Enhanced tolerance of photosynthesis to high-light and drought stress in *Pseudotsuga menziesii* seedlings grown in ultraviolet-B radiation

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Received July 16, 2001; accepted March 2, 2002; published online July 2, 2002

**Summary** We investigated the effects of an ambient dose of ultraviolet-B (UV-B) radiation on chamber-grown *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco (Douglas-fir) seedlings, to determine if the presence of UV-B radiation in the growth light regime induces tolerance to environmental stresses such as high light and drought. Douglas-fir seedlings were grown without UV-B radiation or with 6 kJ m⁻² day⁻¹ of biologically effective UV-B, which is ambient for the intermountain regions of Idaho. Non-stressed seedlings grown with UV-B radiation had 35% lower seedling dry mass, 36% higher concentrations of UV-B absorbing compounds per unit leaf area, 30% lower stomatal frequencies, 25% lower light-saturated photochemical efficiencies of Photosystem II (ΦPSII) and 45% lower light-saturated stomatal conductance than non-stressed seedlings grown without UV-B radiation. After 4 days of high-light stress, seedlings grown with UV-B radiation had 32% higher light-saturated carbon assimilation rates (A_CO₂) than seedlings grown without UV-B radiation. After water was withheld from the seedlings for up to 15 days, seedlings grown with UV-B radiation had 50% higher A_CO₂ and 40% higher seedling water potentials than seedlings grown without UV-B radiation. The results support the hypothesis that UV-B radiation can act as an environmental signal to induce tolerance to high-light and drought stress in Douglas-fir seedlings. Possible mechanisms for the enhanced stress tolerance are discussed.

**Keywords:** environmental stress, photochemical efficiency of Photosystem II, stomatal conductance, stomatal frequency.

**Introduction**

The effects of decreasing stratospheric ozone and a concomitant increase in tropospheric ultraviolet-B (UV-B) radiation on terrestrial plant species have been the focus of many studies (Bornman and Teramura 1993, Caldwell et al. 1995, Björn et al. 1997). Coniferous species possess long-lived foliage and are, therefore, at risk of cumulative UV-B damage (Sullivan and Teramura 1992). Much of the UV-B radiation that coniferous species are exposed to under natural and artificial light regimes is attenuated in leaves (Bornman and Vogelmann 1988, 1991) by UV-B absorbing compounds produced and deposited in leaf epidermal cells in response to UV-B radiation (Robberecht and Caldwell 1983, Day 1993, Schnitzler et al. 1997), or by plant cuticles (Krauss et al. 1997). Despite mechanisms that protect plants from UV-B absorption, high doses of UV-B radiation have been shown to induce numerous effects in conifers, including reductions in seedling biomass and height (Sullivan and Teramura 1989, 1992, Naidu et al. 1993, Yakimchuk and Hoddinott 1994), decreased leaf expansion and increased leaf thickness and width (Sullivan et al. 1996, Laakso et al. 2000) and increased stomatal frequency (Stewart and Hoddinott 1993).

Effects of UV-B exposure on photosynthesis have been determined for several woody species (reviewed in Laakso and Huttunen 1998). For example, studies have shown that UV-B radiation decreases photosynthetic capacity in 1-year-old seedlings of jack pine (*Pinus banksiana* Lamb.) (Stewart and Hoddinott 1993), and in newly emergent needles (Naidu et al. 1993) and needles with low concentrations of UV-B absorbing compounds (Sullivan and Teramura 1989) in field-grown loblolly pine (*P. taeda* L.). At lower doses of UV-B radiation, inhibition of photosynthesis occurs as a result of decreased stomatal conductance (Schumaker et al. 1997, Correia et al. 1999, Nogués, et al. 1999). In contrast, direct UV-B-induced inhibition of photosynthesis is observed at high UV-B irradiances and is frequently associated with damage to the photosynthetic reaction center, Photosystem II (PSII) (e.g., Strid et al. 1994).

Photosystem II is a major target for photoinhibition of photosynthesis, which occurs when the amount of light available exceeds that necessary for photosynthetic processes (e.g., Ögren and Öquist 1985). Plants are particularly susceptible to photoinhibition when high light occurs in conjunction with other stressful environmental conditions, such as low water availability, as would be the case in a clear-cut area where there is little vegetation to prevent light penetration and rapid evaporation from the soil (Cornic 1994). Water availability is
also an important determinant of seedling survival because water stress severely limits conifer seedling growth, photosynthesis and productivity (Cui and Smith 1991, Eastman and Camm 1995, reviewed in Yordanov et al. 2000).

Most studies of the effects of UV-B radiation on plants have focused on the potential adverse effects of high doses (more than twice ambient), e.g., photoinhibition, degradation of DNA or increased oxidative stress (e.g., Stapleton 1992, Jordan 1996). Exposure to lower doses of UV-B radiation, however, may not impair growth or productivity in many plant species (Allen et al. 1998). There is evidence that exposure of plants to near-ambient doses of UV-B radiation can impart high-light and drought tolerance in woody species, including Pinus pinea L. and Pinus halepensis Mill. (Petropoulou et al. 1995, Björn et al. 1997, Manetas et al. 1997).

Understanding responses of coniferous plants to UV-B (280–300 nm) radiation is essential when deciding whether to grow seedlings for reforestation of clear-cut sites in a nursery bed or in a greenhouse where seedlings are shielded from UV-B by UV-B absorbing glass. In the intermountain western regions of the USA, Pseudotsuga menziesii var. glauca (Beissn.) Franco (Douglas-fir) is often harvested by clear-cutting. Typically, seedlings that are used to reforest these areas are grown under glass and, when transplanted, are at risk of damage caused by exposure to full solar radiation, including ambient UV-B radiation (e.g., Klein 1978, Wellman 1983). Mortality rates as high as 95% have been documented for 2-year-old, greenhouse-grown Douglas-fir seedlings planted on a clear-cut area in the Intermountain West (Van Haverbeke 1987). The primary objective of this research was to test the hypothesis that exposure of Douglas-fir seedlings to ambient UV-B radiation enhances seedling tolerance to environmental stresses, such as high light and low water availability.

Materials and methods

Plant material and supplemental UV-B lighting

Douglas-fir seed (Lawyer Nursery, Plains, MT) was wrapped in moist paper towels and stratified at 4 °C for 2 weeks. The seed was then planted in either 50-cm³ centrifuge tubes or D16 Dee Pots (Stuewe and Sons, Corvallis, OR) filled with a 6:1 (v/v) mixture of Premier ProMix PGX (60–70% peat/30–40% vermiculite, Premier Horticulture, Red Hill, PA) and sand, and watered with the fungicide, Banrot (0.33 g l⁻¹, Scotts-Sierra Corp Protection, Marysville, OH). The pots were kept at 4 °C for 2 days before being placed in a growth chamber. Following germination, the pots were watered with nutrient solution (Coleman 1988) and Peters Conifer Starter (Wenny and Dumroese 1992). Seedlings were grown for 10–12 weeks and their primary needles were subsequently used for all experiments.

The environmental chamber (PGV 36, Conviron, Pembina, ND) was maintained at a day/night temperature of 24/21 °C. Relative humidity in the chamber was kept between 10 and 40% RH. Photosynthetically active radiation (PAR) at 300 µmol m⁻² s⁻¹ was provided throughout a 12-h photoperiod by a combination of incandescent and fluorescent lights. Supplemental ultraviolet radiation was provided by UV-B 313 bulbs (Q-panel Lab Products, Cleveland, OH) that were filtered with polyester or cellulose acetate sheeting (Comographics, Logan, UT). Polyester does not transmit UV-B (wavelengths < 320 nm) and was used for the control treatment. Cellulose acetate transmits wavelengths from 290 to 313 nm. Both polyester and cellulose acetate filter out ultraviolet-C (UV-C) radiation (wavelengths < 290 nm), but allow transmission of ultraviolet-A (UV-A) radiation (wavelengths > 320 nm). The UV-B dose, weighted in accordance with a biological action spectrum (Caldwell 1971) amounted to 6 KJ m⁻² day⁻¹ delivered during a 6-h period centered on solar noon. This is equal to the ambient dose in the Intermountain regions of Idaho (R.A. Donahue, unpublished results). Although a near-ambient dose of UV-B radiation was desired, PAR for this experiment (300 µmol m⁻² s⁻¹) was lower than in the field, with the result that the UV-B/PAR ratio in the growth chamber was higher than in the field. The UV-B dose was adjusted daily to within 3% of the desired dose with a UV-B radiation sensor (Solar Light, Philadelphia, PA) calibrated with a spectroradiometer (Optronics 752, Optronics Laboratories, Orlando, FL). Cellulose acetate and polyester filters were changed monthly or as needed to ensure that the plant to lamp distance did not change by more than 10% during the experiment.

Morphological and biochemical characteristics of seedlings

Leaves were harvested from the third node of the main stem of 20 seedlings. Leaf area was measured from images created with a flatbed scanner (Hewlett-Packard, ScanJet 5100C, Greeley, CO) calibrated with Sigma Scan software (Version 4.0, SPSS, Chicago, IL). Leaves were then dried at 70 °C to constant mass. Specific leaf area (SLA) was calculated as leaf area per unit leaf dry mass (cm² g⁻¹). For total aboveground dry mass, total belowground dry mass and total seedling dry mass, whole seedlings were harvested, divided into component tissues, oven-dried and weighed. Root/shoot ratios were determined from dry masses of individual seedlings. For needle length and width, leaves were harvested from 10 seedlings and total length and width of the needles were measured with the aid of a dissecting scope. Seedling height was measured to the nearest mm from the ground line to the shoot apex.

Chlorophyll, UV-B absorbing compounds and soluble protein were extracted and quantified from 1 cm² of leaf tissue harvested from five seedlings. Chlorophylls a and b were extracted with 80% (v/v) acetone and quantified spectrophotometrically (Shimadzu UV-2401PC, Columbia, MD) based on the extinction coefficients determined by Porra et al. (1989). Ultraviolet-B absorbing compounds were extracted from leaves with acidified methanol (methanol:HCl:water, 90:1:1) and quantified spectrophotometrically (A[subscript 313]) (Bradford 1976), with bovine serum albumin as the standard.
Leaf anatomical characteristics

Leaf anatomical parameters, including stomatal frequency and individual cell layer thicknesses, were determined on leaves collected from the third node of the seedling main stem. The number of stomata per mm² was determined from clear nail polish prints made from the epidermal surface.

Photomicrographs of the abaxial leaf surface were made from leaves clarified with a 1:1 (v/v) solution of 30% hydrogen peroxide and glacial acetic acid (Franklin 1945) and subsequently stained with toluidine blue. Digital images were obtained from wet mounts of the leaves with a high-resolution video camera (SPOT Digital, Diagnostic Instruments, Sterling Heights, MI) mounted on a light microscope (Leica DM-R, Wetzlar, Switzerland) to document the difference in stomatal frequencies from actual leaf samples, as opposed to nail polish prints. Leaf thickness, adaxial and abaxial epidermis thickness, palisade parenchyma and spongy parenchyma thickness were determined at the center of toluidine blue-stained thin (1 µm), transverse sections of leaves that had been fixed in 50% for-
determined at the center of toluidine blue-stained thin (1 µm), transverse sections of leaves that had been fixed in 50% for-

Photosynthesis measurement

Photosynthetic carbon assimilation \( (A_{CO_2}) \) and stomatal conductance to water vapor \( (g_s) \) under ambient CO₂ conditions \( (350 \mu l l^{-1}) \) were measured with an open infrared gas analysis
ductance to water vapor \( (A_{CO_2}) \). Photosynthetic carbon assimilation \( (A_{CO_2}) \) was calculated based on values ob-
tained from \( \phi_{PSII} \) measurements. Parameter \( J_o \), a function of \( \phi_{PSII} \), times the amount of incident light absorbed \( (L_i; \text{ assumed to be } 0.85) \times 10^3 \) times a factor for the partitioning of photons between photosystem I \( (PSI) \) and PSII \( (F_o; \text{ assumed to be } 0.5) \) di-
vided by four (considering 4 e-\( O_2 \) evolved by PSII). There-

The photosynthetic response of Douglas-fir leaves to irradi-

Fluorescence measurements

Chlorophyll fluorescence was measured with a pulse-modu-

High-light and water stress treatments

High-light stress was imposed by exposing seedlings to 2000 \( \mu mol m^{-2} s^{-1} \) of PAR from two 150-W fixed output metal halogen lamps at 25 °C (0 UV-B) for 4 h centered around solar noon for four consecutive days. Irradiances were monitored throughout the treatments to ensure that all of the seedlings re-

Water stress was induced by withholding water from seed-

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tered plants before the water stress treatment (Day 0) and 5, 10 and 15 days after it. Stem water potential ($\Psi_w$) was measured with a pressure chamber (Model 600, PMS Instrument, Corvallis, OR) for a separate set of seedlings under similar drought conditions. The seedlings were maintained in their original growth conditions ($\pm$ UV-B) throughout the duration of the water-stress experiment.

**Statistical analysis**

All experiments were repeated three times with seedlings of approximately the same age but grown at different times. Students t-tests were employed to determine statistically significant differences between treatment and control plants for all parameters measured.

**Results**

**Morphological and biochemical characteristics of seedlings**

Compared with Douglas-fir seedlings grown without UV-B radiation, seedlings grown with ambient UV-B radiation exhibited 40% lower leaf dry mass, but similar leaf area, leading to a 30% lower SLA (Table 1). Ultraviolet-B radiation decreased aboveground dry mass (30%), total belowground dry mass (43%), total seedling dry mass (35%), root/shoot ratio (22%) and seedling height (30%) compared with seedlings grown without UV-B radiation. Seedlings grown with UV-B radiation also had a 36% higher concentration of UV-B absorbing compounds than seedlings grown without UV-B radiation. The UV-B radiation treatment had no effect on needle length or width, chlorophyll parameters or total soluble protein concentration (data not shown).

**Leaf anatomical characteristics**

Ultraviolet-B radiation had no significant effect on thickness of leaves, adaxial or abaxial epidermis, or palisade or spongy mesophyll. Seedlings from both treatments exhibited similar leaf anatomy (Figure 1) and were hypostomatic. No treatment differences were observed in the extent of intercellular air spaces present in the spongy mesophyll, which was assessed both visually and by comparing the fresh mass of leaves to that of leaves infiltrated with water under vacuum (data not shown). Leaves of seedlings grown without UV-B radiation had 30% more stomata per unit area (Table 1 and Figure 2) than leaves of seedlings grown with UV-B radiation.

**Photosynthetic response to light**

The response of $A_{CO_2}$ to incident PAR was similar for Douglas-fir seedlings grown with and without ambient UV-B radiation (Figure 3a). Stomatal conductance to water vapor ($g_s$) was up to 45% higher in control seedlings than in seedlings grown with UV-B radiation for incident PAR irradiances from 0 to full sun (2000 $\mu$mol m$^{-2}$ s$^{-1}$) (Figure 3b). Photochemical efficiency of PSII ($\phi_{PSII}$) and the true rate of photosynthetic O$_2$ evolution ($J_{O_2}$) were similar for seedlings grown with or without UV-B radiation in low light (< 500 $\mu$mol m$^{-2}$ s$^{-1}$). In contrast, in high light (> 500 $\mu$mol m$^{-2}$ s$^{-1}$), seedlings grown without UV-B radiation had up to 25% higher $\phi_{PSII}$ and $J_{O_2}$ values than seedlings grown with UV-B radiation (Figure 4). Seedlings in both treatments showed a similar response of CO$_2$-saturated photosynthesis to PAR ranging from 0 to 2000 $\mu$mol m$^{-2}$ s$^{-1}$ (data not shown).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>-UV-B</th>
<th>+UV-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf area (cm$^2$)</td>
<td>0.23 (0.01)</td>
<td>0.21 (0.01)</td>
</tr>
<tr>
<td>Leaf dry weight (mg)</td>
<td>2.6 (0.9)**</td>
<td>1.6 (0.4)</td>
</tr>
<tr>
<td>Specific leaf area (cm$^2$ g$^{-1}$)</td>
<td>93.3 (10.4)**</td>
<td>134.1 (6.2)</td>
</tr>
<tr>
<td>Total aboveground dry weight (mg)</td>
<td>68.1 (7.0)*</td>
<td>48.5 (5.0)</td>
</tr>
<tr>
<td>Total belowground dry weight (mg)</td>
<td>45.0 (6.0)</td>
<td>26.5 (2.0)</td>
</tr>
<tr>
<td>Total seedling dry weight (mg)</td>
<td>114.0 (10.0)*</td>
<td>75.0 (6.0)</td>
</tr>
<tr>
<td>Root/shoot ratio</td>
<td>0.66 (0.03)*</td>
<td>0.52 (0.09)</td>
</tr>
<tr>
<td>Seedling height (cm)</td>
<td>3.58 (0.27)*</td>
<td>2.56 (0.24)</td>
</tr>
<tr>
<td>UV-B absorbing compounds</td>
<td>2.07 (0.21)**</td>
<td>3.24 (0.24)</td>
</tr>
<tr>
<td>($A_{300nm}$ cm$^{-2}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf thickness (mm)</td>
<td>0.424 (0.02)</td>
<td>0.453 (0.003)</td>
</tr>
<tr>
<td>Stomatal frequency (stomata mm$^{-2}$)</td>
<td>102.3 (20.8)**</td>
<td>70.9 (20.9)</td>
</tr>
</tbody>
</table>

Figure 1. Cross sections of Douglas-fir seedling leaves grown without (a) and with (b) UV-B radiation. Needles from the third node of the main stem apex of seedlings were dehydrated, infiltrated with resin and sectioned with a microtome and used to measure leaf and cell layer thickness. No apparent treatment differences in leaf morphology were observed. Scale bar = 0.1 mm.
Responses of seedlings to high-light and water-stress treatments

The UV-B radiation treatment had no effect on maximum \( A_{\text{CO}_2} \) of the seedlings before exposure to high PAR (Figure 5a, Pre), as shown for non-stressed seedlings in Figure 3. For all seedlings grown with or without UV-B radiation, \( A_{\text{CO}_2} \) was lower immediately after the high-light treatment than before it (Figure 5a, Post). After 3 h of low light and a subsequent dark recovery period, \( A_{\text{CO}_2} \) was 32% higher in seedlings grown with UV-B radiation compared with seedlings grown without UV-B radiation (Figure 5a, Recovery). Before the high-light treatment, \( g_s \) was higher for seedlings grown without UV-B radiation (Figure 5b, Pre), as shown for non-stressed seedlings in Figure 3. Following four consecutive days of high-light treatment and subsequent dark recovery periods, there was no difference in \( g_s \) between dark recovery periods, as indicated by the asterisk (*).

Before the 4-day high-light treatment, \( \phi_{\text{PSII}} \) at 1000 \( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \) was higher in seedlings grown without UV-B radiation than in seedlings grown with UV-B radiation (Figure 6a, Pre), as shown for non-stressed seedlings (Figure 4). Immediately following and after dark recovery from the high-light treatment, \( \phi_{\text{PSII}} \) of seedlings grown with or without UV-B radiation was similar (Figure 6a, Post and Recovery). There was little variation in \( F_v/F_m \) between seedlings grown with and without UV-B radiation during any of the high-light stress measurement intervals (Figure 6b). Seedlings in both treatments exhibited photoinhibition during the high-light treatment as...
indicated by lower values of $F_{v}/F_{m}$ immediately after the high-light treatment (Figure 6b, Post). After the dark recovery period, $F_{v}/F_{m}$ values returned to pre high-light values indicating that PSII reaction centers recovered from photoinhibition during this period (Figure 6b, Recovery).

The UV-B radiation treatment had a large effect on the response of maximum $A_{CO_2}$ to water stress, even after only 5 days of withholding water from the seedlings (Figure 7a). After water stress was imposed, seedlings grown with UV-B radiation had up to 50% higher $A_{CO_2}$ throughout the 15-day water stress treatment than seedlings grown without UV-B radiation (Figure 7a). Both before and 5 days after withholding water, $g_{s}$ was higher in seedlings grown with UV-B radiation than in control seedlings (Figure 7b). After 10 and 15 days of water stress, $g_{s}$ of seedlings from both treatments was significantly reduced but did not differ between seedlings grown with and without UV-B radiation (Figure 7b).

Photochemical efficiency of PSII ($\Phi_{PSII}$) at 1000 µmol m$^{-2}$ s$^{-1}$ remained unchanged throughout the 15-day water-stress treatment for seedlings grown with UV-B radiation (Figure 8a). Before the water-stress treatment, $\Phi_{PSII}$ was lower for seedlings grown with UV-B radiation compared with seedlings grown without UV-B radiation (Figure 8a), as shown for non-stressed seedlings in Figure 4. As water stress was imposed, $\Phi_{PSII}$ for seedlings grown without UV-B radiation declined. After 15 days of water stress, $\Phi_{PSII}$ was 20% lower in seedlings grown without UV-B than in seedlings grown with UV-B radiation (Figure 8a). Maximum photochemical efficiency of PSII ($F_{v}/F_{m}$) for seedlings grown with or without UV-B radiation was similar after 0, 5 and 10 days of water stress, but after 15 days without water, $F_{v}/F_{m}$ was 11% lower for seedlings grown without UV-B radiation (Figure 8b).

Both before and after 5 days without water, seedlings in both treatments had similar $\Psi'_w$. After 10 days of water stress, however, $\Psi'_w$ of seedlings grown without UV-B radiation decreased to 40% of that of seedlings grown with UV-B radiation (Figure 9).

**Discussion**

Phenotypic plasticity in response to stressful environmental conditions has been recognized in a variety of evergreen species (Waring 1991). Compared with Douglas-fir seedlings grown in near-ambient UV-B radiation, decreased leaf area and increased leaf thickness have been reported for seedlings in response to a UV-B radiation dose that was 3–4 times higher than the dose we used (Nagel et al. 1998). In our study, UV-B radiation had no effects on leaf area and leaf thickness.
but caused a large reduction in leaf dry mass. The absence of UV-B radiation effects on leaf cell structure and internal air spaces indicates that changes in leaf anatomy do not explain the higher SLA observed for seedlings grown with UV-B radiation.

Lower aboveground, belowground and total biomass and plant height in response to UV-B radiation is commonly observed in both laboratory- and field-grown conifers (Sullivan and Teramura 1988, 1994, Naidu et al. 1993, Yakimchuk and Hoddinott 1994, Sullivan et al. 1996). Decreased biomass is likely the result of lower rates of CO2 assimilation in plants grown with UV-B radiation. Changes in biomass induced by UV-B radiation are not necessarily detrimental to Douglas-fir and may increase its environmental stress tolerance. For example, decreased plant height often occurs in conjunction with increased stem diameter and self-shading by foliage, which reduces heat load at the base of the seedling and minimizes cellular damage that occurs at high surface soil temperatures (Helgerson 1990).

Douglas-fir seedlings grown with UV-B radiation had 30% fewer stomata per unit leaf area compared with control seedlings. In contrast, Stewart and Hoddinott (1993) reported that a similar dose of UV-B radiation did not significantly change stomatal frequency in jack pine. Inconsistencies in the effect of UV-B radiation on stomatal frequency may reflect interspecific differences in response or differences in leaf age or maturity among studies. Reduced stomatal frequency in response to UV-B radiation may limit water loss by seedlings; however, the same attribute may also decrease the potential for leaf cooling through evapotranspiration, which may be important under field conditions.

In Douglas-fir seedlings, the presence of ambient UV-B radiation in the growth light regime induced production of UV-B absorbing compounds, as has been shown for other coniferous species (e.g., Day 1993, Schnitzler et al. 1997, Hoque and Remus 1999). The presence of UV-B absorbing compounds in epidermal cells attenuates UV-B radiation before it reaches the mesophyll cells providing protection from the
damaging effects of UV-B on DNA, proteins and other macromolecules. The marked increase in UV-B absorbing compounds in response to UV-B radiation in Douglas-fir, unlike ponderosa pine (Pinus ponderosa Douglas. ex Laws.) (Nagel et al. 1998), may result from the absence in Douglas-fir of a hypodermal layer that functions to attenuate UV-B radiation.

Similar rates of CO₂- and light-saturated J₀, in Douglas-fir seedlings grown with or without UV-B radiation indicate that enzymes associated with photosynthetic carbon reduction were unaffected by ambient UV-B radiation. Similarly, Naidu et al. (1993) found no effect of UV-B radiation on CO₂- and light-saturated rates of photosynthesis in 3-year-old needles of loblolly pine. Decreased CO₂-limited rates of photosynthesis in response to enhanced UV-B radiation have been shown for jack pine (Stewart and Hoddinott 1993) and loblolly pine seedlings with low concentrations of UV-B absorbing compounds (Sullivan and Teramura 1989). We found a trend for lower CO₂-limited photosynthetic rates in Douglas-fir seedlings grown with UV-B radiation at a range of irradiances, but these differences were not statistically different.

Decreased light-saturated gₛ of Douglas-fir seedlings grown with UV-B radiation is likely a function of decreased stomatal opening and lower stomatal frequency. Intrinsic water use efficiency (Aₐ₉/gₛ) was consistently higher for seedlings grown with UV-B radiation compared with control seedlings. Increased intrinsic water-use efficiency may be especially important in newly planted seedlings that are likely to experience water stress.

Decreased φₚₛₛ and J₀ values at saturating PAR irradiance indicated lower photochemical efficiency of PSII and linear electron flow in seedlings grown with UV-B radiation. Therefore, the sum of RuBP carboxylase and oxygenase activities for Rubisco (Lal and Edwards 1996) and the efficiency of photochemical reactions under conditions that are likely to lead to photoinhibition (Genty et al. 1989, Edwards and Baker 1993) are lower in seedlings grown with UV-B radiation than in seedlings grown without UV-B radiation. Lower φₚₛₛ and J₀ values for seedlings grown with UV-B radiation may act to suppress photoinhibition and compensate for decreased internal CO₂ concentrations when high light occurs in conjunction with lower gₛ. Recovery of light-saturated Aₐ₉ to 5% of pretreatment rates in seedlings subjected to 4 days of high-light stress indicates that seedlings grown in the presence of UV-B radiation are protected from photoinhibition.

Douglas-fir seedlings grown with ambient UV-B radiation maintained higher maximum Aₐ₉ and higher Ψₚₛₛ throughout the 15-day water-stress treatment than seedlings grown without UV-B radiation. Decreased gₛ in response to water stress caused stomatal limitations to photosynthesis in both types of seedlings. Non-stomatal limitations to photosynthesis, caused by drought-induced metabolic constraints (Yordanov et al. 2000), occurred during the first 5 days of water stress for seedlings grown without UV-B radiation, as indicated by the marked decrease in Aₐ₉ and unchanged gₛ. Lower φₚₛₛ and F₆/F₅ₜm values for water-stressed seedlings grown without UV-B radiation than with UV-B radiation indicates that, although these seedlings were not subjected to high light, loss of photochemical photosynthetic capacity occurred in response to water stress.

Higher Ψₚₛₛ values during a 15-day water-stress regime in seedlings grown with UV-B radiation indicates that these plants were subjected to less water stress than seedlings grown without UV-B radiation. Seedlings grown with UV-B radiation maintained better water status under drought conditions because of decreased gₛ and stomatal frequency and, hence, lower transpiration rate than seedlings grown without UV-B radiation. Growth in the presence of UV-B radiation has been shown to increase drought tolerance by increasing leaf cuticle thickness in European heathland species (Björn et al. 1997). Petropoulou et al. (1995) and Manetas et al. (1997) have shown that drought-stressed Mediterranean pines exhibited a smaller decrease in photosynthetic capacity and higher needle retention in response to enhanced UV-B radiation than in response to ambient UV-B radiation.

Douglas-fir seedlings grown with ambient UV-B radiation in the light regime were more tolerant of stressful environmental conditions than seedlings grown without UV-B radiation. Mechanisms for increased stress tolerance in seedlings grown with UV-B radiation included decreases in stomatal frequency and light-saturated φₚₛₛ and gₛ. We conclude that growth of Douglas-fir seedlings in the presence of UV-B radiation can enhance environmental stress tolerance and may reduce mortality of nursery-grown Douglas-fir seedlings planted in the field.

Acknowledgments

The authors are grateful to Dr. Mark Coleman for providing expertise on the growth of Douglas-fir seedlings. We also thank Roderick Griffeth for collection of the oxygen evolution data. This work was supported by the NSF-Idaho EPSCoR Program and by the National Science Foundation Cooperative Agreement number EPS-9720634.

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