

The effect of drought and enhanced UV-B radiation on the growth and physiological traits of two contrasting poplar species

Jian Ren^{a,b}, Weiran Dai^b, Zuying Xuan^a, Yinan Yao^a,
Helena Korpelainen^c, Chunyang Li^{a,*}

^a Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, Chengdu 610041, China

^b Department of Grassland Sciences, Yunnan Agricultural University, Kunming 650201, China

^c Department of Applied Biology, University of Helsinki, P.O. Box 27, FI-00014, Finland

Received 18 December 2005; received in revised form 20 November 2006; accepted 20 November 2006

Abstract

Cuttings of *Populus kangdingensis* and *P. cathayana* originating from high and low altitudes in south-west China, respectively, were used to determine the effect of drought and enhanced UV-B radiation and their combination on plant growth and physiological traits in a greenhouse during one growing season. In both species, cuttings grown under drought conditions exhibited reduced growth and more abscisic acid (ABA) accumulation than did plants kept under well-watered conditions. Enhanced UV-B radiation significantly reduced plant growth and influenced ascorbate peroxidase (APX) activity, proline concentration, the amount of UV-B absorbing compounds and carbon isotope composition ($\delta^{13}\text{C}$) in both species, while it hardly affected ABA accumulation. However, partial differences in responses to each stress were observed between the two species. In *P. cathayana*, the additive effect of both stresses on plant height and leaf area was observed, and drought significantly increased the free proline concentration. In contrast, distinctly higher APX activity, and ABA and $\delta^{13}\text{C}$ levels were observed in *P. kangdingensis* when compared to *P. cathayana*. Moreover, an increase in the amount of UV-B absorbing compounds was detected in *P. kangdingensis* both after the treatment with UV-B alone as well as after its application to drought-stressed plants. In *P. cathayana*, superoxide dismutase (SOD) activity showed a significant increase under enhanced UV-B, while a pronounced increase in the amount of UV-B absorbing compounds was observed only under the combination of the two stresses. Our results suggest that *P. kangdingensis*, originating from high altitude and being apparently adapted to drought and high levels of UV-B, exhibits greater tolerance to drought and enhanced UV-B radiation than does *P. cathayana* originating from lower altitude.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Abscisic acid; Antioxidant enzymes; Free proline; UV-B absorbing compounds; Water use efficiency

1. Introduction

During the past decades there has been considerable concern over the reduction of stratospheric ozone as a result of anthropogenic pollutants, such as chlorofluorocarbons (CFCs) (Molina and Rowland, 1974). The following ozone depletion could lead to a significant increase in UV-B radiation reaching the surface of the Earth (Blumthaler and Amback, 1990; Ziemke et al., 2000). Recently, the effects of UV-B radiation on terrestrial vegetation have been extensively studied in annual crop and herbaceous plants, while the direct effects on trees and ecosystems have been investigated relatively rarely. On the other hand, the response of plants to changes in UV-B radiation

also depends upon concomitant stresses, such as low and high levels of photosynthetic active radiation, temperature extremes, pollutants, metal toxicity, drought and nutrient limitations (UNEP, 2003). Evidence of interactions between UV-B exposure and other stresses in plants indicates that they can bring various responses that can be additive, synergistic or antagonistic (Petropoulou et al., 1995; Alexieva et al., 2001; Poulson et al., 2002; Zhao et al., 2003). Of the environmental variables, water availability is an important factor affecting UV-B responses in plants (Gwynn-Jones et al., 1999). Water limits could exacerbate UV-B effects (Drilias et al., 1997) and cause, e.g., increases in UV-B absorbing compounds and flavonol glycosides (Hofmann et al., 2003a) or a change in the sensitivity to UV-B (Balakumar et al., 1993; Petropoulou et al., 1995; Nogués et al., 1998; Nogués and Baker, 2000; Schmidt et al., 2000; Hofmann et al., 2003b). However, knowledge of such interactions and underlying functional relationships is still

* Corresponding author. Tel.: +86 28 85221347; fax: +86 28 85222753.

E-mail address: licy@cib.ac.cn (C. Li).

mostly lacking. Elucidation of the interaction between drought and enhanced UV-B radiation would help to understand the potential impact of partial stratospheric ozone depletion on plant adaptation to changing environmental conditions.

Poplars are economically and ecologically important tree species, which have been studied in several field and greenhouse experiments to determine the effect of UV-B radiation on their growth and physiological responses (Schumaker et al., 1997; Nagel et al., 1998; Bassman et al., 2003; Warren et al., 2003). However, there is little information on the response of *Populus* to enhanced UV-B radiation when exposed to drought. In our study, *Populus kangdingensis* and *P. cathayana* from high and low altitudes in south-west China, respectively, were employed as experimental materials. Since the contribution of UV-B to total solar radiation tends to increase with altitude because of atmospheric characteristics and since the high-altitude species typically grow under more intensive physiological drought, we hypothesized that *P. kangdingensis* originating from high altitude could be less affected by drought and enhanced UV-B radiation than *P. cathayana* originating from low altitude. It follows that the aims of our study are (1) to determine whether plant growth and physiological traits are affected by exposure to drought, enhanced UV-B radiation and their combination in the two *Populus* species, (2) to evaluate interspecific differences in responses to drought, enhanced UV-B radiation and their combination in the two contrasting *Populus* species, and (3) to measure whether the responses to enhanced UV-B radiation are affected by drought.

2. Material and methods

2.1. Plant material and experimental design

Dormant cuttings of *P. kangdingensis* C. Wang et Tung and *P. cathayana* Rehder were collected in their natural habitats in south-west China (Table 1). In spring, cuttings of a uniform size were collected from each species, transferred to 5–l plastic pots filled with homogenized soil and grown in a naturally lit greenhouse at the Maoxian Ecological Station (103°53'E, 31°41'N, 1800 m). The average daily biologically effective UV-B for the experimental site, based on spectroradiometric measurements, equaled $5.5 \text{ kJ m}^{-2} \text{ day}^{-1}$. The soil used was brown soil. To avoid the effects of rainfall, a polyethylene film was employed as a cover. The cover transmits 80% of ambient solar UV-B and 85% of visible radiation. The greenhouse was a semi-controlled environment with a day temperature range of 12–31 °C, a night temperature range of 9–15 °C and a relative humidity range of 35–85% during the experiment. The

experiment was conducted during the growing season from May to September 2004.

Experimental designs were as follows: two watering regimes were used. Firstly, in the well-watered treatment, 40 pots of each species were watered to 100% of field capacity every other day by supplying an amount of water equal to transpiration losses. In this case, the soil water content was always kept at 36.0%. Secondly, in the drought treatment, 40 pots of each species were maintained at 50% of field capacity by watering every other day. In this case, the soil water content was always kept at 18.0%. Evaporation from the soil surface was prevented by enclosing the pots in plastic bags that were tied to the stems of the plants. There were two UV-B radiation treatments, with and without UV-B supplementation. For each species in each watering regime, one half of the plants received 80% of ambient UV-B radiation, while another half of the plants received 80% of ambient UV-B radiation plus supplemental levels of UV-B radiation. The daily UV-B supplementation was $4.4 \text{ kJ m}^{-2} \text{ day}^{-1}$ (UV-B_{BE}).

In this experiment, square-wave UV-B supplementation systems were used. Supplemental UV-B radiation was delivered to the plants for an 8-h period centered around solar noon using UV-B fluorescent lights (Beijing Electronic Resource Institute, Beijing, China), which were mounted in metal frames suspended over the pots. The lights were turned off when it rained. In the UV-B supplementation treatment, a cellulose diacetate filter was used to absorb radiation below 290 nm. In the non-UV-B supplementation treatment, the plants were illuminated by lights wrapped with polyester film, which absorbs the radiation below 315 nm. The absolute spectral irradiance was weighted with the generalized plant response action spectrum (Caldwell, 1971) and normalized at 300 nm to obtain the daily biologically effective irradiance. The spectral irradiance from the lights was determined at the top of the plants by HR2000CG-UV-NIR High-resolution composite-grating spectrometer (Ocean Optics Inc., USA) with CC-3-UV Cosine Corrector. The spectrometer was calibrated with DH2000-CAL Radiometric Calibrated Deuterium Tungsten Source (210–1050 nm NIST-traceable Calibration, Ocean Optics Inc., USA). UV-B radiation was maintained at the specified levels (measured at the top of the plants) through the experiment by adjusting the light-to-plant canopy distance. Supplemental UV-B radiation was initiated just prior to leaf-out. Within a treatment, the plants were rotated weekly in order to minimize the effects of the microenvironment. In our study, five replications were used in each species and treatment. Each replication included four cuttings. To investigate growth and physiological traits, all cuttings from the five replications from each species and treatment were measured in early September.

Table 1

The origins of the two *Populus* species examined, and climatic data from the collection areas

Species	Latitude	Longitude	Altitude (m)	Evaporation (mm)	Annual rainfall (mm)	Annual temperature (°C)	UV-B dose ^a ($\text{kJ m}^{-2} \text{ day}^{-1}$)
<i>Populus kangdingensis</i>	30°12'N	102°35'E	3500	1301.7	924.0	7.1	7.2
<i>P. cathayana</i>	32°25'N	104°31'E	1500	1056.8	866.5	14.7	4.7

^a The average daily biologically effective UV-B dose using spectroradiometric measurements for the local site during the growing season.

2.2. Growth measurements

At harvesting, the height and leaf numbers of all cuttings were measured. The leaf area was determined with a portable laser area meter (CI-203, CID Inc., Camas, USA). Leaf samples were dried to a constant weight and weighed. Subsequently, the specific leaf mass (leaf dry weight per unit leaf area, mg cm^{-2}) was calculated.

2.3. Determinations of antioxidant enzymes

2.3.1. Superoxide dismutase (EC 1.15.1.1, SOD)

Collected leaves (0.4 g) were homogenized to a fine powder with a mortar and pestle under liquid nitrogen (likewise below). The total SOD activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) (Beuchamp and Fridovich, 1971), modified as follows: The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 130 mM methionine, 750 μM NBT, 20 μM riboflavin and 0.1 ml enzyme source. Riboflavin was added at last, and the reaction was initiated by placing the glass test tubes under fluorescent lamps. The reaction was terminated after 30 min by removal from the light source. Non-illuminated identical tubes served as blanks. An illuminated blank without protein gave the maximum reduction of NBT, thus, the maximum absorbance at 560 nm. In this assay, 1 unit of SOD was defined as the amount of enzyme inhibiting the photo-reduction of NBT by 50%. The specific activity of SOD was expressed as units g^{-1} FW h^{-1} .

2.3.2. Ascorbate peroxidase (EC 1.11.1.11, APX)

The APX activity was measured using a modification of the procedure of Nakano and Asada (1981). Leaves (0.5 g) were homogenized in chilled extraction medium containing 50 mM sodium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM sodium ascorbate, 1% (w/v) PVP. The reaction mixture of a total volume of 3 ml consisted of 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.1 mM sodium ascorbate, 2.5 mM H_2O_2 and 80 μl enzyme extract. The H_2O_2 -dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm.

2.3.3. Catalase (EC 1.11.1.6, CAT)

The CAT activity was determined by directly measuring the decomposition of H_2O_2 at 240 nm ($0.04 \text{ mM}^{-1} \text{ cm}^{-1}$), as described by Aebi (1984). The reaction mixture contained 0.05 M Na phosphate buffer (pH 7.0) with 1 mM EDTA and H_2O_2 (3%).

2.4. Measurements of UV-B absorbing compounds and free proline concentration

The concentration of methanol-extractable UV-B absorbing compounds was determined at harvesting. UV-B absorbing compounds were extracted from leaves (0.5 cm^2) by immersing them in 10 ml acidified methanol ($\text{MeOH}:\text{H}_2\text{O}:\text{HCl} = 79:20:1$, v/v) (Dai et al., 2004). The relative UV-B absorbing compounds

were determined at 300 nm in an UV-vis spectrophotometer. The values obtained, calculated based on the leaf area, were used as an index of relative concentration of UV-B absorbing compounds.

Leaves (0.2 g) were homogenized in 5 ml of 3% sulphosalicylic acid solution. After centrifugation, 2 ml supernatant, 2 ml glacial acetic acid and 2 ml 2.5% acid ninhydrin solution were added in a test tube covered with Teflon cap. Free proline was measured as described by Bates et al. (1973). The absorbance of the free proline concentration was measured at 520 nm. The proline content was expressed as $\mu\text{g g}^{-1}$ fresh weight.

2.5. Quantitative analysis of abscisic acid

Abscisic acid (ABA) present in apical buds was analyzed as described by Li et al. (2002). The samples were first weighed, frozen in liquid nitrogen and freeze-dried. Then, samples of 30–50 mg of plant material were homogenized in 5 ml of 50 mM sodium phosphate buffer, pH 7.0 with 0.02% sodium diethyldithiocarbamate as antioxidant and 30 ng [$^2\text{H}_4$] ABA as an internal standard. ABA was measured by gas chromatography–mass spectrometry as described by Jensen et al. (1986) with selective ion monitoring (SIM). Ions at 190.1 and 194.1 were monitored, and the amount of ABA in the sample was calculated using a standard curve drawn from the area ratios of known amounts of ABA and [$^2\text{H}_4$] ABA. The ABA level was calculated as $\mu\text{g g}^{-1}$ fresh weight.

2.6. Carbon isotope composition

Leaf samples used for the carbon isotope analysis were oven-dried for 24 h at 80 °C and homogenized by grinding in a ball mill. The carbon isotope composition ($\delta^{13}\text{C}$) in combusted samples was measured with a mass spectrometer (Finnegan MAT Delta-E), as described by Hubick et al. (1986) and Li et al. (2000). $\delta^{13}\text{C}$, expressed relative to the PeeDee Belemnite standard (Craig, 1957). The overall precision of the δ -values was better than 0.1‰, as determined from repeated samples.

2.7. Statistical analyses

One- and two-way analyses of variance (ANOVA) were performed using the SPSS 11.0 for Windows statistical software package. Two-way ANOVAs were used to separate the effects of drought, enhanced UV-B radiation and their combination. Within each species, one-way ANOVAs were used to determine differences among treatments. Differences were considered significant at the $P < 0.05$ level.

3. Results

3.1. Effects of drought and enhanced UV-B on growth traits

In both species, there were significant reductions in the plant height and total leaf area when exposed to drought, enhanced

Table 2

The effects of drought, UV-B radiation and their combination on growth traits in the two contrasting *Populus* species based on ANOVA

Species	Treatment	Plant height (cm)	Leaf nos.	Total area (dm ²)	Specific leaf mass (mg cm ⁻²)
<i>P. kangdingensis</i>	WW	148.90 ± 3.85 a	46.00 ± 1.55 a	32.06 ± 0.74 a	6.30 ± 0.18 c
	WW + UV-B	125.88 ± 0.66 b	45.40 ± 1.47 a	20.50 ± 1.09 b	7.60 ± 0.12 b
	D	105.04 ± 4.83 c	36.20 ± 3.01 b	18.03 ± 1.61 b	6.50 ± 0.09 c
	D + UV-B	83.80 ± 3.22 d	33.80 ± 1.64 b	8.48 ± 1.00 c	9.11 ± 0.31 a
	$P_{(UV-B)}$	0.000	0.441	0.000	0.001
$P_{(drought)}$	0.000	0.000	0.000	0.000	
$P_{(drought \times UV-B)}$	0.813	0.642	0.416	0.005	
<i>P. cathayana</i>	WW	126.90 ± 2.16 a	45.60 ± 2.02 a	27.15 ± 2.88 a	6.16 ± 0.14 c
	WW + UV-B	79.25 ± 1.98 c	45.40 ± 1.17 a	14.37 ± 0.64 b	8.14 ± 0.09 b
	D	98.90 ± 2.91 b	32.60 ± 1.02 b	7.74 ± 0.59 c	8.13 ± 0.18 b
	D + UV-B	70.70 ± 7.51 d	36.50 ± 3.12 b	5.46 ± 0.24 c	9.19 ± 0.18 a
	$P_{(UV-B)}$	0.000	0.338	0.000	0.000
$P_{(drought)}$	0.000	0.000	0.004	0.000	
$P_{(drought \times UV-B)}$	0.003	0.290	0.029	0.011	

Each value is the mean ± S.E. WW, well-watered; WW + UV-B, well-watered with UV-B radiation; D, drought-stressed; D + UV-B, drought-stressed with UV-B radiation; $P_{(UV-B)}$, significance of the UV-B effect; $P_{(drought)}$, significance of the drought effect; $P_{(drought \times UV-B)}$, significance of the drought × UV-B interaction effect. Values followed by the same letter in the same column are not significantly different at the $P < 0.05$ level according to Student–Newman–Keuls multiple range test.

UV-B radiation or to their combination (Table 2). Leaf numbers were not affected by enhanced UV-B radiation but they were significantly reduced by drought. Under enhanced UV-B radiation, the cuttings exhibited a distinctly lower plant height in *P. cathayana* than in *P. kangdingensis*. In both species, the specific leaf mass displayed a significant increase in response to enhanced UV-B radiation, and a further increase occurred when the two stresses were applied together. Significant drought × UV-B interaction effects were detected in the plant height, total leaf area and specific leaf mass of *P. cathayana*, and in the specific leaf mass of *P. kangdingensis* (Table 2).

3.2. Effects of drought and enhanced UV-B on enzyme activity

When subjected to drought and enhanced UV-B radiation, partially different responses in antioxidant enzymes were

observed in the two *Populus* species (Table 3). In both species, the APX activity significantly increased as a response to enhanced UV-B. In contrast, SOD increased only in *P. cathayana* when exposed to enhanced UV-B radiation. CAT increased in *P. kangdingensis* when exposed to enhanced UV-B under well-watered conditions. Some drought effects were detected in the APX and CAT activities, which increased when the plants were exposed to drought.

3.3. Effects of drought and enhanced UV-B on UV-B absorbing compounds and proline

When the cuttings were exposed to enhanced UV-B radiation, the amount of UV-B absorbing compounds significantly increased in *P. kangdingensis* under both watering regimes. In contrast, the UV-B absorbing compounds were hardly affected in *P. cathayana* in the well-watered treatment,

Table 3

The effects of drought and UV-B radiation and their combination on SOD, APX and CAT activity in the two contrasting *Populus* species based on ANOVA

Species	Treatment	SOD (unit g FW ⁻¹)	APX (μmol H ₂ O ₂ min ⁻¹ g FW ⁻¹)	CAT (mmol H ₂ O ₂ min ⁻¹ g FW ⁻¹)
<i>P. kangdingensis</i>	WW	193.58 ± 2.00 a	1.27 ± 0.08 b	0.61 ± 0.11 b
	WW + UV-B	203.61 ± 3.19 a	2.46 ± 0.33 a	1.01 ± 0.05 a
	D	203.11 ± 4.89 a	1.73 ± 0.03 b	1.19 ± 0.01 a
	D + UV-B	199.76 ± 3.04 a	2.70 ± 0.17 a	1.02 ± 0.01 a
	$P_{(UV-B)}$	0.359	0.000	0.119
$P_{(drought)}$	0.433	0.094	0.002	
$P_{(drought \times UV-B)}$	0.088	0.572	0.002	
<i>P. cathayana</i>	WW	183.39 ± 2.52 b	0.30 ± 0.02 c	0.68 ± 0.11 a
	WW + UV-B	205.50 ± 0.50 a	2.27 ± 0.22 b	0.41 ± 0.06 a
	D	175.78 ± 2.10 b	0.41 ± 0.04 c	0.82 ± 0.14 a
	D + UV-B	199.33 ± 6.83 a	2.70 ± 0.03 a	0.82 ± 0.03 a
	$P_{(UV-B)}$	0.001	0.000	0.213
$P_{(drought)}$	0.153	0.044	0.020	
$P_{(drought \times UV-B)}$	0.872	0.203	0.224	

Each value is the mean ± S.E. WW, well-watered; WW + UV-B, well-watered with UV-B radiation; D, drought-stressed; D + UV-B, drought-stressed with UV-B radiation; $P_{(UV-B)}$, significance of the UV-B effect; $P_{(drought)}$, significance of the drought effect; $P_{(drought \times UV-B)}$, significance of the drought × UV-B interaction effect. Values followed by the same letter in the same column are not significantly different at the $P < 0.05$ level according to Student–Newman–Keuls multiple range test.

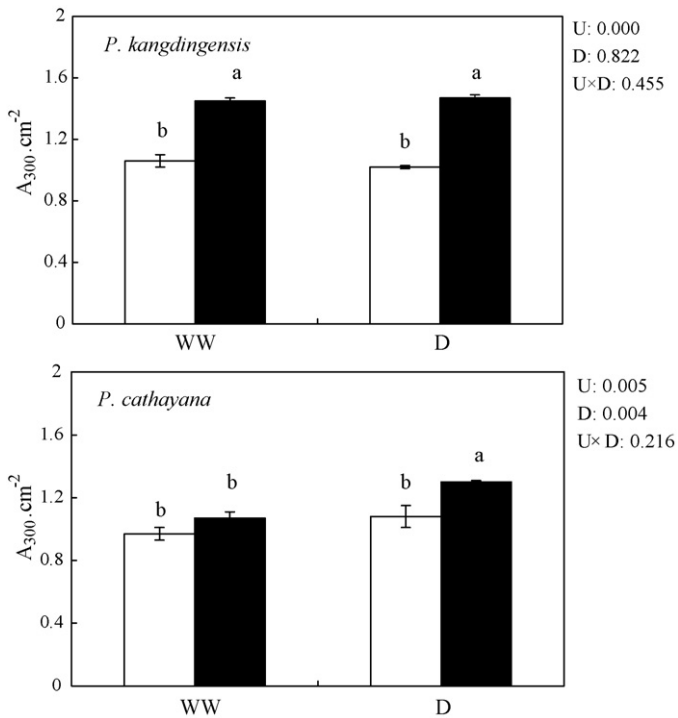


Fig. 1. The relative absorbance of methanol extracts (mean \pm S.E.) at 300 nm in the *Populus* cuttings exposed to ambient (\square) and enhanced UV-B radiation (\blacksquare). Plants were either well-watered (WW) or drought-stressed (D). Different letters above bars within a species denote statistically significant differences between treatments at the $P < 0.05$ level according to Student–Newman–Keuls multiple range test. The significance of the factorial analysis (ANOVA): U, UV-B effect; D, drought effect; U \times D, UV-B \times drought interaction effect.

whereas they significantly increased by the combination of two stresses (Fig. 1). In both species, enhanced UV-B radiation had a significant effect on proline accumulation. However, drought had a significant effect on proline accumulation in *P. cathayana* while only little effect was detected in *P. kangdingensis* (Fig. 2).

3.4. Effects of drought and enhanced UV-B on ABA and $\delta^{13}\text{C}$

In both species, ABA experienced little change to enhanced UV-B radiation but its amount was significantly increased by drought (Fig. 3). Differences in ABA accumulation were found between the two species. ABA increased by 20-fold under drought in *P. kangdingensis*, whereas a nearly 5-fold increase was observed in *P. cathayana*. On the other hand, $\delta^{13}\text{C}$ was significantly affected by enhanced UV-B and drought in both species (Fig. 4).

4. Discussion

In the two studied *Populus* species, drought caused changes in the growth parameters, including decreases in plant height, leaf numbers and leaf area. The results showing the inhibiting growth effects on *Populus* trees caused by drought were similar to those observed in an earlier study (Yin et al., 2005). It is generally believed that water shortage can limit plant growth.

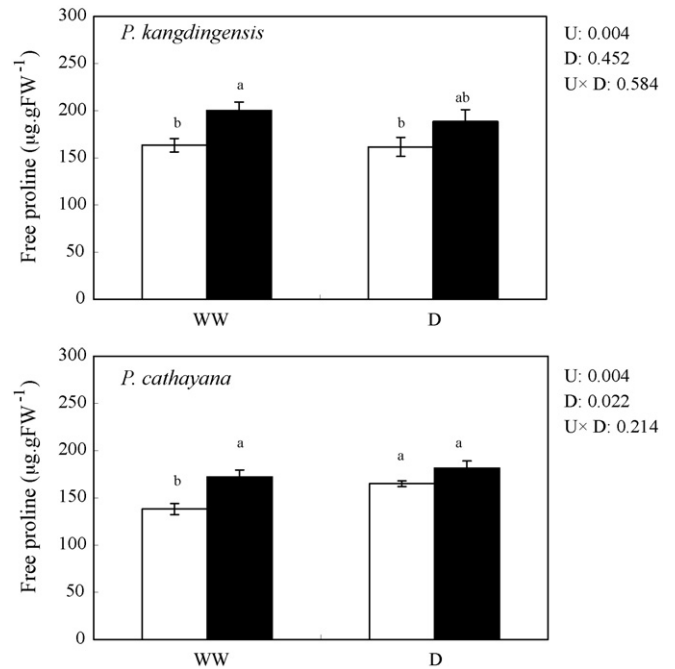


Fig. 2. Free proline concentration (mean \pm S.E.) in the *Populus* cuttings exposed to ambient (\square) and enhanced UV-B radiation (\blacksquare). Plants were either well-watered (WW) or drought-stressed (D). Different letters above bars within a species denote statistically significant differences between treatments at the $P < 0.05$ level according to Student–Newman–Keuls multiple range test. The significance of the factorial analysis (ANOVA): U, UV-B effect; D, drought effect; U \times D, UV-B \times drought interaction effect.

Aside from the reduction of growth, physiological traits were also affected by drought. However, they were partially associated with the species examined. For example, the APX activity significantly increased in *P. cathayana* but not in *P. kangdingensis*. In contrast, SOD and CAT did not show clear interspecific differences to drought stress. In addition, the effect of drought on the UV-B absorbing compounds was variable. The concentration was significantly affected in *P. cathayana* but not in *P. kangdingensis*.

Proline accumulation is a very common response in plants subjected to drought stress (Yancey et al., 1982; Alexieva et al., 2001; Yang et al., 2005). In our study, drought significantly increased proline accumulation in *P. cathayana* while no effect was detected in *P. kangdingensis*. ABA was found to accumulate significantly in the two *Populus* species under drought conditions. ABA is thought to act as a messenger in stress-perception-response pathways under various environmental stresses (Imai et al., 1995; Zhu et al., 1997; Rinne et al., 1998; Guschina et al., 2002; Li et al., 2003). In our experiment, a greater increase in ABA was observed in *P. kangdingensis*, which suggests that *P. kangdingensis* is more responsive to drought than *P. cathayana*. On the other hand, the long-term water use efficiency, assessed by $\delta^{13}\text{C}$, was also significantly influenced by drought. However, *P. kangdingensis* exhibited a distinctly higher $\delta^{13}\text{C}$ as affected by drought than did *P. cathayana*.

Based on the drought-induced effects on the growth parameters and on the levels of free proline, ABA, CAT and

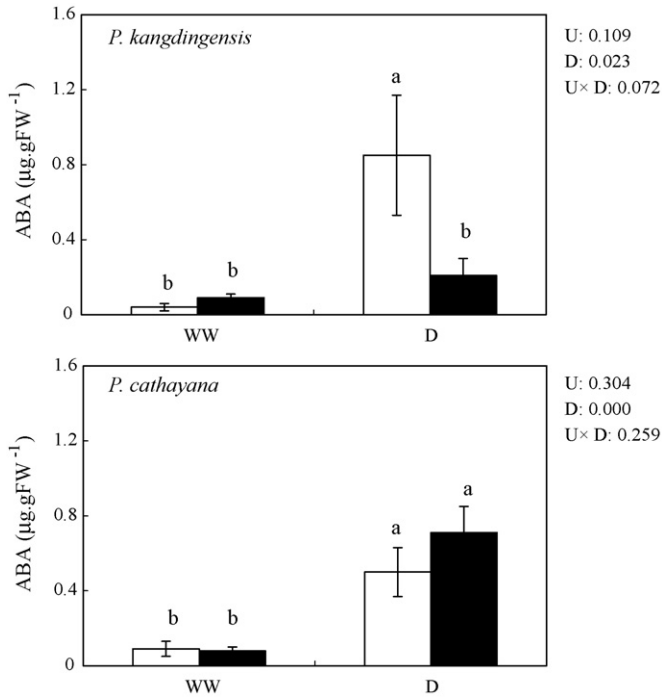


Fig. 3. ABA concentration (mean ± S.E.) in the *Populus* cuttings exposed to ambient (□) and enhanced UV-B radiation (■). Plants were either well-watered (WW) or drought-stressed (D). Different letters above bars within a species denote statistically significant differences between treatments at the $P < 0.05$ level according to Student–Newman–Keuls multiple range test. The significance of the factorial analysis (ANOVA): U, UV-B effect; D, drought effect; U × D, UV-B × drought interaction effect.

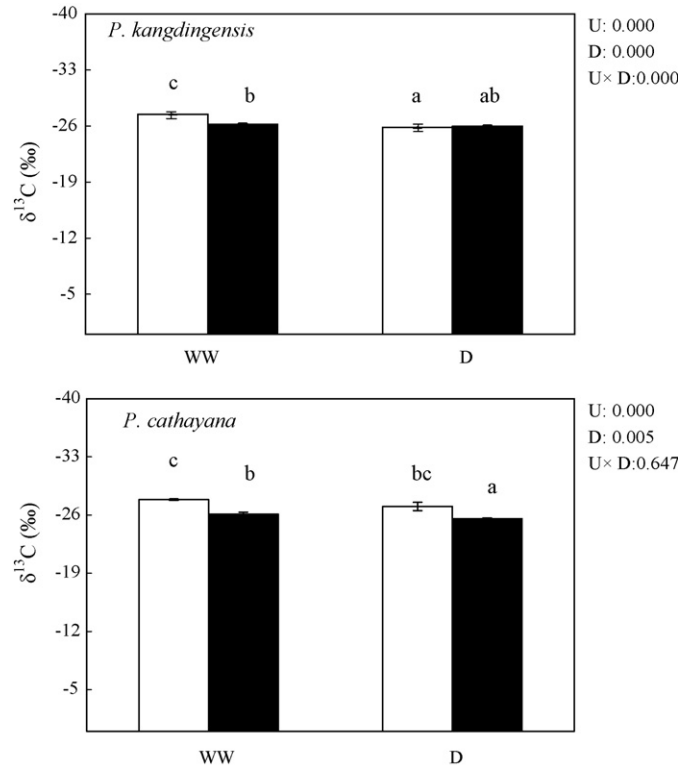


Fig. 4. Carbon isotope compositions ($\delta^{13}\text{C}$) (mean ± S.E.) in the *Populus* cuttings exposed to ambient (□) and enhanced UV-B radiation (■). Plants were either well-watered (WW) or drought-stressed (D). Different letters above bars within a species denote statistically significant differences between treatments at the $P < 0.05$ level according to Student–Newman–Keuls multiple range test. The significance of the factorial analysis (ANOVA): U, UV-B effect; D, drought effect; U × D, UV-B × drought interaction effect.

$\delta^{13}\text{C}$, we suggest that *P. kangdingensis* originating from high altitude possesses higher drought tolerance than does *P. cathayana* originating from low altitude. James et al. (1994) have hypothesized that trees at high altitude are water-stressed. The water stress is caused by damage to the cuticle of the trees due to wind and ice blasting during growing season. One possibility is that colder soils reduce the water uptake of the root system and induce water stress (Magnani and Borghetti, 1995). Another possibility is that colder air temperatures at high altitudes maintain a higher state of frost resistance. Since ABA is a central messenger for both frost resistance and drought, stomatal closure and allocation as apparent acclimations to drought may just be a side effect of a higher level of frost resistance (Li et al., 2004). In both species, the amount of ABA increases under drought conditions, as expected. But in *P. kangdingensis*, the increase is much more pronounced when no UV-B supplementation is provided. This phenomenon may imply that UV-B alleviates drought effects, possibly by causing stomatal closure or thicker cuticles, which result in a better water economy in plants under drought and UV-B treatments. Under these conditions, the plants may suffer from a milder water stress, thus accumulating less ABA compared to plants under drought conditions. Similar trends have been found in a number of other studied species (Petropoulou et al., 1995; Manetas et al., 1997; Poulson et al., 2002; Gitz et al., 2005).

Not only plant growth but also physiological traits are affected by enhanced UV-B radiation, as observed in our study.

Enhanced UV-B radiation significantly decreased plant height and leaf area, and increased leaf thickness. An increase in leaf thickness suggests the possibility that the penetration of UV-B radiation into a deeper mesophyll layer was attenuated (Bornman and Vogelmann, 1991). The absence of a change in leaf numbers but a decrease in plant height due to enhanced UV-B radiation apparently relates to shorter internodes rather than fewer numbers of nodes. A distinctly greater decrease in plant height was observed in *P. cathayana* than in *P. kangdingensis*, as affected by enhanced UV-B radiation. Variation in growth traits as a function of enhanced UV-B radiation has been reported in many previous studies (Sullivan et al., 1996; Nogués et al., 1998; Cuadra et al., 2004; Yang et al., 2005). According to Turunen and Latola (2005), the response of high-altitude plants to UV-B radiation in controlled conditions is often less pronounced compared with low-altitude plants, which shows that the alpine timberline plants are adapted to UV-B.

The APX activity of both species was significantly increased by enhanced UV-B. In addition, an increase in SOD was observed in *P. cathayana*. We hypothesize that an increased capacity to scavenge oxygen free radicals is induced by UV-B radiation. The present results show that *Populus* trees respond to enhanced UV-B radiation with changes in the levels of antioxidant enzymes. The UV-B absorbing compounds have been regarded to have an important role to protect plants, since

they can decrease UV-B penetrability and reduce UV-B damage in plants (Jansen et al., 1996). In our study, a significant increase in UV-B absorbing compounds was induced by enhanced UV-B radiation. This result further proves previous observations (Lavola, 1998; Bieza and Lois, 2001; Warren et al., 2003). However, partially different responses were found between the two *Populus* species. Under well-watered conditions, the UV-B radiation effect was significant in *P. kangdingensis* but not in *P. cathayana*. The different responses may be related to adaptation to native UV-B habitats. According to Wand (1995), UV-B absorbance (280–320 nm) was higher in different types of leaves collected from high elevation. Similar results have been found also in *Quercus ilex* (Filella and Peñuelas, 1999).

In previous studies, enhanced UV-B radiation has been found to increase free proline accumulation in *Trifolium repens* (Hofmann et al., 2003a) and *Vicia faba* (Shetty et al., 2002). Proline is generally assumed to serve as a physiologically compatible solute that increases as needed to maintain a favourable osmotic potential between the cell and its surroundings (Pollard and Wyn-Jones, 1979), and it is known to be involved in alleviating cytosolic acidosis associated with several stresses (Kurkdjian and Guern, 1989). In our study, the accumulation of proline was observed in plants exposed to enhanced UV-B radiation under well-watered conditions, whereas under drought conditions such an increase was not detected. According to Alexieva et al. (2001), the removal of excess H⁺ occurring as a result of proline synthesis may have a positive effect on the reduction of the UV-B-induced damage. In addition, $\delta^{13}\text{C}$ was significantly affected by enhanced UV-B radiation in both species. This result is in agreement with findings by Gitz et al. (2005) in a soybean cultivar.

Some interactive effects of drought and UV-B on growth and physiological traits were detected in our study. Compared with drought or UV-B alone, plant height, total leaf area and specific leaf mass were further affected by the combination of stresses. There were additive effects on the specific leaf area in both species, while the combination of drought and UV-B appeared to have synergistic effects on the plant height and leaf area in *P. cathayana*. Differences in CAT activity and $\delta^{13}\text{C}$ in *P. kangdingensis* as well as in free proline in *P. cathayana* between well-watered and drought-stressed plants were less pronounced when the plants were exposed to enhanced UV-B radiation. This suggests that UV-B exposure may have offset some of the effects of water stress or *vice versa*.

In conclusion, based on the interspecific differences detected primarily in the effects of UV-B on some growth traits, UV-B absorbing compounds as well as on CAT activity, we suggest that *P. kangdingensis* originating from high altitude is adapted to drought and high UV-B habitats. It exhibited a greater tolerance to drought and enhanced UV-B radiation in experimental conditions than did *P. cathayana* originating from low altitude.

Acknowledgements

The research was supported by the Outstanding Young Scientist Program of the National Science Foundation of China

(no. 30525036) and the China National Key Program of the International Cooperation for Science and Technology (no. 2005DFA30620).

References

- Aebi, H., 1984. Catalase in vitro. *Meth. Enzymol.* 105, 121–126.
- Alexieva, V., Sergiev, I., Mapellis, S., Karanov, E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ.* 24, 1337–1344.
- Balakumar, T., Hani, V., Vincent, B., Paliwal, K., 1993. On the interaction of UV-B radiation (280–315 nm) with water stress in crop plants. *Physiol. Plant.* 87, 217–222.
- Bassman, J.H., Edwards, G.E., Robberecht, R., 2003. Photosynthesis and growth in seedlings of five forest tree species with contrasting leaf anatomy subjected to supplemental UV-B radiation. *For. Sci.* 49, 176–187.
- Bates, C.J., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* 39, 205–207.
- Beuchamp, C.H., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Ann. Biochem.* 44, 276–287.
- Bieza, K., Lois, R., 2001. An *Arabidopsis* mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolics. *Plant Physiol.* 126, 1105–1115.
- Blumthaler, M., Ambach, W., 1990. Indication of increasing solar UV-B radiation flux in alpine regions. *Science* 248, 206–208.
- Bornman, J.F., Vogelmann, T.C., 1991. Effect of UV-B radiation on leaf optical properties measured with fiber optics. *J. Exp. Bot.* 42, 547–554.
- Caldwell, M.M., 1971. Solar UV radiation and the growth and development of higher plants. In: Giese, A.G. (Ed.), *Phytophysiology*. Academic Press, New York, pp. 131–177.
- Craig, H., 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochim. Cosmochim. Acta* 12, 133–149.
- Cuadra, P., Herrera, R., Fajardo, V., 2004. Effects of UV-B radiation on the Patagonian *Jaborosa magellanica* Brisben. *J. Photochem. Photobiol. B* 76, 61–68.
- Dai, Q., Furness, N.H., Upadhyaya, M.K., 2004. UV-absorbing compounds and susceptibility of weedy species to UV-B radiation. *Weed Boil. Manage.* 4, 95–102.
- Drilias, P., Karabourniotis, G., Levizou, E., Nikolopoulos, D., Petropoulou, Y., Manetas, Y., 1997. The effects of enhanced UV-B radiation on the Mediterranean evergreen sclerophyll *Nerium oleander* depend on the extent of summer precipitation. *Aust. J. Plant. Physiol.* 24, 301–306.
- Filella, I., Peñuelas, J., 1999. Altitudinal differences in UV absorbance. UV reflectance and related morphological traits of *Quercus ilex* and *Rhododendron ferrugineum* in the Mediterranean region. *Plant Ecol.* 145, 157–165.
- Gitz, D.C., Liu-Gitz, L., Britz, S.J., Sullivan, J.H., 2005. Ultraviolet-B effects on stomatal density, water-use efficiency, and stable carbon isotope discrimination in four glasshouse grown soybean (*Glycine max*) cultivars. *Environ. Exp. Bot.* 53, 343–355.
- Guschina, I.A., Harwood, J.L., Smith, M., Beckett, R.P., 2002. Abscisic acid modifies the changes in lipids brought about by water stress in the moss *Trichum androgynum*. *New Phytol.* 156, 255–264.
- Gwynn-Jones, D., Johanson, U., Phoenix, G.K., Gehrke, C., Callaghan, T.V., Björn, L.O., Sonesson, M., Lee, J.A., 1999. UV-B impacts and interactions with other co-occurring variables of environmental change: an arctic perspective. In: Rozema, J. (Ed.), *Stratospheric Ozone Depletion. The Effects of Enhanced UV-B Radiation on Terrestrial Ecosystems*. Backhuys, Leiden, The Netherlands, pp. 187–201.
- Hofmann, R.W., Campbell, B.D., Bloor, S.J., Swinny, E.E., Markham, K.R., Ryan, K.G., Fountain, D.W., 2003a. Responses to ultraviolet-B radiation in *Trifolium repens* L.-physiological links to plant productivity and water availability. *Plant Cell Environ.* 26, 603–612.
- Hofmann, R.W., Campbell, B.D., Fountain, D.W., 2003b. Sensitivity of white clover to UV-B radiation depends on water availability, plant productivity and duration of stress. *Global Change Boil.* 9, 473–477.

- Hubick, K.T., Farquhar, G.D., Shorter, R., 1986. Correlation between water use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Aust. J. Plant Physiol.* 13, 803–816.
- Imai, R., Moses, M.S., Bray, E.A., 1995. Expression of an ABA-induced gene of tomato in transgenic tobacco during periods of water deficit. *J. Exp. Bot.* 46, 1077–1084.
- James, J.C., Grace, J., Hoad, S.P., 1994. Growth and photosynthesis of *Pinus sylvestris* at its altitudinal limit in Scotland. *J. Ecol.* 82, 297–306.
- Jansen, M.A.K., Babu, T.S., Heller, D., Mattoo, A.K., Edelman, M., 1996. Ultraviolet-B effects on *Spirodela oligorrhiza*: induction of different protection mechanism. *Plant Sci.* 115, 217–223.
- Jensen, E., Rivier, L., Junttila, O., 1986. Abscisic acid and cessation of apical growth in *Salix pentandra*. *Physiol. Plant.* 66, 409–412.
- Kurkdjian, A., Guern, J., 1989. Intracellular pH: measurement and importance in cell activity. *Annu. Rev. Plant Physiol.* 40, 271–303.
- Lavola, A., 1998. Accumulation of flavonoids and related compounds in birch induced by UV-B irradiance. *Tree Physiol.* 18, 53–58.
- Li, C., Berninger, F., Koskela, J., Sonninen, E., 2000. Drought responses of *Eucalyptus microtheca* provenances depend on seasonality of rainfall in their place of origin. *Aust. J. Plant Physiol.* 27, 231–238.
- Li, C., Puhakainen, T., Welling, A., Vihera-Aarnio, A., Ernsten, A., Junttila, O., Heino, P., Palva, E.T., 2002. Cold acclimation in silver birch (*Betula pendula*) development of freezing tolerance in different tissues and climatic ecotypes. *Physiol. Plant.* 116, 478–488.
- Li, C., Junttila, O., Heino, P., Palva, E.T., 2003. Different responses of northern and southern ecotypes of *Betula pendula* to exogenous ABA application. *Tree Physiol.* 23, 481–487.
- Li, C., Liu, S., Berninger, F., 2004. *Picea* seedlings show apparent acclimation to drought with increasing altitude in the eastern Himalaya. *Trees* 18, 277–283.
- Magnani, F., Borghetti, M., 1995. Interpretation of season changes of xylem embolism and plant hydraulic resistance in *Fagus sylvatica*. *Plant Cell Environ.* 18, 689–696.
- Manetas, Y., Petropoulou, Y., Stamatakis, K., Nikolopoulos, D., Levizou, E., Psaras, G., Karabourniotis, G., 1997. Beneficial effects of enhanced UV-B radiation under field conditions: improvement of needle water relations and survival capacity of *Pinus pinea* L. seedlings during the dry Mediterranean summer. *Plant Ecol.* 128, 101–108.
- Molina, M.J., Rowland, F.S., 1974. Stratospheric sink for chlorofluoromethanes: chlorine atom-catalyzed destruction of ozone. *Nature* 249, 810–812.
- Nagel, L.M., Bassman, J.H., Edwards, G.E., Robberecht, R., Franceschi, V.R., 1998. Leaf anatomical changes in *Populus trichocarpa*, *Quercus rubra*, *Pseudotsuga menziesii* and *Pinus ponderosa* exposed to enhanced ultraviolet-B radiation. *Physiol. Plant.* 104, 385–396.
- Nakano, Y., Asada, H., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22, 867–880.
- Nogués, S., Damian, J.A., Morison, J.I.L., Baker, N.R., 1998. Ultraviolet-B radiation effects on water relations, leaf development and photosynthesis in droughted pea plants. *Plant Physiol.* 117, 173–181.
- Nogués, S., Baker, N.R., 2000. Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B radiation. *J. Exp. Bot.* 51, 1309–1317.
- Petropoulou, Y., Kyparissis, A., Nikolopoulos, D., Manetas, Y., 1995. Enhanced UV-B radiation alleviates the adverse effects of summer drought in two Mediterranean pines under field conditions. *Physiol. Plant.* 94, 37–44.
- Pollard, A., Wyn-Jones, R.G., 1979. Enzyme activities in concentrated solutions of glycinebetaine and other solutes. *Planta* 144, 291–298.
- Poulson, E.M., Donahue, R.A., Konvalinka, J., Boeger, M.R.T., 2002. Enhanced tolerance of photosynthesis to high-light and drought stress in *Pseudotsuga menziesii* seedlings grown in ultraviolet-B radiation. *Tree Physiol.* 22, 829–838.
- Rinne, P., Welling, A., Kaikuranta, P., 1998. Onset of freezing tolerance in birch (*Betula pubescens* Ehrh) involves LEA proteins and osmoregulation and is impaired in an ABA-deficient genotype. *Plant Cell Environ.* 21, 601–611.
- Schmidt, A.M., Ormrod, D.P., Livingstone, N.J., Misra, S., 2000. The interaction of Ultraviolet-B radiation on water deficit in two *Arabidopsis thaliana* genotypes. *Ann. Bot.* 85, 571–575.
- Schumaker, A.M., Bassman, J.H., Robberecht, R., Rademaker, G.K., 1997. Growth, leaf anatomy, and physiology of *Populus* clones in response to solar ultraviolet-B radiation. *Tree Physiol.* 17, 617–626.
- Shetty, P., Atallah, M.T., Shetty, K., 2002. Effects of UV treatment on the proline-linked pentose phosphate pathway for phenolics and L-DOPA synthesis in dark germinated *Vicia faba*. *Process Biochem.* 37, 1285–1295.
- Sullivan, J.H., Howells, B.W., Ruhland, C.T., Day, T.A., 1996. Changes in leaf expansion and epidermal screening effectiveness in *Liquidambar styraciflua* and *Pinus taeda* in response to UV-B radiation. *Physiol. Plant.* 98, 349–357.
- Turunen, M., Latola, K., 2005. UV-B radiation and acclimation in timberline plants. *Environ. Pollut.* 137, 390–403.
- UNEP, 2003. Environmental Effects of Ozone Depletion and its Interactions with Climate Change: 2002 Assessment. United Nations Environmental Programme.
- Wand, S.J.E., 1995. Concentration of ultraviolet-B radiation absorbing compounds in leaves of a range of fynbos species. *Vegetatio* 116, 51–60.
- Warren, J.M., Bassman, J.H., Fellman, J.K., Mattinson, D.S., Eigenbrode, S., 2003. Ultraviolet-B radiation alters phenolic salicylate and flavonoid composition of *Populus trichocarpa* leaves. *Tree Physiol.* 23, 527–535.
- Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D., Somero, G.N., 1982. Living with water stress: evolution of osmolyte systems. *Science* 217, 1214–1222.
- Yang, Y., Yao, Y., Xu, G., Li, C., 2005. Growth and physiological responses to drought and elevated ultraviolet-B in two contrasting populations of *Hippophae rhamnoides*. *Physiol. Plant.* 124, 431–440.
- Yin, C., Wang, X., Duan, B., Luo, J., Li, C., 2005. Early growth, dry matter allocation and water use efficiency of two sympatric *Populus* species as affected by water stress. *Environ. Exp. Bot.* 53, 315–322.
- Zhao, D., Reddy, K.R., Kakani, V.G., Read, J., Sullivan, J.H., 2003. Growth and physiological responses of cotton (*Gossypium hirsutum* L.) to elevated carbon dioxide and ultraviolet-B radiation under controlled environment conditions. *Plant Cell Environ.* 26, 771–782.
- Zhu, J., Hasegawa, P.M., Bressan, R.A., 1997. Molecular aspects of osmotic stress in plants. *Crit. Rev. Plant Sci.* 16, 253–277.
- Ziemke, J.R., Chandra, S., Herman, J., 2000. Erythemally weighted UV trends over northern latitudes derived from Nimbus 7 TOMS measurements. *J. Geophys. Res.* 105, 7373–7382.