Xanthophyll-Cycle Pigments and Photosynthetic Capacity in Tropical Forest Species: A Comparative Field Study on Canopy, Gap and Understory Plants

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Xanthophyll-cycle pigments and photosynthetic capacity in tropical forest species: a comparative field study on canopy, gap and understory plants

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Abstract Xanthophyll-cycle pigments and photosynthetic capacity (PS_{max}) were analyzed in 25 species from different light environments (canopy, gap, understory) within a Panamanian tropical forest. (1) Sun-exposed leaves of canopy tree species showed the highest photosynthetic capacities and largest xanthophyll-cycle pools (violaxanthin, antheraxanthin, zeaxanthin) of about 87 mmol mol^{-1} chlorophyll with only small amounts of α-carotene [about 7 mmol mol^{-1} chlorophyll = 8% of total (α+β) carotene pool]. Under high natural photon flux densities (PFDs) canopy leaves rapidly converted up to 96% of the xanthophyll-cycle pool into zeaxanthin. The back reaction to violaxanthin occurred much faster in low light than in complete darkness. At the end of the night, zeaxanthin still accounted for, on average, 14% of the total xanthophyll-cycle pigments. (2) Leaves of gap plants had intermediate values of PS_{max} and a 43% lower total carotenoid content than canopy leaves. The average size of the xanthophyll-cycle pool was 35 mmol mol^{-1} chlorophyll, and α-carotene accounted for up to 66% of the total (α+β) carotene pool. Under high light conditions gap plants converted, on average, 86% of the xanthophyll-cycle pigments into zeaxanthin. The back reaction, following a decrease in ambient PFD, was slower than the forward reaction. At the end of the night, zeaxanthin accounted for, on average, 7% of the xanthophyll-cycle pigments in gap plants. (3) Understory plants showed the lowest values of PS_{max} and the smallest xanthophyll-cycle pool of about 22 mmol mol^{-1} chlorophyll. α-Carotene accounted for up to 70% of total carotene. The conversion of xanthophyll-cycle pigments into zeaxanthin was negligible during short sunflecks of 1–2 min duration and PFDs up to about 400 μmol m^{-2} s^{-1}. At predawn, leaves of understory plants rarely contained any detectable zeaxanthin. *Aechmea magdalenae*, an understory CAM plant, showed exceptionally high rates of PS_{max} per unit leaf area compared to sympatric C_3 understory species.

Key words Photoinhibition · Photosynthesis · Tropical forest · Xanthophyll-cycle pigments

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Introduction

Acclimation of leaves to different light environments involves a variety of physiological adjustments that may involve changes in the leaf biochemistry, photochemistry, morphology and anatomy (Björkman 1981). Some of these processes play a role in photoprotection, preventing photooxidative damage when the absorbed light is in excess of the capacity for carbon assimilation. The xanthophyll-cycle pigments, violaxanthin, antheraxanthin and zeaxanthin, are thought to play a key role in this photoprotection (Demmig et al. 1987; Demmig-Adams 1990). Under conditions of excess light, zeaxanthin accumulates at the expense of violaxanthin, which has been observed to correlate with an increase in the rate constant for heat dissipation (Demmig-Adams et al. 1989; Björkman and Demmig-Adams 1994). Zeaxanthin (and antheraxanthin) appear to act synergistically with the transthylakoid ΔpH gradient in the dissipation of excess excitation energy, but the precise mechanism of photoprotection is not known (Horton and Ruban 1992; Gilmore and Yamamoto 1993; Bilger and Björkman 1994; Hager and Holocher 1994; Osmond 1994). It has been suggested that the concentration of the xanthophyll-cycle pigments determines the maximum amount of zeaxanthin that can be accumulated and therefore the maximum protection available via this dissipative process (Thayer and Björkman 1990; Demmig-Adams and Adams 1992).

Most studies on the photoprotective role of the xanthophyll-cycle pigments have been carried out on plants grown under managed conditions (Demmig-Adams

1990; Thayer and Björkman 1990; Adams and Demmig-Adams 1992; Long et al. 1994). In this field study, xanthophyll pigment composition and dynamics were studied in a variety of species acclimated to different light environments in a tropical forest and exhibiting different photosynthetic capacities. Kinetics of pigment changes were followed during normal diurnal cycles (dawn to dusk) as well as during rapid fluctuations in light intensity (i.e., passing clouds, sunflecks).

Materials and methods

Study sites

Leaf samples of canopy trees were harvested in a semideciduous tropical forest in Parque Natural Metropolitano close to Panama City, Republic of Panama. Average annual rainfall is 1740 mm, most of which falls in the rainy season between May and December, and the average temperature is 28° C. Access to the canopy was possible via a construction crane (Parker et al. 1992). Leaf samples of understory plants and plants in a 40 m^2 forest gap were collected in the tropical moist forest of Barro Colorado Island, Republic of Panama. Average annual rainfall is 2600 mm (Windsor 1990) which falls largely during the rainy season. Samples were taken from fully expanded leaves during the rainy season in 1992 unless stated otherwise.

Species

The species investigated in this study were identified according to Croat (1978): *Aechmea magdalenae* (André) André ex Baker (Bromeliaceae), *Anacardium excelsum* (Bertero and Balb.) Skeels (Anacardiaceae), *Antirrhoea trichantha* (Griseb.) Hemsl. (Rubiaceae), *Calathea panamensis* Rowl. ex Standl. (Marantaceae), *Castilla elastica* Sessé (Moraceae), *Cecropia longipes* Pitt. (Moraceae), *Coccoloba parimensis* Benth. (Polygonaceae), *Dieffenbachia longispatha* Engler and Krause (Araceae), *Ficus insipida* Willd. (Moraceae), *Heliconia latispatha* Benth. (Musaceae), *Herrania purpurea* (Pitt.) R.E. Schult. (Sterculiaceae), *Hybanthus prunifolius* (Schult.) Schulze (Violaceae), *Inga goldmanii* Pitt. (Leguminosae), *Miconia argentea* (Sw.) DC. (Melastomataceae), *Monstera dubia* (H.B.K.) Engler and Krause (Araceae), *Nectandra globosa* (Aubl.) Mez (Lauraceae), *Ouratea lucens* (H.B.K.) Engler (Ochnaceae), *Palicourea guianensis* Aubl. (Rubiaceae), *Paullinia bracteosa* Radlk. (Sapindaceae), *Pharus latifolius* L. (Gramineae), *Philodendron sp.* (Araceae), *Piper cordulatum* C. DC. (Piperaceae), *Pseudobombax septenatum* (Jacq.) Dug. (Bombacaceae), *Psychotria acuminata* Benth. (Rubiaceae), *Psychotria limonensis* Krause (Rubiaceae), *Psychotria marginata* Sw. (Rubiaceae), *Spondias mombin* L. (Anacardiaceae).

Photosynthetic capacity

Capacity of photosynthetic O_2 evolution was determined with a leaf-disc-electrode system (Hansatech, King's Lynn, Norfolk, UK) at saturating CO_2 (5%) and saturating photon flux density (PFD). The leaf-disc chamber was connected to a circulating waterbath set at 30° C.

Fluorescence

Chlorophyll a fluorescence was measured on dark-adapted leaves in the laboratory with a PAM 101 fluorometer (Walz, Effeltrich, Germany; Schreiber et al. 1986) as described in Königer and Winter (1991). Leaves were harvested predawn. The ratio F_v/F_M (variable fluorescence yield/maximum fluorescence yield) was used as a measure of the photochemical efficiency of photosystem II (Kitajima and Butler 1975).

Determination of pigments by high performance liquid chromatography

Discs (2.27 cm^2) were punched from leaves (in situ) and rapidly plunged into liquid nitrogen. Frozen samples were ground in a mortar and extracted with 100% acetone at room temperature under low light. The extract was spun in a 5415C-Eppendorf microcentrifuge at maximum speed for 5 min. The supernatant was removed and the pellet resuspended in 100% acetone and centrifuged again. This procedure was repeated until all pigments were extracted. The combined supernatants were membrane-filtered (0.22 µm PTFE; Alltech, Deerfield, Il., USA) and immediately analyzed.

Separation and quantification of the pigments took place using a Waters high performance liquid chromatography (HPLC) system (Waters Millipore, Milford, Mass., USA). The system consisted of two 510 pumps, U6 K injector, automated gradient controller 680, multiwavelength detector 490E and data module 746. The solvents were degassed with a 3312-ERC instrument (Erma, Tokyo, Japan). A spherisorb ODS-1 5U column (250 mm×4.6 mm) (Alltech, Deerfield, Il., USA) in connection with a micro-Bondapak C_{18} guard-pak pre-column (Waters Millipore) was used for separation. Injection volume was 20 µl.

The pigments were eluted at a flow rate of 2 ml min^{-1} using solvent A [acetonitrile:methanol:Tris-HCl buffer 0.1 M, pH 8.0; 72:12:7 (v:v:v)] isocratically for the first 6 min, followed by a 10 min linear gradient to 100% B [methanol:hexane; 7:1 (v/v)], which was then run isocratically for another 4 min. The column was re-equilibrated between samples for 10 min with solvent A. The detection wavelength for integration was 440 nm. All solvents were of HPLC grade (Fluka, Ronkonkoma, NY, USA) and were filtrated through 0.45 µm PTFE filters (Gelman Sciences, Ann Arbor, Mich., USA). The method allowed for baseline or near baseline separation of chlorophyll a, chlorophyll b and the carotenoids.

For calibration, known quantities of purified pigments were injected. Chlorophyll a, chlorophyll b, α-carotene, β-carotene and lutein were obtained from Sigma (St. Louis, Mo., USA). Zeaxanthin was obtained from Atomergic Chemetals, Farmingdale, NY, USA. Neoxanthin was isolated by thinlayer chromatography according to Czygan (1968) and Weber and Czygan (1972). Violaxanthin was isolated by injecting a very concentrated leaf extract into the HPLC system and collecting the violaxanthin "peak" at the detector outlet. The calibration factor obtained for violaxanthin was also used for antheraxanthin.

The epoxidation state (EPS) is defined as (violaxanthin+0.5 antheraxanthin):(violaxanthin+antheraxanthin+zeaxanthin). EPS incorporates alterations in zeaxanthin as well as antheraxanthin. Recent investigations suggest that antheraxanthin contributes to xanthophyll-dependent energy quenching (Gilmore and Yamamoto 1993).

Photon flux density and temperature

PFD incident on leaves was recorded with a quantum sensor (Li-Cor, Lincoln, Neb., USA). Air and leaf temperatures were determined with copper-constantan thermocouples connected to a TH-65 instrument (Wescor, Logan, Utah, USA).

Dry weight and nitrogen

Leaf samples were dried at 60° C for 48 h for determination of dry weight per unit area. Nitrogen content was determined at the University of Würzburg (Germany) using a CNH analyzer (Heraeus, Hanau, Germany).

Table 1 Photosynthetic capacity (PS_{max}), leaf mass/area, total chlorophyll (*Chl*) content, chlorophyll (a/b ratio and carotenoid composition of leaves of different canopy trees. Samples were harvested before dawn. Data are means (n=2–4) [*V* violaxanthin, *A* antheraxanthin, *Z* zeaxanthin, *EPS* (*V*+0.5 *A*)/(*V*+*A*+*Z*), *DW* dry weight, *Car* carotene, *Neo* neoxanthin]

Species	PS_{max} µmol m^{-2} s^{-1}	Leaf mass /area g DW m^{-2}	Chl a+b µmol m^{-2}	Chl a/b	Carotenoids mmol mol^{-1} Chl a+b						EPS
					total	*V*+*A*+*Z*	β-Car	α-Car	Lutein	Neo	
*Anacardium excelsum**	14.6	67.3	513	2.4	393.7	87.5	77.5	4.1	150.7	73.9	0.82
*Antirrhoea trichanta**	17.0	49.9	556	2.6	325.5	74.5	75.8	5.1	112.2	58.0	0.73
*Castilla elastica**	25.8	68.1	681	3.1	422.9	88.2	76.7	16.1	167.5	74.4	0.91
Cecropia longipes	35.0	84.3	757	3.3	482.4	125.3	97.0	1.2	174.8	84.2	0.88
Ficus insipida	43.3	113.4	1279	3.1	419.6	80.5	92.9	4.9	165.9	75.5	0.88
Pseudobombax septenatum	20.7	96.4	1075	3.1	367.4	81.3	76.2	10.9	128.9	70.2	0.88

* Recently developed leaves

Table 2 Photosynthetic capacity (PS_{max}), leaf mass/area, total chlorophyll (*Chl*) content, chlorophyll a/b ratio and carotenoid composition of leaves of plants in a 40 m^2 rain forest gap. Samples were harvested before dawn. Data are means (n=3) [*V* violaxanthin, *A* antheraxanthin, *Z* zeaxanthin, *EPS* (*V*+0.5 *A*)/(*V*+*A*+*Z*), *DW* dry weight, *Car* carotene, *Neo* neoxanthin]

Species	PS_{max} µmol m^{-2} s^{-1}	Leaf mass /area g DW m^{-2}	Chl a+b µmol m^{-2}	Chl a/b	Carotenoids mmol mol^{-1} Chl a+b						EPS
					total	*V*+*A*+*Z*	β-Car	α-Car	Lutein	Neo	
Coccoloba parimensis	7.9	47.1	761	2.3	283.1	30.7	38.8	30.7	118.4	64.5	1.00
Dieffenbachia longispatha	8.6	53.4	1036	2.1	284.4	36.9	28.6	56.6	101.5	60.8	0.85
Heliconia latispatha	10.2	44.6	964	2.4	275.5	44.5	39.4	36.2	95.9	59.6	0.90
Herrania purpurea	6.8	24.4	787	2.5	306.5	28.4	40.8	50.9	120.3	66.1	1.00
Miconia argentea	7.6	36.3	835	1.8	275.1	26.1	31.2	30.2	123.7	64.0	1.00
Palicourea guianensis	11.2	25.8	697	2.3	252.0	36.8	40.0	30.6	89.1	55.4	0.84
Paullinia bracteosa	6.7	32.4	484	2.4	288.9	33.6	41.5	41.5	107.4	64.8	0.99
Piper cordulatum	8.0	67.1	902	1.9	269.7	42.3	34.4	35.7	96.3	61.1	0.86

Results

Photosynthetic capacity and pigment composition

Sun-exposed leaves of six canopy-tree species were studied in July. Photosynthetic capacity (PS_{max}) ranged from 14.6 to 43.3 µmol m^{-2} s^{-1}, chlorophyll content from 513 to 1279 µmol m^{-2}, chlorophyll a/b ratios from 2.4 to 3.3, and total carotenoid content from 326 to 522 mmol mol^{-1} chl (Table 1). Xanthophyll-cycle pigments per unit chlorophyll were similar among species (violaxanthin+antheraxanthin+zeaxanthin: 73–88 mmol mol^{-1} chl) except for *Cecropia longipes* which had exceptionally high values (125 mmol mol^{-1} chl). Canopy leaves contained small amounts of α-carotene (4–6 mmol mol^{-1} chl) accounting for 1–17% of the (α+β) carotene pool. Predawn EPS, following a sunny day, was on average 0.85, i.e., leaves still contained zeaxanthin and antheraxanthin.

Leaf samples from shrubs, herbs and vines in a gap were harvested in November. PS_{max} was lower than in canopy species (6.7–11.2 µmol m^{-2} s^{-1}) (Table 2). Chlorophyll content ranged from 484 to 1036 µmol m^{-2} and chlorophyll a/b ratios from 1.8 to 2.5. Total carotenoid content was 252–307 mmol mol^{-1} chl and the xanthophyll-cycle pool (26.1–44.5 mmol mol^{-1} chl) was, on average, about 40% of that of canopy trees. α-Carotene was markedly higher in plants from the gap than in canopy trees and accounted for 44–66% of the (α+β) carotene content. Some species contained no zeaxanthin at predawn, while in others 15% of the xanthophyll-cycle pool was in the form of zeaxanthin.

Understory plants were studied between November and December when soil moisture conditions in the understory are still comparable to those earlier in the wet season when the canopy trees were studied. Some of these understory species had also been studied in the gap. Amongst the understory plants was a CAM species, *Aechmea magdalenae*, which is often found in large stands on the forest floor close to riverbeds. *A. magdalenae* had the highest PS_{max} values when expressed per unit leaf area [17.5±6.8 µmol m^{-2} s^{-1} (mean±SD, n=10); Table 3] while all C_3 understory species had consistently lower rates (2.1–6.1 µmol m^{-2} s^{-1}). Chlorophyll content was variable amongst C_3 species (741–1383 µmol m^{-2}), and was notably higher in the CAM species (2295 µmol m^{-2}) The CAM species also had a much higher leaf weight per unit area than the C_3 understory species (Table 3). Therefore, on both a chlorophyll and dry weight basis, PS_{max} of *A. magdalenae* was similar to that of sympatric C_3 species. Chlorophyll a/b ratios ranged from 2.1 to 2.6. Total carotenoid content was 219–318 mmol mol^{-1} chlorophyll and was comparable to that of the gap plants. The understory plants had a small xanthophyll-cycle pool (16.6–32.1 mmol mol^{-1} chl), but a

Table 3 Photosynthetic capacity (PS_{max}), leaf mass/area, total chlorophyll (*Chl*) content, chlorophyll a/b ratio and carotenoid composition of leaves of plants in the rain-forest understory. *Aechmea magdalenae* is a CAM species. All other species are C_3 plants. Samples were harvested before dawn. Data are means (n=3). [*V* violaxanthin, *A* antheraxanthin, *Z* zeaxanthin, *EPS* (*V*+0.5 *A*)/(*V*+*A*+*Z*), *DW* dry weight, *Car* carotene, *Neo* neoxanthin]

Species	PS_{max} µmol m^{-2} s^{-1}	Leaf mass /area g DW m^{-2}	Chl a+b µmol m^{-2}	Chl a/b	Carotenoids mmol mol^{-1} Chl a+b						EPS
					total	*V*+*A*+*Z*	β-Car	α-Car	Lutein	Neo	
Aechmea magdalenae	17.5	111.3	2295	2.4	267.2	20.0	29.0	52.5	104.5	61.2	0.98
Calathea panamensis	5.0	27.8	924	2.4	281.9	18.8	31.6	42.8	122.6	66.1	1.00
Dieffenbachia longispatha	4.8	43.0	1062	2.4	292.5	32.1	34.7	48.9	113.2	63.5	0.95
Hybanthus prunifolius	4.1	24.0	1926	2.5	220.7	16.6	24.7	35.6	96.8	47.0	1.00
Inga goldmanii	6.1	41.4	844	2.3	257.3	19.1	29.0	46.9	94.4	68.0	1.00
Monstera dubia	2.1	27.3	1186	2.3	252.2	26.7	20.5	44.0	96.6	64.4	0.98
Nectandra globosa	5.2	57.5	769	2.1	279.3	18.8	32.0	47.2	119.2	62.0	0.99
Ouratea lucens	3.4	68.8	1363	2.5	241.6	18.7	34.1	45.8	92.3	50.7	0.92
Paullinia bracteosa	5.0	41.2	778	2.6	308.8	23.3	43.1	53.2	120.7	68.5	1.00
Pharus latifolius	5.0	38.2	1069	2.2	247.3	20.7	29.5	35.9	104.3	57.0	0.97
Philodendron sp.	3.3	38.7	943	2.3	285.2	18.6	31.6	55.8	115.1	64.2	1.00
Piper cordulatum	4.2	51.5	1383	2.3	229.8	21.2	21.5	50.2	88.8	47.3	1.00
Psychotria acuminata	5.4	23.0	741	2.2	318.2	28.3	31.6	38.4	137.0	83.0	1.00
Psychotria marginata	4.9	40.7	1068	2.3	219.4	18.1	25.1	35.4	90.6	50.1	1.00

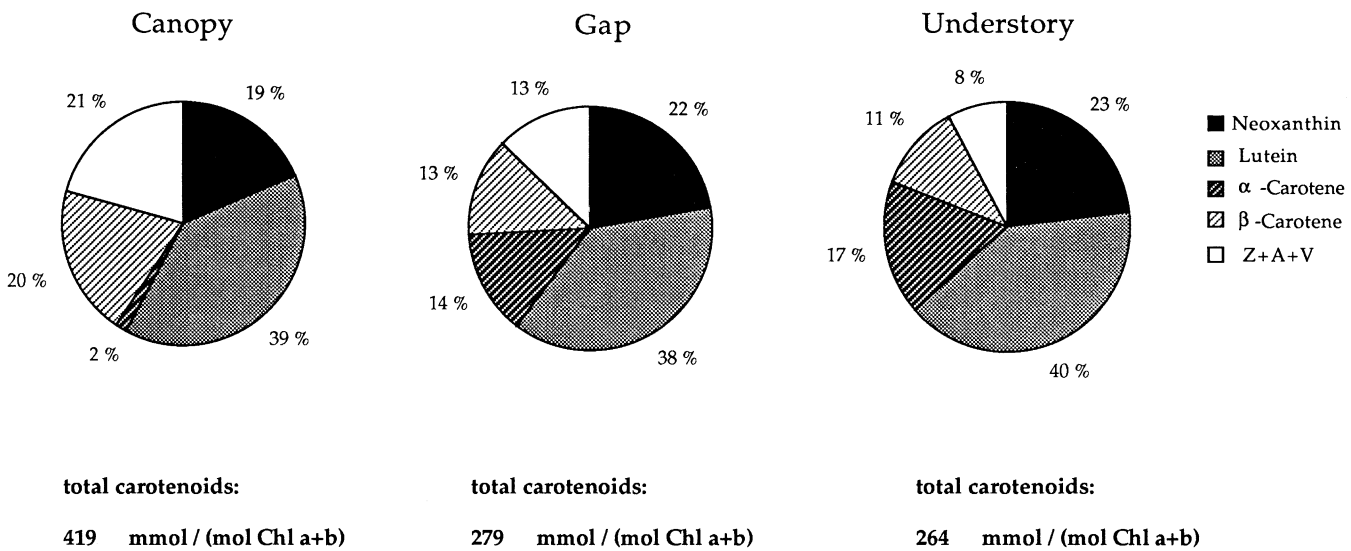

Fig. 1 Percentage contribution of different carotenoid pigments to the total carotenoid pool. Values are means for 6 canopy trees, 8 gap plants and 14 understory plants. Species are listed in Tables 1–3. Leaf samples were harvested before dawn (*Z* zeaxanthin, *A* antheraxanthin, *V* violaxanthin, *Chl* chlorophyll)

high α-carotene content, accounting for 55–70% of the (α+β) carotene pool. The EPS at predawn was high (0.92–1.00).

Relative differences in pigment composition for canopy trees, gap and understory plants are summarized in Fig. 1. Gap and understory plants had similar total carotenoid contents per unit total chlorophyll, whereas relative content of carotenoids was 54% higher in canopy trees. Lutein and neoxanthin were similar for plants of all three habitats. Large differences were seen in the percentage of α-carotene, β-carotene and the xanthophyll-cycle pigments. With increasing average ambient light levels, the xanthophyll-cycle pool increased, while the proportion of α-carotene decreased. There was a strong positive correlation between the xanthophyll-cycle pool and PS_{max} (Fig. 2A, B) and a strong negative correlation between xanthophyll-cycle pool and α-carotene (relative to α+β carotene) (Fig. 3). PS_{max} and nitrogen content were positively correlated (Fig. 2C). However, a closer examination of data shows that the average photosynthetic nitrogen-use efficiency is higher in canopy tree leaves (191±37 µmol O_2 mol^{-1} N s^{-1}, mean of six species±SD) than in gap plants (148±33, n=8) and in understory plants (89±29, n=13) except for *Aechmea magdalenae* (188 µmol O_2 mol^{-1} N s^{-1}) which was associated with the canopy leaves with respect to photosynthetic nitrogen-use efficiency.

Predawn F_V/F_M values of 14 understory species, determined in November after days with integrated PFDs between 19.2 and 37.3 mol m^{-2} above the forest canopy,

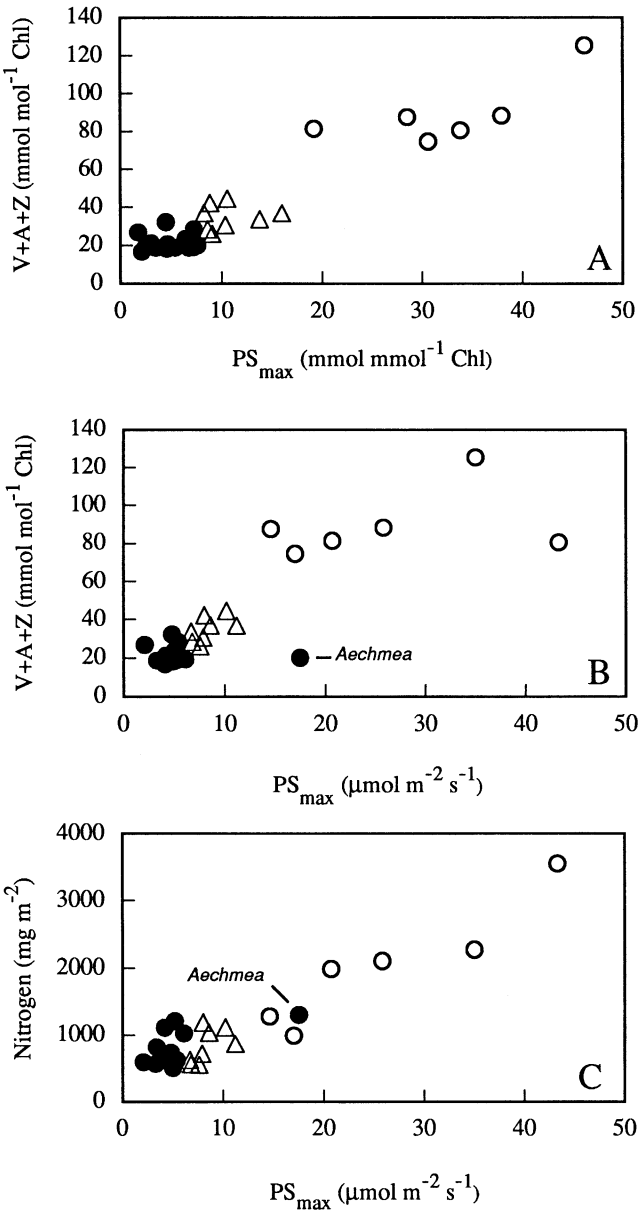

Fig. 2A, B Correlations between the xanthophyll cycle pool (*V+A+Z*; see Fig. 1 for definitions) and photosynthetic capacity (PS_{max}), and **C** correlation between total nitrogen content and PS_{max}. Leaf samples were harvested before dawn from canopy trees (○), gap (△) and understory plants (●)

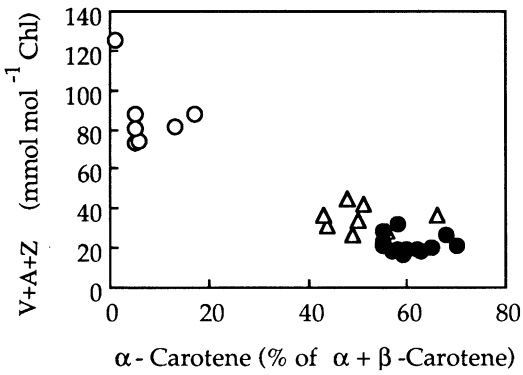

Fig. 3 Correlation between xanthophyll cycle pool (*V+A+Z*; see Fig. 1 for definitions) and relative content of α-carotene for canopy trees (○), gap (△) and understory plants (●). The content of α+β-carotene per unit chlorophyll (*Chl*) was similar in canopy trees (89.7±7.7 mmol mol^{-1} Chl; mean±SD, *n*=6), gap plants (75.9±10.0 mmol mol^{-1} Chl, *n*=8), and understory plants (75.0±10.5 mmol mol^{-1} Chl, *n*=14)

ranged from 0.795 *(Nectandra globosa)* to 0.840 *(Aechmea magdalenae)* with a mean of 0.816, i.e., there was essentially no indication of sustained photoinhibition (Kitajima and Butler 1975; Björkman and Demmig 1987). Measurements on dark-adapted leaves from eight species in the gap during the same period yielded lower F_V/F_M values, ranging from 0.751 *(Piper cordulatum)* to 0.820 *(Heliconia latispatha)* with a mean of 0.790. Canopy leaves showed the largest variability of predawn F_V/F_M. The highest predawn value was obtained for *Cecropia longipes* (0.842). After very bright days, sustained reductions of F_V/F_M as low as 0.698 *(Antirrhoea trichantha)* were detected the following morning.

Pigment kinetics

Diurnal courses of PFD, temperature and pigment composition are shown for leaves of the canopy trees *Antirrhoea trichantha* and *Pseudobombax septenatum* (Fig. 4A–D). *A trichantha* was studied during an exceptionally bright day during the rainy season. PFD stayed above 1500 µmol m^{-2} s^{-1} for nearly 7 h (Fig. 4A). Leaf temperatures reached maximum values of about 40° C (Fig. 4B). Up to 92% of the xanthophyll pool was converted into zeaxanthin (Fig. 4C). Contents of α-carotene, neoxanthin and lutein as well as the xanthophyll-cycle pool were constant (Fig. 4D).

The diurnal course of PFD for *P. septenatum* in Fig. 4E is more typical of the rainy season. PFD reached 2200 µmol m^{-2} s^{-1}, but decreased to 500 µmol m^{-2} s^{-1} with cloud cover. Leaf temperatures reached 35° C (Fig. 4F). Maximum zeaxanthin was observed at 10:00 a.m. when 75% of the xanthophylls were in this form.

Changes in pigment composition were also followed in the canopy tree *Ficus insipida* under three contrasting light regimes. Fig. 5A depicts part of an overcast day when PFDs ranged between 200 und 1000 µmol m^{-2} s^{-1}. There were no marked changes in EPS (0.84–0.87) during this 4 h period. A more detailed analysis of xanthophyll-cycle pigments under fluctuating light conditions is shown in Fig. 5B and C. It was evident that EPS depended on both the level and duration of PFD. For example, in Fig. 5B EPS decreased from 0.48 to 0.32 during a light transient (ΔPFD=1200 µmol m^{-2} s^{-1}) starting at approximately 10:45 a.m.The continuous exposure to high PFD during the subsequent 15 min led to a further decrease in EPS to 0.21. At approximately 12:15 p.m., when PFD decreased from about 2000 to 200 µmol m^{-2}

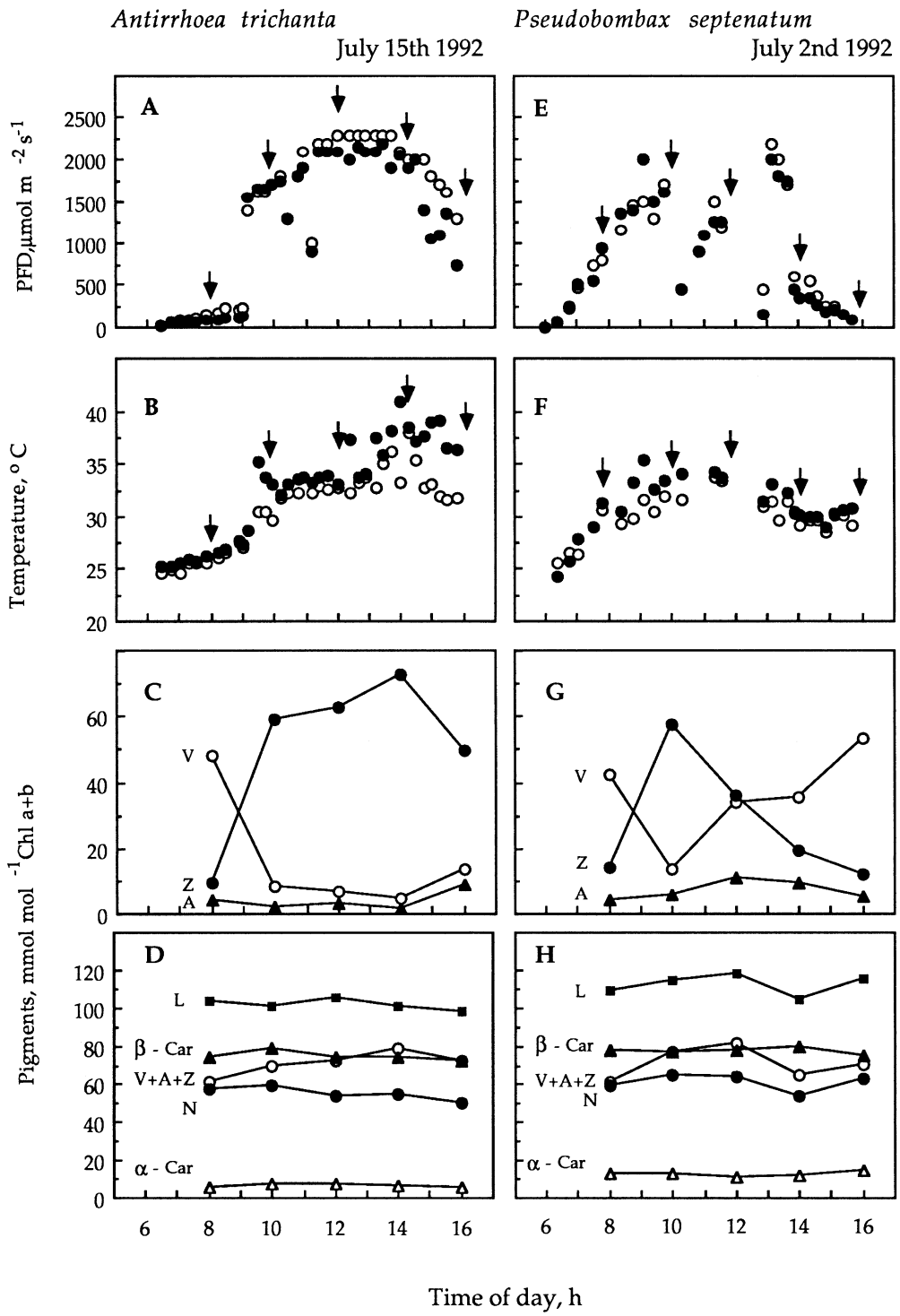

Fig. 4A–H Time courses of different parameters measured for *Antirrhoea trichantha* **A–D** and *Pseudobombax septenatum* **E–H. A, E** Photon flux density (*PFD*) above the canopy (○) and incident on the leaves (●). **B, F** Air (○) and leaf (●) temperatures. **C, G** Changes in violaxanthin (*V*), antheraxanthin (*A*) and zeaxanthin (*Z*). **D, H** changes in lutein (*L*), β-carotene (β-*Car*), violaxanthin+antheraxanthin+zeaxanthin (*V+A+Z*), neoxanthin (*N*) and α-carotene (α-*Car*). *Arrows* indicate the times when the samples for pigment analyses were taken. Pigment data are means of 2–4 samples

s^{-1} over a 21 min period, EPS increased from 0.12 to 0.88. EPS remained relatively constant under rapidly fluctuating PFD between approximately 500 and 2000 µmol m^{-2} s^{-1} (Fig. 5C).

The back conversion of zeaxanthin to violaxanthin was much faster under moderate light conditions of cloudy skies and late afternoons than in complete darkness. In leaves of *F. insipida* collected at noon after a cloudless morning and then kept at either 60–70 µmol photons m^{-2} s^{-1} or in complete darkness for 40 min, EPS increased from 0.1 to 0.25 in darkness and to 0.5 in low light (Fig. 6). In another experiment, in which a *F. insipida* leaf harvested at noon was kept in complete darkness, it took 2 h for EPS to increase from 0.35 to 0.88 (data not shown).

Changes in xanthophyll-cycle composition were followed in three species growing in a rain forest gap (*Palicourea guianensis, Dieffenbachia longispatha, Herrania*

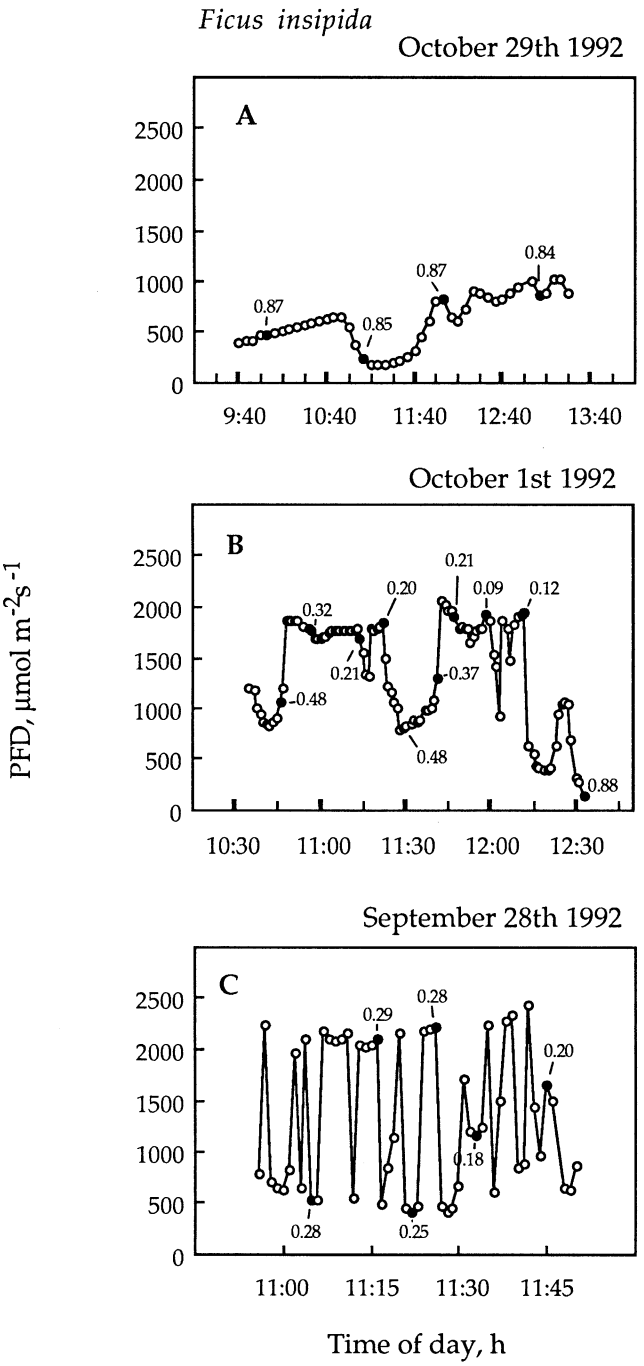

Fig. 5A–C Changes in photon flux density (*PFD*) incident on leaves of *Ficus insipida* on 3 different days. *Filled symbols* indicate the times when samples for pigment analyses were taken. The *given values* indicate the epoxidation state of the leaves at that time. Data are means of 2–3 samples

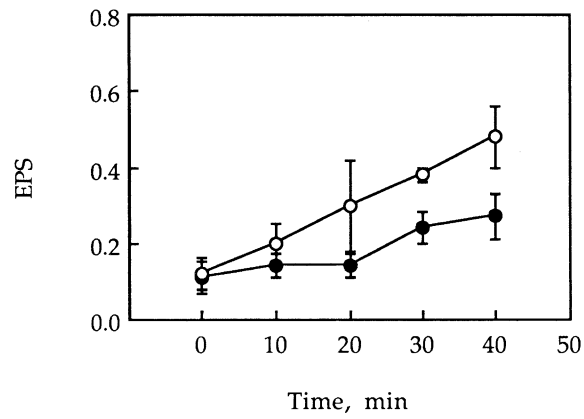

Fig. 6 Increase in epoxidation state (*EPS*) of leaves of *Ficus insipida* kept at 60–70 µmol photons m^{-2} s^{-1} (*open symbols*) or in complete darkness (*closed symbols*). Leaves were harvested at noon on a bright day. Temperature during experimental treatment was 31–33° C. Values are means±SD (n=3)

purpurea) (Fig. 7). On sunny days these plants experienced PFDs up to 1700 µmol m^{-2} s^{-1} for 1–2 h, and low light (20–40 µmol photons m^{-2} s^{-1}) for most of the rest of the day. Leaf temperatures increased by about 10° C during the high light period and reached maxima of 40° C. Under high light conditions, all species showed a marked conversion of violaxanthin to zeaxanthin within 20–30 min. After 1 h at high PFD, EPS values of 0.20–0.13 were measured. The backreaction in low light was much slower than the forward reaction. After 85 min in low light, EPS of *D. longispatha* increased from 0.13 to merely 0.44 (Fig. 7B).

Light in the understory ranged between 1 and 14 µmol m^{-2} s^{-1} and leaves contained very little zeaxanthin (EPS=0.96–0.99). Depending on the canopy structure, the duration and intensity of sunflecks varied markedly. Figure 8A shows the response of EPS in *Psychotria limonensis* to three consecutive sunflecks each lasting approximately 2 min. Maximum PFD was 420 µmol m^{-2} s^{-1}. EPS did not respond to these brief sunflecks. *D. longispatha* received a 16 min sunfleck (Fig. 8B) with PFD up to nearly 400 µmol m^{-2} s^{-1}. During this time, EPS decreased from 0.99 to 0.84. Figure 8C shows the response of EPS to an exceptional sunfleck of 30 min and 1400 µmol photons m^{-2} s^{-1} in *Piper cordulatum*. EPS decreased from 0.84 in low light to 0.34.

Discussion

Pool size of xanthophyll-cycle pigments

Consistent with previous results that violaxanthin+antheraxanthin+zeaxanthin (V+A+Z) increases with the increasing daily photon flux to which leaves are exposed during development (Thayer and Björkman 1990; Demmig-Adams and Adams 1992), canopy tree leaves contained more (V+A+Z)/chlorophyll than leaves from plants growing in a small rain forest gap or in the rain forest understory. Photosynthetic capacity was positively related to increased light availability from understory to gap to canopy, leading to a positive correlation between photosynthetic capacity and V+A+Z across species (Fig. 2).

Chlorophyll a/b and (V+A+Z)/chlorophyll of these tropical forest canopy leaves were, on average, some-

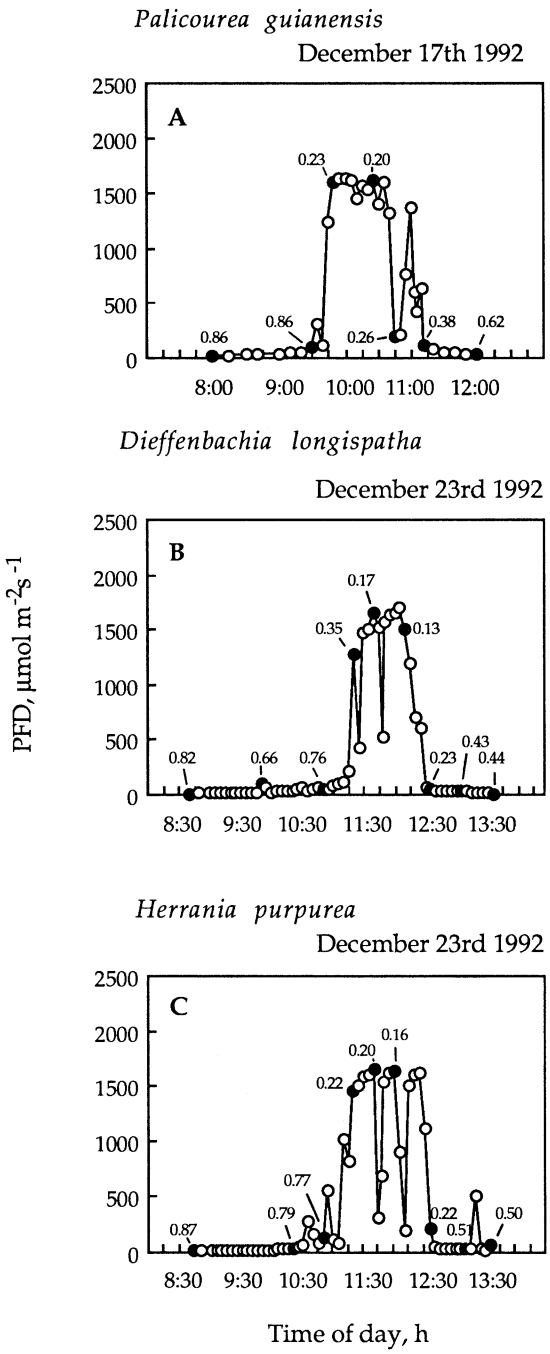

Fig. 7A–C Changes in photon flux density (*PFD*) incident on leaves of plants growing in a rain forest gap: **A** *Palicourea guianensis*; **B** *Dieffenbachia longispatha*; **C** *Herrania purpurea*. *Filled symbols* indicate the times when samples for pigment analyses were taken. The *given values* indicate the epoxidation state of the leaves at that time. Data are means of 2–3 samples

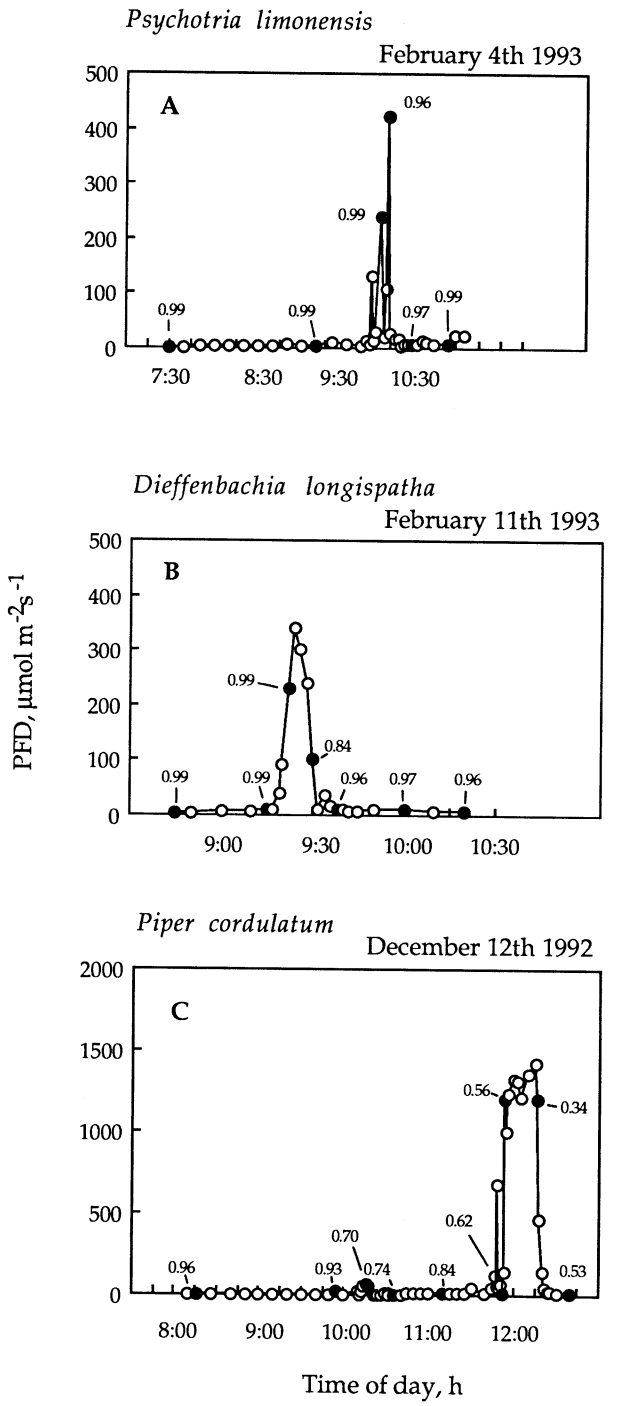

Fig. 8A–C Changes in PFD incident on leaves of plants growing in the rain forest understory: **A** *Psychotria limonensis*; **B** *Dieffenbachia longispatha;* **C** *Piper cordulatum*. *Filled symbols* indicate the times when samples for pigment analyses were taken. The *given values* indicate the epoxidation state of the leaves at that time. Data are means of 2–3 samples

what lower than those of fully sun-grown perennial shrubs and crop plants studied in California and Colorado during summer (Thayer and Björkman 1990; Adams and Demmig-Adams 1992; Demmig-Adams and Adams 1992). In contrast, *V+A+Z*/chlorophyll of canopy tree leaves were slightly higher than in outer canopy leaves of mangrove species from northern Australia which maintained near vertical leaf angles under high solar radiation and, therefore, avoided absorption of sunlight (Lovelock and Clough 1992). Contents of chlorophyll/ar-

ea, lutein/chlorophyll and β-carotene/chlorophyll of tropical forest canopy leaves were similar to those of fully sun-grown shrubs and crops from temperate regions (Thayer and Björkman 1990; Adams and Demmig-Adams 1992).

Relatively high levels of α-carotene (up to 17% of the total α+β carotene pool) were found in canopy leaves. In contrast, α-carotene is hardly detectable in sun leaves of photosynthetically highly active crop plants. The abundance of α-carotene plus the lower *V+A+Z*/chlorophyll compared to other sun plants may be due to the acclimation of leaves to the conditions that prevail during the rainy season.

Increases in α-carotene relative to β-carotene with decreasing growth PFD have been demonstrated for coniferous trees (Ida 1981; Grill and Pfeifhofer 1985) and for *Hibiscus tiliaceus* (Thayer and Björkman 1990). Consistent with these studies and with investigations on shade-grown plants maintained under glasshouse conditions (Demmig-Adams and Adams 1992), understory plants at the forest floor contained high levels of α-carotene, had small xanthophyll-cycle pools, and low PS_{max}. In *Piper cordulatum,* α-carotene exceeded the level of β-carotene more than twofold.

Leaves of plants in the small forest gap had, on average, 86% and 62% higher PS_{max} and xanthophyll-cycle pools, respectively, than plants in the understory. However, consistent with the small gap size, leaves were physiologically more similar to those of understory plants than of canopy trees. For example, PS_{max}, nitrogen use of PS_{max}, (*V+A+Z*)/chl and carotene (as percentage of α+β-carotene) were closer to leaves of understory plants than canopy trees. The functional significance of the relative increase in α-carotene with declining growth PFD, as seen here and in other studies, is not known. In contrast to *V, A* and *Z*, which are mainly associated with the PSI and PSII light harvesting complexes (cotton-thylakoids; Thayer and Björkman 1992), α-carotene seems to be mainly associated with the PSI and PSII reaction centers (Young and Britton 1989; Siefermann-Harms 1994). It remains to be determined whether or not increased levels of α-carotene in low light reflect an advantage at low PFD or a disadvantage at high PFD.

Dynamics of xanthophyll-cycle pigments

The few studies of diurnal changes of xanthophyll-cycle pigments have been performed on plants growing in environments with predictable patterns of solar radiation. In the course of clear days, changes in *Z* more or less followed changes in incident PFD (e.g., Adams and Demmig-Adams 1992). By contrast, daily changes of PFD in tropical environments may be extremely variable. For example, rapidly changing cloud cover leads to variations in solar radiation in the forest canopy, while in the rain forest understory, sunflecks lead to abrupt and strong increases in PFD within seconds. We have used EPS as biochemical indicator of the extent to which these tropical forest leaves are exposed to excess PFD during diurnal cycles, and to gain information about the extent to which the leaves (need to) engage in the xanthophyll cycle for dissipation of solar radiation absorbed that is in excess of photosynthesis requirements.

On relatively clear days during the rainy season, with peak PFDs up to 2300 μmol m^{-2} s^{-1}, outer-canopy leaves of tropical forest trees (e.g., *Antirrhoea trichanta*; Fig. 4A–D) were found to convert more than 90% of xanthophyll-cycle pigments (*V+A+Z*) to *Z*. In contrast, on overcast days with PFDs not exceeding 1000 μmol m^{-2} s^{-1}, violaxanthin remained the major component of the xanthophylls present in *Ficus inspida* (Fig. 5A). This finding clearly demonstrates that EPS responds to excess light, since net CO_2 uptake in *F. insipida* reaches light saturation at approximately 1000 μmol photons m^{-2} s^{-1} in situ (Zotz et al. 1995). During rapid fluctuations in cloud cover where PFD fluctuated between 500 and 2100 μmol m^{-2} s^{-1}, EPS remained low (i.e., *Z* concentrations remained high) (Fig. 5C). Thus, rapidly fluctuating PFD can be similar to continuously high light in terms of its effect on xanthophyll-cycle pigments. Upon sudden exposure of leaves to strong light under controlled conditions, *V*-deepoxidation and *Z*-formation generally proceed much faster than does *Z*-epoxidation during a consequent period in low light (e.g., Hager 1967). Maintenance of high EPS under conditions of rapidly fluctuating PFD (Fig. 5C) is consistent with these deepoxidation-epoxidation kinetics. Similar to early laboratory studies with spinach leaves, yet in contrast to findings with needles of *Taxus baccata* (Hager 1967), *Z*-epoxidation in canopy tree leaves occurred more rapidly in low light than in complete darkness, a phenomenon which may be related to increased NADPH availability in low light promoting the *Z* to *V* conversion (Hager 1980).

Rapid conversions of *V* into *Z* followed by slow back-reactions from *Z* into *V* were also apparent in our studies in a small rain forest gap, where plants received low PFD for most of the day, but were exposed to high PFD (75% of full sunlight) for 1–2 h on bright days around noon when the sun was positioned above the gap. Photoprotection during these prolonged, relatively regularly occurring high light exposures may be particularly important in these plants, whose photosynthetic characteristics were close to those of understory plants. Indeed, they rapidly converted 80% of the xanthophylls into *Z* upon the abrupt transition from low to high PFD (Fig. 7).

In all three gap species shown in Fig. 7, a sustained response of the photochemical apparatus to light stress is suggested (1) because of the very slow change in EPS upon return to low light, and (2) because EPS did not increase above 0.87 overnight. Plants exposed to a combination of high light and other environmental stresses such as drought, have been shown to retain even higher amounts of *Z* overnight, accompanied by an elevated capacity for nonradiative energy dissipation throughout the day/night cycle (Demmig et al. 1988). Predawn F_V/F_M as low as 0.751 in these gap plants are consistent with a sustained down-regulation of photochemical efficiency.

In the rain forest understory, short sunflecks of 1–2 min duration and PFDs up to about 400 μmol m^{-2} s^{-1} had little or no effect on EPS, although 400 μmol m^{-2} s^{-1} is about 25-fold higher than the diffusive PFD normally received by these plants around noon. Detailed long-term records of the light environment in the understory on Barro Colorado Island are not available. However, in the understory of an Hawaiian forest, two-thirds of all sunflecks were less than 0.5 min in length and had peak PFDs between 200 and 500 μmol m^{-2} s^{-1} (Pearcy 1983). Apparently, longer exposures and/or higher PFDs are required for conversion of *V* to *Z* in rain forest understory plants. EPS markedly decreased in *Piper cordulatum* during a 15 min sunfleck of about 1500 μmol m^{-2} s^{-1} (Fig. 8C).

C_3 versus CAM in the understory

The abundance of the CAM species *Aechmea magdalenae* (Pfitsch and Smith 1988) in the rain forest understory on Barro Colorado Island demonstrates that the CAM cycle does not prevent plants from growing in habitats characterized by extremely low PFDs (see also: Winter et al. 1983, 1986; Adams et al. 1986; Adams 1988; Winter and Smith 1995). *A. magdalenae* has the same xanthophyll-cycle pool size per unit chlorophyll as sympatric understory C_3 species but shows much higher rates of PS_{max} per unit leaf area. This is mainly associated with the higher leaf mass/area in *A. magdalenae*. On a dry weight (or chlorophyll basis), PS_{max} in *A. magdalenae* and C_3 understory plants is similarly low compared to gap and canopy plants.

Our comparison of PS_{max} and leaf nitrogen across canopy, gap and understory plants follows the widely recognised positive relation between these two parameters in many plant species (Field and Mooney 1986). The deviation from linearity, which is apparent in a decrease in photosynthetic capacity per unit of nitrogen as PFD decreases from canopy to gap and from gap to understory, is consistent with increased partitioning of nitrogen into thylakoids in low light. This increases the nitrogen cost of light-saturated photosynthesis (Evans 1989). Interestingly, *A. magdalenae* shows a PS_{max}/N (188 μmol O_2 $mol^{-1}N$ s^{-1}) twice as high as that of sympatric C_3 understory species and similar to that of canopy leaves. We do not know whether the reduced nitrogen cost of photosynthesis in *A. magdalenae* is related to the CO_2 concentrating mechanism of CAM. The high N use efficiency of CAM could be analogous to the higher nitrogen-use efficiency of C_4 versus C_3 plants, which holds in shaded as well as in sun-exposed habitats (Pearcy and Calkin 1983). Previous studies with two epiphytic tropical CAM plants from deeply shaded habitats in Australia *(Pyrrosia longifolia* and *Hoya nicholsoniae)* which had similarly high leaf weight per unit area as *A. magdalenae*, revealed a high PS_{max}/N of 129 μmol O_2 mol^{-1} N s^{-1} for *P. longifolia* and a low PS_{max}/N of 73 for *H. nicholsoniae* (Winter et al. 1986), the latter value being similar to the average value observed in the C_3 understory species on Barro Colorado Island. More comparative studies on shade-adapted CAM species are needed to evaluate the extent to which the high N-use efficiency observed for *A. magdalenae* reflects an inherent property of the CAM pathway.

It is possible that the high PS_{max} per unit area enables *A. magdalenae* to use sunflecks more efficiently for CO_2 reduction than sympatric C_3 plants. *A. magdalenae* is found particularly in moist parts of the understory along trails or creeks where sunflecks are characterized by relatively long durations and high PFDs. Elevated intercellular CO_2 concentrations during daytime, resulting from decarboxylation of malic acid, probably also enhance the efficiency to use sunflecks for photosynthesis and may thus lower the requirements for photoprotection (Winter and Demmig 1987).

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