CSIRO PUBLISHING
www.publish.csiro.au/journals/fpb *Functional Plant Biology*, 2003, **30**, 533–542

Capacity of protection against ultraviolet radiation in sun and shade leaves of tropical forest plants

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Abstract. Protection of leaves of tropical forest plants against UV-A and -B radiation was studied in three lowland forests, a montane cloud forest and a mangrove stand in Panama. Leaves were classified as sun or shade leaves according to their chlorophyll *a*/*b* ratio, pool size of xanthophyll cycle pigments and α- and β-carotene contents. The capacity of the leaves for protection against UV radiation was assessed by estimating epidermal UV-A shielding, by a non-invasive fluorometric method, and by the absorbance of ethanolic/aqueous leaf extracts in the UV spectral region. In all sun leaves tested, UV-A shielding by the adaxial epidermis was high, usually above 90%, whereas in shade leaves the epidermal UV-A shielding was markedly lower and varied widely between species. In most cases UV-A shielding by the abaxial epidermis was lower than by the adaxial epidermis. UV absorbance of the leaf extracts was generally higher in sun than in shade leaves, and the absorbance was much higher in the UV-B spectral region at 305 nm than in the UV-A region at 375 nm. The data demonstrate that sun leaves of tropical plants are well protected against solar UV-A and UV-B radiation. However, UV-induced damage may occur when shade leaves become exposed to full solar radiation.

Keywords: carotenoids; chlorophyll *a* and *b*; epidermal UV shielding; UV-A PAM system; UV-absorbing compounds.

Introduction

The anthropogenic decline of the stratospheric ozone (O_3) layer at northern and southern latitudes has stimulated the investigation of detrimental effects of elevated ultraviolet-B (UV-B) radiation on plants (see e.g. Searles *et al.* 2001). A significant reduction of the O_3 layer accompanied by elevated UV-B has not been observed for the tropics (Bojkov and Fioletov 1996). But even in the absence of anthropogenic effects, tropical plants are already exposed to relatively high levels of UV-B radiation because of the larger angle of midday solar radiation and a generally thinner O_3 layer in the tropics than at higher latitudes (Caldwell *et al.* 1989). UV-B (280–320 nm), the most biologically effective UV radiation, is generally at the center of research efforts. Recent evidence indicates that solar UV-A (320–400 nm), which is not absorbed by the O_3 layer, may also adversely affect plants owing to its much higher total energy (Krause *et al.* 1999; Turksanyi and Vass 2000; White and Jahnke 2002; Pfündel 2003). To predict possible future impacts of elevated UV-B on plants, it is helpful to assess potentially detrimental effects of the current levels of solar UV radiation in the tropics, as well as the capacity for protection in tropical plants.

In previous studies, solar UV-B contributed markedly to photoinhibition of PSII in shade-acclimated tropical tree seedlings exposed to full sunlight for short periods. No negative effects on PSII were observed in sun-acclimated outer canopy leaves of tree crowns, except for certain very young and very old, senescent leaves (Krause *et al.* 1999). Sudden exposure of shade leaves to full solar UV-B also decreased quantum yield and capacity of CO_2 assimilation and the photochemical efficiency of PSI (Krause *et al.* 2003). At higher latitude, inhibition of CO_2 assimilation by solar UV-B was also observed in shade-grown leaves of *Vitis vinifera* (Kolb *et al.* 2001).

Protection of plant leaves against solar UV-B and UV-A radiation is achieved predominantly by UV-absorbing

Abbreviations used: Car, carotene; Chl, chlorophyll; Lut, lutein; Neo, neoxanthin; VxAxZx, sum of xanthophyll cycle pigments, viola-, anthera- and zeaxanthin.

 10.1071/FP03047 1445-4408/03/050533

phenolic compounds such as flavonoids and derivatives of hydroxycinnamic acid (see e.g. Burchard *et al.* 2000; Mazza *et al.* 2000; Kolb *et al.* 2001). In numerous field studies, an increase in UV-B-absorbing compounds was the most conspicuous response of plant leaves to artificially elevated UV-B levels simulating a thinning of the ozone layer (Searles *et al.* 2001). UV-absorbing substances are particularly protective when they are accumulated in the adaxial epidermis of leaves (Markstädter *et al.* 2001). Recently, a device ('UV-A PAM') has become available that allows estimation of the fractional absorptance by the leaf epidermis at 375 nm ('epidermal UV-A shielding') in a non-invasive fluorometric manner (Bilger *et al.* 2001). Spectroscopic measurement of UV absorbance of ethanolic/aqueous (or methanolic/aqueous) leaf extracts may serve as another method to assess UV protection (Searles *et al.* 1995; Krause *et al.* 1999, 2001; Mazza *et al.* 2000; Cerovic *et al.* 2002).

In the present study, leaves in different states of light acclimation from numerous species growing in forests near Panama City were screened for UV protection. We compared sun and shade leaves of tree crowns and shade leaves of plants in the forest understory, and assessed UV-A shielding of the adaxial and abaxial epidermis *in vivo* and the absorbance of leaf extracts in the UV-B and UV-A spectral regions The degree of acclimation of the leaves to sun and shade conditions was characterized by analysis of photosynthetic pigments.

Materials and methods

The study was carried out in Panama at the end of the rainy season, during November–December 2001. Analyses of photosynthetic pigments and of extracts of UV-absorbing compounds were performed at the Institute of Plant Biochemistry, Düsseldorf, Germany.

Plant material

Table 1 lists the species studied (nomenclature according to Missouri Botanical Garden's VAST nomenclature database), their life forms and study sites. Sun and shade leaves from crowns of large trees as well as shade leaves from plants growing in the deeply shaded forest understory were analysed as specified in Tables 2 and 3. One species growing at the edge of a tree-fall gap was included. In the absence of cloud cover, midday photosynthetically active radiation was approximately 2000–2300 μmol photons m^{-2} s^{-1} for sun leaves, 200–400 μmol m^{-2} s^{-1} for shade leaves of tree crowns and 20–30 μmol m^{-2} s^{-1} for leaves in the understory. In the tree-fall gap, leaves experienced about 1500–1800 μmol m^{-2} s^{-1} for 1–2 h.

Sites were: (1) Parque Natural Metropolitano (8°58′ N, 79°33′ W), close to Panama City, a seasonally dry, semi-deciduous tropical lowland forest with mostly pioneer species; (2) Fort Sherman (9°17′ N, 79°58′ W), a moist primary lowland forest on the Caribbean slope close to the city of Colon; (3) Cerro Jefe (9°14′ N, 79°23′ W), a montane cloud forest northeast of Panama City, about 1000 m above sea level (see description by Pierce *et al.* 2002); (4) Isla Barro Colorado (9°10′ N, 79°51′ W) in the Panama Canal area (Lago Gatun), a moist, largely secondary lowland forest; (5) Isla Taboga (8°48′ N, 79°33′ W) in the Bay of Panama, where a mangrove species was studied. Leaves high in the forest canopy were accessible from construction cranes located at sites 1 and 2.

Fluorometric assessment of apparent UV-A shielding by leaf epidermis

Apparent UV-A shielding by the leaf epidermis was assessed with a portable UV-A PAM Chlorophyll Fluorometer (Gademann Instruments, Würzburg, Germany, for instrument description see www.gademann.de). This device employs rapidly alternating pulse-modulated UV-A (375 nm) and blue (470 nm) light for quasi-simultaneous measurement of UV-A and blue-excited Chl fluorescence at wavelengths above 650 nm. Both UV-A and blue light can excite Chl fluorescence with high quantum yield in the mesophyll, provided there is no absorption by the epidermis. Accumulation of UV-absorbing substances in the epidermis will lower UV-A-excited fluorescence (F_{375}) relative to blue-excited fluorescence (F_{470}). Basic principles and previous applications of this non-invasive fluorometric method have been described by several authors (Bilger *et al.* 1997, 2001; Barnes *et al.* 2000; Burchard *et al.* 2000; Kolb *et al.* 2001; Markstädter *et al.* 2001; Ounis *et al.* 2001; Pfündel 2003). The UV-A PAM is particularly well suited for field studies, as it is equipped with a flexible UV-transmitting liquid light guide (5 mm active cross-section), at the end of which, a light-weight leaf clip is mounted.

The measuring light intensity of the UV-A PAM is sufficiently weak not to induce variable fluorescence in dark-adapted samples and, hence, measured fluorescence corresponds to the initial, minimal fluorescence yield, F_o. The instrument provides readings of F_{375} and F_{470}, corresponding to the F_o values measured upon UV-A and blue excitation, respectively. The intensity of the blue beam can be adjusted such that $F_{375} = F_{470}$ with a sample known to display no UV protection. Together with the instrument, a blue, plastic fluorescence standard that emits red fluorescence is provided (Filter no. 358 Rose Indigo, Roscolux, Hollywood, CA). This standard was calibrated against a shade leaf of *Vicia faba* (abaxial side), the epidermis of which was removed. For this leaf, the ratio F_{375}/F_{470}, which is calculated by the instrument, was adjusted to 1 by appropriate trimming of the blue LED intensity (blue adjustment). After this adjustment the fluorescence standard showed a ratio $F_{375}/F_{470} = 0.935$. This ratio measured with the fluorescence standard was kept constant throughout the present series of measurements by appropriate blue adjustment. Theoretically, the parameter F_{375}/F_{470} can vary between 0 and 1, with 0 indicating complete UV-A absorption (maximal shielding) and 1 indicating zero UV-A absorption (no UV-A shielding). On the basis of the ratio F_{375}/F_{470}, the parameter, $100 \times (1 - F_{375}/F_{470})$ (%), reflecting the fractional absorptance (1 minus transmittance) of UV-A by the epidermis is designated as apparent 'epidermal UV-A shielding'. This parameter, which is automatically calculated by the instrument, will be applied in the present study to estimate the UV-A shielding (in per cent) by the epidermis.

Epidermis-free mesophyll of the species studied was not available. As the UV-absorbing properties of the mesophyll might vary between and within species, the values of UV-A shielding obtained in the present study should be viewed as approximations (see discussion by Barnes *et al.* 2000; Bilger *et al.* 2001; Markstädter *et al.* 2001). In leaves with high contents of UV-absorbing compounds, UV absorption by the mesophyll cells may contribute to the measured signal of UV-A shielding.

The shielding signal was recorded either *in situ* or on detached leaves stored in moistened plastic bags in dim light for up to 3 h after collection. Measurements were taken on the adaxial (upper) and, subsequently, on the abaxial (lower) epidermis of the leaves studied. The leaf clip of the instrument served for dark adaptation of the sample to attain the F_o state of fluorescence emission. Recordings were made when, after several seconds of dark adaptation, the instrument exhibited a stable signal.

Assessment of soluble UV-absorbing compounds

After recording UV-A shielding, leaf disks (diameter 1 cm) were sampled, frozen in liquid nitrogen and stored at –70 to –80°C or on dry ice. UV-absorbing compounds were extracted with ethanol/water, as described by Krause *et al.* (2001). The extract was centrifuged at 20400 *g* (Microcentrifuge 5415 C, Eppendorf, Hamburg, Germany). UV absorbance spectra of the extracts were recorded. Absorbance at 305 nm and 375 nm served as a relative measure of soluble compounds absorbing in the UV-B and UV-A spectral region, respectively. Values of absorbance (i.e. the logarithm of the reciprocal transmittance) were recalculated on a leaf-area basis, considering the dilution of extracts. Data presented correspond to the UV absorbance of a 1-mL extract from a 1-cm^2 leaf section, measured with a 1-cm light path (*cf.* Cerovic *et al.* 2002), thus, approximating the absorbance of UV radiation *in vivo*.

Analysis of photosynthetic pigments

Disks 1 cm in diameter from leaves on which UV-A shielding was recorded, were frozen (see above). For analysis of chlorophylls and carotenoids, one disk was ground in liquid nitrogen in a mortar and extracted twice with 1 mL acetone. The extract was centrifuged (20400 *g*), adjusted to a final volume of 2 mL and filtered through a membrane filter Anatop 10 (Merck, Darmstadt, Germany). Pigments were separated according to a modified method described by Färber *et al.* (1997) using a HPLC system from Hitachi/Merck (Darmstadt, Germany) with solvent degasser Gastorr 104 (Schambeck, Bad Honnef, Germany) and injection valve 7725i (Cotati, CA, USA). The sample volume analysed was 20 µl. Bands of the HPLC spectrum were identified and band areas calibrated quantitatively with pigment standards of neo-, viola-, anthera- and zeaxanthin, lutein, α- and

Table 1. List of study sites and species

Site and species	Family	Life form
Parque Natural Metropolitano		
Anacardium excelsum (Bertero & Balb. ex Kunth) Skeels	Anacardiaceae	tree
Pseudobombax septenatum (Jacq.) Dugand	Bombacaceae	tree
Castilla elastica Sessé	Moraceae	tree
Ficus insipida Willd.	Moraceae	tree
Antirrhoea trichantha Hemsl.	Rubiaceae	tree
Chrysophyllum cainito L.	Sapotaceae	tree
Fort Sherman		
Aspidospermum cruenta Woodson	Apocynaceae	tree
Calophyllum longifolium Willd.	Clusiaceae	tree
Tachigalia versicolor Standl.& L.O.Williams	Fabaceae	tree
Brosimum utile (Kunth) Pittier	Moraceae	tree
Virola sebifera Aubl.	Myristicaceae	tree
Manilkara bidentata (A.DC.) A.Chev.	Sapotaceae	tree
Vochysia ferruginea Mort.	Vochysiaceae	tree
Cerro Jefe		
Colpothrinax aphanopetala R.J.Evans	Arecaceae	tree
Aechmea dactylina Baker	Bromeliaceae	epiphyte
Werauhia lutheri Pierce & Aranda	Bromeliaceae	epiphyte
Licania jefensis Prance	Chrysobalanaceae	shrub
Calophyllum nubicola D'Arcy & R.C.Keating	Clusiaceae	tree
Clusia spp.	Clusiaceae	tree
Vismia jefensis N.Robson	Clusiaceae	shrub
Terminalia amazonia (J.F.Gmel.) Exell	Combretaceae	tree
Alchornea latifolia Sw.	Euphorbiaceae	shrub
Lisianthus jefensis A.Robyns & T.S.Elias	Gentianaceae	shrub
Eschweilera jacquelyniae S.A.Mori	Lechythidaceae	tree
Miconia oinochrophylla Donn. Sm.	Melastomataceae	shrub
Otoglossum chiriquense (Rchb.f.) Garay & Dunst.	Orchidaceae	epiphyte
Podocarpus oleifolius D.Don ex Lamb.	Podocarpaceae	tree
Isla Barro Colorado		
Aspidospermum cruenta Woodson	Apocynaceae	tree
Dieffenbachia longispatha Engl. & K.Krause	Araceae	herbaceous perennial
Aechmea magdalenae (André) André ex Baker	Bromeliaceae	terrestrial rosette
Calophyllum longifolium Willd.	Clusiaceae	tree
Tachigalia versicolor Standl. & L.O.Williams	Fabaceae	tree
Virola sebifera Aubl.	Myristicaceae	tree
Piper cordulatum C. DC.	Piperaceae	shrub
Tectaria incisa Cav.	Polypodiaceae	herbaceous fern
Isla Taboga		
Laguncularia racemosa (L.) C.F.Gaertn.	Combretaceae	tree

β-carotene, and chlorophyll (Chl) *a* and *b* (DHI Water & Environment, Hørsholm, Denmark).

Results

Pigment characteristics of sun and shade leaves

Analysis of photosynthetic pigments served to characterize the sun and shade leaves of tree crowns and shade leaves from the forest understory (Tables 1, 2). Total Chl content on a leaf-area basis varied strongly between about 200 and 500 μmol m^{-2} and did not show consistent differences between sun and shade leaves. As expected for reduced sizes of light-harvesting Chl *a*, *b*-binding antennae in sun leaves (Anderson and Osmond 1987), the Chl *a*/*b* ratios were highest in outer canopy sun leaves, with values above 3.0 in all 18 species tested (mean ± s.d., 3.43 ± 0.23). In contrast, Chl *a*/*b* ratios were around or below 3.0 in the shade leaves (tree crowns, 2.97 ± 0.11, $n = 6$; understory, 2.79 ± 0.09, $n = 6$). Differences between sun-exposed and shaded leaves from tree crowns were significant ($P<0.01$), as were differences between shaded leaves from tall trees and understory leaves ($P<0.05$).

The ratios of carotenoids to Chl *a*+*b* varied less between species than did Chl (*a*+*b*) content per unit leaf area (Table 2). There were no characteristic differences in pigment composition between sun leaves from lowland and montane forests. But pigment composition did differ between sun and shade leaves (Fig. 1). Sun leaves of tree crowns exhibited the highest and leaves from the understory

Table 2. Photosynthesis pigments in sun and shade leaves of tree crowns, and in leaves of tree seedlings growing in the deeply shaded understory and in a tree-fall gap

Chl content based on leaf area, Chl *a*/Chl *b* ratios and molar ratios of carotenoids to Chl are listed; VxAxZx, sum of xanthophyll cycle pigments, viola-, anthera and zeaxanthin; α-Car, α-carotene; β-Car, β-carotene; Neo, neoxanthin. Means and s.d. from four individual leaves (mostly from different plants) are presented

				Pigments				
Site and species	Leaf type	Chl (*a*+*b*) (μmol m^{-2})	Chl *a*/Chl *b* (mol mol^{-1})	VxAxZx/Chl (*a*+*b*) (mmol mol^{-1})	Lut/Chl (*a*+*b*) (mmol mol^{-1})	α-Car/Chl (*a*+*b*) (mmol mol^{-1})	β-Car/Chl (*a*+*b*) (mmol mol^{-1})	Neo/Chl (*a*+*b*) (mmol mol^{-1})
Parque Metropolitano								
A. excelsum	sun	294 ± 42	3.25 ± 0.14	86.0 ± 4.8	136.9 ± 7.0	7.6 ± 0.7	103.1 ± 4.2	37.6 ± 2.5
	shade	443 ± 49	2.79 ± 0.13	27.6 ± 0.9	122.7 ± 8.1	31.6 ± 2.5	63.5 ± 6.9	40.3 ± 1.6
	understory	268 ± 38	2.67 ± 0.17	20.4 ± 3.0	121.0 ± 12.1	32.3 ± 0.6	53.7 ± 12.1	41.6 ± 1.9
P. septenatum	sun	241 ± 12	3.41 ± 0.29	65.7 ± 7.4	130.1 ± 8.0	9.5 ± 0.5	103.5 ± 8.7	39.1 ± 2.3
	shade	325 ± 52	2.91 ± 0.14	33.0 ± 3.2	106.7 ± 14.9	27.5 ± 4.4	57.7 ± 7.3	34.8 ± 6.3
C. elastica	sun	303 ± 47	3.55 ± 0.34	71.8 ± 26.2	145.6 ± 20.6	20.5 ± 5.1	106.6 ± 10.8	38.9 ± 2.7
	shade	359 ± 73	3.05 ± 0.21	36.9 ± 4.0	117.5 ± 2.0	42.2 ± 4.9	58.8 ± 3.5	39.5 ± 2.4
	understory	234 ± 15	2.70 ± 0.10	27.6 ± 3.6	124.9 ± 14.8	37.3 ± 5.8	45.1 ± 10.4	42.6 ± 2.8
F. insipida	sun	499 ± 48	3.51 ± 0.28	69.9 ± 4.3	115.9 ± 21.0	7.0 ± 1.5	105.2 ± 2.5	36.5 ± 2.2
	shade	453 ± 127	3.10 ± 0.13	41.3 ± 5.3	104.2 ± 2.8	29.1 ± 3.3	71.6 ± 6.2	39.7 ± 1.4
A. trichantha	sun	308 ± 44	3.36 ± 0.18	60.0 ± 9.2	118.5 ± 4.5	9.8 ± 3.6	99.8 ± 8.8	40.3 ± 1.8
	shade	384 ± 46	2.97 ± 0.21	35.2 ± 4.5	116.4 ± 0.8	25.0 ± 8.6	67.0 ± 7.8	40.6 ± 1.2
C. cainito	sun	398 ± 14	3.18 ± 0.05	104.1 ± 8.5	123.3 ± 5.3	20.5 ± 3.0	90.3 ± 6.8	40.5 ± 4.4
	shade	413 ± 73	2.98 ± 0.24	32.4 ± 3.3	125.1 ± 3.9	26.1 ± 1.7	61.3 ± 8.0	42.9 ± 2.0
Fort Sherman								
A. cruenta	sun	347 ± 36	3.51 ± 0.37	72.4 ± 2.5	130.5 ± 6.7	23.4 ± 1.1	88.2 ± 0.5	36.4 ± 0.9
C. longifolium	sun	435 ± 85	3.21 ± 0.22	56.4 ± 14.1	133.0 ± 8.0	13.6 ± 2.0	102.6 ± 6.2	39.6 ± 2.1
T. versicolor	sun	340 ± 41	3.73 ± 0.24	54.7 ± 6.4	122.7 ± 6.3	12.1 ± 2.0	103.1 ± 7.5	37.0 ± 1.0
B. utile	sun	224 ± 40	3.65 ± 0.19	57.5 ± 7.9	170.9 ± 15.8	24.7 ± 6.7	98.1 ± 6.9	36.6 ± 1.3
M. bidentata	sun	338 ± 33	3.32 ± 0.18	82.8 ± 11.4	113.5 ± 3.3	14.1 ± 1.9	86.9 ± 6.3	38.1 ± 0.7
V. sebifera	sun	377 ± 48	3.21 ± 0.12	49.8 ± 4.1	98.0 ± 3.9	42.7 ± 1.5	73.9 ± 11.2	30.2 ± 2.2
Cerro Jefe								
C. aphanopetala	sun	342 ± 35	3.60 ± 0.25	53.0 ± 9.1	147.4 ± 17.2	22.3 ± 1.9	72.4 ± 2.8	38.3 ± 3.0
C. nubicola	sun	342 ± 106	3.18 ± 0.14	59.1 ± 18.5	123.0 ± 14.4	30.7 ± 3.6	75.9 ± 7.4	39.3 ± 2.1
Clusia spp.	sun	342 ± 58	3.02 ± 0.08	74.5 ± 17.0	120.5 ± 19.6	17.8 ± 7.1	86.4 ± 11.9	36.0 ± 2.5
A. latifolia	sun	342 ± 42	3.44 ± 0.09	65.8 ± 8.2	99.5 ± 6.1	17.9 ± 4.1	86.3 ± 4.2	35.2 ± 0.6
E. jacquelyniae	sun	342 ± 51	3.69 ± 0.19	97.5 ± 9.0	156.2 ± 11.6	18.0 ± 1.4	91.1 ± 9.8	37.9 ± 3.0
Isla Barro Colorado								
C. longifolium	understory	332 ± 22	2.85 ± 0.17	18.9 ± 2.4	114.6 ± 6.6	27.9 ± 1.2	52.9 ± 11.1	37.9 ± 0.5
A. cruenta	understory	389 ± 30	2.86 ± 0.13	20.6 ± 0.6	121.7 ± 9.6	42.4 ± 5.5	44.1 ± 3.5	40.7 ± 0.7
V. sebifera	understory	435 ± 62	2.76 ± 0.16	17.2 ± 0.6	114.3 ± 4.6	53.5 ± 1.9	30.1 ± 6.1	39.7 ± 3.1
T. versicolor	understory	281 ± 22	2.83 ± 0.25	22.5 ± 3.1	1 07.1 ± 12.0	43.1 ± 3.4	46.6 ± 6.5	40.0 ± 1.0
T. versicolor	gap	209 ± 17	3.08 ± 0.24	33.5 ± 1.9	125.0 ± 11.4	29.3 ± 2.0	63.6 ± 3.0	39.4 ± 0.9
Isla Taboga								
L. racemosa	sun	301 ± 68	3.88 ± 0.35	71.1 ± 21.1	116.7 ± 9.3	2.2 ± 1.1	134.3 ± 9.1	35.1 ± 3.7

Table 3. Apparent UV-A shielding (%) by the adaxial and abaxial leaf epidermis (measured with a UV-A PAM fluorometer at 375 nm) and UV absorbance (relative units) of leaf extracts at 305 nm (UV-B) and 375 nm (UV-A)

Mature leaves were chosen. For several species, younger, light-green leaves ('young') were included. Means, standard deviation (s.d.) and number of UV-A shielding measurements (*n*) made on different leaves (in part from different plants or tree branches) are given. Data of UV absorbance of leaf extracts are means and s.d. from *n* = 4 leaves; n.d., not determined

Site and species	Leaf type	UV-A shielding at 375 nm			UV absorbance of leaf extracts	
		adaxial	abaxial	*n*	305 nm	375 nm
Parque Metropolitano						
A. excelsum	sun	95.1 ± 2.0	92.0 ± 1.9	10	44.6 ± 3.5	15.3 ± 3.1
	shade	75.8 ± 2.6	67.4 ± 5.9	12	20.0 ± 5.4	9.0 ± 0.8
	understory	32.9 ± 16.6	19.0 ± 8.8	11	n.d.	n.d.
P. septenatum	sun	95.3 ± 2.0	82.5 ± 3.0	10	16.2 ± 4.2	8.0 ± 1.6
	shade	74.2 ± 3.9	67.5 ± 3.8	10	9.0 ± 0.1	5.8 ± 0.3
C. elastica	sun	94.7 ± 0.9	69.1 ± 10.5	10	12.9 ± 1.0	9.0 ± 1.8
	shade	41.9 ± 16.2	30.6 ± 7.7	10	6.2 ± 0.9	3.8 ± 0.6
F. insipida	sun	96.0 ± 1.6	89.8 ± 1.6	10	12.8 ± 2.8	10.3 ± 1.1
	shade	80.0 ± 4.3	75.1 ± 4.7	10	8.9 ± 1.3	7.6 ± 1.2
A. trichantha	sun	90.6 ± 2.2	73.4 ± 7.3	10	20.4 ± 1.7	6.8 ± 0.9
	shade	72.1 ± 9.0	28.6 ± 7.4	10	9.8 ± 1.2	4.5 ± 0.1
C. cainito	sun	91.7 ± 2.4	54.4 ± 5.0	10	42.3 ± 2.7	7.8 ± 1.2
	shade	85.3 ± 9.2	58.8 ± 3.8	10	27.2 ± 2.7	4.6 ± 1.1
Fort Sherman						
A. cruenta	sun	93.4 ± 1.1	89.9 ± 2.1	11	39.8 ± 5.4	14.3 ± 3.9
C. longifolium	sun	88.3 ± 2.8	92.1 ± 1.2	10	28.6 ± 5.7	12.9 ± 2.3
	sun (young)	96.3 ± 0.2	93.7 ± 1.0	10	n.d.	n.d.
T. versicolor	sun	91.9 ± 0.7	77.7 ± 4.3	10	14.4 ± 2.5	7.8 ± 1.5
B. utile	sun	94.2 ± 0.8	91.1 ± 1.8	10	20.9 ± 1.8	6.8 ± 1.7
	understory	31.2 ± 4.3	57.0 ± 5.3	10	n.d.	n.d.
V. sebifera	sun	94.3 ± 1.6	81.6 ± 1.6	8	44.7 ± 18.8	10.7 ± 3.8
	sun (young)	89.1 ± 0.8	86.8 ± 1.0	3	n.d.	n.d.
M. bidentata	sun	97.9 ± 0.2	89.6 ± 1.0	13	20.2 ± 2.0	13.8 ± 0.9
	understory	41.1 ± 5.6	26.0 ± 2.4	10	n.d.	n.d.
V. ferruginea	sun	90.5 ± 1.5	59.3 ± 4.8	10	n.d.	n.d.
	sun (young)	86.1 ± 1.5	56.6 ± 4.9	10	n.d.	n.d.
Cerro Jefe						
C. aphanopetala	sun	93.7 ± 0.5	87.4 ± 6.0	10	n.d.	n.d.
A. dactylina	sun	85.4 ± 12.1	93.2 ± 3.2	3	n.d.	n.d.
W. lutheri	sun	94.0 ± 2.8	90.7 ± 0.8	10	n.d.	n.d.
Licania jefensis	sun	93.1 ± 1.2	65.8 ± 3.8	10	n.d.	n.d.
C. nubicola	sun	93.6 ± 1.6	90.3 ± 1.4	10	24.2 ± 6.1	13.3 ± 1.4
Clusia spp.	sun	94.6 ± 1.1	92.9 ± 1.4	10	11.4 ± 3.9	8.0 ± 1.0
V. jefensis	sun	95.1 ± 0.7	65.1 ± 6.4	10	n.d.	n.d.
T. amazonia	sun	95.0 ± 1.4	91.0 ± 1.4	10	n.d.	n.d.
A. latifolia	sun	94.7 ± 0.4	88.6 ± 1.5	10	96.6 ± 21.1	7.8 ± 1.0
Lisianthus jefensis	sun	95.5 ± 1.3	93.7 ± 1.1	10	n.d.	n.d.
E. jacquelyniae	sun	88.6 ± 2.4	68.9 ± 6.8	10	41.2 ± 8.0	4.9 ± 1.3
M. oinochrophylla	sun	95.7 ± 0.8	86.8 ± 2.8	10	n.d.	n.d.
O. chiriquense	sun	83.9 ± 3.9	93.0 ± 3.1	10	n.d.	n.d.
P. oleifolius	sun	92.5 ± 0.8	94.1 ± 0.5	10	n.d.	n.d.
Isla Barro Colorado						
A. cruenta	understory	23.5 ± 11.1	49.6 ± 6.5	7	10.0 ± 0.7	3.8 ± 0.8
D. longispatha	understory	30.3 ± 4.1	35.7 ± 5.0	6	n.d.	n.d.
A. magdalenae	understory	31.5 ± 4.4	42.3 ± 2.7	5	n.d.	n.d.
C. longifolium	understory	79.9 ± 5.1	82.1 ± 2.1	6	n.d.	n.d.
	understory (young)	45.4 ± 14.1	48.9 ± 14.1	3	8.1 ± 2.0	4.1 ± 0.6
T. versicolor	understory	21.0 ± 7.0	25.9 ± 3.2	4	4.6 ± 1.0	1.7 ± 0.5
	gap	42.1 ± 6.5	34.3 ± 4.1	4	5.8 ± 1.0	2.0 ± 0.3
V. sebifera	understory	66.8 ± 3.8	56.9 ± 1.1	4	18.4 ± 0.3	4.6 ± 0.2
P. cordulata	understory	20.4 ± 4.8	49.9 ± 4.2	10	n.d.	n.d.
T. incisa	understory	43.9 ± 8.7	45.5 ± 4.4	10	n.d.	n.d.
Isla Taboga						
L. racemosa	sun	93.4 ± 0.6	92.5 ± 0.9	10	92.2 ± 7.5	12.6 ± 3.8

the lowest pool sizes of the xanthophyll cycle pigments viola-, anthera- and zeaxanthin (VxAxZx). An understory plant growing in a tree-fall gap (*Tachigalia versicolor*) showed a larger VxAxZx/Chl (*a*+*b*) ratio than leaves from the fully shaded understory (Table 2). Moreover, relative to Chl (*a*+*b*), sun leaves had the highest content of β-carotene (β-Car), whereas α-carotene (α-Car) content was lower in sun than in shade leaves (Fig. 1). Sun leaves from the canopy of the mangrove (*Laguncularia racemosa*) exhibited the lowest α-Car and highest β-Car content of all species tested (Table 2). The contents of lutein (Lut) and neoxanthin (Neo) were similar in the three leaf types. A tendency for increased Lut levels in sun leaves was not significant (Table 1, Fig. 1).

Apparent epidermal UV-A shielding and UV absorbance by leaf extracts

The extent of UV-A shielding by the adaxial and abaxial leaf epidermis, as estimated with a UV-A PAM fluorometer, is listed in Table 3 (left hand columns). With few exceptions, the measurements indicate very high (above 90%) UV-A shielding at 375 nm by the adaxial epidermis of sun leaves. The UV-A shielding by the abaxial epidermis of sun leaves varied widely and was lower than that of the adaxial epidermis in 20 out of 31 species tested. In 10 species, most of them from the Cerro Jefe montane forest, similar UV-A shielding by upper and lower epidermis of sun leaves was found, and in one species (*Otoglossum chiriquense*), the UV-A shielding of the lower epidermis was even higher than that of the upper epidermis.

In shade leaves of tree crowns, and even more so in shade leaves from the forest understory, the epidermal UV-A shielding was generally lower than in sun leaves (Table 3, left hand columns). Differences between adaxial and abaxial epidermis did not show a clear trend. Lower (6 species), similar (8 species) or higher UV-A shielding (4 species) of the abaxial epidermis was observed.

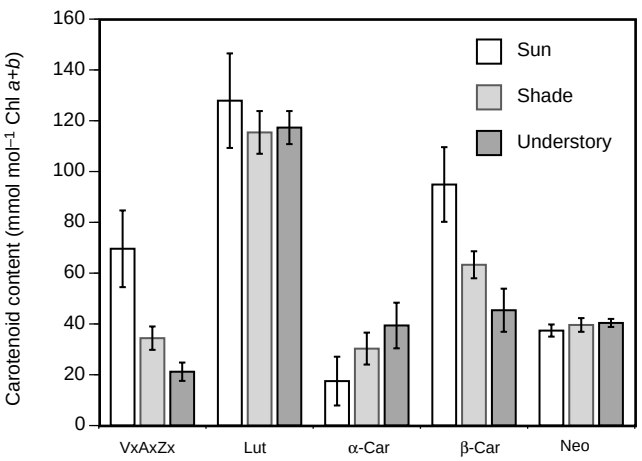

Fig. 1. Comparison of average carotenoid contents of leaves with different states of light acclimation. Contents of total xanthophyll cycle pigments (VxAxZx), lutein (Lut), α-carotene (α-Car), β-carotene (β-Car) and neoxanthin (Neo) per unit Chl *a*+*b* are presented for sun leaves of tree crowns (sun), shade leaves of tree crowns (shade) and shade leaves from the forest understory. Means and s.d. of data from Table 3 are given; number of species: sun, n = 18; shade, n = 6; understory, n = 6.

From several of the species tested for epidermal UV-A shielding, ethanolic/aqueous leaf extracts were prepared and their absorbance in the UV spectral region determined (Table 3, right hand columns). The absorbance spectra of the extracts usually exhibited a broad band with a maximum between about 270 and 290 nm. In most cases, a second broad band with a peak between about 320 and 340 nm was observed. Examples of spectra are depicted in Fig. 2. To represent the absorbance of UV-B, potentially the most damaging component of solar radiation reaching the earth's surface, the absorbance by the extracts at 305 nm is given in Table 3. In addition, the absorbance values in the UV-A region at 375 nm, at the measuring wavelength of the UV-A PAM, are shown. In most spectra, the absorbance at 305 nm was much higher than at 375 nm, indicating a better protection against UV-B than against UV-A radiation. In sun leaves of two species, the shrub *Alchomea latifolia* and the mangrove *L. racemosa*, extremely high absorbance values, close to three times the mean value of 15 other species, were found at 305 nm. Low values were observed at both wavelengths in extracts from leaves in the understory and also in the shaded canopy leaves of some tree species.

Figure 3 shows a comparison between data obtained with the UV-A PAM and by spectroscopy of leaf extracts for all species tested. From the understory, via shaded tree crowns, to the outer tree canopy, a gradual increase in adaxial epidermal UV-A shielding is apparent. These differences are reflected by the absorbance of leaf extracts both at 305 and 375 nm. A plot of the adaxial epidermal UV-A shielding of all species and leaf types *versus* the UV-A absorbance of leaf extracts (Figs 4*A*, *B*) shows characteristics of a saturation curve. Saturation of the epidermal UV-A shielding was reached in practically all sun leaves, indicating a high degree of protection. For low to medium absorbance values of leaf extracts (about 5–9 units at 375 nm, 10–20 units at 305 nm), UV-A shielding was lower in shade than in sun leaves, and shade leaves generally did not attain maximum shielding observed in sun leaves (Figs 4*A*, *B*).

Discussion

The photosynthetic pigment composition of the three leaf types tested (canopy sun, canopy shade and understory) exhibited typical characteristics of sun and shade leaves (*cf.* Demmig-Adams 1998). The shade leaf character was less pronounced in the shaded tree crowns than in leaves from the understory, where photon flux densities are lowest (Table 2, Fig. 1). Noticeably, sun leaves from different habitats (Table 1), such as young and old tropical lowland and montane cloud forests were very similar in their pigment

composition. Low Chl *a*/*b* ratios in the shade leaves indicate efficient light absorption for photosynthesis, owing to a larger number of Chl *a*, *b*-binding light-harvesting complexes (Anderson and Osmond 1987). The larger pool sizes (relative to Chl *a*+*b*) of xanthophyll cycle pigments in the sun leaves is in accordance with acclimation to high-light stress (Demmig-Adams and Adams III 1992; Thiele *et al.* 1996, 1998; Demmig-Adams 1998; Krause *et al.* 2001). The large amount of β-Car in the sun leaves (Fig. 1) may also play a photoprotective role. The particularly high β-Car content in leaves of the mangrove, *L. racemosa* (Table 2) is possibly related to salt stress in addition to high light levels prevailing in the habitat of this plant. As discussed previously (Krause *et al.* 2001), a larger conjugated double bond system probably makes β-Car better suited than α-Car to deactivate triplet Chl and singlet oxygen. In contrast, α-Car, which is present in large pools in shade leaves (Fig. 1), may function better as a light-harvesting pigment than β-Car.

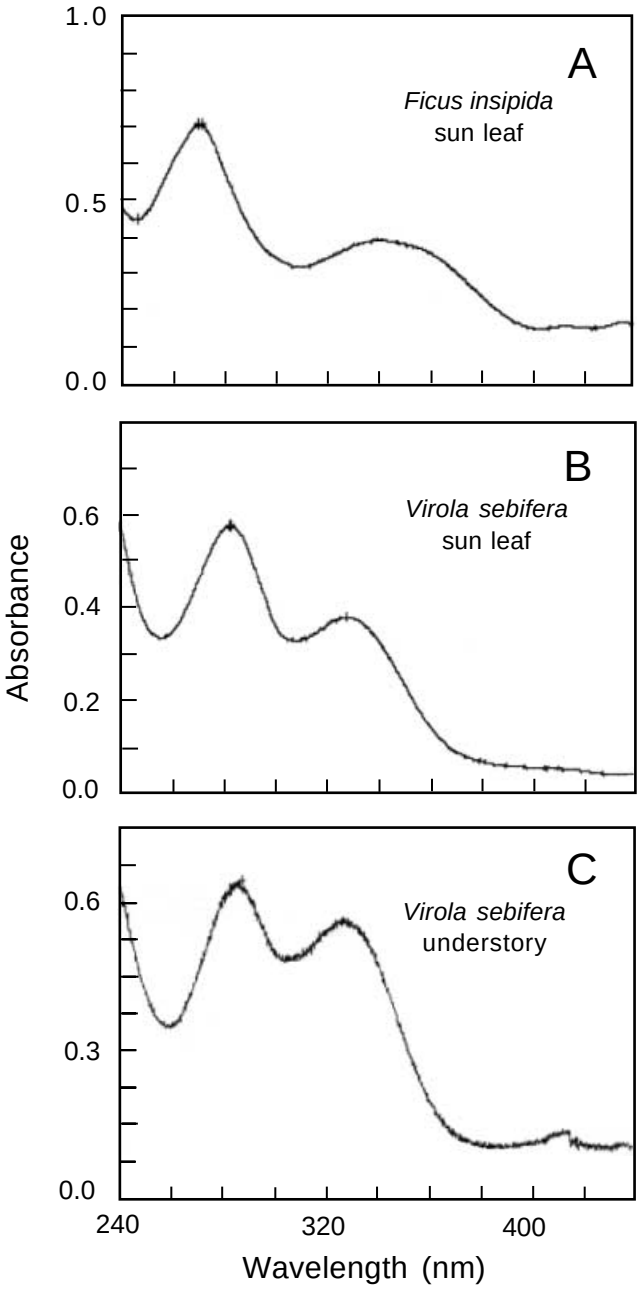

Fig. 2. Examples of absorbance spectra of leaf extracts in the UV spectral region. Spectra from sun leaves of two species (*A*, *B*) and a leaf from the forest understory (*C*) are shown. Leaf disks (0.8 cm^2) were extracted in 3 mL 80% ethanol. For spectroscopy, the extracts were diluted 10-fold (*A*, *C*) and 20-fold (*B*), respectively. For collection sites see Table 1.

Given the high (mostly above 90%) UV-A shielding by the adaxial epidermis of sun leaves (Table 3, Fig. 3), it can be concluded that sun leaves are efficiently protected against solar UV-A radiation. The lower UV-A shielding by the abaxial epidermis, seen in the majority of sun leaves (Table 3), indicates an adjustment to milder UV stress at the lower leaf side. The high UV-A shielding observed in the abaxial epidermis of sun leaves in the montane cloud forest is probably related to increased scattering of UV radiation in this habitat. In shade leaves, the generally low and highly variable UV-A shielding is consistent with a modest requirement for UV protection.

The spectra of leaf extracts show a high content of UV-absorbing compounds in sun leaves (Table 3, Figs 3, 4). Strong absorbance by leaf extracts from sun leaves was observed in the UV-A spectral region at 375 nm. In many cases, sun leaf extracts showed an absorbance of UV-B at 305 nm several fold-higher than of UV-A at 375 nm. We

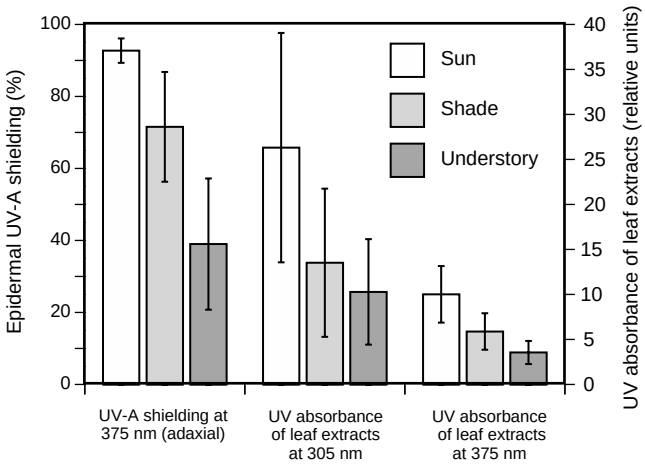

Fig. 3. Comparison of apparent UV-A shielding at 375 nm by the adaxial leaf epidermis and UV absorbance of leaf extracts at 305 and 375 nm for leaves with different status of light acclimation. Means and s.d. of data from Table 3 are presented for sun leaves of tree crowns (sun), shade leaves of tree crowns (shade) and leaves from the forest understory. Number of species for epidermal UV-A shielding: sun, $n = 31$; shade, $n = 6$; understory, $n = 12$; for absorbance of leaf extracts: sun, $n = 15$; shade, $n = 6$; understory, $n = 4$. Two species (*A. latifolia* and *L. racemosa*) with extremely high absorbance of extracts at 305 nm were omitted from average absorbance values.

conclude that sun leaves are protected at least as effectively against solar UV-B as against UV-A. This supports previous studies with tropical trees showing that in mature sun leaves, a contribution of solar UV-B to photoinhibition of PSII during exposure to full sunlight could not be detected (Krause *et al.* 1999). Light-green, nearly fully-expanded young sun leaves included in the present study exhibited epidermal UV-A shielding as high as mature sun leaves (Table 3). Thus, enhanced photoinhibition of PSII observed in such young sun leaves *in situ* (Krause *et al.* 1995) is obviously not related to an increased impact of solar UV radiation.

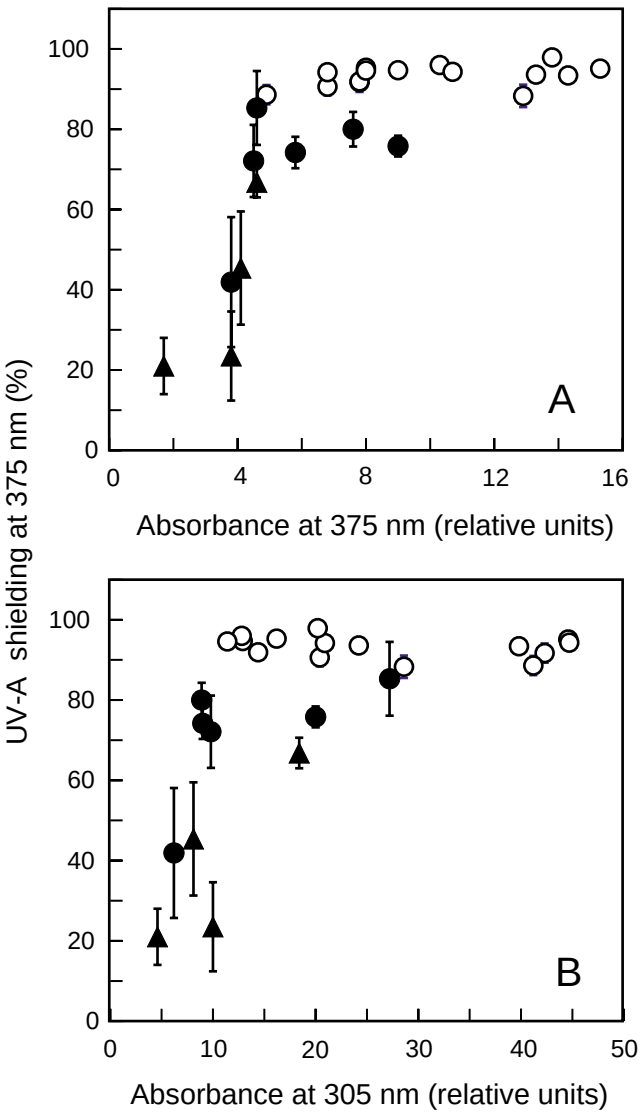

Fig. 4. Relationship between apparent UV-A shielding at 375 nm and absorbance of leaf extracts in the UV-A spectral region at 375 nm (*A*) and in the UV-B region at 305 nm (*B*). Data from Table 3 are depicted for sun leaves of tree crowns (○), shade leaves of tree crowns (●) and leaves from the forest understory (▲). Each symbol represents means and s.d. for an individual species (*cf.* Table 3); s.d. not shown where smaller than symbols; number of leaves per species, n = 3–13. Data from two species (*A. latifolia* and *L. racemosa*) with extremely high absorbance values of extracts at 305 nm have been omitted.

Shade leaves, particularly those from the forest understory, extracts of which exhibited low absorbance at 375 and 305 nm (Table 3, Figs 3, 4), appear to be insufficiently protected against full solar UV radiation. Even when the UV absorbance of leaf extracts was relatively high, shade leaves usually did not reach saturation of UV-A shielding by the adaxial epidermis (Fig. 4). Thus, compared with sun leaves, the UV-absorbing compounds are probably less concentrated in the adaxial epidermis of shade leaves. This may contribute to the high sensitivity of shade-grown tree seedlings to even short periods (less than 1 h) of direct sun exposure, during which ambient UV-B adversely affected PSII (Krause *et al.* 1999), the capacity of net CO_2 assimilation and the photochemical efficiency of PSI (Krause *et al.* 2003).

The chemical nature of the substances involved in UV protection has not been identified in this study. The spectra (examples in Fig. 2) with peaks at 270–290 nm and 320–340 nm did not allow us to clearly differentiate between classes of compounds. The peak at 270–290 nm can be attributed to flavonoids, whereas at 320–340 nm the spectra of flavonoids and hydroxycinnamate derivatives overlap (*cf.* Bilger *et al.* 2001; Cerovic *et al.* 2002). The high absorbance of leaf extracts and strong epidermal UV shielding in the long wavelength UV-A region at 375 nm, where absorbance by hydroxycinnamates is low (Burchard *et al.* 2000; Bilger *et al.* 2001), indicates a substantial, if not major, contribution of flavonoids to UV protection.

The approximate correlation between UV-A shielding by the adaxial epidermis and UV-A absorbance of leaf extracts at 375 nm (Figs 3, 4*A*) demonstrates that the non-invasive technique of the UV-A PAM system provides a useful assessment of leaf protection against solar UV-A radiation. Although Figs 3 and 4*B* show a similar relationship between UV-A shielding and absorbance of leaf extracts in the UV-B region (305 nm), one can only roughly extrapolate from the measurements of UV-A shielding to the degree of UV-B protection. There was no close correlation between absorbance of leaf extracts at 375 and 305 nm (graph not shown) in data from Table 3; that is, the ratio between absorbance at 305 and 375 nm calculated for individual species was not constant. This may be attributable to different compositions of UV-screening compounds in the species tested and/or in shade and sun leaves. A comparative study of epidermal UV shielding with a Xenon PAM operating in the UV-B region (Bilger *et al.* 1997; Barnes *et al.* 2000; Kolb *et al.* 2001) and the UV-A PAM might clarify this point. Substantial differences in classes of UV-shielding compounds between eight species (*Arabidopsis thaliana* and various crop plants) were apparent from complete Chl fluorescence excitation spectra in the wavelength range of 220–600 nm (Cerovic *et al.* 2002). UV-absorbing substances, in addition to protecting

against solar UV radiation, have further important functions in the plant, such as defense against pathogens and provision of precursors for lignin synthesis. Functions other than UV protection could thus, explain the extremely high absorbance values found at 305 nm in sun leaves of two species, *A. latifolia* and *L. racemosa* (Table 3).

A comparison of mean values of data from the three leaf types in Figs 1 and 3 shows a relationship between contents of specific carotenoids (xanthophyll cycle pigments and β-Car) and UV absorbance by leaf extracts. Such a relationship was also observed in leaves of tree seedlings grown in simulated tree-fall gaps of different size (Krause *et al.* 2001). However, when the individual data in Tables 2 and 3 are compared, a close correlation between contents of carotenoids and UV-absorbing compounds cannot be found, as was also observed in a previous study (Krause *et al.* 1999). Taken together, present evidence suggests that the increase in contents of certain carotenoids and in UV-absorbing substances are two regularly occurring, but independent, responses (based on entirely different biochemical pathways) of plant leaves to high levels of solar radiation.

In conclusion, the data on UV-A shielding obtained *in vivo* with the UV-A PAM, along with the absorbance values of leaf extracts in the UV-A and UV-B spectral regions, demonstrate that mature sun leaves are well, and probably fully, protected against solar UV-A and UV-B radiation. This is in agreement with our previous photoinhibition studies. In shade leaves, however, UV protection was only modest, consistent with observations of strong inhibitory effects on several photosynthetic parameters upon sudden exposure of shade leaves to full sunlight.

Acknowledgments

The authors thank Barbara Krause, Ingrid Prikulis and Aurelio Virgo for valuable assistance, Elisabeth King for reading the manuscript, and Ulrich Schreiber for discussion of technical details of the UV-A PAM fluorometer. The study was supported by the Deutsche Forschungsgemeinschaft, the Andrew W. Mellon Foundation and the Smithsonian Tropical Research Institute.

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Manuscript received 17 March 2003, accepted 16 May 2003