Sudden Exposure to Solar UV-B Radiation Reduces Net CO_2 Uptake and Photosystem I Efficiency in Shade-Acclimated Tropical Tree Seedlings[1]

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Tree seedlings developing in the understory of the tropical forest have to endure short periods of high-light stress when tree-fall gaps are formed, and direct solar radiation, including substantial UV light, reaches the leaves. In experiments simulating the opening of a tree-fall gap, the response of photosynthesis in leaves of shade-acclimated seedlings (*Anacardium excelsum*, *Virola surinamensis*, and *Calophyllum longifolium*) to exposure to direct sunlight (for 20–50 min) was investigated in Panama (9°N). To assess the effects of solar UV-B radiation (280–320 nm), the sunlight was filtered through plastic films that selectively absorbed UV-B or transmitted the complete spectrum. The results document a strong inhibition of CO_2 assimilation by sun exposure. Light-limited and light-saturated rates of photosynthetic CO_2 uptake by the leaves were affected, which apparently occurred independently of a simultaneous inhibition of potential photosystem (PS) II efficiency. The ambient UV-B light substantially contributed to these effects. The photochemical capacity of PSI, measured as absorbance change at 810 nm in saturating far-red light, was not significantly affected by sun exposure of the seedlings. However, a decrease in the efficiency of P700 photooxidation by far-red light was observed, which was strongly promoted by solar UV-B radiation. The decrease in PSI efficiency may result from enhanced charge recombination in the reaction center, which might represent an incipient inactivation of PSI, but contributes to thermal dissipation of excessive light energy and thereby to photoprotection.

The thinning of the stratospheric ozone layer and the resulting increase in solar UV-B radiation (280–320 nm) at the earth's surface have led to major research efforts in studying the effects of UV-B on photosynthetic organisms. Based on a survey of a large number of long-term field studies of vascular plants published between 1976 and 1999, Searles et al. (2001) concluded that artificially elevated UV-B induced an increase in content of UV-B-absorbing compounds, but had little or no effect on morphological parameters and leaf photosynthesis as measured by means of gas exchange and chlorophyll (Chl) fluorescence. More recent studies also did not show effects of substantially elevated UV-B on photosynthetic performance of several plant species (Nogués and Baker, 2000; Lud et al., 2001; Bassman et al., 2002). It appears that in many cases, plants are capable of acclimating and, thereby, achieve efficient protection against increased UV-B levels (for review, see Allen et al., 1998). In leaves of tropical tree seedlings grown in simulated, differently sized tree-fall gaps, the level of UV screening substances was positively related to the radiation dose determined by the duration of daily sun exposure (Krause et al., 2001). However, when the protective mechanisms are overtaxed, detrimental effects of elevated UV-B radiation on photosynthesis do occur (for review, see Teramura and Ziska, 1996). In particular, photosystem (PS) II has been found to be UV-B-sensitive, whereas PSI appeared to be unaffected by UV-B. Inhibition of photosynthetic CO_2 assimilation and, specifically, effects on the activity, synthesis, and degradation of Rubisco were seen in a number of studies (Strid et al., 1990; Huang et al., 1993; Nogués and Baker, 1995; Rao et al., 1995; Allen et al., 1997; Bassman et al., 2001; Keiller and Holmes, 2001; Takeuchi et al., 2002).

In most investigations, artificial UV-B supplemental to the ambient solar UV-B level was applied. Fewer studies have been designed to evaluate the effects of present levels of solar UV-B radiation on photosynthesis. With suitable optical filters, one can test whether the ambient UV-B (and UV-A) contributes to photoinhibition of photosynthesis caused by exposure of plant leaves to full sunlight. In particular, the high UV-B flux within tropical latitudes may adversely affect photosynthesis (Madronich et al., 1995; Ziska, 1996). When shade-acclimated tropical tree seedlings were suddenly exposed for short periods (15–75 min) to direct sunlight, as may occur when tree-fall gaps open in the forest, PSII was found to be sensitive to ambient UV-B (and UV-A) radiation (Krause et al., 1998, 1999). In sun leaves from the

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outer canopy of mature tropical trees, UV-B effects on PSII were also observed in certain cases, depending on acclimation and developmental stage of the leaves (Krause et al., 1999). A number of studies have shown that in aquatic organisms, solar UV-B may inhibit photosynthetic reactions, predominantly those of PSII (e.g. Herrmann et al., 1997; Figueroa and Gómez, 2001; Häder et al., 2001). In a study of shade-grown grape (*Vitis vinifera*) plants, performed at mid-latitude (49°N), leaves responded to the UV-B component of full sunlight by enhanced inhibition of potential PSII efficiency and, perhaps independently, of CO_2 assimilation (Kolb et al., 2001). In Antarctic vascular plants studied during periods of ozone depletion, ambient UV-B did not reduce the rates of photosynthetic O_2 evolution per unit leaf area, but rates were diminished on a Chl or dry mass basis (Xiong and Day, 2001). The authors concluded from fluorescence analyses that, independent of PSII limitation, inhibition of photosynthetic enzyme reactions had occurred in the upper mesophyll of the leaves. It should be noted that solar UV-B has also been shown to cause DNA damage in photosynthetic organisms (Ballaré et al., 2001; Buma et al., 2001).

The present study aims to clarify whether the high solar UV-B of tropical latitudes causes a primary inactivating effect on photosynthetic CO_2 assimilation independent of (or in parallel with) inactivation of PSII. In addition, we investigated the response of PSI to ambient UV-B. Under controlled conditions, exposure of shade leaves of seedlings and sun leaves of mature tropical trees to excessive visible light caused a decrease in the efficiency of the photooxidation of the PSI reaction center pigment, P700, by far-red light, whereas the photochemical capacity of PSI remained largely unaffected (Barth et al., 2001). The decline in the efficiency of the P700 photoreaction is probably a universal response of PSI to high-light stress and may be caused by enhanced charge recombination in the PSI reaction center that confers photoprotection (Barth et al., 2001). Thus far, it is unknown whether such alteration of PSI photochemistry is also induced by the UV-B component of solar radiation. Therefore, in the present investigation, the effects of solar UV-B on PSI, as well as on the rate of net CO_2 assimilation in shade-acclimated tree seedlings, were assessed and compared with the effects on PSII.

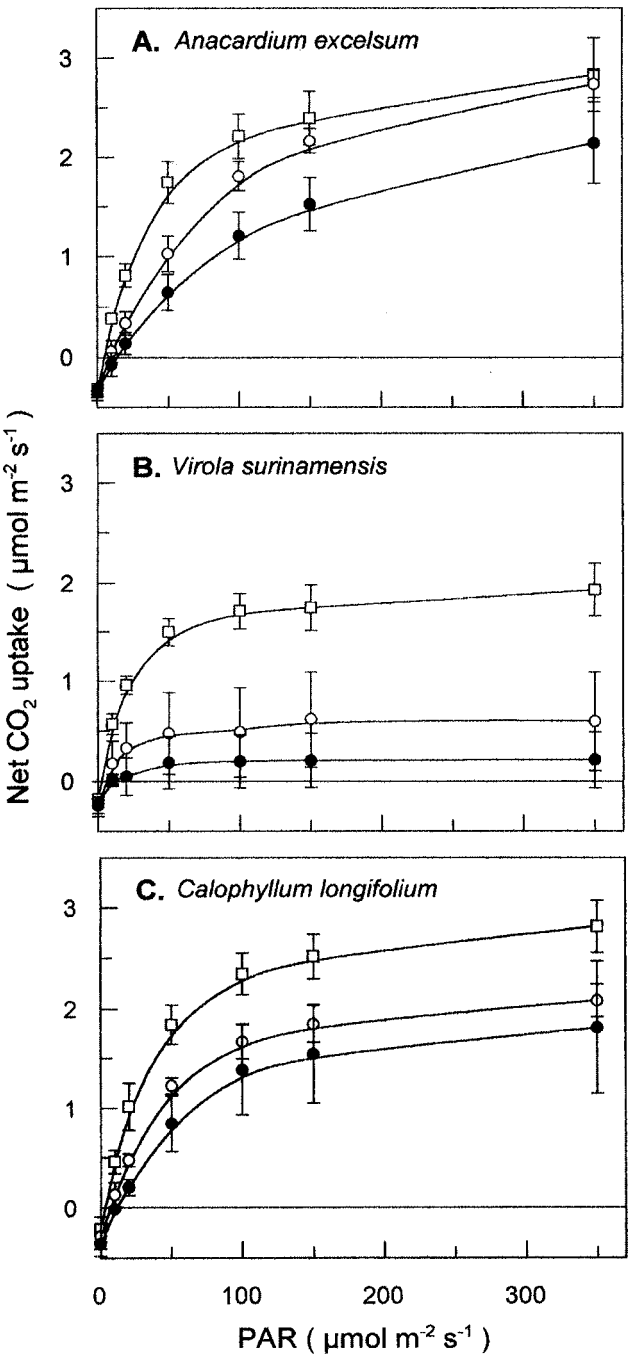

Figure 1. Effects of sun exposure of shade-acclimated seedlings of tropical trees on net CO_2 assimilation. Rates are depicted as a function of PAR. Leaves of one pioneer species (*Anacardium excelsum*) and two late successional species (*Virola surinamensis* and *Calophyllum longifolium*) were examined before (□) and after the plants were exposed for short periods to direct sunlight in the presence (●) and absence (○) of ambient UV-B radiation. Experimental conditions for A, B, and C, respectively, are exposure time, 34, 24, and 45 min; PAR dose, 3.0, 2.5, and 2.8 mol m^{-2}; and UV-B dose, 3.9, 3.1, and 4.0 kJ m^{-2}. Means and SD are presented; controls: n = 4 (A), n = 6 (B and C); sun-exposed plants, n = 3.

RESULTS AND DISCUSSION

CO_2 Assimilation in Comparison with PSII

When shade-acclimated leaves of tropical tree seedlings were exposed around midday for short periods to direct sunlight, subsequently measured net CO_2 assimilation rates were substantially reduced. Figure 1 shows data from typical experiments obtained prior to high light treatments (controls) and from the same leaves after exposure to direct sunlight

in the presence or absence of UV-B. In leaves of *A. excelsum* (Bertero and Balb.) Skeels (Fig. 1A), a significant UV-B effect on net CO_2 uptake can be seen at intermediate light intensities. Similar patterns were observed in leaves of *V. surinamensis* (Rol.) Warb. (Fig. 1B) and *C. longifolium* Willd. (Fig. 1C), although the UV-B effect was not significant. There was no marked difference in the overall response of CO_2 assimilation between *A. excelsum*, a pioneer tree, and the late successional species, *V. surinamensis* and *C. longifolium*. Seedlings of the three species are known to be shade tolerant and had been acclimated to the same shade conditions. In agreement with the literature (e.g. Bassman et al., 2001), no effect of sun exposure on rates of dark respiration was detectable for any of these species (Fig. 1, A–C).

A strong tendency to enhanced photoinhibition of CO_2 assimilation in the presence of solar UV-B was observed in all experiments conducted as part of the present investigation, although leaf responses varied widely. To evaluate all CO_2 assimilation and PSII measurements concurrently, data from individual leaves were ranked according to the degree of remaining activity determined after sun exposure in the presence and absence of UV-B. The resulting graphs (Figs. 2 and 3) clearly demonstrate the enhancing effect of ambient UV-B on photoinhibition of net CO_2 assimilation and of PSII. Figure 2 depicts CO_2 uptake rates from eight independent experiments (four with *V. surinamensis* and two each with *A. excelsum* and *C. longifolium*). The top panels show the control rates measured prior to sun exposure at 20 μmol m^{-2} s^{-1} PAR (Fig. 2A) and near-light saturation at 350 μmol m^{-2} s^{-1} (Fig. 2B). The bottom panels show the normalized rates after sun exposure (Fig. 2, C and D). In general, CO_2 uptake rates of leaves that had been shielded from UV-B were substantially greater than those from leaves exposed in the presence of ambient UV-B radiation. Only in the extreme case where solar visible plus UV-A light had lowered CO_2 uptake close to the compensation point, little additional UV-B effect was apparent (Fig. 2, C and D, right-hand parts).

The response of PSII to sun exposure in the shade-acclimated tree seedlings is shown in Figure 3. In the control leaves prior to treatments, maximum photochemical efficiency of PSII in the dark-adapted state (F_V/F_M) ratios were very uniform (see legend to Fig. 3), but varied strongly after sun exposure. Data were obtained from the same leaves (and one additional leaf of each plant) used for CO_2 uptake measurements (compare with Fig. 2). All data points from UV-B-shielded leaves indicated a higher potential PSII efficiency than in leaves exposed in the presence of UV-B (Fig. 3, A and B). The UV-B effect was most distinct when the degree of photoinhibition was intermediate. The comparison of F_V/F_M recorded 15 to 20 min (Fig. 3A) and about 5 h (Fig. 3B) subsequent to sun exposure indicated a greater UV-B effect after the

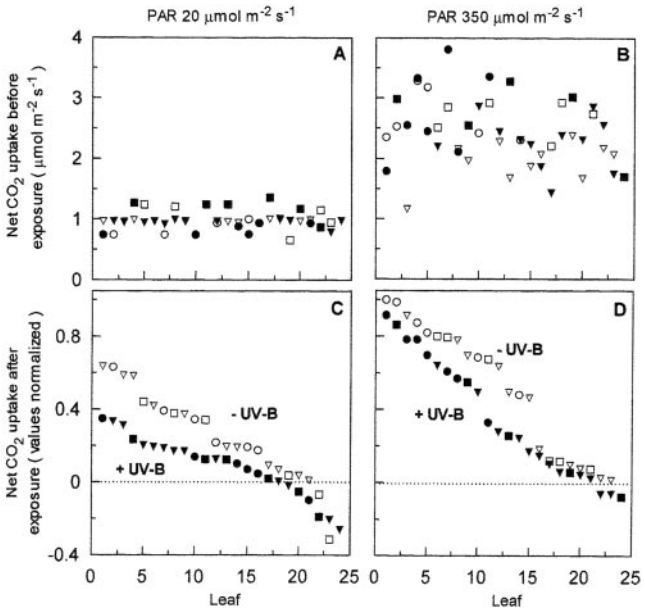

Figure 2. Net CO_2 uptake of shade-acclimated tree seedlings measured at 20 μmol m^{-2} s^{-1} (A and C) and 350 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR; B and D) before sun exposure (controls, A and B) and after exposure in the presence and absence of ambient UV-B radiation (C and D). Rates recorded subsequent to sun exposure have been normalized based on the respective control values. Negative values indicate rates below the compensation point. Data are from eight independent sun exposure experiments using seedlings from three tree species. The mean value obtained at 20 μmol m^{-2} s^{-1} PAR was significantly lower after sun exposure in the presence than in the absence of UV-B ($P < 0.01$). Data points representing individual leaves from different plants have been ordered according to increasing degree of inhibition of CO_2 assimilation caused by the preceding exposure to direct sunlight. For experimental details of sun exposure see "Materials and Methods." Circles, *A. excelsum*; triangles, *V. surinamensis*; squares, *C. longifolium*; open symbols, exposure in the absence of UV-B; closed symbols, exposure in the presence of UV-B.

5-h period in low light. This resulted from lack of recovery of potential PSII efficiency of the UV-B-exposed leaves, whereas after 5 h, onset of recovery was seen in leaves pre-exposed in the absence of UV-B. The fluorescence data confirm results of an earlier study (Krause et al., 1999). A comparison of Figures 2 and 3 shows that inhibition of net CO_2 assimilation did not correlate with the decline of F_V/F_M ratios. Measurement in limiting light (20 μmol photons m^{-2} s^{-1}) revealed the strongest UV-B effect on net CO_2 uptake in the data region where F_V/F_M was relatively mildly influenced by UV-B (compare left-hand parts of Fig. 2C and 3, A and B). This may be interpreted as a reduction in quantum yield of CO_2 assimilation (Φ_P), whereas electron transport activity is presumably only slightly inhibited. Moreover, the CO_2 data obtained under saturating light (Fig. 2D) exhibited a steeper decline than the F_V/F_M data (Fig. 3). Thus, in the presence and (to a lesser extent) in the absence of UV-B, a photoinhibition of CO_2 assimilation obviously occurred independently

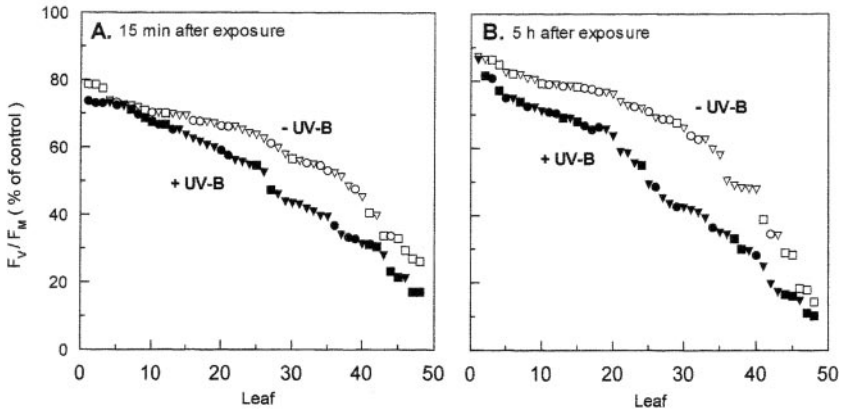

Figure 3. Potential PSII efficiency (F_V/F_M) in leaves of shade-acclimated tree seedlings after sun exposure in the presence and absence of ambient UV-B radiation. F_V/F_M ratios were recorded 15 to 20 min (A) and about 5 h (B) subsequent to sun exposure and are given in the percentage of the control values of the respective leaves determined prior to exposure. Open symbols, absence of UV-B; closed symbols, presence of UV-B. Symbols for plant species as for Figure 2. The data points were ranked from lowest to highest degree of PSII photoinhibition and represent 96 individual leaves from a total of 48 plants (two leaves per plant). Control values of F_V/F_M (means ± SD) were: *A. excelsum*, 0.805 ± 0.001 (n = 24); *V. surinamensis*, 0.779 ± 0.008 (n = 48); and *C. longifolium*, 0.799 ± 0.010 (n = 24). Mean values of F_V/F_M were significantly more reduced by sun exposure in the presence than in the absence of UV-B (15 min after exposure, $P < 0.05$; 5 h after exposure, $P < 0.01$).

of the partial inactivation of the electron transport system. These results were obtained when shade-acclimated leaves were exposed to full sunlight for only short periods (less than 1 h), and they demonstrate a direct impact of solar UV-B radiation on photosynthetic parameters. The only other investigation that shows an effect of solar UV-B on rates of net CO_2 assimilation per unit leaf area was that of Kolb et al. (2001). In that study with shade-acclimated grape leaves, the UV-B effect was seen only after several days of exposure to full sunlight, whereas in our case, the UV-B-induced reduction in CO_2 uptake was instantaneous.

The above conclusion is supported by the plots of Φ_P (obtained under strictly limiting light) versus F_V/F_M (Fig. 4). The data, recorded after the plants had been exposed to full solar radiation (Fig. 4A) or in the absence of UV-B (Fig. 4B), were expressed relative to control values measured prior to exposure. Slopes of calculated regression lines were steep and could not be extrapolated to the origin or to the pair of control values (100%). Correlation coefficients were low, particularly in the UV-B-exposed leaves (see legend to Fig. 4). In total, the Φ_P values depicted in Figure 4A (presence of UV-B) were significantly lower ($P < 0.05$) than in Figure 4B (minus UV-B). In the presence of UV-B, Φ_P was more severely inhibited than F_V/F_M ($P < 0.01$). This difference was also seen, but was less distinct ($P < 0.05$), in the absence of UV-B (see legend to Fig. 4). When rates of CO_2 uptake were plotted versus F_V/F_M, pictures similar to Figure 4 were obtained (data not shown). These results contrast with those of studies using artificial visible light, where a close empirical correlation between optimal quantum yield of photosynthesis and F_V/F_M (at room temperature) was observed, with regression lines extending to the origin (Leverenz and Öquist, 1987; Krause and Somersalo, 1989; Giersch and Krause, 1991; Krause and Weis, 1991). Figure 4 indicates a large increase in the degree of inhibition of CO_2 assimilation when only small changes in potential PSII efficiency occur.

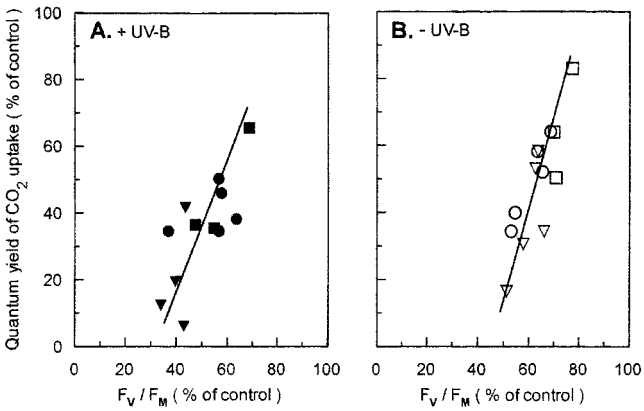

Figure 4. Quantum yield of CO_2 uptake (Φ_P) depicted as function of F_V/F_M. Data were obtained from shade-acclimated leaves of tree seedlings after short-term sun exposure in the presence (A) and absence (B) of ambient UV-B. Relative values of Φ_P were calculated from the slope of the linear part of the light-saturation curves of CO_2 exchange (compare with Fig. 1) and are presented in the percentage of the respective values determined prior to sun exposure. Symbols for plant species as for Figure 2. Control values of Φ_P (means ± SD) were: *A. excelsum*, 0.059 ± 0.005 (n = 4); *V. surinamensis*, 0.056 ± 0.006 (n = 6); and *C. longifolium*, 0.061 ± 0.009 (n = 8). Correlation coefficients (r^2) of the regression lines were: A, 0.55; B, 0.72. Means of data determined subsequent to sun exposure were: Φ_P = 35.1% ± 15.6%, F_V/F_M = 50.6% ± 10.7% (A); Φ_P = 49.4% ± 17.6%, F_V/F_M = 63.4% ± 7.6% (B). According to t tests, the difference between A and B was significant for Φ_P ($P < 0.01$) and F_V/F_M ($P < 0.01$). Means of Φ_P were significantly lower than means of F_V/F_M in A ($P < 0.01$) and B ($P < 0.05$).

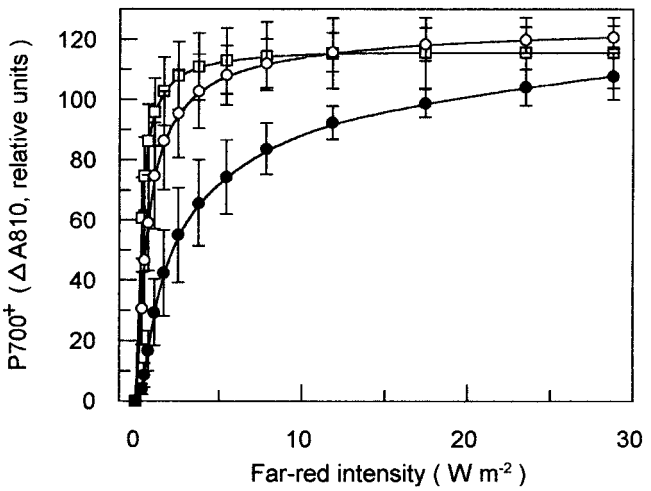

Figure 5. P700 oxidation as a function of far-red light intensity measured as absorbance change at 810 nm in shade-acclimated leaves of *A. excelsum* before and after exposure to direct sunlight. Exposure time, 18 min; PAR dose, 2.2 mol m^{-2}; UV-B dose, 2.6 kJ m^{-2}. Means and SD are given. □, control values prior to exposure ($n = 6$); ○, absence of UV-B ($n = 3$); ●, presence of ambient UV-B ($n = 3$).

The gas exchange analysis (measured transpiration rates and calculated intercellular CO_2 concentration) did not indicate increased stomatal limitation of CO_2 uptake in response to light stress (data not shown). Thus, the inhibition of CO_2 assimilation presumably was caused by restriction of enzymatic reactions in the chloroplasts. Damage to enzyme proteins such as Rubisco (see "Introduction") could result from active oxygen species formed under UV light (Hideg et al., 2000). The absence of stomatal limitation and loss of Rubisco was observed in greenhouse-grown oilseed rape (*Brassica napus*) exposed to high supplemental UV-B (Allen et al., 1997). Moreover, active oxygen species might affect the complex thiol-mediated light activation system of the Calvin cycle. Reductions in light activation could, to a lesser extent, also be caused by solar UV-A and even by visible light. Strong white light was reported to inhibit CO_2 assimilation in isolated intact chloroplasts, which was related to an accumulation of Fru-1.6-bisphosphate and a decrease in the ribulose-1.5-bisphosphate pool size (Giersch and Robinson, 1987). An inhibition of light activation of the Calvin cycle that is partly overcome by saturating light could explain why the UV-B effect on CO_2 assimilation was strongest when CO_2 uptake was measured in limiting light (Fig. 2C).

Response of PSI to Solar UV-B Radiation

Exposure of shade-grown leaves to natural sunlight led to a decrease in the efficiency of P700 photooxidation (Figs. 5 and 6), similar to results from previous experiments with artificial white light (Barth et al., 2001). The alteration in the efficiency of P700 oxidation was strongly enhanced by ambient UV-B radiation. Figure 5 demonstrates the dependence of P700 oxidation on the intensity of far-red light for an experiment with seedlings of *A. excelsum.* Exposure of leaves to direct sunlight altered the slopes of the light-response curves, indicating a reduction in the efficiency of P700 oxidation, which was substantially more prominent after sun exposure in the presence than in the absence of ambient UV-B.

In Figure 6, the sunlight-induced decrease in the efficiency of P700 oxidation under far-red light is expressed as an increase in the saturation constant, K_S, i.e. the far-red intensity required for half-maximal P700 absorbance change in the steady state (compare with Barth et al., 2001). The data were obtained from eight experiments in which the response of CO_2 assimilation and PSII were also investigated (compare with Figs. 2–4). The relative increases in the values of K_S (with reference to control measurements prior to sun exposure) have been arranged from lowest to highest. The resulting graph (Fig. 6) indicates a much stronger increase in K_S induced by exposure to full sunlight than in the absence of UV-B. The UV-B effect is strongest in the region where a substantial increase in K_S is seen also in leaves exposed to visible plus

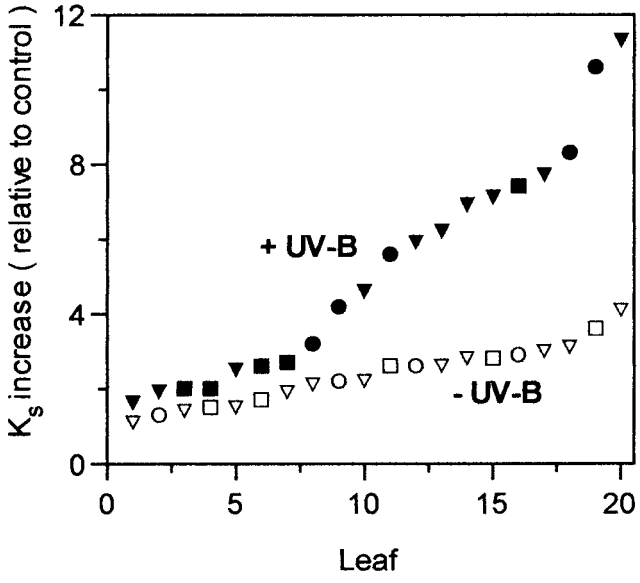

Figure 6. Increase of K_S of P700 oxidation under far-red light caused by exposure of shade-grown tree seedlings to direct sunlight in the presence (closed symbols) and absence (open symbols) of ambient UV-B radiation. The relative increase in K_S is expressed as the ratio of K_S values determined after sun exposure to the corresponding control values prior to exposure. Ratios obtained from individual leaves were ranked from low to high values. Four pairs of extremely high K_S values ranging from 14- to 51-fold (presence of UV-B) and 6.5- to 11-fold increase (absence of UV-B) have been omitted. Symbols for plant species: circles, *A. excelsum*; triangles, *V. surinamensis*; and squares, *C. longifolium*. Control K_S values (determined prior to sun exposure) were (W m^{-2}): *A. excelsum*, 0.3 ± 0.1 ($n = 12$); *V. surinamensis*, 0.4 ± 0.2 ($n = 24$); and *C. longifolium*, 0.4 ± 0.1 ($n = 6$). The mean value of K_S increase was significantly higher after sun exposure in the presence than in the absence of UV-B ($P < 0.01$).

UV-A light (right-hand part of Fig. 6). Highest K_S values (not shown) reached a nearly 50-fold increase in the presence, and an 11-fold increase in the absence, of ambient UV-B. There was a negative correlation between K_S values and F_V/F_M, i.e. K_S tended to increase when the potential PSII efficiency decreased due to light stress (data not shown), but recovery studies (Barth et al., 2001) indicated that changes in K_S and F_V/F_M occur independently.

As in the laboratory study by Barth et al. (2001), exposure to sunlight did not significantly affect the photochemical capacity of PSI represented by the maximum P700 absorbance change, $\Delta A_{810\ max}$ (Fig. 5). Thus, the increase in K_S does not result from a population of fully inactive PSI, but might be related to changes in the function of the FeS centers that delay electron transfer to ferredoxin. Such effect may facilitate recombination of radical pairs and, thereby, dissipation of excessive photon energy (for discussion, see Barth et al., 2001). The FeS centers of PSI are known to be the primary targets of PSI photoinhibition (Sonoike et al., 1995; Tjus et al., 1999). Charge recombination in the PSI reaction center has been reviewed by Brettel (1997) and studied by Teicher et al. (2000) in thylakoid membranes from barley (*Hordeum vulgare*) leaves subjected to illumination at chilling temperatures. Recombination in PSI was also suggested to occur in leaves of cucumber (*Cucumis sativus*) in response to chilling under low visible light (Kim et al., 2001). Enhanced recombination manifested by an increase in K_S, as observed here, may indicate an incipient inactivation of PSI that prevents severe damage to the reaction center.

The present investigation shows that exposure of shade-acclimated leaves to direct sunlight, simulating the opening of a tree-fall gap in the tropical forest, strongly affects quantum yield and light-saturated rate of net CO_2 assimilation. It is apparent that this occurs independently of a simultaneous photoinhibition of PSII. The solar UV-B component of sunlight contributes substantially to these effects. Moreover, solar UV-B promotes a decrease in the efficiency of P700 oxidation by far-red light that might be caused by accelerated charge recombination in the PSI reaction center and probably represents a photoprotective response.

Our data on short-term responses to high solar UV-B radiation do not contradict previous conclusions by Searles et al. (2001) that, on average, photosynthesis is not significantly affected in terrestrial plants acclimated to elevated levels of UV-B, which simulate a realistic stratospheric ozone depletion. However, when drastic changes in light conditions occur, e.g. upon formation of a gap in the forest as simulated in the present study, a UV-B dosage not previously experienced by the leaves evidently does affect the photosynthetic apparatus.

The changes in photosynthetic activities reported here were slowly reversible. Daily sun exposure with photon dosages similar (or higher) to those applied here led to an acclimative adjustment of photosynthetic pigment composition and to an increase in the level of UV-absorbing compounds in seedlings of the three species studied (Krause et al., 1999, and G.H. Krause, E. Grube, O.Y. Koroleva, and K. Winter, unpublished data).

MATERIALS AND METHODS

Experiments were performed at the Tupper Center, Smithsonian Tropical Research Institute, in Panama City (9°N, 49°W).

Plant Material

Seedlings of *Anacardium excelsum* (Bertero and Balb.) Skeels (Anacardiaceae), *Virola surinamensis* (Rol.) Warb. (Myristicaceae), and *Calophyllum longifolium* Willd. (Clusiaceae; nomenclature according to Croat, 1978) were cultivated in a shaded greenhouse (neutral shade, PAR 10–60 μmol m^{-2} s^{-1}) in pots (40 cm high, 15 × 15-cm wide) filled with soil without fertilizer. At the time of the experiments, the seedlings were 6 to 9 months old, 20 to 30 cm tall, and possessed three to nine leaves. Measurements were taken on the youngest mature leaves. *A. excelsum* is a pioneer tree, whereas *V. surinamensis* and *C. longifolium* are late-successional trees of tropical forests in Central and South America. Seedlings of all three species are shade tolerant. Contents of photosynthetic pigments (determined with HPLC) and soluble UV-B-absorbing compounds (extracted with ethanol/water mixtures) of *A. excelsum* and *C. longifolium* did not show significant differences between leaves from the shaded greenhouse and from the shaded understory of natural forests in Central Panama (G.H. Krause, E. Grube, O.Y. Koroleva, and K. Winter, unpublished data). Understory seedlings of *V. surinamensis* were not investigated.

Sun Exposure

Plants were exposed to direct sunlight for 20 to 50 min on days of low cloudiness between 11:00 AM and 1:30 PM, local time, simulating the conditions in a typical forest gap caused by the fall of a single tree. Exposure times were varied depending on the degree of sunlight fluctuations caused by clouds. Three plants were placed under a frame covered with 0.13-mm polyester plastic film (Mylar D; DuPont, Wilmington, DE) that excluded most UV-B but had a high transmission for UV-A and visible light. Three additional plants were placed under 0.08-mm Aclar film (22A; Allied Signal, Pottsville, PA) with high transmittance of UV-B, UV-A (320–400 nm), and PAR (400–700 nm). For details of the optical properties of these plastic films, see Krause et al. (1999). The plastic covers had slits for air circulation, which was enhanced by fans. The pots were shielded from the sun with aluminum foil.

PAR was measured with a quantum sensor (LI 189 B; LI-COR, Lincoln, NE) and UV-B was measured with a radiometer (IL 1400 A; International Light, Newburyport, MA). Measured integrated PAR doses of exposure varied from 2.2 to 3.6 mol m^{-2} and UV-B doses under Aclar film from 2.6 to 4.8 kJ m^{-2}. The UV-B sensor has a fixed wavelength sensitivity (with a tail reaching in the UV-A region); it provided an approximate measure of UV-B energy. Weighted biologically effective UV-B radiation was not determined. Leaf temperatures were measured on the lower leaf surface with a leaf clip holder (2030-B) of a fluorometer system (PAM 2000; Walz, Effeltrich, Germany). In eight experiments, leaf temperatures (mean ± SD) in full sun were 40°C ± 2°C and 39°C ± 3°C in the presence and the absence of UV-B, respectively. Corresponding air temperature under the plastic films was 33°C ± 2°C, and ambient air temperature was 32°C ± 2°C. Subsequent to sun exposure, the plants were returned to the shaded greenhouse until the measurements of photosynthetic activities (below) were performed.

CO_2 Gas Exchange

About 1 to 2 h before and 1 to 3 h after sun exposure of plants, CO_2 gas exchange was measured on one leaf per plant using a portable photosynthesis system (LI-6400; LI-COR) equipped with a light source (6400–02B LED; LI-COR). In addition, transpiration was recorded. Leaf temperature in

the chamber was maintained at 28°C. Prior to measurements, plants were preilluminated in a controlled-environment chamber (EGC, Chagrin Falls, OH) for 30 min at about 150 μmol m^{-2} s^{-1} PAR and at an air temperature of 24°C. Air flow through the photosynthesis cuvette was 250 μmol s^{-1}; ambient air containing 360 to 380 μL L^{-1} CO_2 was used.

Prior to sun exposure, net CO_2 uptake at 350 μmol m^{-2} s^{-1} PAR and dark respiration were recorded for 6 min each on four plants. Light saturation curves of CO_2 assimilation were determined on two plants using the following protocol: 6 min at 350 μmol m^{-2} s^{-1}; 2 min at 150, 100, 50, 20, and 10 μmol m^{-2} s^{-1}; and 6 min in the dark. The rates obtained served as control values. Subsequent to sun exposure, light-saturation curves were determined for all six plants.

Φ_P was calculated from the initial slopes of the light saturation curves; only curves were considered that exhibited at low actinic light an approximately linear phase with high regression coefficient ($r^2 > 0.9$). In particular, in strongly inhibited leaves (e.g. see Fig. 1B), such a linear phase was absent, and an initial slope could not be determined.

Chl *a* Fluorescence

F_V/F_M served as a measure of potential efficiency of PSII (see Giersch and Krause, 1991; Krause and Weis, 1991). The F_V/F_M ratio was determined with a chlorophyll fluorometer (PAM 2000; Walz, Effeltrich, Germany) equipped with a portable personal computer (Poquet Computer, Santa Clara, CA) and data acquisition software (DA-2000; Walz). Measurements were performed on the same leaves used for gas exchange measurements and on an additional leaf of each plant of similar orientation and age. Control measurements were done about 1 h prior to sun exposure, after 10 min of dark adaptation. After the end of sun exposure, the leaves were dark adapted for 15 min, and F_V/F_M was measured again. An additional measurement was performed about 5 h after exposure (after a 10-min dark adaptation). Immediately before F_V/F_M recording, weak far-red light from a diode of the PAM system was applied for 3 s to fully oxidize intersystem electron carriers for exact determination of initial fluorescence, F_O. The decrease in the "dark-adapted" F_V/F_M ratio caused by sun exposure indicates the degree of PSII photoinhibition (Giersch and Krause, 1991). For further details of the measuring protocol, see Barth and Krause (1999).

Photooxidation of P700

Photochemical activity of the PSI reaction center was assessed as described by Barth et al. (2001), by means of P700 absorbance changes at 810 nm (Klughammer and Schreiber, 1998) using a dual-wavelength emitter-detector unit (ED-P700DW; Walz) connected to a fluorometer system (PAM 101/102/103; Walz). Far-red light to photooxidize P700 was supplied by a diode with maximum emission at 735 nm (model 102-FR; Walz) controlled by the PAM 102, supplemented by a lamp (KL-150; Schott, Mainz, Germany) with optical filters RG9 (Schott) and Calflex C (Balzers, Liechtenstein; maximum intensity at 738 nm). Leaves were preilluminated for 5 min with white light (about 170 μmol m^{-2} s^{-1}) from a lamp (KL-1500; Schott). Relative absorbance changes were subsequently recorded in the steady state as a function of far-red intensity. From the data, a "saturation constant" (K_S), defined as the far-red flux at which one-half of the maximum absorbance change ($\Delta A810_{max/2}$) in the steady state is reached (Barth et al., 2001), was calculated. The maximum absorbance change ($\Delta A810_{max}$) served as a relative measure of the photochemical capacity of PSI.

Measurements were performed 1 to 3 h before and 2 to 4 h after sun exposure of plants (subsequent to recording of gas exchange) with leaf discs (diameter of 1.45 cm) placed into a temperature-controlled cuvette (24°C) and ventilated with a moistened air stream. The changes in PSI activity induced by sun exposure did not show any tendency of reversal during the measuring period.

Statistics

To assess the significance of UV-B effects on photosynthetic parameters, the data from eight experiments with three species (sun-exposed in the presence and absence of UV-B, respectively) were averaged, and *t* tests were made. Probabilities of error (*P*) are given in the legends of Figures 2 through 4 and 6. In Figures 1 and 5, typical experiments with three plants each are depicted; for reasons of clarity, means and SD are presented instead of raw data.

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LITERATURE CITED

Allen DJ, McKee IF, Farage PK, Baker NR (1997) Analysis of limitations to CO_2 assimilation on exposure of leaves of two *Brassica napus* cultivars to UV-B. Plant Cell Environ **20:** 633–640

Allen DJ, Nogués S, Baker NR (1998) Ozone depletion and increased UV-B radiation: Is there a real threat to photosynthesis? J Exp Bot **49:** 1775–1788

Ballaré CL, Rousseaux MC, Searles PS, Zaller JG, Giordano CV, Robson TM, Caldwell MM, Sala OE, Scopel AL (2001) Impacts of ultraviolet-B radiation on terrestrial ecosystems of Tierra del Fuego (southern Argentina): an overview of recent progress. J Photochem Photobiol B-Biol **62:** 67–77

Barth C, Krause GH (1999) Inhibition of photosystem I and II in chilling-sensitive and chilling-tolerant plants under light and low-temperature stress. Z Naturforsch **54c:** 645–657

Barth C, Krause GH, Winter K (2001) Responses of photosystem I compared with photosystem II to high-light stress in tropical shade and sun leaves. Plant Cell Environ **24:** 163–176

Bassman JH, Edwards GE, Roberrecht R (2002) Long-term exposure to enhanced UV-B radiation is not detrimental to growth and photosynthesis in Douglas fir. New Phytol **154:** 107–120

Bassman JH, Roberrecht R, Edwards GE (2001) Effects of enhanced UV-B radiation on growth and gas exchange in *Populus deltoides* Bartr. ex Marsh. Int J Plant Sci **162:** 103–110

Brettel K (1997) Electron transfer and arrangement of the redox cofactors in photosystem I. Biochim Biophys Acta **1318:** 322–373

Buma AGJ, Helbling EW, de Boer MK, Villafane VE (2001) Patterns of DNA damage and photoinhibition in temperate South Atlantic picophytoplankton exposed to solar ultraviolet radiation. J Photochem Photobiol B-Biol **62:** 9–18

Croat TB (1978) Flora of Barro Colorado Island. Stanford University Press, Stanford, CA

Figueroa FL, Gómez I (2001) Photosynthetic acclimation to solar UV radiation or marine red algae from warm-temperature coast of southern Spain: a review. J Applied Phycol **13:** 233–245

Giersch C, Krause GH (1991) A simple model relating photoinhibitory fluorescence quenching in chloroplasts to a population of altered photosystem II reaction centers. Photosynth Res **30:** 115–121

Giersch C, Robinson SP (1987) Effects of photoinhibition on photosynthetic carbon metabolism in intact isolated spinach chloroplasts. Aust J Plant Physiol **14:** 439–449

Häder DP, Porst M, Lebert M (2001) Photoinhibition in common Atlantic macroalgae measured on site in Gran Canaria. Helgol Mar Res **55:** 67–76

Herrmann H, Häder D-P, Ghetti F (1997) Inhibition of photosynthesis by solar radiation in *Dunaliella salina*: relative efficiencies of UV-B, UV-A and PAR. Plant Cell Environ **20:** 359–365

Hideg É, Kalai T, Hideg K, Vass I (2000) Do oxidative stress conditions impairing photosynthesis in the light manifest as photoinhibition? Phil Trans R Soc Lond **B 355:** 1511–1516

Huang L-K, Chow WS, Whitecross MI, Anderson JM (1993) Responses of detached rice leaves (*Oryza sativa* L.) to moderate supplementary ultraviolet-B radiation allow early screening for relative sensitivity to ultraviolet-B irradiation. Aust J Plant Physiol **20:** 285–297

Keiller DR, Holmes MG (2001) Effects of long-term exposure to elevated UV-B radiation on the photosynthetic performance of five broad-leaved tree species. Photosynth Res **67:** 229–240

Kim S-J, Lee C-H, Hope AB, Chow WS (2001) Inhibition of photosystems I and II and enhanced back flow of photosystem I electrons in cucumber leaf discs chilled in the light. Plant Cell Physiol **42:** 842–848

Klughammer C, Schreiber U (1998) Measuring P700 absorbance changes in the near infrared spectral region with a dual wavelength pulse modula-

tion system. *In* G Garab, ed, Photosynthesis: Mechanisms and Effects, Vol 5, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 4357–4360

Kolb CA, Käser MA, Kopecky J, Zotz G, Riederer M, Pfündel EE (2001) Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grapevine leaves (*Vitis vinifera* cv. Silvaner). Plant Physiol **127:** 863–875

Krause GH, Garden H, Schmude C, Koroleva OY, Winter K (1998) Contribution of ambient UV light to photoinhibition of photosystem II in tropical plants. *In* G Garab, ed, Photosynthesis: Mechanisms and Effects, Vol 3. Kluwer Academic Publisjers, Dordrecht, The Netherlands, pp 2409–2412

Krause GH, Koroleva OY, Dalling JW, Winter K (2001) Acclimation of tropical tree seedlings to excessive light in simulated tree-fall gaps. Plant Cell Environ **24:** 1345–1352

Krause GH, Schmude C, Garden H, Koroleva OY, Winter K (1999) Effects of solar ultraviolet radiation on the potential efficiency of photosystem II in leaves of tropical plants. Plant Physiol **121:** 1349–1358

Krause GH, Somersalo S (1989) Fluorescence as a tool in photosynthesis research: application in studies of photoinhibition, cold acclimation and freezing stress. Phil Trans R Soc Lond B **323:** 281–293

Krause GH, Weis E (1991) Chlorophyll fluorescence: the basics. Annu Rev Plant Physiol Plant Mol Biol **42:** 313–349

Leverenz JW, Öquist G (1987) Quantum yields of photosynthesis at temperatures between 2°C and 35°C in a cold-tolerant C_3 plant (*Pinus sylvestris*) during the course of one year. Plant Cell Environ **10:** 287–295

Lud D, Huiskes AHL, Moerdijk TCW, Rozema J (2001) The effects of altered levels of UV-B radiation on an Antarctic grass and lichen. Plant Ecol **154:** 89–99

Madronich S, McKenzie RL, Caldwell MM, Björn LO (1995) Changes in ultraviolet radiation reaching the earth's surface. Ambio **24:** 143–152

Nogués S, Baker NR (1995) Evaluation of the role of damage to photosystem II in the inhibition of CO_2 assimilation in pea leaves on exposure to UV-B radiation. Plant Cell Environ **18:** 781–787

Nogués S, Baker NR (2000) Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B. J Exp Bot **51:** 1309–1317

Rao MV, Paliyath G, Ormrod DP (1995) Differential response of photosynthetic pigments, Rubisco activity and Rubisco protein of *Arabidopsis thaliana* exposed to UVB and ozone. Photochem Photobiol **62:** 727–735

Searles PS, Flint SD, Caldwell MM (2001) A meta-analysis of plant field studies simulating stratospheric ozone depletion. Oecologia **127:** 1–10

Sonoike K, Terashima I, Iwaki M, Itoh S (1995) Destruction of photosystem I iron-sulfur centers in leaves of *Cucumis sativus* L. by weak illumination at chilling temperatures. FEBS Lett **362:** 235–238

Strid A, Chow WS, Anderson JM (1990) Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum.* Biochim Biophys Acta **1020:** 260–268

Takeuchi A, Yamaguchi T, Hidema J, Strid A, Kumagai T (2002) Changes in synthesis and degradation of Rubisco and LHCII with leaf age in rice (*Oriza sativa* L.) growing under supplementary UV-B radiation. Plant Call Environ **25:** 695–706

Teicher HB, Møller BL, Scheller HV (2000) Photoinhibition of photosystem I in field-grown barley (*Hordeum vulgare* L.): induction, recovery and acclimation. Photosynth Res **64:** 53–61

Teramura AH, Ziska LH (1996) Ultraviolet-B radiation and photosynthesis. *In* NR Baker, ed, Photosynthesis and the Environment, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 435–450

Tjus SE, Møller BL, Scheller HV (1999) Photoinhibition of photosystem I damages both reaction centre proteins PSI-A and PSI-B and acceptor-side located small photosystem I polypeptides. Photosynth Res **60:** 75–86

Xiong FS, Day TA (2001) Effect of solar ultraviolet-B radiation during springtime ozone depletion on photosynthesis and biomass production of Antarctic vascular plants. Plant Physiol **125:** 738–751

Ziska LH (1996) The potential sensitivity of tropical plants to increased ultraviolet-B radiation. J Plant Physiol **148:** 35–41