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Do mature shade leaves of tropical tree seedlings acclimate to high sunlight and UV radiation?

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Abstract. Seedlings of neotropical forest trees grown in low light were exposed to 0.5–9 h d^{-1} direct sunlight, for up to 3 months, to test the capability of mature shade leaves to acclimate to full solar visible and UV radiation. Photosynthetic pigments and the antioxidant, ascorbate, were analysed in leaves of two pioneer and two late-succession species. Seedlings of one or two of these species were used to assess further acclimative responses. Sun-exposure for 0.5 or 1 h d^{-1} resulted in strongly decreased α-carotene and increased β-carotene and lutein levels. The pool size of xanthophyll-cycle pigments (sum of viola-, anthera- and zeaxanthin) was increased and their turnover was enhanced. These changes were associated with an increase in the capacity of non-photochemical fluorescence quenching and its 'energy-dependent' component, qE, and with reduced susceptibility to photoinhibition of PSII. Prolonged exposure to full direct sunlight (approximately 4 or 9 h d^{-1}) resulted in a marked decrease of chlorophyll $a + b$ content and increase in chlorophyll a/b ratios and the pool of xanthophyll-cycle pigments (based on chlorophyll), leading to extremely high zeaxanthin levels during high-light periods. Contents of ascorbate and UV-B-absorbing substances were substantially increased. PSI activity exhibited a response to full sunlight that is characteristic of sun leaves. Rates of net photosynthetic CO_2 assimilation under saturating light were increased. The data show that mature shade leaves of seedlings of both early- and late-succession tree species can substantially acclimate to full-sunlight conditions by employing similar physiological mechanisms.

Keywords: *Anacardium excelsum*, ascorbate, *Calophyllum longifolium*, chlorophyll a/b, *Ficus insipida*, photosynthetic pigments, *Virola surinamensis*.

Introduction

Numerous studies have investigated the physiological acclimation of vascular plant leaves to specific light conditions during their development (e.g. Anderson *et al.* 1995; Demmig-Adams 1998; Kurasová *et al.* 2002). Sun leaves have higher chlorophyll (chl) a/b ratios than shade leaves, indicating a lower number of light-harvesting chl $a + b$-binding antenna complexes (Anderson *et al.* 1995). The specific composition of carotenoids in the chloroplast thylakoid membranes of sun leaves is considered essential to protect the photosynthetic apparatus from adverse effects of excessive light. Compared with shade leaves, increased levels of xanthophyll-cycle pigments (viola-, anthera- and zeaxanthin) and, in several cases, of β-carotene (β-car) and/or lutein (Lut), have been reported, while the α-carotene (α-car) content is reduced (Thayer and Björkman 1990; Demmig-Adams and Adams 1992, 1996*a*, *b*; Krause *et al.* 2003*a*). Moreover, the content of protective UV-absorbing compounds is increased in sun leaves (Krause *et al.* 2003*a*), as well as in leaves of plants exposed to supplementary UV-B radiation (Searles *et al.* 2001).

Sun leaves of plants in tropical climate zones have to cope with extremely high levels of visible and ultraviolet (UV) radiation (Caldwell *et al.* 1989; Madronich *et al.* 1995),

Abbreviations used: AsA, ascorbate; Ax, antheraxanthin; car, carotene; chl, chlorophyll; DHA dehydroascorbate; Lut, lutein; Neo, neoxanthin; Vx, violaxanthin, VxAxZx, sum of viola-, anthera- and zeaxanthin; Zx, zeaxanthin.

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often together with high leaf temperatures. Furthermore, in the understorey of the tropical forest, leaves grow in deep shade, but when canopy gaps are created by tree fall, shade leaves may become exposed to strong direct sunlight for one to several hours a day. Leaves produced under such gap conditions acclimate well to their particular environment in terms of their photosynthetic pigments (Königer *et al.* 1995) and content of substances known to protect against UV-B radiation (Krause *et al.* 2001). During periods of direct sun exposure, these leaves exhibit distinct photoinhibition of PSII, which is largely reversed when the leaves are shaded again during the course of the day (Krause and Winter 1996; Thiele *et al.* 1998; Krause *et al.* 2001). A fast phase of 'recovery' from photoinhibition (approximately 1 h) is probably related to the light-dependent turnover of the xanthophyll-cycle pigments, indicating a protective regulatory response to high-light stress (Thiele *et al.* 1996, 1998). A slower recovery phase (requiring several hours) appears to be associated with turnover of the D1 protein in the PSII reaction centre (Leitsch *et al.* 1994; Thiele *et al.* 1996, 1998). Similar diurnal responses of PSII have also been observed in outer canopy sun leaves of tall trees (Krause *et al.* 1995). In contrast, when mature shade leaves of tropical plants were exposed for the first time to full solar radiation, strong photoinhibition of PSII occurred, which required extremely long recovery periods (up to 2 weeks) in low light (Mulkey and Pearcy 1992; Krause *et al.* 1999*a*) indicating sustained damage. Differences in the role of D1 protein turnover in sun and shade leaves under light stress have been reported (Öquist *et al.* 1992).

Surprisingly little is known about the capability of fully developed shade leaves to acclimate to changes in the light regime *in situ*. Several studies show that mature leaves can acclimate to seasonal climatic changes, such as the transition from warm to chilling or freezing temperatures in cold and temperate climate zones (e.g. Somersalo and Krause 1990; Krause 1994*a*; Adams *et al.* 1995, 2002; Krause *et al.* 1999*b*; Gilmore *et al.* 2003; Kirchgeßner *et al.* 2003). Besides alterations in photosynthetic pigment levels and in organisation and function of the photosynthetic apparatus, such acclimation includes increases in the activities of antioxidative systems (Schöner and Krause 1990; Krause 1994*b*; Streb *et al.* 2003). However, acclimation of mature shade leaves to full solar radiation, which may be required in leaves of tropical forest seedlings upon formation of tree-fall gaps, has not been extensively investigated, although several studies indicate that at least partial acclimation is possible.

Demmig-Adams *et al.* (1989) observed that leaves of a crop plant, *Gossypium hirsutum* L., and a mangrove, *Rhizophora mangle* L., grown under low PAR, exhibited substantial increases in the levels of total carotenoids, xanthophyll-cycle pigments and, correspondingly, of zeaxanthin (Zx) within 7 d under high PAR. In leaves of *G. hirsutum*, the capacity of photosynthetic CO_2 assimilation was increased. Acclimative responses of CO_2 assimilation were also reported for a tropical understorey herb, *Alocasia macrorrhiza* (L.) G.Don subjected to simulated gap conditions (Mulkey and Pearcy 1992) and for plants of three tropical rain forest species grown in the shade and transferred to full sunlight (Lovelock *et al.* 1994). Shade-grown seedlings of *Anacardium excelsum* (Bertero and Balb.) Skeels, a neotropical pioneer tree species, exposed to direct sunlight (in the presence of either near-ambient or strongly reduced UV-B) for 1 h per day, exhibited a gradual increase in the pool size of xanthophyll-cycle pigments and content of Lut (on a chl $a + b$ basis) and a decrease in the α-car/β-car ratio within 8 d (Krause *et al.* 1999*a*). Moreover, increases in xanthophyll-cycle pool size, midday deepoxidation levels and photosynthetic capacity were observed in leaves of shade-grown tree seedlings of four dipterocarp species (from the south-east Asian rain forest) exposed to full solar irradiance (Bungard *et al.* 2000). The high initial susceptibility of PSII to photoinhibition in shade leaves was reduced upon high-light treatments (Mulkey and Pearcy 1992; Lovelock *et al.* 1994; Bungard *et al.* 2000; Kolb *et al.* 2001). Changes in chloroplast structure and levels of chloroplast proteins were found upon high-light acclimation of mature leaves of the tropical epiphytic species, *Guzmania monostachia* (L.) Rusby ex Mez (Maxwell *et al.* 1999).

In the present study, we tested mature shade leaves of four neotropical tree species, both pioneer and late-succession types, for their acclimative responses to direct sun exposure. We examined a wide array of physiological parameters, such as the composition of photosynthetic pigments, levels of UV-absorbing substances and of the antioxidant ascorbate, photochemical efficiency of PSII, capacity of non-photochemical chl *a* fluorescence quenching and photosynthetic CO_2 assimilation.

Materials and methods

The study was conducted at the Smithsonian Tropical Research Institute in Panama City (9°N, 80°W), Panama. Photosynthetic pigments, UV-absorbing compounds, ascorbate and dehydroascorbate of frozen leaf samples were analysed at the Institute of Plant Biochemistry, Düsseldorf, Germany.

Plant material and experimental procedure

Seedlings of four neotropical tree species were studied: *Ficus insipida* Willd. (Moraceae) and *Anacardium excelsum* (Bertero and Balb.) Skeels (Anacardiaceae), two pioneer species common in secondary forests near Panama City; *Virola surinamensis* (Rol.) Warb. (Myristicaceae) and *Calophyllum longifolium* Willd. (Clusiaceae), two late-succession species, common in lowland forests of Central and South America.

Seeds were germinated in Pro-Mix Bx soil (Les Tourbieres Premier LTEE, Quebec, Canada) in a deeply shaded greenhouse (midday PAR approximately 10–30 μmol m^{-2} s^{-1}, mean temperature approximately 30°C), except for *F. insipida*, which was germinated in moderate light (300–500 μmol photons m^{-2} s^{-1}). Seedlings were cultivated in deep shade in pots (7.5 L; height 40 cm) filled with Pro-Mix soil.

Table 1. Chl contents and chl *a* / *b* ratios of leaves of two pioneer species (*Ficus insipida* and *Anacardium excelsum*) and two late-succession species (*Virola surinamensis* and *Calophyllum longifolium*) in different states of light acclimation

Means and SDs for *n* samples are given (*n'* = number of plants tested; canopy sun leaves were sampled from one mature tree). Two sets of experiments were performed. *Experiment 1*: shade-grown seedlings (*F. insipida*, *A. excelsum*) were sun-exposed at midday for 1 h d^{-1}. Subsequent to sun exposure, plants were returned to deep shade. Control plants were kept continuously in deep shade. *Experiment 2*: seedlings were sun-exposed for 0.5 h d^{-1} (*C. longifolium*) or 1 h d^{-1} (*V. surinamensis*). Control plants were kept in deep and moderate shade. After sun exposure, plants were returned to moderate shade. Plants of *C. longifolium* were, subsequent to 0.5 h d^{-1} exposure, sun-exposed for 4 h d^{-1}. Shade-grown plants of *A. excelsum* were stepwise acclimated to whole-day full sun exposure (approximately 9 h d^{-1}). Data from leaves collected from the forest (*C. longifolium*, *A. excelsum*) are presented for comparison. For details see Materials and methods. Pigment contents were determined at the end of sun exposure treatments as given in the table

PAR conditions	Chl *a* + *b* (μmol m^{-2})	Chl *a* / chl *b* (mol mol^{-1})	*n*	*n'*
Experiment 1				
Ficus insipida				
Deep shade	296 ± 28	2.66 ± 0.05	3	3
Sun-exposed 1 h d^{-1} (33 d)	228 ± 62	2.89 ± 0.02	3	3
Anacardium excelsum				
Deep shade	423 ± 34	2.84 ± 0.06	3	3
Sun-exposed 1 h d^{-1} (35 d)	388 ± 71	2.74 ± 0.13	3	3
Experiment 2				
Virola surinamensis				
Deep shade	310 ± 64	2.76 ± 0.06	20	5
Moderate shade	258 ± 43	2.94 ± 0.10	20	5
Sun-exposed 1 h d^{-1} (17 d)	274 ± 32	2.89 ± 0.13	20	5
Calophyllum longifolium				
Deep shade	467 ± 52	2.76 ± 0.13	24	6
Moderate shade	448 ± 40	2.83 ± 0.14	23	6
Sun-exposed 0.5 h d^{-1} (21 d)	420 ± 50	2.77 ± 0.10	24	6
Sun-exposed 4 h d^{-1} (3 months)	150 ± 24	3.27 ± 0.19	4	3
Canopy sun leaves	512 ± 68	3.36 ± 0.03	5	1
Anacardium excelsum				
Deep shade (greenhouse)	371 ± 81	2.75 ± 0.15	6	6
Deep shade (forest)	293 ± 52	2.94 ± 0.13	5	5
Moderate shade (forest)	280 ± 42	3.10 ± 0.20	5	5
Fully sun-exposed 9 h d^{-1} (7 weeks)	153 ± 26	3.35 ± 0.36	9	9
Canopy sun leaves	268 ± 60	3.35 ± 0.41	5	1

Homogeneous populations of plants were selected for two sets of experiments.

Experiment 1

Seedlings of *F. insipida* (approximately 5 months old) and *A. excelsum* (approximately 6 months old) were separated into two groups each: (1) plants remaining in deep shade for control; (2) plants subjected to unshaded outdoor conditions around midday for 10–50 min (15 d) and subsequently for 1 h d^{-1}, as specified in Table 1 and figure legends. PAR doses received during 1 h sun exposure varied between approximately 3 and 7 mol m^{-2}, depending on cloudiness. After sun exposure, the plants were returned to deep shade to simulate light conditions in small to medium-sized forest gaps with low levels of scattered light.

Experiment 2

To consider acclimation to conditions prevailing in larger gaps, shade-grown seedlings of *V. surinamensis* (8 months old) and *C. longifolium* (5 months old) were separated into three groups each: (1) plants remaining in deep shade; (2) plants placed at a moderately shaded site in the greenhouse (midday PAR approximately 40–90 μmol m^{-2} s^{-1}); (3) plants exposed daily to sun around midday for specified periods (*V. surinamensis*, 17 d, 1 h d^{-1}; *C. longifolium*, 21 d, approximately 0.5 h d^{-1}, with PAR doses varying between 2.5 and 3.5 mol m^{-2}). Subsequent to sun exposure, plants were returned to moderate shade. To test the response to prolonged daily periods of sun exposure, as occurs in the outer forest canopy, shade-grown plants of *A. excelsum* (9 months old) were subjected to stepwise reduction in shading within 6 weeks and then kept for 7 weeks at an open, fully sun-exposed site (approximately 9 h d^{-1} direct sun exposure on clear days). Plants of *C. longifolium*, which had previously been sun-exposed 21 d for 0.5 h d^{-1} (see above), were kept for 3 more months at an open site allowing 4 h d^{-1} direct sun exposure. For these light treatments of *A. excelsum* and *C. longifolium*, plants were transferred to larger pots (15 L; height 45 cm), containing forest soil mixed with 50% (v/v) leaf litter. Shorter daily exposure times for *C. longifolium* were used because previous observations showed a substantially higher

susceptibility to photoinhibition of PSII of late-succession compared with pioneer species under simulated forest gap conditions (Krause *et al.* 2001). To compare plants cultivated in the greenhouse with those growing in the forest, leaves of *A. excelsum* seedlings were collected from the forest understory in Parque Natural Metropolitano near Panama City from sites with similar light conditions (deep and moderate shade) as in the greenhouse. In addition, canopy sun leaves from mature trees of *A. excelsum* and *C. longifolium* were included in the analyses.

PAR was measured with quantum sensors LI 189 B (LI-COR, Lincoln, NE) and UV-B radiation with a radiometer IL 400 A (International Light, Newburyport, MA). The UV-B sensor of the latter has a fixed wavelength sensitivity with maximum at 291 nm (half-band width 275–313 nm) and provides an approximate measure of ambient UV-B energy.

Quantification of photosynthetic pigments and assessment of soluble UV-absorbing substances

Leaf disks (0.57 cm^2) were sampled, frozen in liquid nitrogen and stored at –70 to –80°C or on dry ice. Photosynthetic pigments were quantified by HPLC following a modified method of Färber *et al.* (1997), outlined by Krause *et al.* (2003*a*).

The absorbance in the UV-B spectral region of ethanolic–aqueous extracts of the leaf disks served as a measure of soluble UV-absorbing compounds. Extracts were prepared and absorbance data recalculated on a leaf-area basis as described previously (Krause *et al.* 2001, 2003*a*).

Analysis of ascorbate and dehydroascorbate

Ascorbate (AsA) and dehydroascorbate (DHA) were determined according to Law *et al.* (1983) from leaf samples of approximately 0.2 g fresh weight, as described by Barth and Krause (1999).

Chlorophyll fluorescence

The ratio of maximum variable to maximum total chl *a* fluorescence emission, F_v/F_m, served as a measure of potential efficiency of PSII (Krause and Weis 1991; Krause and Jahns 2003). A decline in this ratio remaining after dark adaptation of light-exposed leaves indicates photoinhibition of PSII. The F_v/F_m ratio was determined with a PAM 2000 portable fluorometer (Walz, Effeltrich, Germany) after 10 min dark adaptation using DLC-8 aluminum leaf clips (Walz). For details about the measurements see Barth and Krause (1999).

Fluorescence quenching in dark-adapted leaf disks (1.65 cm^2) in a moistened air stream (temperature 24°C) was measured with a PAM 101/102/103 fluorometer system (Walz). The fluorescence induction signal was recorded for 20 min under 1500 μmol photons m^{-2} s^{-1} actinic white light. The decline in maximum total fluorescence was monitored by means of saturating pulses (1 s) of white light applied every 100 s. After 20 min, a steady-state of maximum fluorescence, F'_m, and of fluorescence induced by the actinic light was reached. The actinic light was switched off and relaxation of the quenching of maximum fluorescence was recorded by applying further saturating light pulses (1 s) every 100 s for 10 min, when a new steady-state of 'relaxed' maximum fluorescence, F_{mr}, was obtained. Frequency of the weak modulated 'measuring light' was 1.6 kHz during 'dark' periods and 100 kHz during irradiation with actinic light and saturating pulses. From the initial maximum fluorescence, F_m and the value at the end of the actinic irradiation period, F'_m, total Stern–Volmer-type non-photochemical fluorescence quenching was calculated as $qN_{SV} = F_m/F'_m - 1$. An approximation for Stern–Volmer-type energy-dependent quenching, qE_{SV}, which depends on the trans-thylakoid proton gradient and the formation of zeaxanthin, was calculated from the extent of relaxation of maximum fluorescence during the 10 min dark period, $qE_{SV} = F_m/F'_m - F_m/F_{mr}$. For details about the measuring technique and modes of calculation see Thiele *et al.* (1997) and Krause and Jahns (2003).

Photochemical activity of PSI

Photooxidation of P700, the reaction centre chl of PSI, by far-red light (735 nm), was monitored by means of P700 absorbance changes at 810 nm (Klughammer and Schreiber 1998) with a dual-wavelength emitter-detector unit, ED-P700DW (Walz.), connected to a PAM 101/102/103 fluorometer system (Walz). For details of the method see Barth *et al.* (2001) and Krause *et al.* (2003*b*). From the light-response curve of P700 photooxidation, a 'saturation constant', K_s, was calculated, defined as the far-red intensity causing half-maximal absorbance change at 810 nm in the steady-state (i.e. maintaining 50% of P700 in the oxidized state). K_s values served as an indicator of photochemical activity of the PSI reaction centre (Barth *et al.* 2001). Measurements were performed with leaf disks (1.65 cm^2) ventilated with a moistened air stream in a temperature-controlled (24°C) cuvette.

CO_2 gas exchange

Photosynthetic net CO_2 assimilation and dark respiration were measured on attached leaves with a LI-6400 portable photosynthesis system (LI-COR). After exposing the plants to moderate PAR (50–500 μmol m^{-2} s^{-1}, depending on their light-acclimation state) for at least 30 min, light-response curves of CO_2 exchange were determined, using a light source with red and blue LED (6400-02B LED, LI-COR). Flow of ambient air (360–380 μL L^{-1} CO_2) passing through the leaf chamber was 250 or 500 μmol s^{-1}, and the temperature was controlled at 28°C. Light saturation of net CO_2 assimilation was reached at 400–500 μmol photons m^{-2} s^{-1} PAR. Light use efficiency of CO_2 assimilation was calculated from the initial linear slopes of the light-response curves obtained under strictly limiting light. Coefficients of linear regression of these slopes were r^2=0.98–1.00.

Results

Chlorophyll contents and chlorophyll a/b ratios

In response to direct sun exposure of shade-grown tropical tree seedlings for 0.5 or 1 h d^{-1}, simulating a small or medium-sized tree-fall canopy gap, chl $a+b$ contents of leaves did not decline significantly (Table 1). After longer exposure periods (approximately 9 h d^{-1} for *A. excelsum* and 4 h d^{-1} for *C. longifolium*), leaves exhibited a very pronounced decline of chl content, which decreased below levels determined in canopy sun leaves of tall trees (Table 1). As apparent from the increase in chl *a*/chl *b* ratios occurring in parallel to that reduction in chl content (Table 1), the chl $a+b$-binding light-harvesting complexes were diminished as a response to highly excessive light absorption. Qualitatively, there was no conspicuous difference between the response of the pioneer species, *A. excelsum*, and late-succession species, *C. longifolium*. Leaves of *A. excelsum* that developed in the deep shade of the greenhouse had similar chl contents and chl *a*/*b* ratios as leaves from seedlings of similar age growing in the forest understory (Table 1).

Carotenoid contents

Figure 1 shows alterations in carotenoid contents (on a chl $a+b$ basis) upon 1 h daily sun exposure of shade leaves of two pioneer species, *F. insipida* and *A. excelsum*. In both species, the most distinct change was a decrease in α-car and

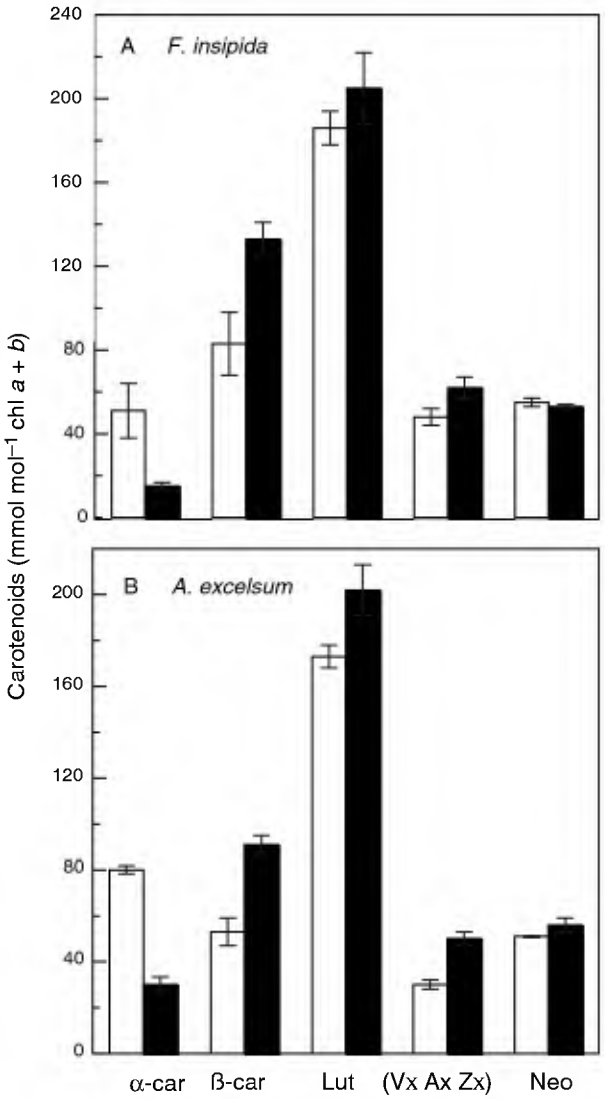

Fig. 1. Response of carotenoid levels in leaves of shade-grown seedlings of *F. insipida* (*A*) and *A. excelsum* (*B*) to 1 h d^{-1} direct sun exposure. Samples were taken after 30 d (*A*) or 18 d (*B*) of daily exposure (black bars). Control plants (white bars) were kept in deep shade throughout the experiment. Pigment contents are based on chl *a* + *b*. Means ± SD of $n = 3$ leaves from different plants are given.

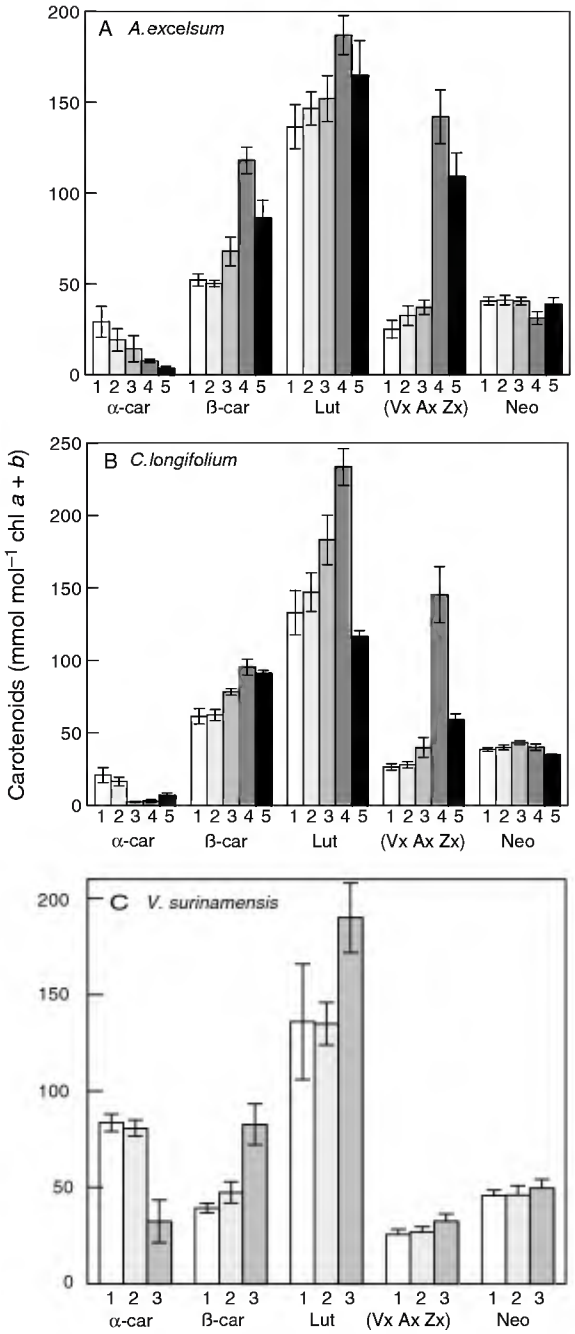

Fig. 2. Carotenoid contents of leaves in different states of light acclimation. Numbered bars denote different acclimation states. (*A*) Plants of *A. excelsum* grown in deep shade in the greenhouse (1), collected from the deeply shaded (2) or moderately shaded (3) forest understorey; shade-grown plants acclimated stepwise to whole-day (approximately 9 h d^{-1}) sun exposure (4); canopy sun leaves from tall trees (5). (*B*) Plants of *C. longifolium* kept in deep shade (1) or moderate shade (2) in the greenhouse and sun exposed for 0.5 h d^{-1} (3) or 4 h d^{-1} (4); canopy sun leaves from a mature tree (5). (*C*) Seedlings of *V. surinamensis* grown in deep shade of the greenhouse (1), acclimated to moderate shade (2) and sun-exposed for 1 h d^{-1} (3). Pigment contents are based on chl *a* + *b*. Means ± SDs are given for *n* leaf samples: *A*, $n = 3$–9; *B*, $n = 23$–24 (bars 1–3), $n = 4$–5 (bars 4, 5); *C*, $n = 20$.

increase in β-car contents. The sum of carotenes increased slightly. Additionally, there was a relatively small increase in Lut levels and a moderate, but significant increase in the sum of xanthophyll-cycle pigments (VxAxZx). The contents of neoxanthin (Neo) remained remarkably stable.

In Fig. 2*A*, the carotenoid contents in shade-grown leaves of the pioneer species *A. excelsum* after long-term full sun exposure (approximately 9 h d^{-1}) are depicted in comparison with either control plants kept in the shade or with leaves in different states of light acclimation from the forest. The decrease in the α/β-car ratio and increase in Lut content

in sun-acclimated shade-grown leaves were similar as after 1 h d^{-1} sun exposure (*cf.* Fig. 1*B*). However, there was a dramatic increase in the VxAxZx pool (based on chl), which even surpassed that of canopy sun leaves from the forest. As the chl content had decreased by approximately 60% (Table 1), the increase in VxAxZx based on leaf area was smaller but still substantial.

Carotenoid data from the two late-succession species are presented in Fig. 2*B*, *C*. The carotenoid content of shade-grown leaves of *C. longifolium* (Fig. 2*B*) was very similar to that found previously (Krause *et al.* 2003*a*) in leaves collected from the forest understorey. Brief midday sun exposure of shade-grown *C. longifolium* and *V. surinamensis* seedlings (0.5 h d^{-1} for 21 d or 1 h d^{-1} for 17 d, respectively) led to similar changes in the levels of the two carotenes as in the pioneer species (*cf.* Fig. 1). In leaves of *C. longifolium*, longer daily sun exposure (4 h d^{-1} for 3 months) caused, much like in *A. excelsum*, a strong increase in the VxAxZx pool size and, to a lesser extent, in Lut content reaching values above those measured in canopy sun leaves. Neo levels remained unaltered (Fig. 2*A–C*), except for a small decrease in fully sun-exposed leaves of *A. excelsum*. The strong increase in VxAxZx levels occurring upon long exposure periods does not seem to be an indiscriminate effect resulting from the decline in chl content, as indicated by the constancy of Neo and only moderate increases in β-car amounts (Fig. 2*A*, *B*).

Xanthophyll cycle activity

As shown for *F. insipida*, 1 h d^{-1} sun exposure for 30 d resulted in an increased xanthophyll cycle activity (Fig. 3). When transferred from shade to full sun at midday, faster formation of Zx + Ax from Vx occurred compared to plants not previously sun-exposed, yielding higher Zx + Ax levels, both expressed in percent of the VxAxZx pool (Fig. 3*A*) and on a chl basis (Fig. 3*B*). Moreover, re-conversion of Zx + Ax to Vx by epoxidation during the subsequent shade period proceeded faster in the plants that had experienced daily sun exposure (Fig. 3*A*, *B*). Similar results were obtained with seedlings of *A. excelsum* grown in deep shade and sun exposed for 1 h d^{-1} (data not shown).

Plants of both a pioneer (*A. excelsum*) and a late-succession species (*C. longifolium*) that had experienced full sunlight for prolonged periods per day, exhibited very high Zx levels around 120 mmol mol^{-1} chl *a* + *b*, when maximum de-epoxidation was tested in high PAR at midday (Fig. 4*A*). Only a small percentage of the VxAxZx pool was left as Vx, corresponding to an extremely high de-epoxidation state around 0.95 (Fig. 4*B*). As shown for comparison, young leaves of *C. longifolium*, which had developed after initiating the daily sun exposure, responded in a similar fashion as did the mature ('old') leaves. This is in agreement with a previous study (Krause *et al.* 1995) showing that young sun leaves of mature tropical trees with low chl content possess

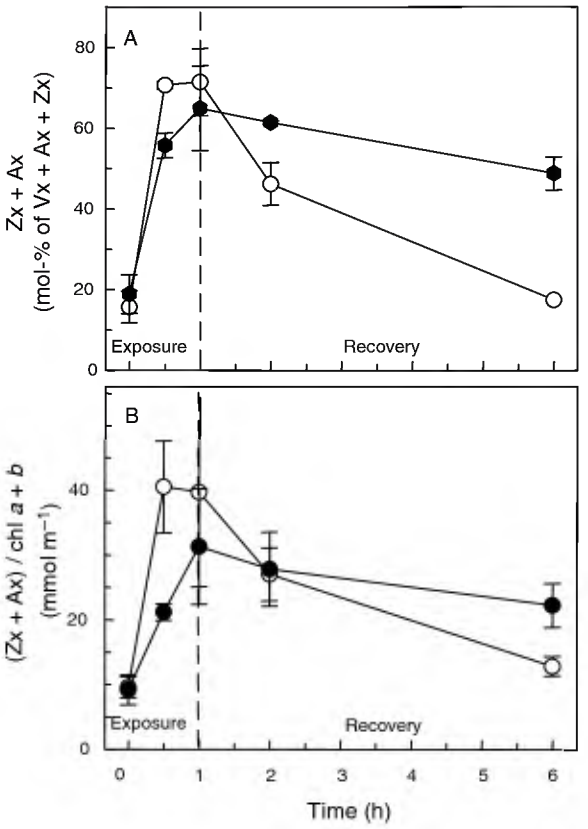

Fig. 3. Xanthophyll cycle activity induced by 1 h direct sun exposure at midday and subsequent 'recovery' in deep shade in shade-grown leaves of *F. insipida*. Plants acclimated to 1 h d^{-1} (for 30 d) sun exposure (○) are compared with control plants (●) kept continuously in deep shade. PAR dose during the 1 h exposure period on the day of the experiment was 7.0 ± 0.1 mol m^{-2}. Levels of zeaxanthin plus antheraxanthin (Zx + Ax) are given in mol-% of the VxAxZx pool (*A*) and on a chl *a* + *b* basis (*B*). Means ± SDs are presented (n = 3 leaf samples from different plants).

particularly large pools of xanthophyll-cycle pigments (on a chl *a* + *b* basis).

Photoinhibition of PSII

When shade leaves were sun-exposed for the first time around midday for 1 h, considerable photoinhibition of PSII occurred, as seen from a decline in potential PSII efficiency (F_v/F_m ratio). Photoinhibition, measured in leaves of *F. insipida* and *A. excelsum* (Fig. 5*A*, *B*), was reduced in plants that had been acclimated to such sun exposure, compared with non-acclimated control plants kept continuously in the shade. During the subsequent recovery period in deep shade, photoinhibition was reversed much faster in the acclimated than in the non-acclimated leaves (Fig. 5*A*, *B*). The faster recovery seemed to be associated with an enhanced rate of epoxidation of Zx + Ax (compare Figs 3 and 5*A*). The stronger photoinhibition effect in

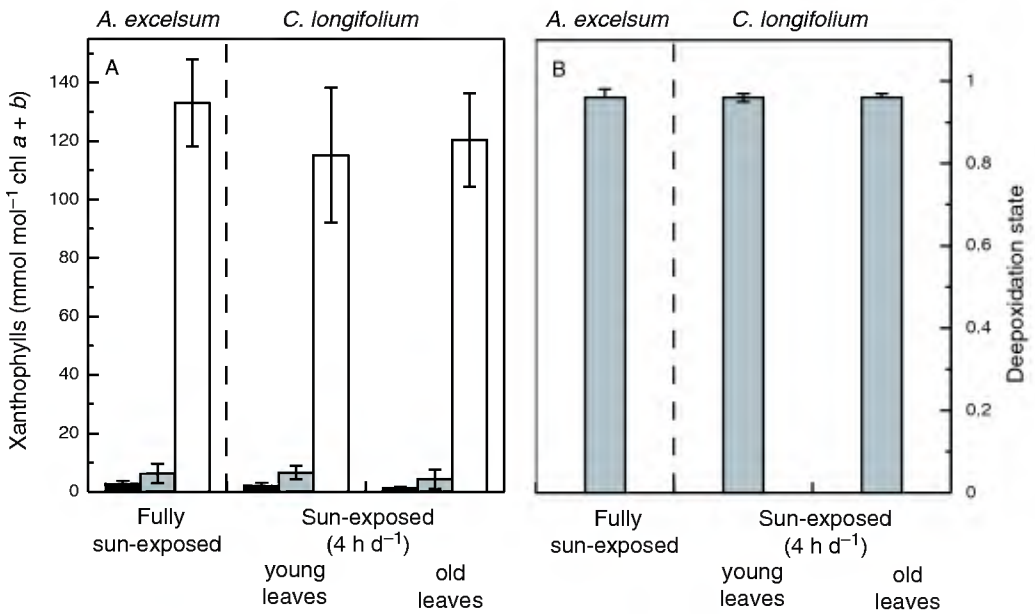

Fig. 4. Maximum degree of violaxanthin (Vx) de-epoxidation in shade-grown leaves after acclimation to full sunlight. Leaf samples of 9 h d^{-1} (7 weeks) sun-exposed plants of *A. excelsum* and 4 h d^{-1} (3 months) exposed plants of *C. longifolium* were taken around midday after approximately 3 h exposure to bright sunlight. (*A*) Xanthophyll-cycle pigments based on chl *a* + *b*: Vx (black bars), Ax (grey bars), Zx (white bars). (*B*) Deepoxidation state, defined as the molar ratio (Zx + 0.5 Ax) / (Vx + Ax + Zx), of leaf samples as for (*A*). Young leaves of *C. longifolium* had developed during the 3-month acclimation period to 4 h d^{-1} sun exposure. Means ± SDs are given (*A. excelsum*, $n = 9$; *C. longifolium*, $n = 8$). Light conditions at the time of sampling: *C. longifolium*, 2000–2400 μmol m^{-2} s^{-1} PAR; *A. excelsum*, 1900–2300 μmol m^{-2} s^{-1} PAR; 2.6–2.8 W m^{-2} UV-B; leaf temperature approximately 39°C, air temperature approximately 33°C.

Fig. 5*A* compared with Fig. 5*B* resulted from the higher photon dose received by leaves of *F. insipida* (see legend to Fig. 5).

Non-photochemical chl fluorescence quenching

The capacity of total non-photochemical fluorescence quenching (Stern–Volmer type), qN_{SV}, and of its 'energy-dependent' component, qE_{SV}, determined after 20 min under high PAR, was tested in leaves of *C. longifolium* (Fig. 6). Energy-dependent quenching is known to require a high trans-thylakoid proton gradient and is enhanced by the presence of Zx (Thiele and Krause 1994; Ruban and Horton 1995). Here, it was estimated, based on the portion of the total fluorescence decline that relaxes within 10 min in the dark. A proton gradient sustained in the dark (Gilmore and Björkman 1994) could be involved in the component of qN_{SV} that does not relax within 10 min. Control plants growing in deep or moderate shade were compared with plants exposed for 0.5 h d^{-1} and 4 h d^{-1} to direct solar radiation. Both quenching components increased substantially in leaves of exposed plants and reached highest levels after sun exposure had been prolonged to 4 h d^{-1}. However, the increase in quenching was not strictly proportional to the increase in xanthophyll-cycle pool size (see Fig. 2*B*), which increased moderately upon 0.5 h d^{-1}, but strongly upon 4 h d^{-1} exposure time.

Response of PSI

As described in a previous study (Barth *et al.* 2001), PSI responds to excessive light (under laboratory conditions) by a characteristic increase in the 'saturation constant', K_s, of photochemical P700 oxidation under far-red light (K_s was defined as the far-red intensity leading to half-maximal P700 oxidation in the steady-state). This phenomenon has been interpreted as an incipient and reversible photoinhibition of PSI that reflects enhanced thermal energy dissipation in the PSI reaction centre. It was shown that in contrast to sun leaves, the reversal of this effect under low light was very slow in shade leaves of tropical tree seedlings and not complete within 5 d after return to shade conditions (Barth *et al.* 2001). Figure 7 demonstrates that after acclimation of shade-grown leaves of *C. longifolium* to 4 h d^{-1} sun exposure, the K_s value of P700 quite closely followed the diurnal pattern of PAR; subsequent to a strong increase when the leaves became exposed to full solar radiation, the K_s values declined again in the afternoon with declining PAR, as would be expected for sun leaves. A similar response of PSI activity as in *C. longifolium* was observed during 1 h sun exposure of

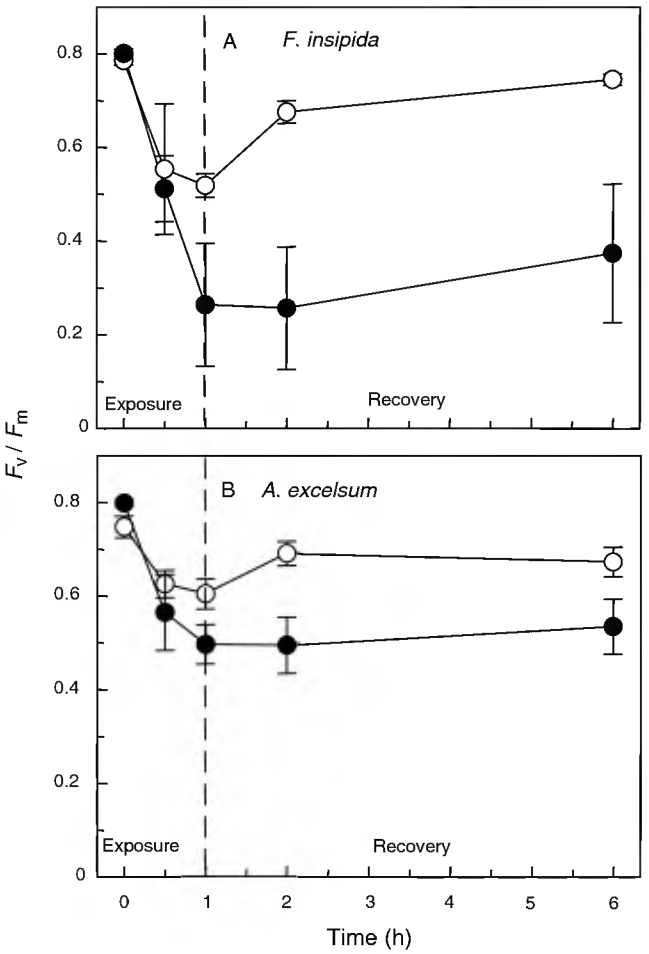

Fig. 5. Photoinhibition of PS11 during 1 h sun exposure and subsequent recovery in deep shade of shade leaves of *F. insipida* (*A*) and *A. excelsum* (*B*) acclimated (○) and non-acclimated (●) to daily 1 h exposure to direct sunlight for 30 d and 18 d, respectively. PAR doses received during the exposure period on the day of the measurement were (*A*) 7.0 ± 0.1 mol m^{-2} and (*B*) 3.0 ± 0.4 mol m^{-2}. Photoinhibition is expressed as decline (and subsequent recovery as increase) in the chl fluorescence ratio F_v/F_m of leaf disks (0.78 cm^2) removed at the specified times and dark-adapted for 10 min. Means ± SDs are given ($n = 3$ leaf samples from different plants).

A excelsum plants, which had experienced 1 h d^{-1} direct sunlight for 18 d (data not shown).

Ascorbate content

Figure 8 depicts the ascorbate (ascorbic acid, AsA) and AsA plus dehydroascorbate (DHA) contents (expressed on a fresh-weight basis) in leaves of the four species investigated. The AsA and AsA + DHA values varied considerably between species and did not show characteristic differences between pioneers (Fig. 8*A*, *B*) and late-succession plants (Fig. 8*C*, *D*). Relatively high proportions of DHA were seen in the pioneer species *F. insipida* and the late-succession species *V. surinamensis*. In all four species, sun exposure caused a substantial increase in AsA and/or AsA + DHA levels

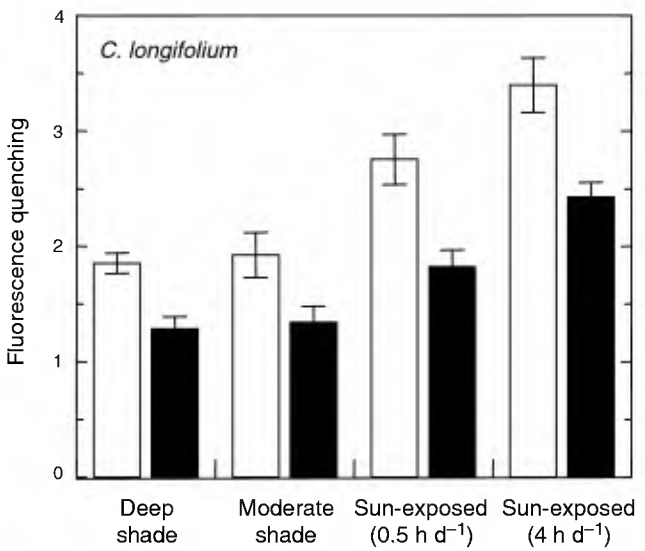

Fig. 6. Capacity of non-photochemical chl fluorescence quenching in shade-grown leaves of *C. longifolium* in different states of light acclimation. White bars: total non-photochemical quenching (qN_{sv}), black bars: energy-dependent quenching (qE_{sv}). Plants had been kept in deep or moderate shade or acclimated to 0.5 h d^{-1} (21 d) or 4 h d^{-1} (3 months) sun exposure (see Materials and methods). Leaf disks (1.65 cm^2) sampled in the morning were, after dark adaptation, irradiated for 20 min with 1500 μmol m^{-2} s^{-1} PAR. qN_{SV} was determined in the steady state at the end of the irradiation period. qE_{SV} was estimated as the quenching component relaxing during a subsequent 10-min dark period. Data are expressed as Stern–Volmer-type quenching. Means ± SDs are given (n = 3–6 leaf samples from different plants).

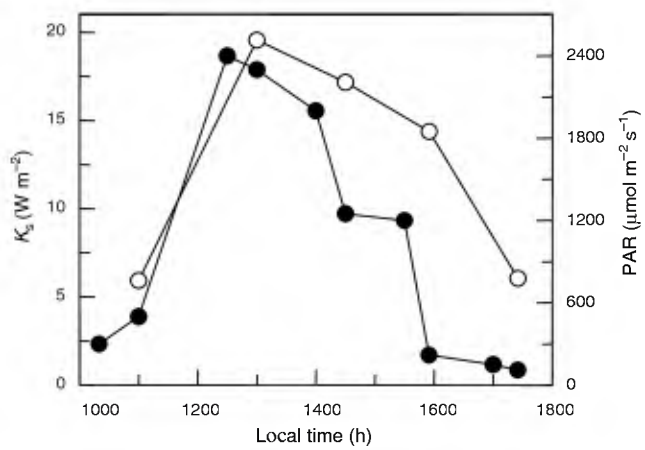

Fig. 7. Response of PS1 to 4 h sun exposure of shade-grown *C. longifolium* plants acclimated for 3 months to 4 h d^{-1} direct solar radiation. The increase in the 'saturation constant', K_s (i.e. the intensity of far-red light inducing half-saturation of P700 oxidation), denotes a decrease in photochemical efficiency of PS1. Leaf disks (1.65 cm^2) were removed for K_s determination (see Materials and methods) at the specified times. (○) K_s values; (●) incident PAR.

(based on fresh weight), compared with controls kept in deep shade (10–30 μmol m^{-2} s^{-1} PAR). Already in moderate shade (40–90 μmol m^{-2} s^{-1}PAR), slightly increased AsA levels were seen (Fig. 8*B–D*). Shade leaves of *A. excelsum* collected from the forest understory had higher AsA levels than those from the shaded greenhouse (Fig. 8*B*), possibly due to the effect of sun flecks in the forest. 'Fully sun-exposed' leaves of *A. excelsum* (approximately 9 h d^{-1} exposure time) did not reach the AsA level of outer canopy sun leaves of mature trees (Fig. 8*B*). By contrast, in shade-grown leaves of *C. longifolium*, sun exposure for 4 h d^{-1} led to higher AsA levels than found in canopy sun leaves (Fig. 8*D*).

UV-absorbing compounds

Canopy sun leaves of tropical trees are known to have a high content of secondary plant substances absorbing

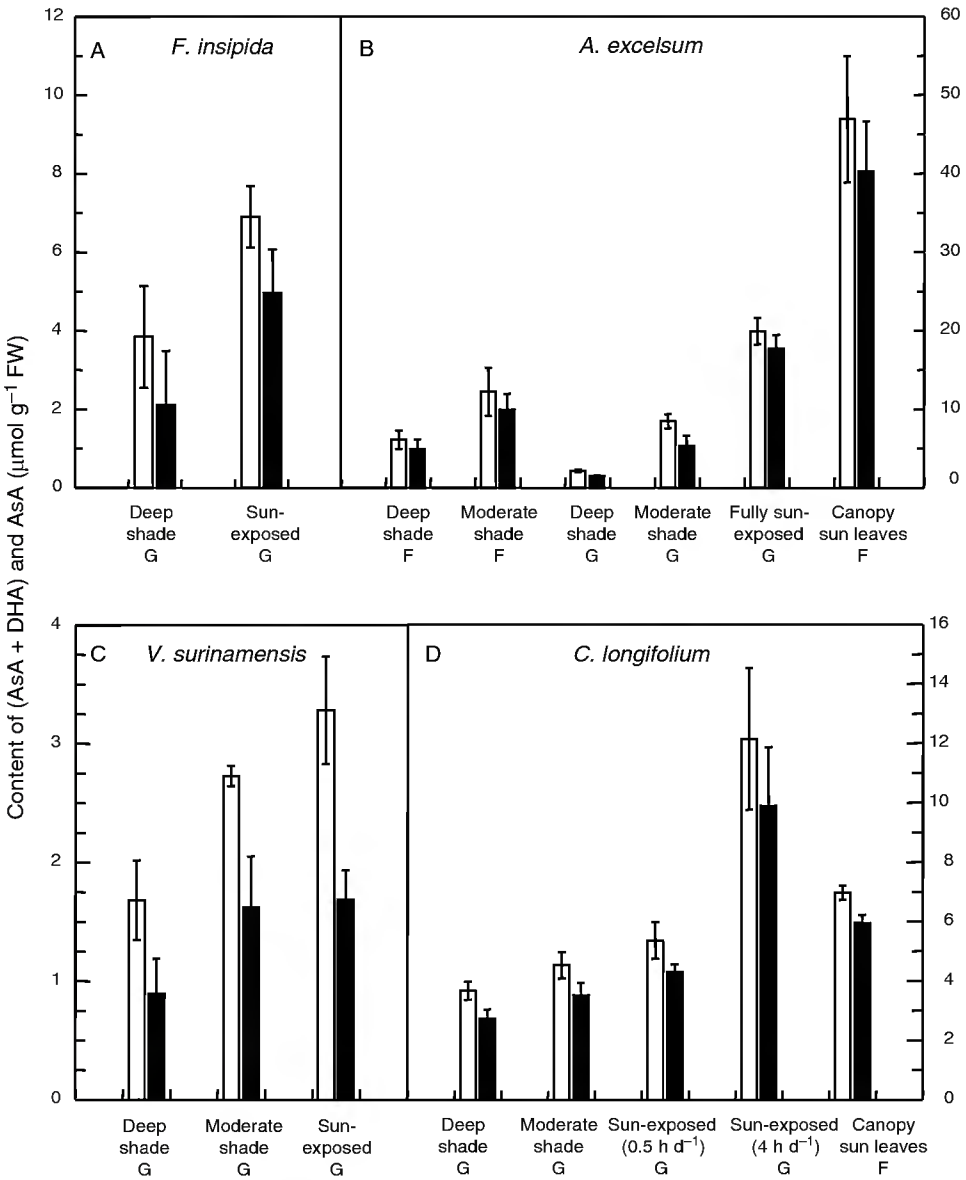

Fig. 8. Contents of ascorbate plus dehydroascorbate and ascorbate (based on fresh weight) in leaves of *F. insipida* (*A*), *A. excelsum* (*B*), *V. surinamensis* (*C*) and *C. longifolium* (*D*) in different states of light acclimation. Seedlings were grown in deep shade and in part acclimated to moderate shade in the greenhouse (G) and were sun-exposed daily for 1 h (*A*, 33 d; *C*, 17 d), approximately 9 h (*B*, 'fully sun-exposed' for 7 weeks) or 0.5 h (*D*, 21 d) and 4 h (*D*, 3 months). For comparison, leaves from the forest (F) were analysed, collected from the deeply and moderately shaded understorey (*B*) or outer canopy of tall trees (*B* and *D*). Sum of ascorbate plus dehydroascorbate (white bars); ascorbate (black bars). Means ± SDs are given ($n = 3$–7 leaf samples); note different scales of the four panels.

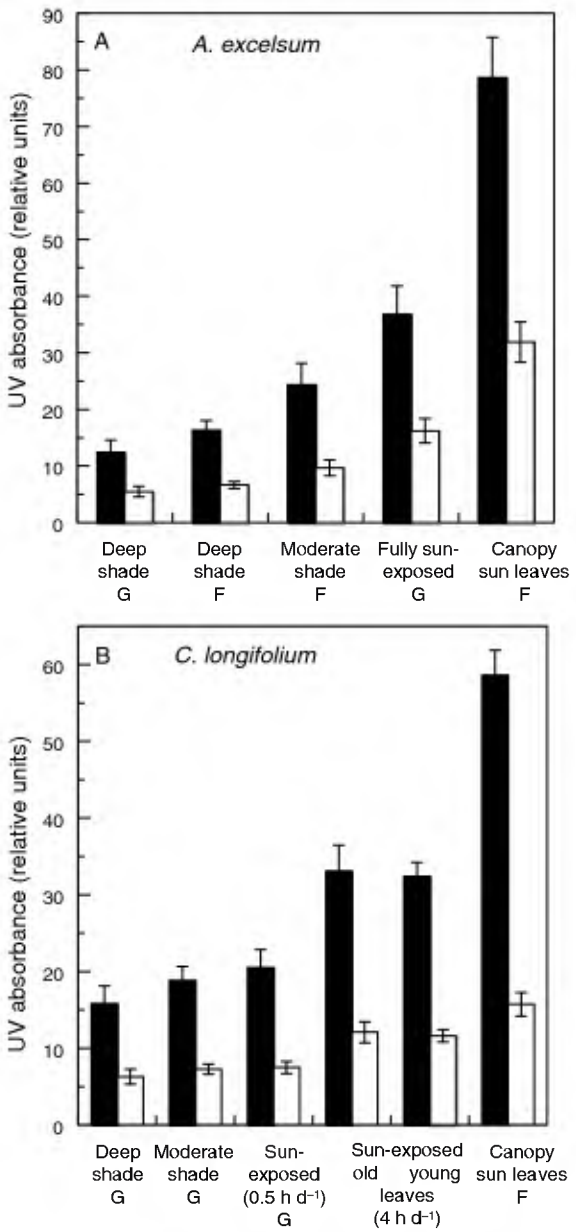

Fig. 9. Contents of soluble UV-absorbing compounds in leaves of *A. excelsum* (*A*) and *C. longifolium* (*B*) in different states of light acclimation. Seedlings were grown in deep shade and in (*B*) were acclimated to moderate shade in the greenhouse (G), and then were exposed daily to direct solar irradiation for 9 h, 'fully sun-exposed' (*A*) or 0.5 h and 4 h (*B*). Young leaves (*B*) had developed during the 4 h d^{-1} exposure period. For comparison, leaves from the forest (F) were analysed, obtained from the shaded understorey (*A*) and outer canopy of mature trees (*A, B*). Means ± SDs ($n = 4$–6) of relative absorbance values of leaf extracts (based on leaf area) are presented. Absorbance maximum (between 275 and 280 nm, black bars); absorbance at 305 nm (white bars).

UV light (Krause *et al.* 2003*a*). This is demonstrated in Fig. 9*A*, *B* (right-hand bar-pairs) for *A. excelsum* and *C. longifolium*. UV spectra of ethanolic–aqueous leaf extracts exhibited a high peak at approximately 275–280 nm and much lower absorbance at 305 nm, i.e. in the wavelength region relevant for UV-B protection. The absorbance peaks at 275–280 nm and absorbance values at 305 nm both increased upon sun exposure for long daily periods, but did not reach the high values of canopy sun leaves from the forest (Fig. 9*A*, *B*). Extracts from young leaves of *C. longifolium* that had developed during the high-light treatment, exhibited very similar absorbance values as mature ('old') leaves (Fig. 9 *B*). Short daily sun exposure did not induce synthesis of UV-absorbing compounds significantly in *A. excelsum* (not shown) and *C. longifolium* (Fig. 9*B*), but did so in a previous experiment with *V. surinamensis* (Krause *et al.* 1999*a*). Leaves of *A. excelsum* from the forest understorey showed higher contents of UV-absorbing substances than shade leaves from the greenhouse, as also found for AsA levels (*cf.* Fig. 8*B*).

Photosynthetic CO_2 assimilation

Net CO_2 exchange was measured with leaves of *C. longifolium* and *A. excelsum*. High-light acclimation of mature shade leaves of *C. longifolium* led to an increased capacity of net CO_2 assimilation determined under saturating PAR (Fig. 10), although maximum rates of sun leaves developed at an unshaded site (7–10 μmol CO_2 m^{-2} s^{-1})

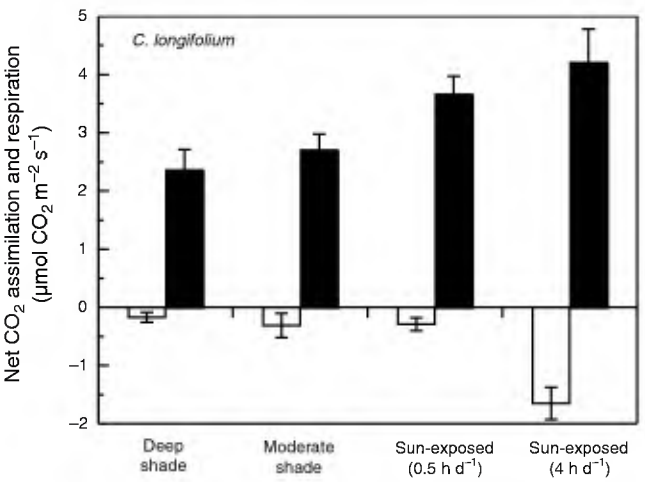

Fig. 10. Maximum rates of photosynthetic net CO_2 assimilation and rates of dark respiration in ambient air of *C. longifolium* leaves in different states of light acclimation. Rates were obtained from light-response curves determined on attached leaves of shade-grown seedlings maintained in deep shade and acclimated to moderate shade and daily sun exposure for 0.5 and 4 h, respectively. Light saturation of photosynthesis was reached at 400–500 μmol m^{-2} s^{-1} PAR. Negative values denote dark respiration. Light-use efficiency was 0.061 ± 0.002 in leaves from deep shade and 0.076 ± 0.010 in leaves of 4 h d^{-1} sun-exposed plants. Means ± SDs are given ($n = 5$–6 leaves).

were not reached. This effect was also observed with *A. excelsum* (data not shown). The light use efficiency of CO_2 assimilation, measured under strictly limiting PAR, was not decreased in leaves of *C. longifolium* (legend to Fig. 10), which received daily sun exposure for 4 h and in which chl content had strongly declined (*cf.* Table 1). Rates of respiration in these leaves were increased.

Discussion

The present study of four tropical tree species shows that leaves of shade-grown seedlings are, within limits, capable of acclimating to full sunlight, including solar UV-B radiation. Mature shade leaves of all four species exhibited acclimative responses towards characteristics of sun leaves. Conspicuous changes were observed in the composition and content of photosynthetic pigments (Table 1; Figs 1, 2). Direct sun exposure caused a strong decline in α-car and increase in β-car. Low α-car/β-car ratios are well known for sun leaves (see Introduction). Possibly, β-car located in the core antennae of both photosystems and in the reaction centre of PSII, due to its larger conjugated π-electron system, is better suited than α-car to convert excess excitation energy to heat. In contrast, α-car might be beneficial for accessory light absorption in shade leaves (*cf.* Krause *et al.* 2001). The increase in Lut, seen upon sun exposure, might improve photoprotection. It has been suggested that Lut plays a role in the qE mechanism of energy dissipation in PSII in addition to its structural functions in the chl *a*/*b* binding light-harvesting complexes (Niyogi *et al.* 2001). A general antioxidative function of Lut also appears feasible.

Short periods of daily sun exposure (1 h or 0.5 h) caused a moderate significant increase in the pool size of xanthophyll-cycle pigments (Figs 1, 2). As shown for *F. insipida*, in previously sun-exposed leaves the conversion of Vx to Zx + Ax under direct sunlight was enhanced, as was the re-conversion of Zx + Ax to Vx in the shade (Fig. 3). The improved photoprotection of these leaves, tested with *F. insipida* and *A. excelsum* (Fig. 5) was apparent from the reduced photoinhibition of PSII under direct sunlight and faster 'recovery' in the shade. As recorded in leaves of *C. longifolium*, the increased pool of VxAxZx was associated with an increased capacity of the qE component of non-photochemical chl fluorescence quenching (Fig. 6), indicating a higher capacity for harmless dissipation of excessively absorbed photon energy.

When daily sun exposure was extended to long periods of 4 or 9 h, drastic changes in pigment composition occurred, as observed with *C. longifolium* and *A. excelsum*. A large decline in chl *a* + *b* content of the leaves was accompanied by a substantial increase in chl *a*/*b* ratios, the latter reaching values identical with those of canopy sun leaves of mature trees (Table 1). Obviously, under long-lasting high-light stress, total chl content and number of light-harvesting complexes were adjusted to diminish excessive photon absorption. In contrast, when excessive light conditions prevailed for brief periods only, the shade-leaf characteristics of chl contents and chl *a*/chl *b* ratios were not significantly changed. Long periods of sun exposure led to a strong increase in the content of VxAxZx and to a lesser extent of Lut, based on chl *a* + *b* (Fig. 2). The extremely high deepoxidation state seen in high light (Fig. 4) indicates that approximately 95% of Vx is accessible to the violaxanthin deepoxidase. This value exceeds the 70–80% Vx turnover normally observed in sun leaves (Demmig-Adams 1998).

The capacity for total non-photochemical quenching and its qE component (in leaves of *C. longifolium,* Fig. 6) increased further, when sun exposure had been extended from 0.5 to 4 h d^{-1}; but the increase was much less than proportional to the VxAxZx pool. The high level of Zx present under excess light (Fig. 4*A*) suggests that, in addition to its function in the qE mechanism, which probably involves Zx binding to the chl *a*/*b* binding complexes of PSII, a considerable proportion of this xanthophyll resides freely in the thylakoid membrane. That fraction probably provides additional photoprotection. There is mounting evidence that Zx, besides promoting qE, exerts general antioxidative functions in the thylakoids (Havaux and Niyogi 1999).

The decline in the photochemical activity of PSI observed upon strong illumination of leaves has been suggested to represent a photoprotective response facilitating thermal energy dissipation (Barth *et al.* 2001). In shade-grown leaves of *C. longifolium* sun-exposed for 4 h d^{-1}, this effect, which was monitored by an increase in the 'saturation constant' (K_s) of P700 oxidation, exhibited sun-leaf characteristics. During the course of the day, the increase in K_s was quickly and completely reversed, when the leaves became shaded in the afternoon (Fig. 7), while in shade leaves such reversion was shown to be extremely slow (Barth *et al.* 2001). The level of the antioxidant AsA in tree leaves has been reported to increase with the average PAR received in the canopy (Hansen *et al.* 2002). In the present study, shade leaves of all four species tested responded to changes in light conditions with respect to their pools of AsA + DHA (Fig. 8). Compared with deep shade, moderately increased PAR (40–90 μmol m^{-2} s^{-1}) resulted in an increase in AsA + DHA. Direct sun exposure led to further increases in AsA + DHA, corroborating the importance of AsA and the associated reaction system (Asada 1994) in photoprotection.

Previous investigations have shown that in shade leaves of tropical tree seedlings, solar UV-B radiation may enhance photoinhibition of PSII and also affect PSI and photosynthetic CO_2 assimilation (Krause *et al.* 1999*a*, 2003*b*). Large differences in the contents of UV-absorbing compounds between shade and sun leaves of tropical plants *in situ* have been reported (Krause *et al.* 2003*a*). In the experiments with *C. longifolium* and *A. excelsum*, only long daily periods

(4 or 9 h) of direct sun exposure caused a substantial increase in the level of UV-B absorbing compounds in shade-grown leaves (Fig. 9). But the high levels of such substances present in canopy sun leaves were not achieved. In an earlier study (Krause *et al.* 1999*a*), a small increase in UV-absorbing compounds obtained by 0.5 h d^{-1} sun exposure of shade-grown plants of *Virola surinamensis* resulted in effective protection of PSII against solar UV-B. Probably relatively small amounts of protective substances suffice when they are accumulated in the adaxial leaf epidermis (Markstädter *et al.* 2001). As recently reported by Liakoura *et al.* (2003), shade leaves of Mediterranean woody plants were perfectly protected against UV-B by their epidermis, although leaf extracts exhibited much lower UV absorbance than those obtained from sun leaves. This shows that the absorbance of whole-leaf extracts does not necessarily correlate with UV protection. However, shade leaves of the present study had developed under substantially lower light than those investigated by Liakoura *et al.* (2003) and were known to be UV-B-sensitive. Thus, the increase in UV absorbance of whole-leaf extracts (Fig. 9) can be viewed as an indication of improved UV-B protection.

The increase in maximum rate of CO_2 assimilation observed as a long-term response to direct sun exposure of shade leaves (Fig. 10) shows that the shade leaves improved photosynthetic performance in response to increased light flux. This effect and the absence of a decline in light use efficiency of CO_2 assimilation indicate that the massive reduction in chl content occurring upon prolonged daily sun exposure (Table 1) is not associated with a general destruction of the photosynthetic apparatus. Leaf anatomy of the tree seedlings was not investigated. The fact that light-saturated rates of net CO_2 assimilation in the sun-exposed leaves remained below those of mature canopy sun leaves, might be due to limiting leaf thickness (*cf.* Oguchi *et al.* 2003).

Our study was not designed to document quantitative differences between pioneer and late-succession species in their acclimative responses. However, the investigation shows that mature shade leaves of both plant types adjusted physiologically to direct solar irradiation in similar fashion. Differences in acclimative responses between pioneer and late-succession plants are to be expected in leaves that develop under light conditions of forest gaps. In a recent study of tree seedlings growing in simulated tree-fall gaps of different sizes, fast-growing pioneer species exhibited a better adjustment of β-car levels and chl *a*/chl *b* ratios to light conditions and were less susceptible to photoinhibition of PSII than the late-succession plants (Krause *et al.* 2001).

In conclusion, our data demonstrate that leaves of tree seedlings of both pioneer and late-succession species that matured in the shade of the tropical forest understorey may be capable of markedly acclimating to direct solar visible and UV radiation when light flux strongly increases in the habitat of the plants. The acclimation allows them to maintain or even improve photosynthetic performance before new leaves develop under the altered light regime.

Acknowledgments

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