



# Photoprotection, photosynthesis and growth of tropical tree seedlings under near-ambient and strongly reduced solar ultraviolet-B radiation

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## Summary

Seedlings of two late-successional tropical rainforest tree species, *Tetragastris panamensis* (Engler) O. Kuntze and *Calophyllum longifolium* (Willd.), were field grown for 3–4 months at an open site near Panama City (9°N), Panama, under plastic films that either transmitted or excluded most solar UV-B radiation. Experiments were designed to test whether leaves developing under bright sunlight with strongly reduced UV-B are capable of acclimating to near-ambient UV-B conditions. Leaves of *T. panamensis* that developed under near-ambient UV-B contained higher amounts of UV-absorbing substances than leaves of seedlings grown under reduced UV-B. Photosynthetic pigment composition, content of  $\alpha$ -tocopherol, CO<sub>2</sub> assimilation, potential photosystem II (PSII) efficiency (evaluated by  $F_v/F_m$  ratios) and growth of *T. panamensis* and *C. longifolium* did not differ between seedlings developed under near-ambient and reduced solar UV-B. When seedlings were transferred from the reduced UV-B treatment to the near-ambient UV-B treatment, a pronounced inhibition of photosynthetic capacity was observed initially in both species. UV-B-mediated inhibition of photosynthetic capacity nearly fully recovered within 1 week of the transfer in *C. longifolium*, whereas in *T. panamensis* an about 35% reduced capacity of CO<sub>2</sub> uptake was maintained. A marked increase in UV-absorbing substances was observed in foliage of transferred *T. panamensis* seedlings. Both

**Abbreviations:**  $A_{max}$ , maximum rate of net CO<sub>2</sub> assimilation; Ax, antheraxanthin; Car, carotene; Chl, chlorophyll;  $c_i/c_a$ , ratio of intercellular-to-ambient CO<sub>2</sub> concentration; PAR, photosynthetically active radiation (400–700 nm); PSII, photosystem II; Vx, violaxanthin; Zx, zeaxanthin

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species exhibited enhanced mid-day photoinhibition of PSII immediately after being transferred from the reduced UV-B to the near-ambient UV-B treatment. This effect was fully reversible within 1 d in *T. panamensis* and within a few days in *C. longifolium*. The data show that leaves of these tropical tree seedlings, when developing in full-spectrum sunlight, are effectively protected against high solar UV-B radiation. In contrast, leaves developing under conditions of low UV-B lacked sufficient UV protection. They experienced a decline in photosynthetic competence when suddenly exposed to near-ambient UV-B levels, but exhibited pronounced acclimative responses.

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## Introduction

High-energy ultraviolet (UV) solar radiation, especially in the UV-B band (280–320 nm) is potentially damaging to terrestrial plants. UV-B varies greatly at the earth's surface over both temporal and spatial dimensions. Given the tremendous variation in solar UV-B, it is important to understand the acclimation potential of plants to changes in UV-B exposure. The question of UV-B acclimation potential is of particular interest for tropical plants owing to the relatively high UV-B present at tropical latitudes and the dramatic variation in local light environments within tropical forests (Caldwell et al., 1980; Flint and Caldwell, 1998; Ziska, 1996).

The effect of UV-B on plants is studied experimentally by either using filters to attenuate the UV-B exposure or by using supplementary lights to increase the UV-B exposure (e.g., Albert et al., 2005; Ballaré et al., 2001; Bassman et al., 2001; Day and Neale, 2002; Flint et al., 2003; Qaderi and Reid, 2005; Rousseaux et al., 2004; Searles et al., 1995; Tevini, 2004). These reports reveal a great variety of UV-B effects on photosynthesis, morphology, and growth. UV-B effects on plants vary among these studies in part because of species differences and in part due to differences in experimental conditions (Kakani et al., 2003). Nevertheless, it is clear that terrestrial plants have a broad capacity to tolerate relatively high levels of UV-B light (Allen et al., 1998). UV-absorbing compounds such as flavonoids and hydroxycinnamic acids are thought to be essential for UV protection (Burchard et al., 2000; Caldwell et al., 1983; Cockell and Knowland, 1999; Kolb et al., 2001; Mazza et al., 2000; Reuber et al., 1993). They act most effectively when accumulated in the upper (adaxial) leaf epidermis (Flint et al., 1985; Markstädter et al., 2001). Reviewing numerous published studies, Searles et al. (2001) reported that plants exposed to supplementary UV-B generally exhibited conspicuous increases in UV-absorbing compounds with little

or no treatment effects on leaf morphology or photosynthesis. This suggests that UV-B acclimation involves accumulation of UV-absorbing compounds, which minimizes deleterious UV-B effects on the photosynthetic apparatus.

In support of this suggestion, shade-acclimated leaves of tropical plants are known to contain low levels of UV-absorbing compounds (Krause et al., 2003a; Lovelock et al., 1992) and when these leaves are suddenly exposed to full sunlight, the UV-B wavelengths strongly amplify the photoinhibitory reductions in photosystems II and I (PSII and PSI) efficiency as well as the capacity for net CO<sub>2</sub> assimilation (Krause et al., 1999, 2003b). Recently, it has been shown that brief daily exposures of shade-grown leaves of tropical tree seedlings to full sunlight were sufficient to promote the accumulation of high levels of UV-absorbing compounds and carotenoids which, in turn, protected the photosynthetic apparatus against UV radiation and excess visible light (Krause et al., 2004). Although these findings verify the importance of UV-absorbing compounds for photoprotection against UV-B, they partially confound the acclimation responses of leaves to increases in UV light with the well-known acclimation responses of shade leaves to increases in visible light. It is unknown to which extent leaves grown under bright visible light but reduced UV-B are able to acclimate to sudden increases in the UV-B light.

The objective of the present study was to characterize the photosynthetic and growth responses of sun-grown tropical tree seedlings to a sudden shift from a low UV-B level to near-ambient UV-B levels. Seedlings of two tree species native to the rainforests of Panama, *Tetragastris panamensis* and *Calophyllum longifolium*, were grown in the field at an open sun-exposed site under plastic films which either transmitted or absorbed most of the ambient solar UV-B. After 3–4 months, some individuals were transferred from the reduced UV-B to the near-ambient UV-B conditions to characterize the physiological responses to that

strong increase in solar UV-B. Photosynthetic CO<sub>2</sub> assimilation, chlorophyll (Chl) *a* fluorescence emission, contents of UV-absorbing compounds, photosynthetic pigments and  $\alpha$ -tocopherol were evaluated in mature leaves and overall seedling biomass accumulation was assessed in order to obtain a broad picture of the effects of ambient UV-B radiation on both UV-B acclimated and non-acclimated plants.

## Materials and methods

The experiments were performed at the Santa Cruz Experimental Field Facility of the Smithsonian Tropical Research Institute in Gamboa near Panama City (9°N, 35 m above sea level), Republic of Panama. Analyses of photosynthetic pigments and  $\alpha$ -tocopherol were carried out at the Institute of Plant Biochemistry, University Düsseldorf, Germany.

### Plant material

Seedlings of *T. panamensis* (Engler) O. Kuntze (Burseraceae) and *C. longifolium* Willd. (Clusiaceae) were grown from seeds collected in the forest. Both species are late-successional trees in neotropical forests. Their seedlings are shade-tolerant.

### Experimental procedure

Two long-term experiments were performed in 2003/2004 and 2004/2005 with *C. longifolium* and *T. panamensis*, respectively.

*T. panamensis*: Seedlings were grown in 15 L pots (height 50 cm) in forest soil mixed with 50% leaf litter. Photosynthetically active radiation (PAR) was about 46% of ambient levels during the first 6 weeks after sowing and was subsequently increased to 80% for 2 months. On 9 November 2004, 22 pots containing one plant per pot were placed under a metal frame (4 m × 1 m × 1.8 m) covered with polyester film (0.13 mm Melinex 516, Conservation Support Systems, Santa Barbara, CA, USA), which reduced UV-B to about 6% of ambient levels. Further 22 pots were placed under a second frame covered with Aclar film (0.038 mm Aclar 22A, Honyewell, Pottsville, PA, USA), which maintained UV-B at 88% of ambient levels. Polyester and Aclar films reduced PAR by 9% and 5%, respectively. The chambers covered by the plastic films were located at an open, non-shaded site. Extractor fans at the two top ends of the chambers maintained close to

ambient air temperatures; on bright days at mid-day, temperatures inside the chambers did not exceed ambient temperatures by more than 1–3 °C.

After 3.8 months, six plants were transferred from the polyester chamber (“strongly reduced UV-B”) to the Aclar chamber (“near-ambient UV-B”) and physiological responses (i.e., Chl *a* fluorescence, CO<sub>2</sub> assimilation and content of UV-absorbing substances) of mature leaves to the increase in UV-B irradiance were monitored for 7 d following the transfer. After about 4 months (8 March 2005) under the specified UV-B conditions, plants from both chambers were harvested for determination of growth parameters.

*C. longifolium*: On 23 October 2003, 20 pots each (with four seeds per pot) were placed under polyester and Aclar films, as described above for *T. panamensis*. After germination (early December 2003), the number of seedlings was reduced to one per pot. On 26 February 2004, five plants were transferred from strongly reduced (polyester) to near-ambient (Aclar) UV-B conditions. Chl *a* fluorescence and CO<sub>2</sub> gas exchange of the mature second leaf pair were monitored for 7 d following the transfer. On 10 March 2004, plants from both chambers were harvested and growth parameters determined.

### Measurement of UV irradiance and PAR

Spectra of ambient UV-B (280–320 nm) and UV-A (320–400 nm) radiation energy were recorded with a spectroradiometer (OL 754-0-PMT, Optronics, Orlando, FL, USA). PAR was measured at 5 min intervals with quantum sensors (LI-190SA, LI-COR, Lincoln, NE), connected to a data logger (CR10X Measurement and Control System, Campbell Scientific Inc., Logan, Utah).

### Assessment of soluble UV-absorbing compounds

Leaf disks (area 0.9 cm<sup>2</sup>) were frozen in liquid nitrogen, stored at –70 to –80 °C in a deep freezer and transported on dry ice. Soluble UV-absorbing substances were extracted with ethanol/water (Krause et al., 2001, 2003a). Absorbance spectra of the extracts in the UV spectral region were recorded. Absorbance values at 280 and 305 nm (UV-B) or 360 and 375 nm (UV-A) were taken as relative measure of the content of UV-absorbing substances. Absorbance at 280 nm is not relevant for screening of solar UV-B radiation; it was included in Table 1 because the highest absorbance peak in the whole UV-B region was frequently

**Table 1.** UV absorbance by ethanolic/aqueous extracts of leaves of *Tetragastris panamensis* grown in the presence of either near-ambient or strongly reduced UV-B radiation

$\lambda$ (nm)	Growth conditions		
	Near-ambient UV-B	Reduced UV-B (% <sup>a</sup> )	Transferred from reduced to near-ambient UV-B (% <sup>a</sup> )
280	20.9 ± 4.9 <sup>b</sup>	12.0 ± 2.1 (57.4)	15.6 ± 4.6 (74.6)
305	18.9 ± 4.3 <sup>b</sup>	7.9 ± 3.0 (41.8)	13.9 ± 5.8 <sup>c</sup> (73.5)
360	17.3 ± 2.5 <sup>b</sup>	2.5 ± 1.3 (14.5)	7.1 ± 2.1 <sup>b</sup> (41.0)
375	16.9 ± 4.2 <sup>b</sup>	1.6 ± 0.9 (9.5)	3.8 ± 0.8 <sup>b</sup> (22.5)

After a period of 3.8 months, samples were taken and six plants were transferred from reduced to near-ambient UV-B conditions and further samples taken 7 d after transfer. Means ± SD of absorbance values at four wavelengths ( $\lambda$ ) in the UV spectral region are shown ( $n = 6$ ; leaves from different plants). Absorbance values are given in relative units (referred to 1 mL extract per 1 cm<sup>2</sup> leaf area).

<sup>a</sup>Percentage of absorbance values under near-ambient UV-B.

<sup>b</sup>Significant difference from 'reduced UV-B' ( $P < 0.01$ ).

<sup>c</sup>Significant difference from 'reduced UV-B' ( $P < 0.05$ ).

observed close to that wavelength (see Krause et al., 2003a, for examples of spectra). The wavelengths 305 and 360 nm were selected as being central in the spectral regions of UV-B and UV-A, respectively. Absorbance at 375 nm allows comparison with data obtained with the portable UV-A-PAM fluorometer, which records epidermal UV-A screening at 375 nm (Kolb et al., 2005; Krause et al., 2003a). Presented absorbance data correspond to 1 mL extract per 1 cm<sup>2</sup> leaf area (Cerovic et al., 2002).

### Quantification of photosynthetic pigments and $\alpha$ -tocopherol

Photosynthetic pigments in leaf disks (see above) were quantified by HPLC using a modified method of Färber et al. (1997), described by Krause et al. (2003a). The content of  $\alpha$ -tocopherol in the same leaf extract as used for pigment analysis was determined by HPLC (Krause et al., 2006) according to García-Plazaola and Becerril (1999).

### Net CO<sub>2</sub> gas exchange

A portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA) was used to measure CO<sub>2</sub> gas exchange, as described in detail by Krause et al. (2006). In the experiment with *T. panamensis*, maximum rates of photosynthetic net CO<sub>2</sub> uptake ( $A_{\max}$ ) were recorded under saturating natural sunlight (PAR > 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). In case of *C. longifolium*,  $A_{\max}$  values were obtained from light-response curves of net CO<sub>2</sub> uptake measured under artificial light emitted by a 6400-02B LED (LI-COR). Light saturation was reached at about 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR.

### Assessment of potential photosystem II efficiency

The ratio of maximum variable to maximum total Chl *a* fluorescence emission from attached leaves,  $F_v/F_m$ , recorded at the adaxial leaf side after 10 min dark adaptation, served as a measure of potential PSII efficiency (Krause and Weis, 1991; Krause and Winter, 1996). Ratios were determined with a portable fluorometer (MINI-PAM, Walz, Effeltrich, Germany). For dark adaptation, DLC-8 leaf clips (Walz) were used. Details of the measuring mode have been described previously (Krause et al., 2006).

### Growth parameters

Upon harvest of the seedlings, the area of leaf blades per plant was measured with an area meter (LI-3100, LI-COR, Lincoln, NE, USA). For dry mass determination, leaf blades, stems (including leaf petioles) and roots were dried separately at 70 °C for at least 72 h. Specific leaf area (SLA) was calculated as leaf area per leaf dry mass.

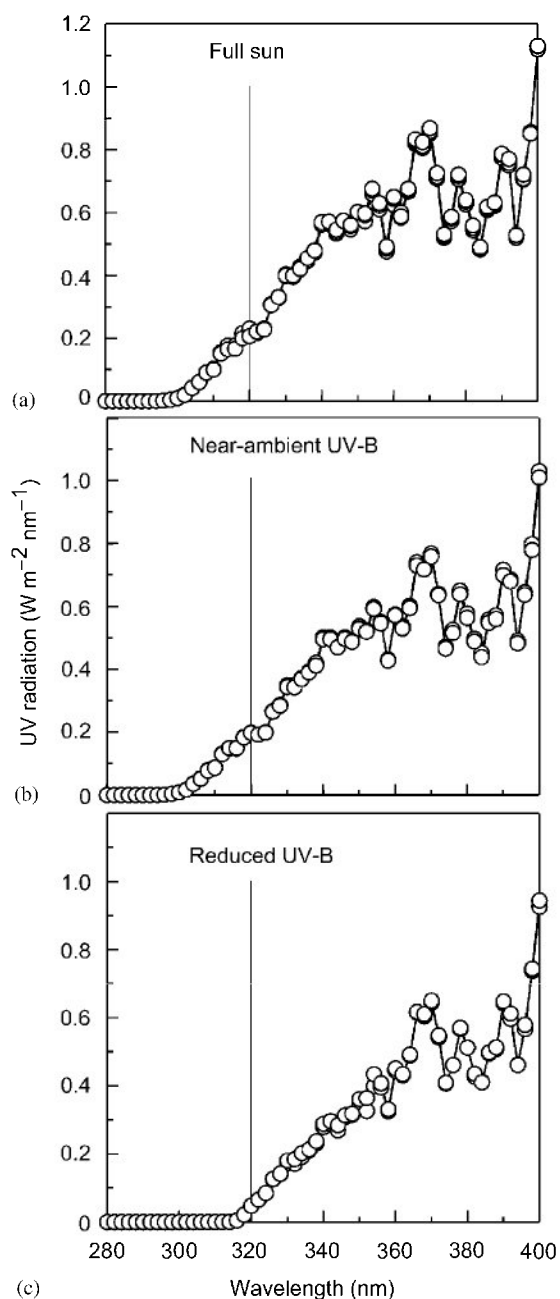
### Statistics

Mean values and standard deviations (SD) of recorded data are presented. Significance of differences between treatment datasets were evaluated by unpaired *t*-tests. Where appropriate, the probabilities of error (*P*) are noted.

## Results

### Light conditions

Spectra of solar UV radiation recorded close to mid-day under clear sky are depicted in Fig. 1.



**Figure 1.** Spectra of solar UV radiation recorded in 2 nm intervals. Measurements were done in Gamboa near Panama City (9°N) on 4 Feb. 2004 between 11:30 and 12:50 h, local time; (a) recording under full sunlight (data from three spectra pooled); (b) recording under Aclar film ('near-ambient UV-B', data from two spectra); (c) recording under polyester film ('reduced UV-B', data from two spectra). Integration of the spectra showed UV-B (280–320 nm) and UV-A (320–400 nm) energies in full sunlight (a) of  $2.52 \pm 0.07$  and  $47.3 \pm 0.5$   $\text{W m}^{-2}$ , respectively. Mean transmittance of UV radiation energy was 88.2% UV-B and 90.0% UV-A by Aclar film and 5.9% UV-B and 69.4% UV-A by polyester film.

Photosynthetically active radiation (PAR) during the measurements ranged between 1910 and 1980  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . As calculated from the spectra, under full sun (Fig. 1a), integrated UV-B radiation energy (280–320 nm) was  $2.52 \pm 0.07$   $\text{W m}^{-2}$  and UV-A energy (320–400 nm)  $47.3 \pm 0.5$   $\text{W m}^{-2}$ . Plants growing under Aclar film (near-ambient UV-B, Fig. 1b) received about 88% of ambient UV-B and 90% of ambient UV-A, whereas plants under polyester film (reduced UV-B, Fig. 1c) received 6% and 69% of ambient UV-B and UV-A energy, respectively (see Legend to Fig. 1). As the polyester film excluded part of UV-A, particularly in the shorter wavelength region, that fraction of UV-A might have contributed to the effects observed when plants were transferred from reduced (polyester) to near-ambient (Aclar) UV conditions.

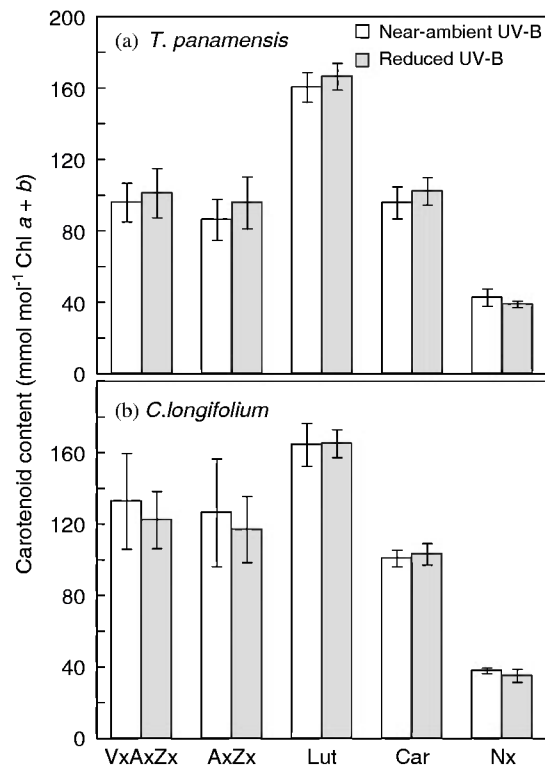
Due to changing cloudiness, daily solar radiation doses varied strongly during the time of the experiment and were significantly lower ( $P < 0.01$ ) in November and December (rainy season) than from January to March (dry season). Mean daily ambient PAR doses ( $\text{mol photons m}^{-2} \text{d}^{-1}$ ) during the experimental periods were  $29.0 \pm 11.0$  in Nov./December 2003,  $28.9 \pm 9.7$  in November/December 2004,  $40.3 \pm 6.4$  from January to March 2004, and  $37.0 \pm 8.2$  from January to March 2005. Plants under Aclar and polyester films received about 95% and 91% of ambient PAR, respectively.

### Protection against UV and excessive visible light

Absorbance characteristics in the UV spectral region of ethanolic/aqueous leaf extracts of *T. panamensis* were used to assess the level of UV-absorbing compounds (Table 1). UV-B and UV-A absorbance were much lower for leaves grown under reduced compared to near-ambient UV-B radiation. Reduction of UV-B had a larger effect on absorbance in the UV-A (360 and 375 nm) than in the UV-B region (280 and 305 nm). Within 7 d of transferring plants from the reduced to the near-ambient UV-B conditions, a significant increase in UV absorbing compounds was observed in *T. panamensis* leaf extracts (Table 1). This effect was most pronounced in the UV-B region.

Carotenoid contents in leaves of *T. panamensis* and *C. longifolium* did not respond to the level of UV-B radiation during growth (Fig. 2). Leaves of both species exhibited relatively high contents of xanthophyll cycle pigments, viola-, anthera- and zeaxanthin (VxAxZx), high contents of  $\beta$ -carotene ( $\beta$ -Car), and low  $\alpha/\beta$ -Car ratios (see Legend to Fig. 2), all characteristic of typical sun-acclimated

leaves (Krause et al., 2003a; Thayer and Björkman, 1990). Leaf samples for pigment analyses were collected under bright sun conditions at which time



**Figure 2.** Contents of carotenoids in leaves of *T. panamensis* (a) and *C. longifolium* (b) after 3–4 months growth under near-ambient or strongly reduced UV-B radiation. Samples were taken at 11 h, local time, when the sun was not obscured by clouds. Means  $\pm$  SD are presented ( $n = 6$  [a];  $n = 4-5$  [b]; leaves of different plants). Open bars, near-ambient UV-B; closed bars, reduced UV-B. VxAxZx, sum of viola-, anthera- and zeaxanthin; AxZx, sum of anthera- and zeaxanthin; Lut, lutein; Car, sum of  $\alpha$ - and  $\beta$ -carotene; Nx, neoxanthin. In (a),  $\alpha$ - and  $\beta$ -carotene were not separated; in (b), the molar  $\alpha/\beta$ -carotene ratio was  $0.061 \pm 0.028$  and  $0.067 \pm 0.009$  under near-ambient and reduced UV-B, respectively.

the de-epoxidation state of the xanthophyll cycle pigments was expected to be at its maximum. As seen from the high level of the sum of anthera- and zeaxanthin (AxZx), a very high de-epoxidation state was reached in both species under near-ambient and reduced UV-B conditions (Fig. 2). Similarly, between leaves developed under the two growth regimes, no significant differences were detected in Chl *a/b* ratios and contents of Chl *a+b* and  $\alpha$ -tocopherol expressed per unit leaf area (Table 2).

### CO<sub>2</sub> assimilation and potential PS II efficiency

Maximum rates of net CO<sub>2</sub> uptake ( $A_{max}$ ) did not differ significantly between leaves which had developed under near-ambient or reduced UV-B radiation, as shown for *T. panamensis* in Table 3 (see Legend to Fig. 4 for *C. longifolium*). Under clear or partly cloudy sky, mid-day depression of CO<sub>2</sub> uptake occurred to a similar extent under both UV-B conditions (Table 3).

Upon transfer of plants from strongly reduced to near-ambient UV-B, significant photoinhibition of PSII (decrease in  $F_v/F_m$  ratio) was observed close to mid-day on the day of transfer (Fig. 3). For comparison, the response of PSII in leaves remaining under their respective light regimes is shown (Fig. 3). A decreased  $F_v/F_m$  ratio was seen in all leaves tested. There was a tendency (not statistically significant) of stronger PSII photoinhibition in leaves under near-ambient compared with reduced UV-B in both species, but the largest  $F_v/F_m$  decline was seen in leaves of the transferred plants. In the experiment with *T. panamensis* (Fig. 3a), the day of transfer was relatively cloudy, resulting in a lower than average PAR dose (see Legend to Fig. 3). Full recovery of PSII efficiency occurred overnight. *C. longifolium* plants were transferred on a day with little cloud cover; only partial recovery of PSII efficiency had occurred by sunset (Fig. 3b) or by the next morning (Fig. 4b).

**Table 2.** Contents of chlorophyll and  $\alpha$ -tocopherol in leaves of *T. panamensis* and *C. longifolium* seedlings after 3–4 months growth under near-ambient or reduced UV-B radiation

Parameter	Species	Growth condition	
		Near-ambient UV-B	Reduced UV-B
Chl <i>a/b</i> (mol mol <sup>-1</sup> )	<i>T. panamensis</i>	$3.34 \pm 0.29$	$3.27 \pm 0.18$
	<i>C. longifolium</i>	$3.15 \pm 0.22$	$3.34 \pm 0.13$
Chl <i>a+b</i> ( $\mu$ mol m <sup>-2</sup> )	<i>T. panamensis</i>	$255.4 \pm 44.0$	$218.3 \pm 44.3$
	<i>C. longifolium</i>	$301.9 \pm 23.7$	$308.0 \pm 27.6$
$\alpha$ -Tocopherol ( $\mu$ mol m <sup>-2</sup> )	<i>T. panamensis</i>	$151.5 \pm 42.9$	$143.0 \pm 29.3$
	<i>C. longifolium</i>	$92.0 \pm 20.7$	$88.3 \pm 18.5$

Means  $\pm$  SD are presented ( $n = 4-6$ ; leaves of different plants).

**Table 3.** Maximum rates of net CO<sub>2</sub> assimilation ( $A_{\max}$ ) by leaves of *T. panamensis* seedlings grown for about 3.3 months in the presence of near-ambient or reduced UV-B radiation

Experimental conditions	$A_{\max}$ ( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ )	
	Morning	Mid-day
Near-ambient UV-B	$8.0 \pm 1.1^a$	$4.6 \pm 1.2$
Reduced UV-B	$8.3 \pm 0.4^a$	$4.8 \pm 1.2$

Measurements were performed, when PAR was saturating (PAR at leaf level above  $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) in the morning (8:30 h, local time) and close to mid-day (11:40 h). High-light periods ( $1000\text{--}2390 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) occurred between 10:20 and 11:40 h. During that time, the plants received PAR doses of about 7.0 (under near-ambient UV-B) or  $6.2 \text{ mol photons m}^{-2}$  (under reduced UV-B), recorded with horizontally placed sensors. Means  $\pm$  SD are presented ( $n = 6$ ; leaves of different plants).

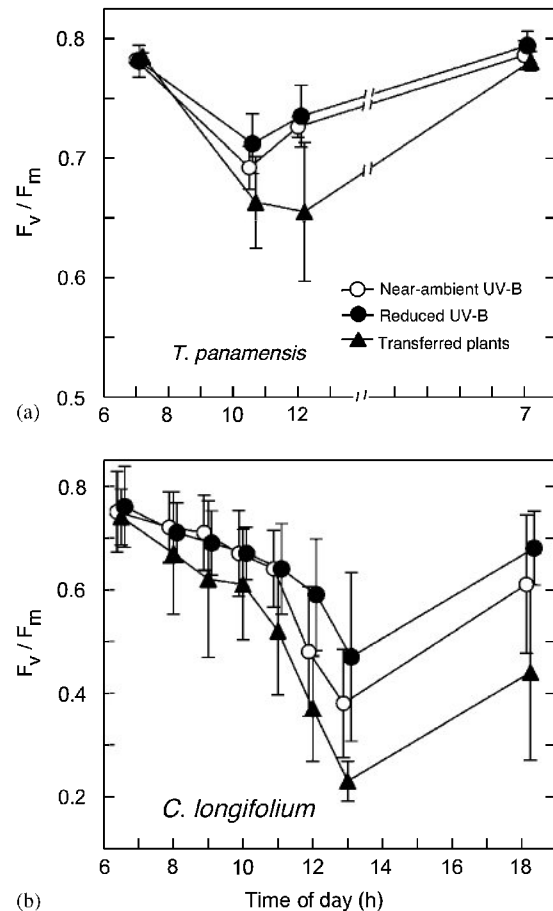
<sup>a</sup>Significant difference between morning and mid-day ( $P < 0.01$ ).

$A_{\max}$  and potential PSII efficiency of leaves of seedlings transferred from reduced to near-ambient UV-B were followed for 7 d (Fig. 4). In leaves of *T. panamensis* (Fig. 4a),  $A_{\max}$  was reduced by approximately 35% in response to the strong increase in UV-B radiation. The lowered  $A_{\max}$  was maintained, but did not significantly recover within 7 d after the transfer. There was no indication that the reduction in  $A_{\max}$  was caused by decreased stomatal conductance. The ratio of intercellular to ambient CO<sub>2</sub> concentration,  $c_i/c_a$ , increased from  $0.680 \pm 0.041$  1 d before transfer (control) to  $0.748 \pm 0.055$  ( $P < 0.05$ ) on the day after transfer. The  $c_i/c_a$  ratio was not significantly different from the control 4 and 7 d after transfer. Morning values of  $F_v/F_m$  were not affected by the transfer.

Leaves of *C. longifolium* (Fig. 4b) also exhibited a sharp reduction in  $A_{\max}$  1 d after transfer. Within 7 d, those leaves showed a substantial recovery of CO<sub>2</sub> uptake, reaching  $A_{\max}$  values close to the controls. Potential PSII efficiency remained inhibited in the morning after 1 d of high UV-B exposure but recovered almost completely during the following days.

### Biomass accumulation

Total biomass accumulation and its allocation to leaves, stems and roots per plant are shown in Fig. 5. These data indicate that there were no discernable effects of the different UV-B treatments on the growth or allocation patterns for either species. Other measures of plant size and allocation (stem height, leaf number, leaf area and specific leaf area) shown in Table 4 also indicate an absence of UV-B effects on plant and leaf morphology except for an approximately

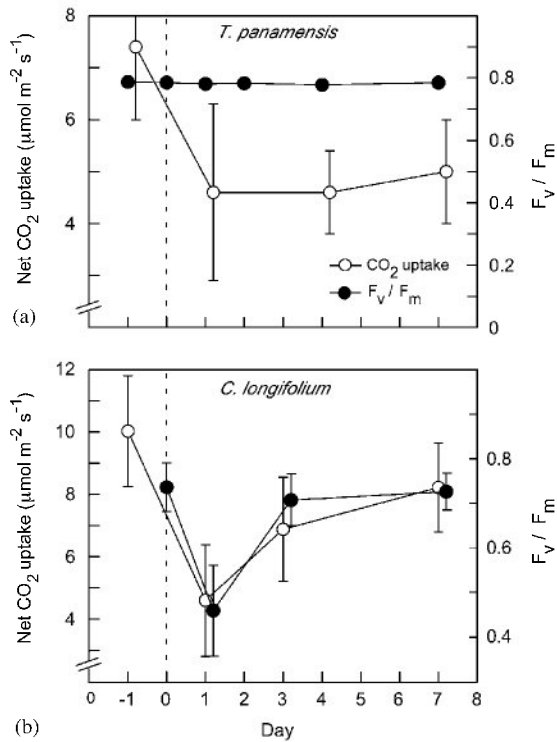


**Figure 3.** Day course of potential photosystem II efficiency ( $F_v/F_m$  ratio) of *T. panamensis* (a) and *C. longifolium* (b) seedlings cultivated for 3–4 months under conditions of near-ambient or strongly reduced UV-B radiation. On the day of measurements at 7 h, local time, 5–6 plants were transferred from reduced to near-ambient UV conditions. Means  $\pm$  SD are shown ( $n = 5$  [a],  $n = 5\text{--}8$  [b]; leaves of different plants). Open circles, seedlings under near-ambient UV-B; closed circles, seedlings under strongly reduced UV-B; triangles, seedlings transferred from reduced to near-ambient UV-B. Incident PAR dose under reduced UV-B conditions was  $25.7 \text{ mol m}^{-2} \text{ d}^{-1}$  in (a) and  $39.4 \text{ mol m}^{-2} \text{ d}^{-1}$  in (b); about 6% higher PAR doses were recorded under near-ambient UV-B conditions. The difference in  $F_v/F_m$  between leaves of plants under reduced UV-B and transferred plants was significant in (a) at 10:30 h and 12 h ( $P < 0.05$ ) and in (b) at 13 h and 18 h, local time ( $P < 0.01$ ).

15% greater stem height in *T. panamensis* plants cultivated under near-ambient compared to reduced UV-B.

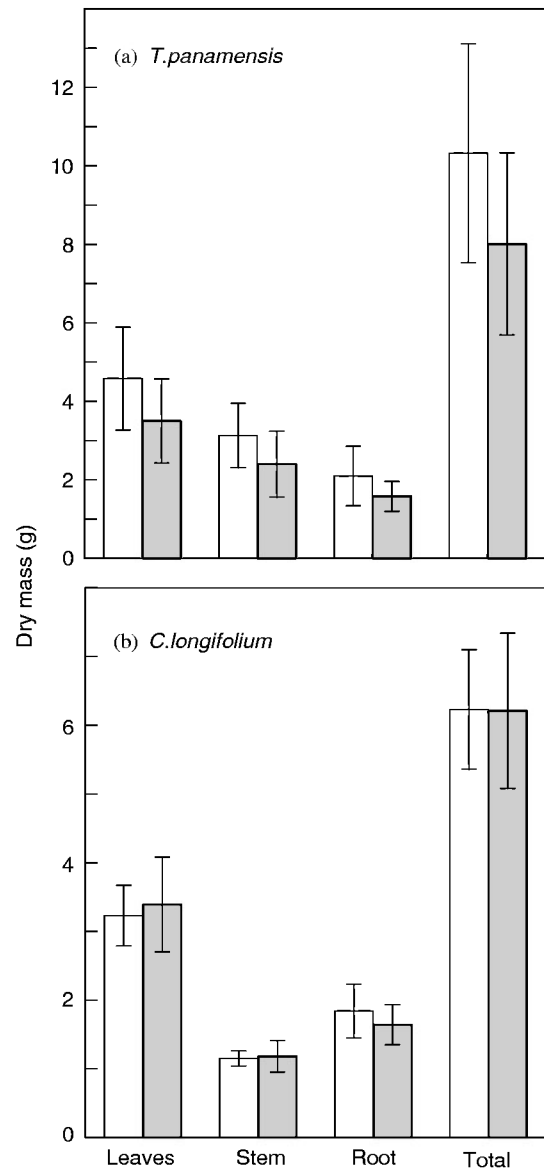
### Discussion

The seedlings growing under the two types of plastic films received strongly different UV-B



**Fig. 4.** Maximum net CO<sub>2</sub> assimilation and potential photosystem II efficiency ( $F_v/F_m$ ) of leaves of *T. panamensis* (a) and *C. longifolium* (b) seedlings before and after transfer from strongly reduced to near-ambient UV-B conditions. Data were obtained in the morning in the absence of inhibitory levels of irradiance. Means  $\pm$  SD (where larger than symbols) are shown ( $n = 5-6$  [a],  $n = 5$  [b]; leaves of different plants). Open symbols, rates of net CO<sub>2</sub> uptake; closed symbols,  $F_v/F_m$  ratios. Day-1 denotes 1 d before transfer; day 0, day of transfer. Maximum rates of net CO<sub>2</sub> uptake by *C. longifolium* leaves grown under near-ambient UV-B were  $10.3 \pm 2.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  ( $n = 5$ ), i.e. not different from maximum rates under reduced UV-B. Compared to rates measured 1 d before transfer, net CO<sub>2</sub> assimilation in (a) was significantly reduced 1 d ( $P < 0.05$ ), 4 d ( $P < 0.01$ ) and 7 d ( $P < 0.05$ ) after transfer; in (b), net CO<sub>2</sub> uptake was significantly reduced after 1 d ( $P < 0.01$ ) and 3 d ( $P < 0.05$ ), but not after 7 d.  $F_v/F_m$  ratios were significantly reduced only in (b) 1 d after transfer ( $P < 0.01$ ).

radiation energies (Fig. 1). Polyester film cuts off UV-B radiation sharply below 320 nm; the few percent solar UV-B penetrating the film belong largely to the longer, biologically less effective UV-B wavelength region between 316 and 320 nm (Fig. 1c). Thus, the seedlings cultivated under polyester film can be regarded as non-acclimated to solar UV-B. In leaves of *T. panamensis* (Table 1), this is evident from the reduced content of soluble UV-B absorbing compounds. Absorbance of leaf extracts at 305 nm was in the same range as found for leaves of eight rainforest species devel-



**Figure 5.** Dry mass of *T. panamensis* and *C. longifolium* seedlings after 3–4 months of growth under conditions of near-ambient or strongly reduced UV-B radiation. Means  $\pm$  SD are presented ( $n = 10$  plants). Open bars, near-ambient UV-B; closed bars, reduced UV-B. Leaf blades, stems (including leaf petioles) and roots were separated before drying. For *T. panamensis*, only leaves developed after the plants were subjected to the respective growth conditions were considered; at the start of the experiment, the seedlings had a total dry mass of  $1.09 \pm 0.18$  g ( $n = 5$  plants). *C. longifolium* was sown under the two growth conditions.

oped in the deep shade of the forest understorey (Krause et al., 2003a).

In a previous investigation of tropical plants including seedlings of *T. panamensis* and *C. longifolium* (Searles et al., 1995), a similar reduction of UV-B absorbing compounds as reported in the



**Table 4.** Growth parameters of *T. panamensis* and *C. longifolium* seedlings after 3–4 months of growth under near-ambient or strongly reduced UV-B radiation

Parameter	Growth condition		
	Species	Near-ambient UV-B	Reduced UV-B
Stem height (cm)	<i>T. panamensis</i>	28.3 ± 3.8 <sup>a</sup>	24.7 ± 2.3 <sup>b</sup>
	<i>C. longifolium</i>	24.1 ± 1.2	24.8 ± 2.6
Leaf number	<i>T. panamensis</i>	17.8 ± 4.1	15.1 ± 4.8
	<i>C. longifolium</i>	8.40 ± 0.84	8.00 ± 0.94
Leaf area (cm <sup>2</sup> )	<i>T. panamensis</i>	573 ± 143	493 ± 140
	<i>C. longifolium</i>	319 ± 50	349 ± 77
SLA (cm <sup>2</sup> g <sup>-1</sup> ) <sup>c</sup>	<i>T. panamensis</i>	126.5 ± 10.9	143.1 ± 17.2
	<i>C. longifolium</i>	99.3 ± 9.0	103.4 ± 8.0

Means ± SD are presented ( $n = 10$  plants). At the start of the experiment, the stem height of *T. panamensis* was 15.8 ± 1.7 cm and seedling had 12.8 ± 0.8 leaves ( $n = 5$  plants); only leaves developed after start of the experiment are considered in the table. *C. longifolium* was sown under the two growth conditions.

<sup>a,b</sup>Significant difference,  $P < 0.05$ .

<sup>c</sup>Specific leaf area, i.e. leaf area referred to leaf dry mass per plant.

present study (Table 1) was observed under strongly reduced UV-B in leaves of *T. panamensis*, but not in leaves of *C. longifolium* (not assessed here). However, in situ, much higher contents of UV-B absorbing compounds were found in canopy sun leaves of a *C. longifolium* forest tree than in mature leaves of seedlings grown in deep shade of the understorey (Krause et al., 2004). The content of UV-absorbing compounds in seedlings of pioneer and late-successional tree species cultivated in simulated tree-fall gaps responded positively to the gap size, i.e. the daily period of high PAR and UV exposure (Krause et al., 2001). In several reports (Liu et al., 2005; Lovelock et al., 1992; Lud et al., 2001; Rousseaux et al., 2004; Searles et al., 2002), not all tested plant species responded with a reduction in the level of UV-B absorbing compounds, when solar UV-B was attenuated by means of filters. The significance of these different responses among species is unclear at present. It should be noted that the level of extractable UV-absorbing compounds was not always found to be closely related to UV screening of the leaf epidermis (Liakoura et al., 2003), but may serve as an approximate measure of the capacity of UV protection of many plant species and various leaf ecotypes (Cerovic et al., 2002; Kolb and Pfündel, 2005; Krause et al., 2003a).

Except for the low content of UV absorbing compounds (observed in *T. panamensis*, Table 1), leaves that developed under strongly reduced UV-B had typical sun-leaf characteristics. They were indistinguishable from leaves grown under near-ambient UV-B with respect to Chl *a/b* ratios, contents of total Chl and the antioxidant  $\alpha$ -tocopherol (Table 2). This also applied to carote-

noid contents (based on Chl *a+b*),  $\alpha/\beta$ -Car ratios and the de-epoxidation state of the violaxanthin cycle xanthophylls (VxAxZx) at mid-day under clear sky (Fig. 2).

Inhibitory effects on photosynthetic performance following transfer of seedlings from strongly reduced to near-ambient UV-B conditions (Figs. 3 and 4) were expected. Previous experiments with shade-grown tree seedlings (Krause et al., 1999, 2003b) had shown that inhibition of photosynthetic parameters by exposure to nearly full sunlight was caused partly by the solar UV component. The present experimental procedure was designed to separate the effects of high solar UV-B from those of excessive PAR and, moreover, to test possible acclimative responses of the plants to increased solar UV-B. Substantial acclimation regarding UV-absorbing compounds in leaves of *T. panamensis* was observed 7 d after transfer, when absorbance of leaf extracts at 305 nm had reached more than 70% of that seen in leaves grown under near-ambient UV-B (Table 1). It is of interest that absorbance in the UV-A region (360 and 375 nm) was also strongly influenced by the level of UV-B irradiance (Table 1). Overall, the data of Table 1 confirm the induction of synthesis of UV-absorbing compounds by solar UV-B radiation. However, the relatively high absorbance (about 40% at 305 nm) remaining in the absence of most solar UV-B might indicate that synthesis of UV-absorbing compounds is in part constitutive or induced by UV-A/blue light receptor systems (Jenkins, 1997; Treutter, 2005; Vogt et al., 1991; Wade et al., 2001).

In both species, photoinhibition of PSII at mid-day, shown by the decrease in  $F_v/F_m$ , was enhanced on the day of transfer (Fig. 3). The strong inhibition

of  $A_{\max}$  (Fig. 4) appears to have been independent of the reduction in PSII efficiency. In leaves of *T. panamensis* (Fig. 4a), UV-B effects on morning values of  $F_v/F_m$  were absent already 1 d after transfer, whereas  $\text{CO}_2$  assimilation remained partly inhibited for 7 d. In leaves of *C. longifolium*, PSII 'recovered' faster than  $A_{\max}$  (Fig. 4b), similar to recovery responses of  $\text{CO}_2$  assimilation and  $F_v/F_m$  in leaves of grapevine (*Vitis vinifera* L.) that were exposed to full sunlight following growth in the greenhouse under low UV irradiance and PAR (Kolb et al., 2001). Decreased  $F_v/F_m$  ratios recorded at the adaxial leaf side indicate photoinhibition occurring predominantly in the upper chloroplast layers (Lichtenthaler et al., 2005), which might be partly responsible for the differences between the response of  $F_v/F_m$  and  $A_{\max}$ . Calculated  $c_i/c_a$  ratios indicated that the decrease in  $A_{\max}$  did not result from increased stomatal limitation of  $\text{CO}_2$  uptake. According to several investigations that used supplemental UV-B to simulate stratospheric ozone depletion (Allen et al., 1997; Keiller and Holmes, 2001; Keiller et al., 2003; Nogués and Baker, 1995; Rao et al., 1995; Takeuchi et al., 2002),  $\text{CO}_2$  assimilation is a highly UV-B-sensitive photosynthetic reaction. Enzyme activities of the carbon reduction cycle, in particular of ribulose-1.5-bisphosphate carboxylase/oxygenase, seem to be inhibited.

Leaves of both *T. panamensis* and *C. longifolium* that had developed under near-ambient UV-B conditions were adequately protected against damaging effects of high solar UV-B, consistent with studies in which acclimated plants had been irradiated with supplemental UV-B (Allen et al., 1998; Searles et al., 2001). Between seedlings grown under strongly reduced UV-B and near-ambient UV-B, there were no significant differences in maximum rates and degree of 'mid-day depression' of  $\text{CO}_2$  assimilation (Table 3), in potential PSII efficiency (Fig. 3) and biomass production (Table 4, Fig. 5). In contrast to observations by Searles et al. (1995), we noted an increased stem height accompanied by a tendency (albeit statistically non-significant) for greater leaf area (Table 4) and biomass accumulation in *T. panamensis* (Fig. 5a) grown under near-ambient UV-B. At present, we cannot explain this discrepancy.

The absence of inhibitory effects of solar UV-B on total dry mass production by acclimated plants found in the present and former (Searles et al., 1995) investigations on tropical tree seedlings, as well as in other studies, e.g. on the moss *Sphagnum magellanicum* (Searles et al., 2002) and crop plants such as oat (*Avena sativa* L.) and lettuce (*Lactuca sativa* L.) (Rousseaux et al., 2004), cannot be

generalized among species. Substantial inhibition of dry matter productivity by solar UV-B has been shown in a number of reports (see Day and Neale, 2002, for review; Liu et al., 2005; Zavala and Ravetta, 2002).

In conclusion, leaves of tropical tree seedlings grown in high light from which UV-B was excluded were highly UV-B sensitive. Photosynthetic  $\text{CO}_2$  assimilation and potential PSII efficiency declined strongly when plants were transferred to ambient UV-B conditions. However, photosynthetic recovery and increase in the content of UV absorbing compounds occurred rapidly. A high susceptibility to UV-B mediated photoinhibition in sun-acclimated leaves, combined with the capacity for rapid UV-B acclimation, has not been demonstrated before. The results are remarkable because the plants studied are late-successional tropical species which, in the seedling state, are shade-tolerant and widely considered to be intolerant to full sunlight.

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