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The Potential for Photoinhibition of *Pinus sylvestris* L. Seedlings Exposed to High Light and Low Soil Temperature

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ABSTRACT

The effect of high light and root chilling on gas exchange, chlorophyll fluorescence, and bulk shoot water potential (ψ_{shoot}) was examined for *Pinus sylvestris* seedlings. Transferring plants from low light (200 μmol m^{-2} s^{-1}, *PAR*) and a soil temperature of 15 °C to high light (850 μmol m^{-2} s^{-1}) and 1 °C caused >90% decrease in net photosynthesis and leaf conductance measured at 350 mm^3 dm^{-3} CO_2, and a decrease in the ratio of variable to maximum fluorescence (F_v/F_m) from 0·83 to 0·63. The decrease in F_v/F_m was, however, only marginally greater than when seedlings were transferred from low to high light but kept at a soil temperature of 15 °C. Thus, photoinhibition was a minor component of the substantial decrease observed for net photosynthesis at 1 °C soil temperature. The decrease in net photosynthesis and ψ_{shoot} at 1 °C was associated with an increase in calculated intracellular CO_2 concentration, suggesting that non-stomatal factors related to water stress were involved in inhibiting carbon assimilation. Measurements at saturating external CO_2 concentration, however, indicate that stomatal closure was the dominant factor limiting net photosynthesis at low soil temperature. This interpretation was confirmed with additional experiments using *Pinus taeda* and *Picea engelmannii* seedlings. Decreases in gas-exchange variables at 5 °C soil temperature were not associated with changes in ψ_{shoot}. Thus, hormonal factors, localized decreases in ψ_{needle}, or changes in xylem flux may mediate the response to moderate root chilling.

Key words: Conifer, photoinhibition, photosynthesis, *Picea*, *Pinus*, root temperature, stomatal conductance, water potential.

INTRODUCTION

Low temperature is a dominant abiotic limitation to photosynthesis of boreal and subalpine trees. The potential for positive carbon gain on an annual basis is defined by the period with daily maximum air temperatures greater than 0 °C. However, intermittent night-time frosts may further reduce photosynthesis on subsequent days during summer (DeLucia and Smith, 1987; Strand and Lundmark, 1987). Recently it has been shown that low soil temperature also limits stomatal conductance and photosynthesis in conifers well after the cessation of frost. In the central Rocky Mountains (USA) air temperature rises rapidly through May and June, but, depending on snow depth and elevation, soil-temperature limitations to photosynthesis may occur through late July (Day, DeLucia, and Smith, 1989; DeLucia and Smith, 1987). Low soil temperature delays the onset of root growth (Lopushinsky and Kaufmann, 1984), increases root hydraulic resistance (Running and Reid, 1980), and reduces transpiration and stomatal conductance (Kaufmann, 1984; Kramer, 1942). Low soil temperature can reduce net photosynthesis either by reducing stomatal conductance or by other mechanisms acting directly at the biochemical level (DeLucia, 1986).

The combination of low soil temperature caused by persistent snow cover creates a situation early in the season where air temperature may be optimal but net photosynthesis is limited by low soil temperature. Moreover, snow has relatively high albedo (Rosenberg, Blad, and Verma, 1983) and greatly increases reflected shortwave irradiance on foliage. The sum of downward and reflected photosynthetically active radiation (*PAR*, 400–700 nm) incident on a branch over clean snow can exceed

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3500 μmol m^{-2} s^{-1} (Day *et al.*, 1989). The combination of high irradiance and reduced rates of photosynthesis may cause photoinhibition, evident as a reduction in quantum yield and, in many cases, the maximum capacity for net photosynthesis (Powles, 1984). The primary damage of photoinhibition occurs in photosystem II, however, the precise molecular mechanisms are not well understood. Photoinhibition may occur in different situations where excitation energy exceeds the capacity for photochemistry such as the combination of high light and drought (Björkman and Powles, 1984; Ögren and Öquist, 1985), chilling or freezing temperatures (Farage and Long, 1987; Öquist and Ögren, 1985; Strand and Lundmark, 1987), or high temperatures (Ludlow and Björkman, 1984; Adams, Smith, and Osmond, 1987). The interaction of light and low soil temperature have not been investigated.

The objective of this study was to test the prediction that low soil temperature can act synergistically with high irradiance to cause photoinhibition of photosynthesis of *Pinus sylvestris*. Parameters derived from the induction kinetics of chlorophyll fluorescence have proven useful in examining the extent of photoinhibition (Adams, Demmig-Adams, Winter, and Schreiber, 1990; Bolhar-Nordenkampf, Long, Baker, Öquist, Schreiber, and Lechner, 1989; Ögren and Öquist, 1984). We used the ratio of variable/maximum fluorescence (F_v/F_m) measured at room temperature, which provides an estimate of the photochemical efficiency of photosystem II (Kitajima and Butler, 1975), and apparent quantum yield of O_2 evolution (Demmig and Björkman, 1987) to evaluate photoinhibition under root-chilling conditions. Additional experiments were conducted using *Pinus taeda* and *Picea engelmannii*, a root-chilling susceptible and tolerant species, respectively, to examine potential mechanisms of soil temperature limitations to photosynthesis. Experiments were done under controlled growth chamber conditions by manipulating root and shoot temperature independently for non-hardened seedlings.

MATERIALS AND METHODS

Plant material

Pinus sylvestris L. (Scots pine; 65°17′N, 16°43′E, altitude 500 m, open pollinated, Ostteg) was grown from seed and frost hardened according to Strand and Öquist (1985). Twenty-week old seedlings were planted in 250 cm^3 pots in peat/loam soil mix (1:1 by vol.). Plants were grown in a glasshouse for 6 weeks at approximately 23 °C. The photoperiod was extended to 16 h with supplemental light (150 μmol m^{-2} s^{-1}, *PPFD*, at the top of the shoot) provided by fluorescent tubes. Seedlings were transferred to a growth chamber 2 weeks before the start of experiments. The day and night air temperatures in the chamber were 25 °C and 15 °C, respectively. Light was provided by metal halogen lamps (Osram HAI-T 400 W D/H; 200 μmol m^{-2} s^{-1}, *PPFD*) for 17 h d^{-1} and relative humidity was 50%. A second experiment was conducted with *Pinus taeda* (loblolly pine) and *Picea engelmannii* (Engelmann spruce) seedlings grown from seeds for approximately 6 months in a growth chamber. *Pinus taeda* seeds were collected from open-pollinated trees growing on low elevation coastal plain in North Carolina (35°N, 79°W), and *Picea engelmannii* seeds were from trees growing at approximately 2800 m above sea-level in the Rocky Mountains (41°21′N, 106°13′W). Seeds were planted in 500 cm^3 pots in a peat:sand:vermiculite mix (1:1:1 by vol.). The *PPFD* at the top of seedlings in the growth chamber was 500 μmol m^{-2} s^{-1} for a 14 h photoperiod. Day and night air temperature were 24 °C and 20 °C, respectively, and the relative humidity was 50%. Seedlings were fertilized regularly and, by the beginning of the experiments, seedlings had produced mature fascicles or needles. *Pinus sylvestris* and *Picea engelmannii* had set terminal buds.

Experimental treatments

For experiments with *Pinus sylvestris*, root temperature was controlled by inserting pots through the insulated lid of a temperature-controlled water bath (Hetofrig, Birkerod, Denmark). Prior to immersion pots were watered and allowed to drain to field capacity and the bottoms were placed in plastic bags to prevent inundation of the root systems. Shoots were insulated from roots by placing loosely fitted 2·0 cm styrofoam collars around the stems at the soil surface. Root temperature was measured at the centre of each pot with a copper–constantan thermocouple and foliage temperature was measured by looping fine copper–crommel thermocouples around individual needles. Variation in soil temperature within and between pots was $\leq$0·5 °C. For high light treatments the water bath was placed under two additional metal halogen lamps (Osram HQI-T 400 W/DH) that were suspended from the ceiling of the growth chamber. A plexiglass bath containing 10 cm of water was used as a heat filter between the lights and seedlings. Because of rapid air flow in the growth chamber, needle temperature under the high light treatment was $\leq$1·5 °C above air temperature.

The experimental protocol consisted of measuring control rates of gas exchange for seedlings in the growth chamber at 10.00 h and then transferring seedlings to the treatment conditions. Gas exchange was measured again at 10.00 h and 20.00 h on the following day. The same protocol was used for the treatments in a second experiment but gas exchange was measured at 20.00 h on day 2 only. The treatments consisted of manipulating root temperature at constant shoot temperature with high or low irradiance. Control plants were kept at 15 °C root temperature and low light (200 μmol m^{-2} s^{-1}, *PPFD*). Roots were chilled to 5 °C or 1 °C and the high light treatment was 850 μmol m^{-2} s^{-1} measured at the top of the shoots. Seedlings transferred from low to high light, but kept at 15 °C root temperature, served as a control for changing the light regime at constant temperature. Stem collars were used to examine the effect of stem chilling on gas exchange. Temperature at the stem surface was maintained at 1 °C, while roots and shoots were at 15 °C and 25 °C, respectively. To examine the effects of root chilling on photosynthesis at high shoot water potential, a subset of plants were misted with deionized water continuously during root-chilling treatments.

Root-chilling treatments for *Pinus taeda* and *Picea engelmannii* were administered by circulating a refrigerated polyethylene glycol 4000 solution through insulated copper coils surrounding the pots (Day, Heckathorn, and DeLucia, 1990). Plants were kept at their growth light condition 500 μmol m^{-2} s^{-1} (*PPFD*) during the root chilling treatments and photosynthetic characteristics were measured before and after 30 h at a root temperature of 1 °C. Root chilling treatments were administered at 50% RH or with shoots exposed to continuous misting.

Physiological measurements

Carbon dioxide and water vapour exchange were measured with a closed IR gas analysis system (model LI-6200, LICOR Inc., Lincoln, NE, USA). The entire shoot was enclosed in a well-mixed 4·0 dm^3 cuvette. Humidity in the cuvette was generated by transpiration and maintained at $40 \pm 5\%$ by diverting a portion of the incoming air through a column containing magnesium perchlorate (anhydrous). Transpiration was calculated by measuring the rate of change in humidity in the cuvette (which was maintained close to 0) and correcting for the addition of dry air. Flow rate through the desiccant and into the cuvette was measured with a mass flow meter. The initial CO_2 concentration in the cuvette was approximately 400 $mm^3\ dm^{-3}$ and the measurement was initiated once the plant had depleted the atmosphere to $<360\ mm^3\ dm^{-3}$. Measurements were made over a 5 $mm^3\ dm^{-3}$ depletion and typically took 20 to 60 s. The cuvette was cooled with an external fan and heat-exchanger so foliage temperature inside the cuvette was $20 \pm 1{\cdot}5$ °C. Measurements were made at an irradiance of 500 $\mu mol\ m^{-2}\ s^{-1}$ (*PPFD*; Osram HQI-T400 W/DH) provided to each side of the cuvette to minimize self-shading (Leverenz and Jarvis, 1979). Net photosynthesis, transpiration, and stomatal conductance to water vapour were calculated as in von Caemmerer and Farquhar (1981) on a projected needle area basis. We assumed negligible cuticular transpiration and a boundary layer conductance of 1·3 $mol\ m^{-2}\ s^{-1}$.

Apparent quantum yield and maximum rates of O_2 evolution at light saturation (880 $\mu mol\ m^{-2}\ s^{-1}$, *PPFD*) were measured at 5% CO_2 with a leaf disc electrode (LD2, Hansatech Ltd., Norfolk, England) according to Delieu and Walker (1981). Needles were placed in the cuvette in the same orientation relative to the light as they had on the plant and were illuminated with a metal halogen lamp (LS2, Hansatech). Irradiance was manipulated with neutral density filters and the cuvette was flushed with humidified 5% CO_2 in air before each measurement. Recent results suggest that 5% CO_2 may be insufficient to overcome completely stomatal limitations to gas exchange for stressed conifer needles (Day *et al.*, 1990). Measurements of O_2 evolution for *P. taeda* and *P. engelmannii* were, therefore, made at 10% CO_2.

Room temperature fluorescence induction kinetics expressed as F_v/F_m were measured with a microprocessor controlled bifurcated fibre optic system described by Öquist and Wass (1988) (PSM, BioMonitor S.C.I. AB, Umeå). Excitation was with a broad-band filter (5·0 mm Schott, BG 39) which transmits between *c.* 330 and 660 nm with a peak at 500 nm. The excitation light was 400 $\mu mol\ m^{-2}\ s^{-1}$ (*PPFD*), and the high values of F_v/F_m (>0·80) for control plants suggested that this light intensity was sufficient to saturate all electron acceptors of photosystem II. Fluorescence from photosystem II was measured at 691 mn (Ealing, half band width 12·1 nm) on attached needles that were dark acclimated for 30 min. We did not report values for F_o because total needle area varied among measurements. Following gas-exchange and fluorescence measurements, bulk shoot water potential was measured with a Scholander-type pressure chamber (Ritchie and Hinckley, 1975).

RESULTS

Chilling the root systems of *Pinus sylvestris* seedlings for 24 to 34 h caused significant reductions in net photosynthesis (A_{max}) and stomatal-conductance to water vapour (g) measured at *c.* 350 $mm^3\ dm^{-3}$ CO_2 and saturating irradiance (Fig. 1). Inhibition was greatest for seedlings transferred to high light and a root temperature of 1 °C.

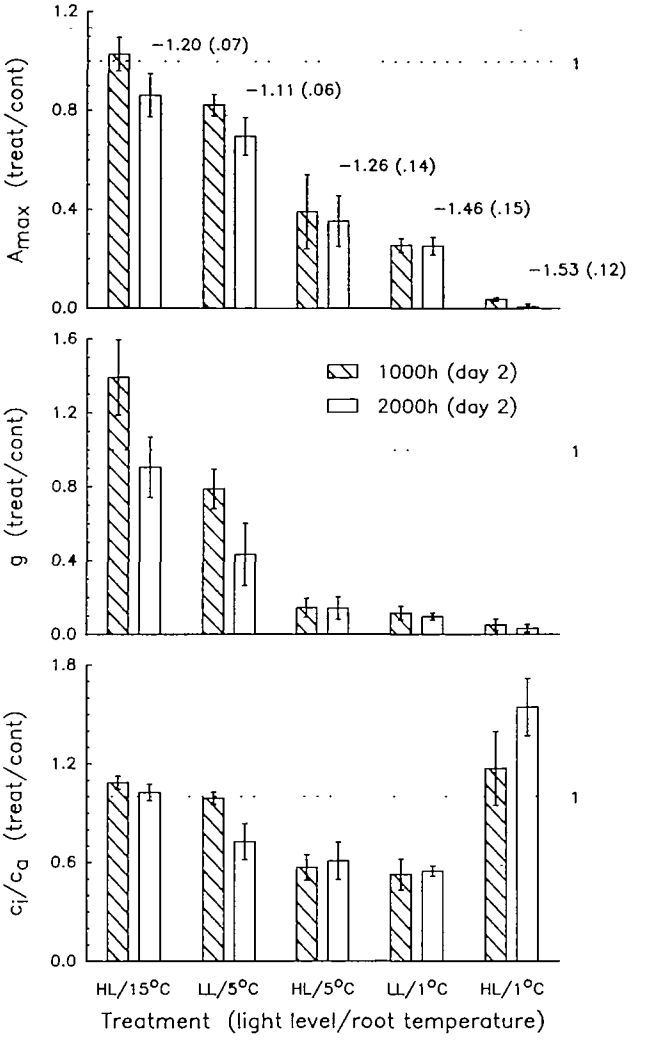

FIG. 1. The effect of soil temperature and light level on net photosynthesis (A_{max}), bulk shoot water potential, stomatal conductance to water vapour (g), and the ratio of calculated intercellular to ambient CO_2 concentration (c_i/c_a) for potted seedlings of *Pinus sylvestris*. The dependent variables ($n=4$, ±1 s.d.) are expressed as fractions of the control values, which were measured at 10.00 h on the previous day. Mean values of water potential (MPa) ±1 s.d. measured at 20.00 h are indicated above the open bars in the top panel. Measurements were made with an IRGA-system at *c.* 355 $mm^3\ dm^{-3}$ CO_2. The high light (HL) and low light (LL) treatments were 850 and 200 $\mu mol\ m^{-2}\ s^{-1}$ (*PPFD*), respectively. Typical control values of A_{max}, g, and c_i were $3{\cdot}65 \pm 0{\cdot}16\ \mu mol\ m^{-2}\ s^{-1}$, $62 \pm 10\ mmol\ m^{-2}\ s^{-1}$, and $240 \pm 14\ mm^3\ dm^{-3}$, respectively.

Transferring low light grown (200 $\mu mol\ m^{-2}\ s^{-1}$, *PPFD*) seedlings to high light (850 $\mu mol\ m^{-2}\ s^{-1}$) but maintaining high root temperature (15 °C), caused a small but significant reduction in A_{max} and g after 24 h of treatment (Fig. 1). Similar results were observed for A_{max} and apparent quantum yield for O_2 evolution measured at high CO_2 concentration (Table 1). The decreases in gas-exchange parameters associated with the transfer from low to high light may have been partially caused by photoinhibition, as indicated by the decrease in F_v/F_m (Fig. 2, Table 1).

TABLE 1. *Effect of soil temperature treatments for 24 h and light level* (PPFD) *on light-saturated rate of photosynthesis* (A_{max}, $\mu mol\ m^{-2}\ s^{-1}$), *apparent quantum yield* (ϕ, $\mu mol/\mu mol$), *ratio of variable maximum fluorescence* (F_v/F_m), *and bulk shoot water potential* (ψ_{shoot}, *MPa*) *of* Pinus sylvestris *seedlings*

Net photosynthesis was measured as O_2 evolution at 5% (v/v) CO_2. The treatments were: Control—15 °C soil temperature and low light (200 $\mu mol\ m^{-2}\ s^{-1}$); Control, HL—15 °C soil temperature and high light (800 $\mu mol\ m^{-2}\ s^{-1}$); Stem chill, HL—1 °C stem temperature and high light; 1 °C soil, HL—root chilling and high light; 1 °C soil, HL, Mist—continuous misting of foliage during the soil chilling treatment. Values are the mean of four plants (±1 s.d.).

Treatment	A_{max}	ϕ	F_v/F_m	ψ_{shoot}
Control	20·5 (3·1)	0·0984 (0·0021)	0·851 (0·014)	−0·82 (0·11)
Control, HL	19·4 (1·1)	0·0890 (0·0080)	0·723 (0·048)	−0·95 (0·08)
Stem chill, HL	18·1 (2·5)	0·0954 (0·0129)	0·712 (0·031)	−0·70 (0·04)
1 °C soil, HL	15·3 (1·7)	0·0758 (0·0058)	0·670 (0·062)	−1·81 (0·21)
1 °C soil, HL, Mist	20·5 (3·9)	0·0799 (0·0188)	0·698 (0·032)	−0·27 (0·09)

Root chilling treatments under low light conditions caused significant reductions in A_{max} and g (Fig. 1) but had no effect on F_v/F_m (Fig. 2). The reduction in intercellular CO_2 content (c_i) suggests that lower A_{max} was caused by reduced g under these conditions. Net photosynthesis and g were strongly reduced by root chilling to 5 °C or 1 °C and high light. However, the slight increase in c_i in seedlings exposed to high light at a root temperature of 1 °C indicates that non-stomatal limitations may have contributed to the inhibition of photosynthesis under more extreme conditions. In contrast to low light, the combination of root chilling and high light caused a reduction in F_v/F_m. The decrease in F_v/F_m was slightly greater when seedlings were exposed to high light and low root temperatures than when exposed to high light only, indicating an interaction between light and root temperature.

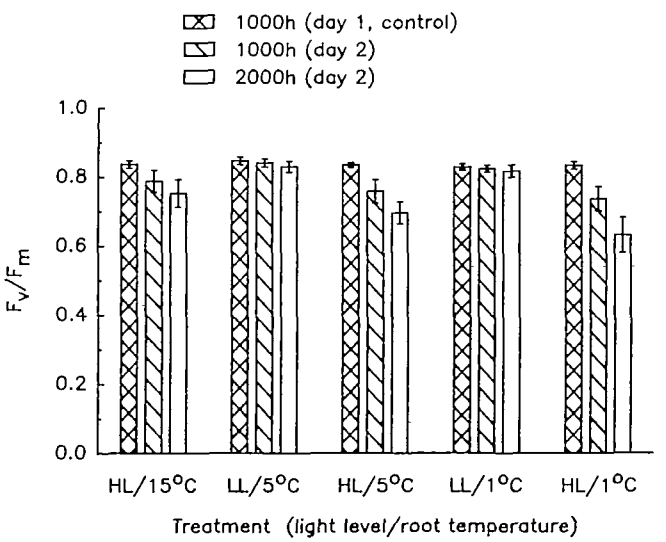

FIG. 2. The effect of soil temperature and light level on the ratio of variable to maximum chorophyll fluorescence (F_v/F_m) measured at room-temperature ($n=4$, ±1 s.d.). The treatments were the same as in Fig. 1.

The ψ_{shoot} of control plants at a root temperature of 15 °C and low light was −1·23 (±0·03) MPa. Exposure to high light or high light and root temperatures of 5 °C had no effect on ψ_{shoot}, which decreased significantly after exposure to 1 °C root temperatures (Fig. 1).

Using the same experimental protocol but measuring rates of O_2 evolution at 5% CO_2, 1 °C root temperature caused *c.* 25% and 15% reductions in A_{max} and apparent quantum yield, respectively, relative to the high light control (Table 1). These reductions were substantially less than observed for measurements of CO_2 exchange, indicating that the primary cause of reduced photosynthetic rates at 1 °C root temperature was stomatal closure. Misting foliage during the root chilling treatment maintained high ψ_{shoot} and prevented inhibition of A_{max} and apparent quantum yield, though there was a slight reduction in apparent quantum yield and F_v/F_m associated with the transfer to high light (Table 1). Stem chilling had no effect on gas-exchange variables or F_v/F_m. Thus, feedback inhibition of photosynthesis by carbohydrates probably was not a significant factor reducing photosynthesis.

Root chilling (1 °C) caused significant reductions in ψ_{shoot} for *Pinus taeda* and *Picea engelmannii* but had no effect on A_{max} for O_2 evolution measured at 10% CO_2 (Table 2). Dark respiration of both species was consistently lower after root chilling, which also caused a slight reduction in apparent quantum yield for *P. engelmannii*. Misting caused large increases in ψ_{shoot} and A_{max} for *Pinus taeda* (Table 2). This suggests that under control temperature conditions total plant resistance to water flow may have limited photosynthesis for this species. We suspect that the decrease in A_{max} following misting for *Picea engelmannii* was caused by leaching of foliar nutrients. Repeated experiments with *Pinus taeda* and *Picea engelmannii* yielded similar results.

TABLE 2. *The effect of root chilling and misting on the light-saturated rate of photosynthesis* (A_{max}, $\mu mol^{-2} s^{-1}$), *apparent quantum yield* (ϕ, $\mu mol/\mu mol$), *dark respiration* (R_d, $\mu mol\, m^{-2} s^{-1}$), *and bulk shoot water potential* (ψ_{shoot}, *MPa*) *for* Pinus taeda *and* Picea engelmannii

Net photosynthesis was measured as O_2 evolution at 10% (v/v) CO_2. Values are the mean of four plants (±1 s.d.).

	A_{max}	ϕ	R_d	ψ_{shoot}
Pinus taeda				
20 °C air/20 °C soil				
No Mist	21·6 (3·8)	0·0868 (0·0123)	2·5 (0·3)	−0·87 (0·18)
Mist	29·1 (2·5)	0·1023 (0·0079)	1·4 (0·4)	−0·44 (0·05)
20 °C air/1 °C soil				
No Mist	19·6 (1·3)	0·0905 (0·0044)	1·9 (0·3)	−1·70 (0·15)
Mist	19·6 (2·8)	0·0769 (0·0058)	2·5 (0·1)	−0·42 (0·10)
Picea engelmannii				
20 °C air/20 °C soil				
No Mist	18·2 (1·4)	0·0558 (0·0039)	2·6 (0·4)	−0·77 (0·07)
Mist	19·7 (0·9)	0·0433 (0·0043)	1·2 (0·1)	−0·33 (0·06)
20 °C air/1 °C soil				
No Mist	18·4 (1·6)	0·0426 (0·0039)	1·2 (0·2)	−1·38 (0·27)
Mist	12·5 (1·5)	0·0353 (0·0059)	36·9 (14·2)	−0·36 (0·04)

DISCUSSION

Low soil temperature caused dramatic reductions in A_{max} and g for potted seedlings of *Pinus sylvestris* measured at ambient CO_2 concentrations. Similar results have been reported for other conifers, including *Picea engelmannii*, *Pinus contorta*, *Pinus strobus*, and *Pinus taeda* (Day *et al.*, 1989; DeLucia, 1986; Kramer, 1942; Running and Reid, 1980). In contrast to this study, Linder (1972) found no effect of soil temperature on net photosynthesis of *P. sylvestris* at soil temperatures above 2 °C. This discrepancy is explained by differences in experimental protocol. Linder (1972) exposed seedlings to decreasing soil temperatures for relatively short durations; less than 1 hour per treatment temperature. More than 4 h at a root temperature of *c.* 1 °C was necessary to initiate a reduction in gas-exchange parameters for *Picea engelmannii* (DeLucia, 1986) and a longer time may be necessary depending on the size of the seedling. The size dependence of the response to soil temperature may be a function of stem capacitance (Day *et al.*, 1990).

The combination of low soil temperature and high light caused significant reductions of F_v/F_m and apparent quantum yield for *Pinus sylvestris* (Fig. 2, Table 1), indicating the occurrence of photoinhibition. However, the decrease in these variables for seedlings at 1 °C soil temperature and high light was only marginally greater than for seedlings transferred to high light at control soil temperature. Thus, photoinhibition played a minor role in soil temperature-induced reductions in net photosynthesis of *Pinus sylvestris*. In contrast to low soil temperature, a foliage temperature of 1 °C would induce very strong photoinhibition under the light regime used in this study (Öquist and Huner, 1990). This implies that it is the low temperature effect on the photosynthetic apparatus *per se* that triggers increased susceptibility to photoinhibition rather than the suppression of the photosynthetic process.

The mechanisms of low soil temperature-induced reductions in net photosynthesis are complex. The decrease in c_i/c_a at a soil temperature of 5 °C suggests that the reduction in net photosynthesis was caused by the concomitant reduction in g (Fig. 1). Low soil temperature causes a decrease in root hydraulic conductivity (Running and Reid, 1980; Smit-Spinks, Swanson, and Markhart, 1984) and may reduce transpiration and ψ_{shoot}. However, there was no significant reduction in ψ_{shoot} at 5 °C, indicating that the decrease in g may have been mediated by hormonal interactions rather than a direct response to leaf water potential. Endogenous signals from roots have been implicated in regulating stomatal conductance under conditions of limiting and excessive soil moisture, and may also be important under moderate root chilling (Belding and Young, 1989; Davies, Metcalfe, Lodge, and da Costa, 1986).

Exposure to soil temperatures of 1 °C caused significant reductions in ψ_{shoot} for all three species (−1·53 to −1·81 MPa), and stomatal closure in conifers is generally associated with values of ψ_{shoot} in this range (Lassoie, Hinckley, and Grier, 1985; Smith, 1985). As observed at 5 °C, the decrease in A_{max} for *Pinus sylvestris* at 1 °C and low light was caused by the decrease in g. At 1 °C and high light, however, the increase in c_i/c_a indicates an increase in non-stomatal limitations to photosynthesis associated with water stress. Although the high light treatment caused only a 1·5 °C increase in needle temperature, this would be sufficient to cause a 16% increase in

transpiration under the conditions of this experiment. The combination of increased transpiration and severely reduced root hydraulic conductivity contribute to low ψ_{shoot} and reduced g observed under these conditions. Non-stomatal limitations of photosynthesis have been documented in water-stressed plants (Bradford and Hsiao, 1982; DeLucia and Heckathorn, 1989; Ehleringer and Cook, 1984; Jones and Faujul, 1982; Ögren and Öquist, 1985). Inferences based on c_i calculated for severely stressed plants must, however, be viewed with caution. At very low g, cuticular transpiration (Kirshbaum and Pearcy, 1988) and stem respiration may cause significant errors in estimation of c_i. Moreover, the possibility of errors caused by heterogeneous stomatal closing (Downton, Loveys, and Grant, 1988; Terashima, Wong, Osmond, and Farquhar, 1988) have not been assessed for most conifers, though our results indicate that stomata closed uniformly when roots of *Pinus taeda* were chilled (Day *et al.*, 1990).

We conclude that photoinhibition occurs when the root system of *Pinus sylvestris* is chilled under high light conditions, but the effect is minor. The minimal effects of root chilling on A_{max} (O_2 evolution) and apparent quantum yield measured at saturating external CO_2 concentration indicate that low g was the dominant limitation to photosynthesis at low soil temperatures. Although a causal relationship can not be inferred from these data, under moderate root-chilling (5 °C) stomata may respond to hormonal signals from the roots (Benzioni and Dunstone, 1988; Davies *et al.*, 1986). Teskey, Hinckley, and Grier (1983) suggested that stomatal conductance may also decrease in direct response to reductions in xylem water flux caused by increased root resistance under chilling conditions. Changes in water flux may cause localized release of ABA in foliage or decreases in water potential in the epidermis. Under more severe root chilling (1 °C) stomata appear to be responding to changes in ψ_{shoot} as well.

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