidase enzyme system has been observed in spruce along an elevational gradient (Grant & Mitton, 1977). Furthermore, it is well established that growth under different climatic regimes often cause acclimatory changes in various metabolic processes, including photosynthesis (see Berry & Björkman, 1980; Öquist, 1983 for review). The ability of spruce to form elevational ecotypes and the effect of growth temperature on the photosynthetic response to freezing nights was investigated. The contribution of stomatal resistance in reducing photosynthesis after a freezing night was also assessed.

Experiments were performed in growth chambers in the Duke University Phytotron where root and shoot temperatures could be controlled independently.

## Materials and methods

Experiments were conducted on 1-year-old seedlings from seeds grown in the Duke University greenhouses. Seeds were collected in the fall of 1984 at three elevations in the Medicine Bow Mountains, Wyoming. The three elevations, 2704 m, 3048 m, and 3315 m above sea level, encompass the elevational range where spruce forms a characteristic spruce or spruce-fir canopy (DeLucia & Smith, 1987). Plants

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were grown in 0.5 dm<sup>3</sup> 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, Hellmers & Downs, 1970). Plants were maintained under a 14-h photo- and thermal-period. The day/night temperatures in the warm and cool chamber were 24°C/19°C and 14°C/9°C, respectively. Irradiance at plant height was 350 ± 10 μmol s<sup>-1</sup> m<sup>-2</sup> (PPFD), and the relative humidity was maintained at 70% during the day.

Shoot and root temperatures were manipulated independently. At the end of the photoperiod, potted seedlings were placed inside a refrigerated box inside a growth chamber. The pots were inserted through holes in the bottom of the insulated box so that root temperature could be maintained at 10°C during the shoot-freezing treatments. Root temperatures from 10°C to 20°C have no effect on photosynthesis and stomatal conductance in spruce seedlings (DeLucia, 1987). Shoot temperature was controlled with a thermostat that regulated the flow of cold ethylene glycol through a radiator inside the insulated box. The shoots were insulated from the roots by placing loosely fitted 2 cm-thick styrofoam collars around the stems at the soil surface. The air inside the box was mixed with a high velocity fan.

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 temperature inside the cuvette was measured by looping fine-wire thermocouples around individual needles. Transpiration was measured simultaneously with dewpoint hygrometers placed in series before and after the cuvette. Photosynthetic measurements were made under saturating irradiance (1200 μmol s<sup>-1</sup> m<sup>-2</sup>, PPFD) and at an air temperature and VPD of 20°C and 1 kPa, respectively. Net photosynthesis, stomatal conductance, and internal CO<sub>2</sub> concentration (C<sub>i</sub>) were calculated on a total leaf surface area basis (Carter & Smith, 1985) using the equations of von Caemmerer & Farquhar (1981).

Net photosynthesis and other gas exchange parameters were measured during eight successive nights of exposure to 0, -2.5 or -5.0°C and after a 5-day recovery period for seedlings grown from seeds collected at 3048 m. Seedlings were exposed to the various freezing treatments for 10 h beginning at the end of the photoperiod. Air temperature was lowered at a rate of approximately 0.2°C min<sup>-1</sup> until the desired temperature was attained. At the end of the freezing treatment plants were placed directly into darkened chambers at the pretreatment temperature for 15 min before the lights went on. Photosynthesis and transpiration were measured before the freezing treatment and after a 2-hour recovery period in the light at the pretreatment temperature. In a second

experiment, the effect of exposure to progressively lower temperatures on the gas exchange of seedlings grown from seeds collected at three elevations was examined. Night temperature was decreased from 19°C to 2°C after the first night, and in 2°C increments thereafter. Seedlings were grown from seeds collected at 2704 m, 3048 m, and 3316 m above sea level. To select representative seedlings from each elevation, the height of all seedlings within a population (*n* = 50) was measured and five seedlings in the mean size class were selected from each population.

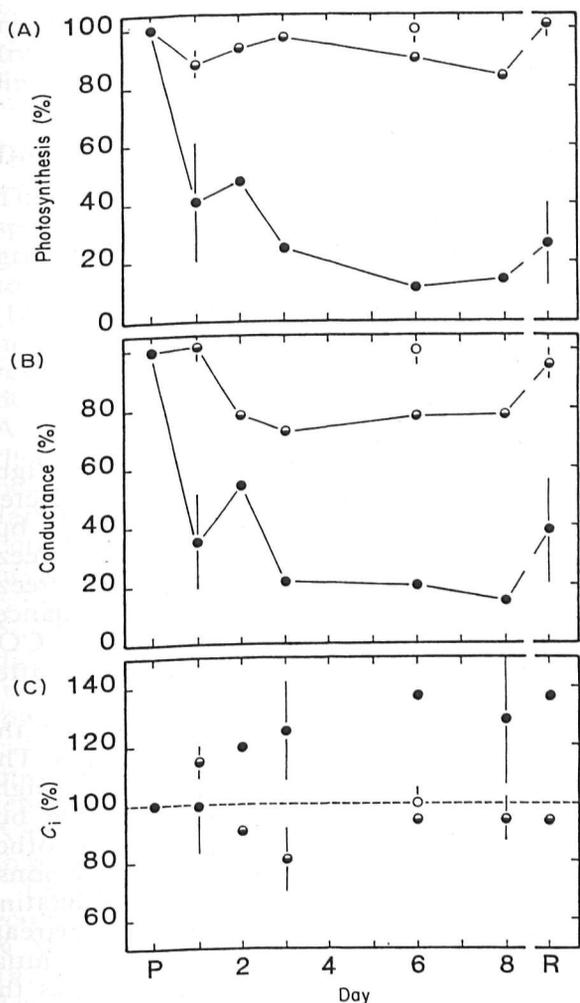
The effect of growth at warm or cool temperatures on the photosynthetic response to a hard freeze was examined in seedlings grown from seeds collected at 3048 m. Plants were acclimated for 8 weeks at either 24°C/19°C or 14°C/9°C. These temperatures approximate the mean maximum summer temperatures observed at 2704 m and 3316 m above sea level, respectively. Photosynthetic rates were measured before and after 8 d at 24°C/-5°C or 14°C/-5°C and after a 5-day recovery period at the pretreatment temperatures.

The effect of freezing night temperatures on the photosynthetic light response, and on the relationship between net photosynthesis and internal CO<sub>2</sub> concentration, was also examined. Measurements were made on seedlings (from 3048 m) before and after exposure to -2.5°C and -5°C. The photosynthesis vs C<sub>i</sub> response was replicated three times; representative data are presented. The gas phase limitation to photosynthesis before and after the freezing treatments was calculated by the differential method (Jones, 1985). Apparent quantum yield was calculated as the slope of the photosynthetic light response at low irradiances.

## Results

Exposure to a night temperature of 0°C for six successive nights had no effect on photosynthesis, stomatal conductance, or C<sub>i</sub> (Fig. 1). A light freeze (-2.5°C) caused a 2–10% reduction in photosynthesis on the following day. The decrease in stomatal conductance was somewhat greater, between 20% and 25% of the initial value. The freeze-induced inhibition of both parameters was completely alleviated after a 5-day recovery period under the pretreatment conditions. Internal CO<sub>2</sub> concentration decreased slightly after the second and third days of the freezing treatment, but was not significantly different from the control value by Days 6 and 8.

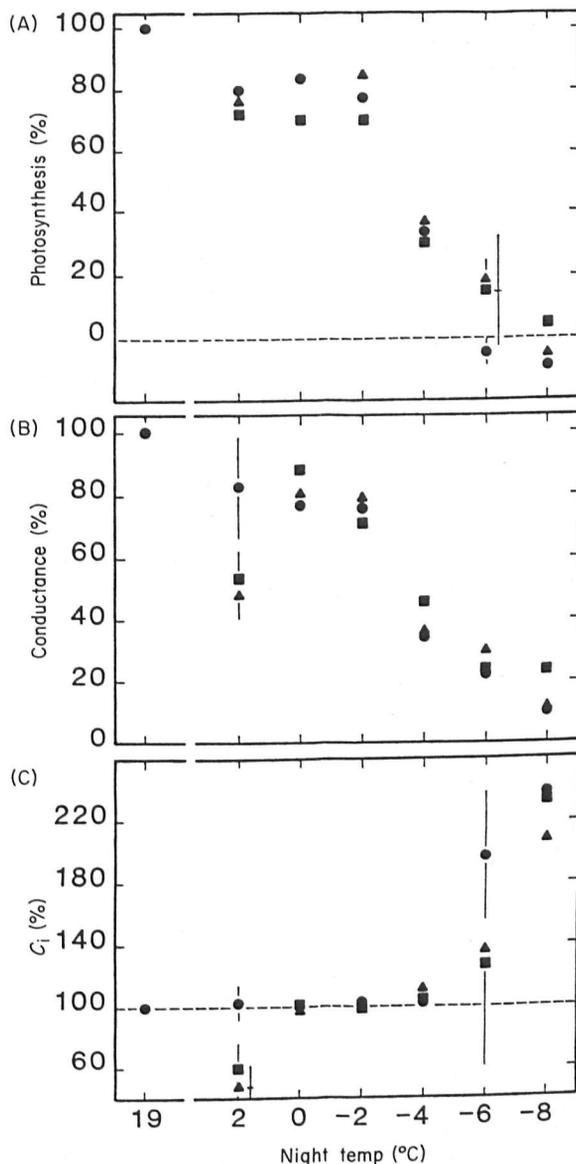
Exposure to a hard freeze (-5°C) caused substantial and irreversible decreases in photosynthesis and conductance (Fig. 1A, B). Both parameters were reduced by 35–40% of the pretreatment values after one night. There was a slight recovery after the second night, but rates continued to drop on successive days to 20% or less



**Figure 1.** The effect of successive freezing nights at the same temperature on photosynthesis (A), stomatal conductance (B), and  $C_i$  (C) in Engelmann spruce seedlings. Seedlings were grown at 24°C/19°C day/night temperature and were transferred to either 24°C/0°C (○) 24°C/−2.5°C (◐) or 24°C/−5.0°C (●). The initial values for photosynthesis, stomatal conductance, and  $C_i$  were 3.73  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 44.1  $\text{mmol m}^{-2} \text{s}^{-1}$ , and 217.6  $\text{mm}^3 \text{dm}^{-3}$ , respectively ( $n=4$ , and the largest standard error is shown). The pretreatment value (100%) is designated with a 'P', and the value after the 5-d recovery period with a 'R'.

of the control level. Only a slight recovery of photosynthesis and conductance occurred in seedlings that were returned to the pretreatment temperatures. Internal  $\text{CO}_2$  concentration was consistently higher than the initial value after the first night of treatment (Fig. 1C).

Photosynthesis decreased slightly when seedlings from various elevations were transferred from 24°C/19°C day/night temperature to 24°C/2°C (Fig. 2A). Exposure to progressively lower night temperatures, from 2°C to −2°C, did not cause a further reduction. However, a strong decrease in photosynthesis and conductance was observed in seedlings from all elevations after exposure to night temperatures of −4°C or lower. Differences in the



**Figure 2.** The effect of progressively lower night temperatures at a constant day temperature (24°C) on photosynthesis (A), stomatal conductance (B), and  $C_i$  (C) in Engelmann spruce seedlings. The seedlings were grown from seeds collected at 2704 m (●), 3048 m (▲), and 3316 m (■) above sea level and were acclimated to 24°C/19°C day/night temperature ( $n=4-5$ , and the largest standard error is shown).

response of seedlings from different elevations were not apparent.

Growth temperature had a significant effect on the photosynthetic and stomatal response to freezing nights (−5.0°C) (Table 1). Photosynthesis and conductance in warm-acclimated plants (24°C/19°C) were reduced to 6.6% and 10.4% of initial rates, respectively. In cool-acclimated seedlings (14°C/19°C) photosynthesis and conductance were reduced to 38.5% and 39.8% of the initial values, respectively. Furthermore, recovery was greater in the cool-acclimated seedlings.

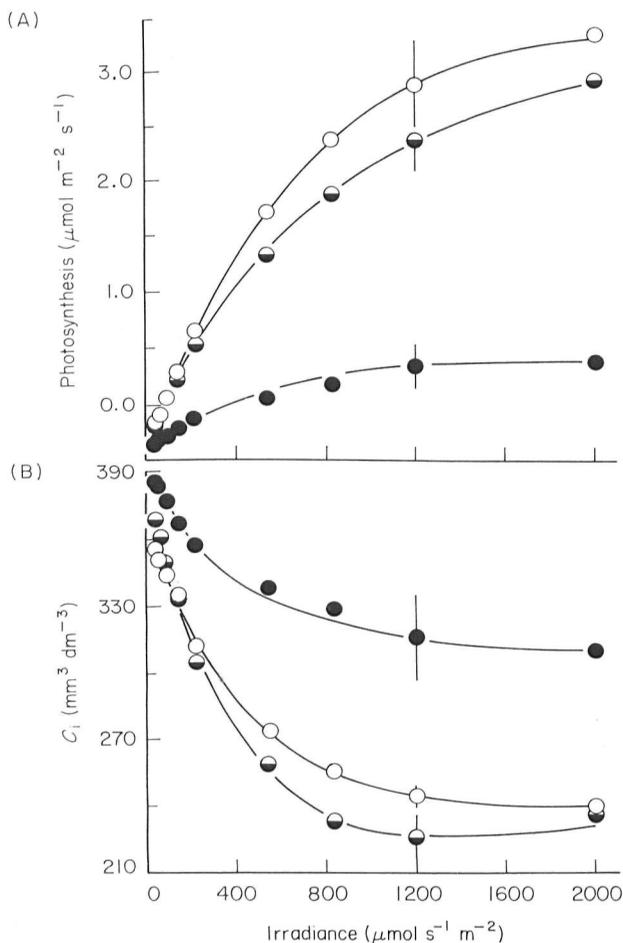
**Table 1.** The effect of freezing night temperature ( $-5.0^{\circ}\text{C}$ ) on net photosynthesis, stomatal conductance, and internal  $\text{CO}_2$  concentration in Engelmann spruce seedlings grown at warm ( $24^{\circ}\text{C}/19^{\circ}\text{C}$ ) or cool ( $14^{\circ}\text{C}/9^{\circ}\text{C}$ ) day/night temperatures

|                               | Net Photosynthesis<br>( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) |                 | Conductance<br>( $\text{mmol m}^{-2} \text{s}^{-1}$ ) |                  | Internal $\text{CO}_2$<br>( $\text{mm}^3 \text{dm}^{-3}$ ) |                             |
|-------------------------------|--|-----------------|---|------------------|--|-----------------------------|
|                               | Warm   | Cool            | Warm  | Cool             | Warm   | Cool                        |
| Initial<br>(absolute<br>rate) | $3.87 \pm 0.24$  | $3.32 \pm 0.27$ | $48.7 \pm 1.9$  | $62.8 \pm 1.7$   | $203.3 \pm 7.2$  | $247.2 \pm 13.1$            |
| Freeze (%)                    | $6.6 \pm 5.7$  | $38.5 \pm 4.1$  | $10.4 \pm 3.0$  | $39.8 \pm 2.4$   | $142.4 \pm 21.8^{\text{NS}}$                               | $102.9 \pm 4.5^{\text{NS}}$ |
| Recoveries (%)                | $18.0 \pm 13.3$  | $65.9 \pm 5.2$  | $26.2 \pm 15.1^*$                                     | $75.6 \pm 7.5^*$ | $144.4 \pm 10.6$   | $107.9 \pm 3.4$             |

The initial rates (absolute value) and the percentage of the initial values after eight consecutive freezing nights and 5 d of recovery at the pretreatment temperatures are shown. The mean values were compared with a *t*-test.

<sup>NS</sup>Not significant; \**P* < 0.1; all other values significant at *P* < 0.05.

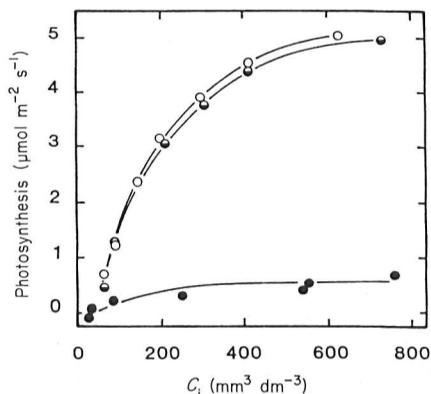
Exposure to a light freeze ( $-2.5^{\circ}\text{C}$ ) followed by a hard freeze ( $-5.0^{\circ}\text{C}$ ) caused a reduction in apparent quantum yield, carboxylation efficiency, and the maximum photosynthetic rate under  $\text{CO}_2$  saturating conditions (Figs 3A & 4). Apparent quantum yield,



**Figure 3.** The effect of exposure for one night to  $24^{\circ}\text{C}/-2.5^{\circ}\text{C}$  (●) followed by one night at  $24^{\circ}\text{C}/-5.0^{\circ}\text{C}$  (●) day/night temperature on the photosynthetic light response (A) in Engelmann spruce seedlings acclimated to  $24^{\circ}\text{C}/19^{\circ}\text{C}$  (○). The effect of freezing night temperatures on the relationship between  $C_i$  and irradiance is shown in B. ( $n = 3 \pm 1$  SEM).

defined as the slope of the photosynthetic light response at low incident irradiances, was not affected by a light freeze ( $0.0046$  vs  $0.0042 \text{ mol mol}^{-1}$ ), but was substantially reduced following a hard freeze ( $0.0046$  vs  $0.0015 \text{ mol mol}^{-1}$ ). A light freeze ( $-2.5^{\circ}\text{C}$ ) caused a reduction in  $C_i$  at irradiances above  $200 \mu\text{mol s}^{-1} \text{m}^{-2}$  (Fig. 3B). Internal  $\text{CO}_2$  concentration increased at all irradiances after exposure to a hard freeze ( $-5.0^{\circ}\text{C}$ ).

The effect of freezing night temperatures on the photosynthetic response to  $C_i$  is shown in Fig. 4. The example illustrates that exposure to a  $-2.5^{\circ}\text{C}$  night had no effect on the photosynthetic/ $C_i$  response, but this was somewhat variable. A light freeze in other cases caused a slight downward shift in the response curve; however, the photosynthetic rate at saturating  $C_i$  was generally greater than 90% of the pretreatment level. After exposure to  $-5.0^{\circ}\text{C}$ , the initial slope of the photosynthetic/ $C_i$  curve, as well as the maximum photosynthetic rate at saturating  $C_i$ , was greatly reduced. The stomatal limitation to  $\text{CO}_2$  diffusion before and after exposure to  $-2.5^{\circ}\text{C}$  was approximately 26% and decreased to approximately 14% after exposure to  $-5.0^{\circ}\text{C}$ . At an internal  $\text{CO}_2$  concentration of  $350 \text{ mm}^3 \text{dm}^{-3}$ , there was an 88%



**Figure 4.** The effect of exposure for one night to  $24^{\circ}\text{C}/-2.5^{\circ}\text{C}$  (●) followed by one night at  $24^{\circ}\text{C}/-5.0^{\circ}\text{C}$  (●) day/night temperature on the relationship between photosynthesis and  $C_i$  in Engelmann spruce seedlings acclimated to  $24^{\circ}\text{C}/19^{\circ}\text{C}$  (○). The curves were fitted by hand.

reduction in photosynthesis after the  $-5.0^{\circ}\text{C}$  treatment that was attributable to non-stomatal limitations.

## Discussion

The photosynthetic response to freezing nights in spruce seedlings grown under greenhouse and growth-chamber conditions was qualitatively similar to the response observed in field-collected tissue (DeLucia & Smith, 1987). Exposure to successive nights of  $0^{\circ}\text{C}$  or  $-2.5^{\circ}\text{C}$  caused a slight though reversible decrease in photosynthesis. Photosynthesis declined precipitously after exposure to  $-4^{\circ}\text{C}$ . A similar threshold response for photosynthesis was observed for *Picea sitchensis* exposed to below freezing temperatures (Neilson, Ludlow & Jarvis, 1972). Although no attempt was made to detect an exotherm associated with ice formation, needle water generally freezes in this temperature range (Pisek, 1973; Bauer, Larcher & Walker, 1975). Nucleation of intercellular ice crystals causes the disruption of cell membranes and the breakdown of intercellular compartmentalization. In addition, the lower free energy of ice causes dehydration of the cytosol as water diffuses to the growing crystal. The minimal response of photosynthesis to night temperatures from 2 to  $-2.5^{\circ}\text{C}$  indicates that spruce is relatively insensitive to temperatures above those that cause freezing of intra- and extra-cellular water.

Despite the potential for extensive outcrossing, ecotypic differentiation of Engelmann spruce (Grant & Mitton, 1977) and other conifers (Fryer & Ledig, 1972) along an elevational gradient has been demonstrated. Strong selective pressures operating over relatively short elevational distances produce changes in the peroxidase enzymes in spruce (Grant & Mitton, 1977). There was no evidence, however, for differentiation of the photosynthetic response to freezing nights in seedlings from elevationally disjunct populations. This was not entirely unexpected because of the observation that photosynthesis in spruce was insensitive to summer freezes in the range that occurs with the highest frequency ( $0^{\circ}\text{C}$  to  $-2.5^{\circ}\text{C}$ ). Furthermore, two potentially important environmental limitations to photosynthesis during the summer, freezing nights and low maximum air temperature, may operate as similar selective forces at different elevations. The frequency and severity of freezing night temperatures during the summer are greatest at low elevations, whereas maximum daily temperature is lowest at high elevations. It is possible that low maximum air temperature, and the onset of severe frost during the late fall and early winter, are the overriding selective factors operating on photosynthesis in spruce.

Although there was no conclusive evidence for genetic differentiation of the photosynthetic response to freezing nights, there was a phenotypic or acclimatory response. Net photosynthesis and

stomatal conductance in spruce were inhibited more strongly in seedlings grown at  $24^{\circ}\text{C}/19^{\circ}\text{C}$  than in those grown at  $14^{\circ}\text{C}/9^{\circ}\text{C}$ , day/night temperatures. It is well established that growth under different temperature regimes can result in compensatory changes in photosynthesis and respiration that will maximize the rate of carbon assimilation at the specific growth conditions (see Berry & Björkman, 1980; Öquist, 1983 for review). The mechanism of temperature acclimation is complex and involves many structural and biochemical changes. The metabolic changes associated with growth of spruce at warm temperatures resulted in a greater sensitivity to freezing nights. An increased sensitivity, and a higher frequency and severity of freezing nights during the summer at low elevations, suggests that freezing nights may pose a greater limitation to carbon assimilation during the summer at low rather than at high elevations.

Exposure to night temperatures low enough to inhibit photosynthesis caused a parallel decrease in stomatal conductance (Figs 1 & 2); however, photosynthesis was not limited by stomatal conductance. The stomatal limitation to gas exchange, calculated from the relationship between photosynthesis and  $C_i$ , actually decreased from 26 to 14% after exposure to a hard freeze. In contrast, one night at  $-5.0^{\circ}\text{C}$  caused an 88% decrease in photosynthesis at an internal  $\text{CO}_2$  concentration of  $350\text{ mm}^3\text{ dm}^{-3}$  indicating a strong non-stomatal limitation of photosynthesis. A hard freeze also caused a decrease in carboxylation efficiency and apparent quantum yield. Carboxylation efficiency is defined as the initial slope of the photosynthesis vs  $C_i$  curve (Ku & Edwards, 1977) and is closely correlated with the RuBP saturated kinetics of RuBP carboxylase (von Caemmerer & Farquhar, 1981; Farquhar, von Caemmerer & Berry, 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 decrease in photosynthesis at high  $C_i$  and the decrease in apparent quantum yield suggest that a hard freeze inhibits the rate of photosynthetic electron transport. Freezing may inhibit photosynthesis at several sites on the subcellular level. Rumich-Bayer & Krause (1986) found a hierarchy of susceptibility to freezing in several photosynthetic processes. In a study examining the effect of freezing on protoplasts from *Valerianella locusta* they found that  $\text{CO}_2$ -dependent  $\text{O}_2$  evolution was inhibited first, followed by ATP synthesis and photosynthetic electron transport.

A light freeze ( $-2.5^{\circ}\text{C}$ ) caused a slight reduction in the photosynthetic rate at high irradiances and a decrease in  $C_i$  in spruce (Fig. 3) suggesting that photosynthesis was diffusion limited. However, an increase in  $C_i$  at high irradiances, and the decrease in photosynthesis at a given  $C_i$  (Fig. 4), indicate that a hard freeze inhibits photosynthesis by increasing the

internal or biochemical resistance to carbon assimilation. Lower photosynthetic rates as a result of high internal or biochemical resistances have been observed in other conifers in response to subfreezing air temperature (Zelawski & Kucharska, 1967; Neilson *et al.*, 1972).

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