

# Acclimation of tropical tree seedlings to excessive light in simulated tree-fall gaps

G. H. KRAUSE,<sup>1</sup> O. Y. KOROLEVA,<sup>1</sup> J. W. DALLING<sup>2</sup> & K. WINTER<sup>3</sup>

<sup>1</sup>Institute of Plant Biochemistry, Heinrich Heine University Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany,

<sup>2</sup>Department of Plant Biology, University of Illinois, Champaign-Urbana, 265 Morrill Hall, 505 S. Goodwin Avenue, Urbana IL 61801, USA and <sup>3</sup>Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Ancon, Panama

## ABSTRACT

Acclimation to periodic high-light stress was studied in tree seedlings from a neotropical forest. Seedlings of several pioneer and late-succession species were cultivated under simulated tree-fall gap conditions; they were placed under frames covered with shade cloth with apertures of different widths that permitted defined periods of daily leaf exposure to direct sunlight. During direct sun exposure, all plants exhibited a marked reversible decline in potential photosystem II (PSII) efficiency, determined by means of the ratio of variable to maximum Chl *a* fluorescence ( $F_v/F_m$ ). The decline in  $F_v/F_m$  under full sunlight was much stronger in late-succession than in pioneer species. For each gap size, all species exhibited a similar degree of de-epoxidation of violaxanthin in direct sunlight and similar pool sizes of xanthophyll cycle pigments. Pool sizes increased with increasing gap size. Pioneer plants possessed high levels of  $\beta$ -carotene that also increased with gap size, whereas  $\alpha$ -carotene decreased. In contrast to late-succession plants, pioneer plants were capable of adjusting their Chl *alb* ratio to a high value in wide gaps. The content of extractable UV-B-absorbing compounds was highest in the plants acclimated to large gaps and did not depend on the successional status of the plants. The results demonstrate a better performance of pioneer species under high-light conditions as compared with late-succession plants, manifested by reduced photoinhibition of PSII in pioneer species. This was not related to increased pool size and turnover of xanthophyll cycle pigments, nor to higher contents of UV-B-absorbing substances. High  $\beta$ -carotene levels and increased Chl *alb* ratios, i.e. reduced size of the Chl *a* and *b* binding antennae, may contribute to photoprotection in pioneer species.

**Key-words:** Carotenoids; chlorophyll fluorescence; photoinhibition; photosystem II; UV-B-absorbing compounds; xanthophyll cycle.

**Abbreviations:** Ax, antheraxanthin; Car, carotene; Chl, chlorophyll;  $F_v/F_m$ , ratio of maximum variable to maximum total Chl *a* fluorescence yield; PAR, photosynthetically active radiation; PSI (II), photosystem I (II); UV-B, ultraviolet-B radiation (280–320 nm); Vx, violaxanthin; Zx, zeaxanthin.

Correspondence: G. H. Krause. Fax: +49 211 81 13706; e-mail: ghkrause@uni-duesseldorf.de

## INTRODUCTION

Green plants are capable of adjusting their photosynthetic apparatus, within limits of genetic constitution, to the light conditions experienced during growth. Acclimative features of leaves growing in sun or shade have been studied extensively (e.g. Anderson, Chow & Goodchild 1988; Öquist *et al.* 1992; Walters & Horton 1999; Barth, Krause & Winter 2001). Sun leaves usually possess a larger pool of xanthophyll cycle pigments than shade leaves and, in high light, exhibit faster kinetics and a higher degree of de-epoxidation of Vx to Ax and Zx (Demmig-Adams & Adams 1992a; Demmig-Adams 1998). Sun leaves also show lower ratios of  $\alpha$ -carotene ( $\alpha$ -Car) to  $\beta$ -carotene ( $\beta$ -Car) than shade leaves (Thayer & Björkman 1990; Demmig-Adams & Adams 1992a; Königer *et al.* 1995; Brugnoli *et al.* 1998; Demmig-Adams 1998). In some cases, increased  $\beta$ -Car and/or lutein levels, based on Chl *a* and *b*, have been observed upon acclimation to high light (e.g. Demmig-Adams & Adams 1996; Barker *et al.* 1998; Krause *et al.* 1999a).

In the tropics, plants frequently have to cope with extremely high sunlight, including ultraviolet (UV) radiation. This is true not only for outer-canopy sun leaves but also for shade-acclimated plants growing in the forest understorey. The latter possess a highly efficient light-harvesting system that permits net photosynthetic carbon gain in deep shade. However, these plants may have to endure periodic exposure to highly excessive light when the leaves are exposed to direct solar radiation in the form of large sunflecks (Watling *et al.* 1997), and even more so when tree-fall gaps are created (Krause & Winter 1996; Thiele, Krause & Winter 1998). Absorption of light in excess of photon utilization in photosynthesis may cause photoinhibition, as measured by a decline in potential efficiency of PSII. Reversible photoinhibition of PSII by direct exposure of leaves to natural sunlight has been observed in both canopy sun leaves (Krause, Virgo & Winter 1995) and leaves of plants growing in tree-fall gaps of the tropical forest (Krause & Winter 1996; Thiele *et al.* 1998). Ambient UV radiation contributes substantially to the photoinhibition of PSII in leaves of tropical plants exposed to full sunlight (Krause *et al.* 1999a). Compared with PSII, the capacity of PSI-driven electron transport is very stable in tropical plants under highly excessive light. However, a distinct decrease in the efficiency of far-red light to oxidize the PSI

reaction centre pigment (P700) has been discovered as a general response to strong illumination (Barth *et al.* 2001). The acclimation of tree seedlings of the tropical forest to the light conditions of tree-fall gaps results in a characteristic composition of photosynthetic pigments that is intermediate between those of deep-shade and sun leaves (Königer *et al.* 1995; Thiele *et al.* 1998).

The formation of gaps is important for maintaining the high diversity of plant species in tropical forests (Hubbell *et al.* 1999). The eventual occupants of gaps may be either fast-growing pioneer species that germinate in response to gap formation or shade-tolerant late-succession species present at the site as seedlings and saplings before the gap was formed. Differences in acclimation potential and susceptibility to photoinhibition in new gaps may be critical in determining the outcome of competition for space between these groups of species. To date, the different responses of the photosynthetic apparatus to specific light regimes in forest gaps in pioneer and late-succession trees have not been investigated in detail. The wide variation of light conditions within and between natural gaps makes it difficult to obtain conclusive information on putative differences in the acclimation between the two functional groups. Therefore, in the present study, seedlings of pioneer and shade-tolerant late-succession species were cultivated under controlled conditions that allowed direct sunlight exposure on clear days for defined periods, simulating narrow, medium and wide tree-fall gaps. To evaluate the performance and acclimation state of the leaves, the extent of photoinhibition of PSII during direct sun exposure and subsequent recovery in the shade was followed by means of Chl fluorescence recording. Leaf samples were taken during the course of the day to analyse chloroplast pigments and assess UV-B-absorbing substances.

## MATERIALS AND METHODS

Experiments were performed at Summit Botanical Garden, situated adjacent to the moist, seasonally dry lowland forest of Soberania National Park (9°05'N, 79°45'W) near Gamboa, Panama. Pigment analyses were carried out at the Institute of Plant Biochemistry, University Düsseldorf, Germany.

### Plant material

Seeds were collected on Barro Colorado Island (Gatun Lake, Panama) and germinated in flats containing forest soil in a growing house under approximately 10% full sunlight. The tree seedlings were transferred to 8-L-capacity, 30-cm-tall tree pots (Stuewe Sons Inc., Corvallis, OR, USA) filled with soil (without added fertilizer) obtained from a creek bed near Summit Botanical Garden. The pots were placed into artificial gaps constructed with steel frames that were covered (except for a central aperture) with shade cloth rated at 70% light interception and Gold Point ST7-SLT-60 dye-impregnated energy film (Gold

Point, Panama City, Panama). The shading simulated the reduced red to far-red ratio of the forest understorey (red/far-red transmittance = 0.15). The apertures were aligned in North–South direction. Details of this gap simulation method have been described earlier (Dalling *et al.* 1999). Three sizes of apertures were used (width 5.6, 16.0 and 31.9 cm), which resulted in the absence of cloud cover in direct sun exposure around midday for approximately 0.5, 1.5 and 3 h, respectively. This set-up simulated the light conditions in idealized circular forest gaps of 25 m<sup>2</sup> ('small gap'), 200 m<sup>2</sup> ('medium gap') and 800 m<sup>2</sup> ('wide gap').

Seedlings of three pioneer species and three late-succession species were chosen for the experiments. The pioneer species were: *Ficus insipida* Willd. (Moraceae), a fast-growing tree up to 30 or 40 m tall, abundant in younger forest, the seedlings requiring tree-fall gaps or clearings for growth; *Cecropia obtusifolia* Bertol. (Moraceae), a tree common in secondary moist forest in Panama, growing 5–10 m tall, and *Ochroma pyramidale* (Cav. Ex Lam.) Urban (Bombacaceae), a very fast-growing common tree at forest edges and in disturbed areas, rare in older forest, up to 30 m tall and known for its lightweight wood (balsa). The late-succession plants were *Drypetes standleyi* Webster (Euphorbiaceae) and *Quararibea asterolepis* Pitt (Bombacaceae), both found as trees of up to 30 m height in old forest, and *Faramea occidentalis* (L) A. Rich. (Rubiaceae), an abundant understorey treelet that grows to a height of around about 10 m (Croat 1978).

After 2 months' cultivation in the simulated gaps, measurements and the collection of leaf samples were started in the dry season (February 1999). The stems of the plants had reached heights of between 3 cm (*F. occidentalis* in wide gap) and 28 cm (*F. insipida* in wide gap), the heights of most plants ranging between 7 and 20 cm. Three to six plants of each species and gap width were available for the experiments, with the exception of *F. occidentalis* in the wide gaps, in which only the leaves of two plants survived.

### Recording of PAR, Chl *a* fluorescence and leaf temperatures

PAR (400–700 nm) was measured at leaf levels during the course of the day with a quantum sensor (LI-189B, Li-Cor, Lincoln, NE, USA). Leaf discs (diameter 8.5 mm) were punched out (at intervals given in Figs 1 & 2, lower panels) and darkened for 10 min, before  $F_v/F_m$ , which served as a measure of potential PSII efficiency, was determined. Earlier studies have shown that 10 min dark adaptation of pre-illuminated leaves is sufficient to relax  $\Delta$ pH-dependent fluorescence quenching (qE) and probably most of quenching related to state transition (qT). Thus, the decrease in  $F_v/F_m$  persisting after 10 min in the dark, compared with the  $F_v/F_m$  ratio of non-stressed control leaves, provides an approximate measure of photoinhibition of PSII (Giersch & Krause 1991; Krause & Weis 1991; Leitsch *et al.* 1994; Thiele, Winter & Krause 1997). The quenching related to photoinhibition is known to relax more readily in low light than in total darkness (Leitsch *et al.* 1994). A portable flu-

rometer system (PAM 2000; Walz, Effeltrich, Germany) equipped with a Poquet computer (Poquet Computer Corp., Santa Clara, CA, USA) was used for fluorescence recording. The measuring procedure has been described previously (Barth & Krause 1999).

Leaf temperatures were measured on the lower leaf surfaces with a leaf clip holder (2030-B) of the PAM 2000 system (Walz).

## Pigment analyses

Immediately after fluorescence recording, the leaf discs were frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  for later pigment analysis. Photosynthetic pigments were quantified by high-performance liquid chromatography (HPLC) according to a published method (Färber *et al.* 1997). For the calculation of  $\alpha$ -Car content, the same conversion factor (peak area per pmol) as for  $\beta$ -Car, determined by Färber *et al.* (1997), was used. UV-absorbing compounds were extracted from leaf discs (diameter 8.5 mm), ground in a mortar in the presence of 80% (v/v) ethanol (final volume 3 mL). After centrifugation (20 400 g), the pellet was extracted consecutively with 1 mL of 60%, 40% and 20% (v/v) ethanol. The maximum absorbance of the extracts (between 270 and 280 nm) in the UV spectral region was taken as a relative measure of the content of UV-absorbing substances. Qualitatively similar data (but with higher standard deviations) were obtained when the absorbance in the spectral region between 290 and 320 nm – relevant for solar UV-B reaching the earth surface – was integrated (see also Krause *et al.* 1999a).

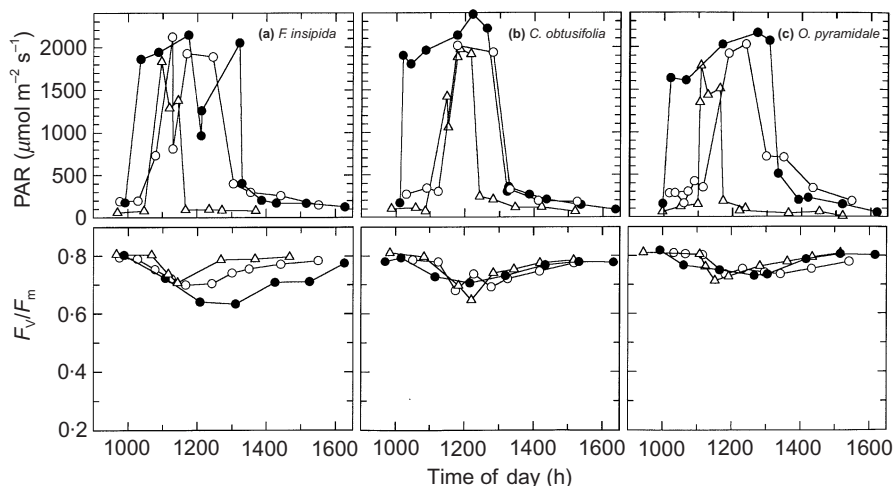
## RESULTS

Leaf temperatures measured during direct sun exposure varied between 33 and 39  $^{\circ}\text{C}$  and tended to be slightly lower in pioneer than in late-succession species. Air temperatures within the simulated gaps were several degrees lower (31–

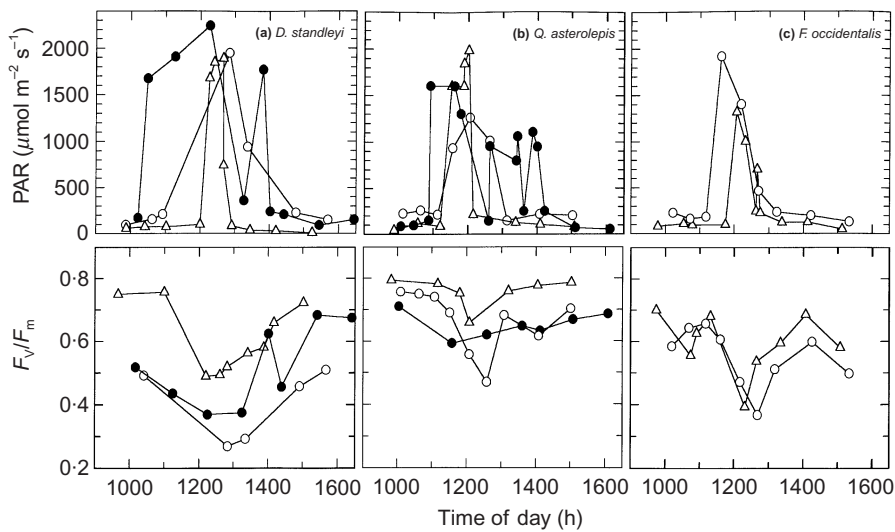
37  $^{\circ}\text{C}$ ), while ambient air temperatures around midday were between 28 and 33  $^{\circ}\text{C}$ .

During periods of direct sun exposure (Figs 1 & 2, upper panels), all plants exhibited significant photoinhibition of PSII – i.e. a decline in potential PSII efficiency (decrease in  $F_v/F_m$  ratio) that persisted after 10 min dark adaptation of leaf samples (Figs 1 & 2, lower panels). The decline in PSII efficiency was detected just a few minutes after direct sunlight had reached the leaves. There was no correlation between gap width and maximum extent of photoinhibition. Recovery from photoinhibition (increase in  $F_v/F_m$  ratio) started in all tested leaves soon after the end of the sun exposure period (compare the upper and lower panels in Figs 1 & 2). However, there was a distinct difference between pioneer and late-succession seedlings in the response to high light. In the leaves of pioneers (Fig. 1),  $F_v/F_m$  ratios were close to 0.8 in the morning, which is characteristic of non-stressed leaves. During direct sun exposure,  $F_v/F_m$  ratios declined much less than in the late-succession plants. The smallest decrease in  $F_v/F_m$  was observed in *O. pyramidale*, a pioneer tree occurring mostly in large gaps or clearings. In the late afternoon, the leaves of the pioneers had recovered completely. In contrast, in leaves of late-succession seedlings (Fig. 2),  $F_v/F_m$  ratios were already substantially below 0.8 in the morning (with the exception of *Q. asterolepis* in the small gap), indicating a ‘chronic’ photoinhibition of PSII. During sun exposure,  $F_v/F_m$  values declined strongly and considerable photoinhibition persisted in the afternoon. *Quararibea asterolepis* exhibited a fast leaf movement in response to high light. Within a few minutes of the start of direct sun exposure, the leaves tilted towards the stem into a vertical position. This reduced the effective PAR (Fig. 2b, upper panel) in comparison with the other species. Nevertheless, the  $F_v/F_m$  ratio in leaves of *Q. asterolepis* declined strongly.

The changes in  $F_v/F_m$  were accompanied by the turnover of the xanthophyll cycle pigments, Vx, Ax and Zx. High levels of Ax + Zx were formed by de-epoxidation of Vx during direct sun exposure and declined again during the following



**Figure 1.** Response of leaves of three pioneer species to direct sun exposure in simulated tree-fall gaps of different sizes, allowing exposure periods around midday of approximately 0.5 ('small gap'), 1.5 ('medium gap') and 3 h ('wide gap'), respectively. (a) *Ficus insipida*; (b) *Cecropia obtusifolia*; (c) *Ochroma pyramidale*. Upper panels: PAR measured at leaf level during the course of the day. Lower panels:  $F_v/F_m$  ratios (determined after 10 min dark adaptation) indicating the potential efficiency of photosystem II. Symbols:  $\Delta$ , small gap;  $\circ$ , medium gap;  $\bullet$ , wide gap.



**Figure 2.** Response of leaves of three late-succession species to direct sun exposure in simulated tree-fall gaps of different sizes. (a) *Drypetes standleyi*; (b) *Quararibea asterolepis*; (c) *Faramaea occidentalis*. Upper panels: PAR measured at leaf levels during the course of the day. Lower panels:  $F_v/F_m$  ratios determined after 10 min dark adaptation. Symbols:  $\Delta$ , small gap;  $\circ$ , medium gap;  $\bullet$ , wide gap.

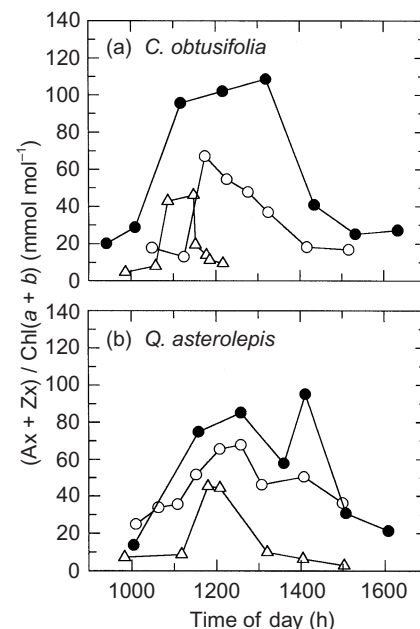
shade period. Based on Chl  $a + b$  content, the levels of Ax + Zx accumulating under direct sunlight depended on the gap width to which the plants were accustomed. There were no significant differences between pioneer and late-succession plants. In Fig. 3, the responses of Ax + Zx levels in the leaves of a pioneer (*C. obtusifolia*) and late-succession (*Q. asterolepis*) plant are depicted as examples (other data not shown). A remarkable adjustment of the pool sizes of xanthophyll cycle pigments, based on Chl content, to the light conditions in differently sized gaps was observed (Fig. 4). All plants exhibited a significantly larger pool of Vx + Ax + Zx in the wide compared with the small gaps. Again, no differences between pioneers (Fig. 4a–c) and late-succession species (Fig. 4d–f) could be detected.

The three pioneer species were characterized by high  $\beta$ -Car contents, expressed on a Chl  $a + b$  basis, and low  $\alpha/\beta$ -Car ratios. Acclimation to increasing gap size resulted in decreased levels of  $\alpha$ - and increased levels of  $\beta$ -Car, whereas the total carotene content remained approximately constant (Fig. 5a–c). Leaves of the late-succession species (Fig. 5d–f) exhibited lower  $\beta$ -Car levels than the pioneers (with the exception of *D. standleyi* in small gaps, Fig. 5d), and the  $\alpha/\beta$ -Car ratios were correlated less closely with the gap size. Also, the total carotene content in late-succession species was lower than that in pioneer plants (Fig. 5d–f).

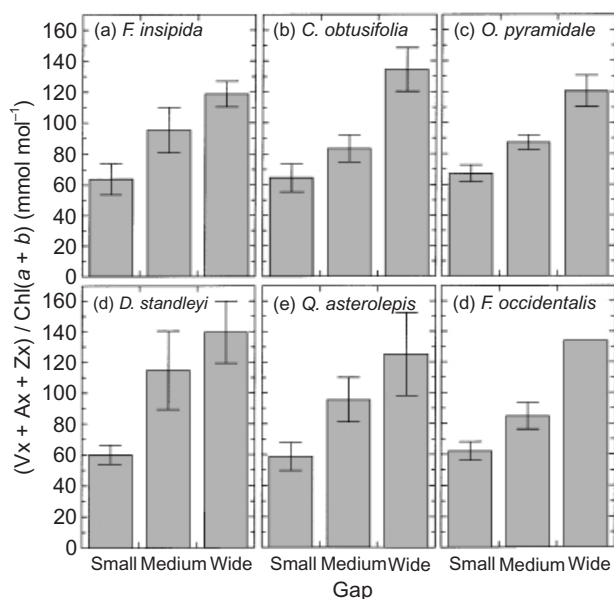
Figure 6 indicates that the pioneer species were more efficient than the late-succession plants in adjusting their Chl  $a/b$  ratios to the light regimes prevailing in the gaps. In all three pioneers, the Chl  $a/b$  ratio increased with gap size and reached average values of over 3.0 in the wide gaps (Fig. 6a–c). Such acclimation was not seen in the late-succession plants (Fig. 6c–f), except for a tendency in *F. occidentalis* (Fig. 5f); in the three species tested, the Chl  $a/b$  ratio remained significantly below 3.0.

Further data on photosynthetic pigments are given in Table 1. The Chl  $a + b$  content per unit leaf area varied with leaf age: younger leaves of light-green appearance exhib-

ited lower Chl contents (e.g. *D. standleyi* in the medium gap, *Q. asterolepis* in the wide gap) than older, dark-green leaves. However, this variation did not seem to influence Chl  $a/b$  ratios (cf. Fig. 6). The contents of lutein based on Chl (Table 1) were considerably higher in the late-succession than in the pioneer species. Moreover, lutein levels tended to increase with gap size in leaves of the late-succession plants, which was not observed in the pioneers. Neoxanthin to Chl  $a + b$  ratios (Table 1) did not vary with light conditions, but tended to be larger in the late-succes-



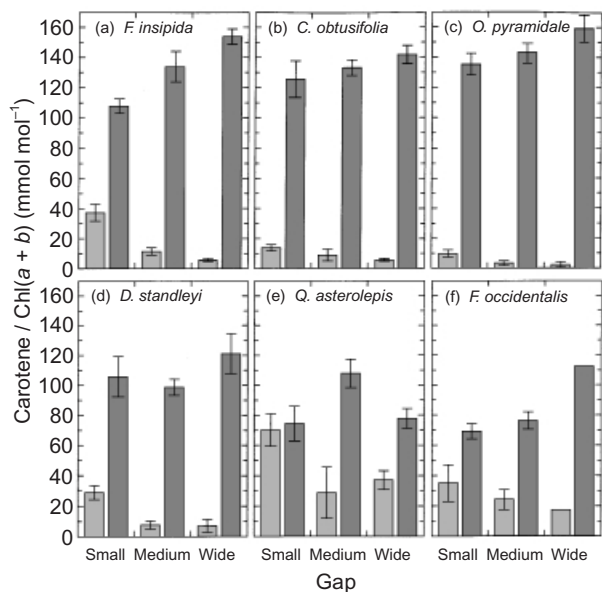
**Figure 3.** Changes during the course of the day in the content of de-epoxidized xanthophylls (Ax + Zx) in response to direct sun exposure in leaves of (a) the pioneer species *C. obtusifolia* and (b) the late-succession species *Q. asterolepis*, growing in simulated tree-fall gaps of different sizes. Symbols:  $\Delta$ , small gap;  $\circ$ , medium gap;  $\bullet$ , wide gap.



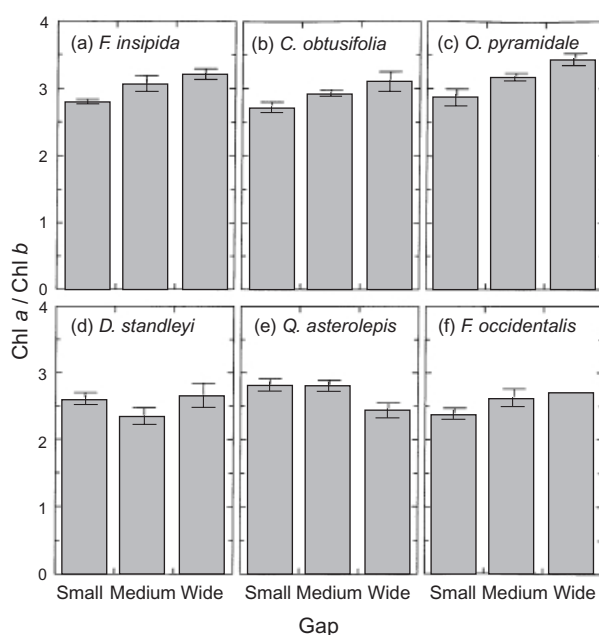
**Figure 4.** Pool sizes of xanthophyll cycle pigments in leaves of (a–c) three pioneer species and (d–f) three late-succession species acclimated to simulated gaps of different sizes. Bars represent the sum of Vx, Ax and Zx. Mean values  $\pm$  standard deviations (SD) of five to 10 leaf samples (mean of two samples for *F. occidentalis*, wide gap) are given.

sion seedlings grown in medium-sized and wide gaps than in the pioneer plants grown under the same conditions.

The spectra of ethanolic/aqueous leaf extracts showed significantly higher contents of soluble UV-B-absorbing



**Figure 5.** Content of  $\alpha$ - and  $\beta$ -Car in leaves of (a–c) three pioneer species and (d–f) three late-succession species acclimated to artificial gaps of different sizes. Means  $\pm$  SD are depicted for five to 10 leaf samples (means of two samples for *F. occidentalis*, wide gap). Lighter bars,  $\alpha$ -Car; darker bars,  $\beta$ -Car.



**Figure 6.** Chlorophyll *a/b* ratios in leaves of (a–c) three pioneer species and (d–f) three late-succession species acclimated to simulated gaps of different sizes. Data are given in molar ratios. Bars represent means  $\pm$  SD for five to 10 leaf samples (mean of two samples for *F. occidentalis*, wide gap).

substances per unit leaf area in wide compared with small gaps. This was found in the leaves of the three pioneer species (Fig. 7a–c) and in two late-succession species (Fig. 7d,e). Leaves of *F. occidentalis* in wide gaps could not be analysed due to lack of material. The data did not reveal a difference between the two ecotypes in the amounts of extractable UV-B-absorbing compounds.

The growth responses of the seedlings were not investigated systematically. From visual appearance, the pioneer plants grew considerably faster and produced larger and more numerous leaves than the late-succession plants during the 2 month cultivation period (cf. Dalling *et al.* 2001). The pioneer plants grew fastest in the wide gap, and still faster than the late-succession plants in the small gap. The late-succession plants did not show much dependence of growth on gap size, with the exception of *F. occidentalis* in the wide gap (3 h direct sun exposure), in which seedlings remained small and the leaves of all but two seedlings were lost due to severe photodamage.

## DISCUSSION

The data presented here reveal characteristic differences between seedlings of pioneer plant species and shade-tolerant late-succession seedlings in their response to varying light conditions in simulated, differently sized tree-fall gaps. However, certain acclimative alterations of pigment content induced by the light stress experienced in the simulated gaps were similar in the leaves of all species studied.

The constitutive adaptation of pioneer species to high

**Table 1.** Content of Chl *a + b*, lutein (Lut) and neoxanthin (Neo) in the leaves of three pioneer species (*F. insipida*, *C. obtusifolia* and *O. pyramidale*) and three late-succession species (*D. standleyi*, *Q. asterolepis* and *F. occidentalis*) acclimated to simulated gaps of different sizes. The data are means  $\pm$  SD for five to 10 leaf samples (means of two samples for *F. occidentalis*, wide gap)

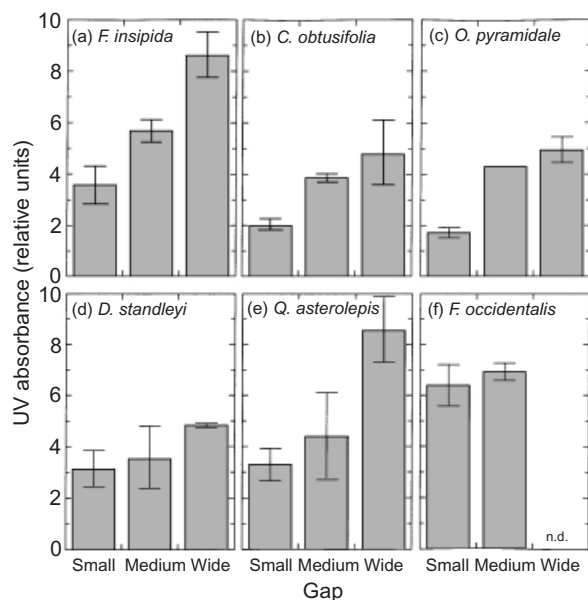
	Gap	<i>F. insipida</i>	<i>C. obtusifolia</i>	<i>O. pyramidale</i>	<i>D. standleyi</i>	<i>Q. asterolepis</i>	<i>F. occidentalis</i>
Chl <i>a + b</i> ( $\mu\text{mol m}^{-2}$ )	Small	338 $\pm$ 25	301 $\pm$ 35	295 $\pm$ 63	263 $\pm$ 53	416 $\pm$ 36	315 $\pm$ 44
	Medium	334 $\pm$ 46	396 $\pm$ 27	329 $\pm$ 38	195 $\pm$ 49	266 $\pm$ 27	325 $\pm$ 40
	Wide	383 $\pm$ 20	381 $\pm$ 30	348 $\pm$ 34	257 $\pm$ 31	225 $\pm$ 33	221
Lut/Chl <i>a + b</i> ( $\text{mmol mol}^{-1}$ )	Small	180 $\pm$ 10	165 $\pm$ 9	164 $\pm$ 8	205 $\pm$ 16	191 $\pm$ 18	245 $\pm$ 17
	Medium	156 $\pm$ 14	162 $\pm$ 11	154 $\pm$ 8	326 $\pm$ 35	234 $\pm$ 24	270 $\pm$ 18
	Wide	172 $\pm$ 7	174 $\pm$ 7	160 $\pm$ 5	291 $\pm$ 23	243 $\pm$ 14	290
Neo/Chl <i>a + b</i> ( $\text{mmol mol}^{-1}$ )	Small	54 $\pm$ 4.6	52 $\pm$ 6.1	51 $\pm$ 4.3	61 $\pm$ 3.5	54 $\pm$ 3.0	62 $\pm$ 5.1
	Medium	50 $\pm$ 3.2	51 $\pm$ 5.6	53 $\pm$ 2.5	72 $\pm$ 4.9	56 $\pm$ 4.5	60 $\pm$ 3.0
	Wide	52 $\pm$ 2.7	51 $\pm$ 3.7	49 $\pm$ 5.9	64 $\pm$ 4.7	61 $\pm$ 7.1	63

light was reflected by the low degree of photoinhibition of PSII occurring during periods of direct sun exposure (Fig. 1). In comparison, the late-succession species showed a much stronger decline in potential PSII efficiency during sun exposure and also chronic photoinhibition, persisting overnight. (Fig. 2). In earlier studies on PSII photoinhibition in natural forest gaps on Barro Colorado Island (located close to the site of the present study), clear differences between pioneers and various understorey plants including late-succession trees were not detected (Krause & Winter 1996; Thiele *et al.* 1998). This may be explained by the great variance in light conditions within a gap, depending on its size, density of vegetation, height of surrounding

trees, location of plants within the gap and orientation of their leaves.

Photoinhibition of PSII observed here may include an inactivation of the D1 protein in the PSII reaction centre, followed by degradation and resynthesis of this protein (see Aro *et al.* 1993). However, in several earlier studies, the photoinhibitory decline in the  $F_v/F_m$  ratio has been found to be unrelated, at least in part, to D1 protein inactivation (Leitsch *et al.* 1994; Thiele *et al.* 1996, 1997). D1 protein inactivation appeared to be low or negligible, particularly in plants that were well acclimated to high-light conditions and possessed a high pool size and turnover activity of the xanthophyll cycle (Thiele *et al.* 1996, 1997). It was observed both in the laboratory (Thiele *et al.* 1996) and in natural tree-fall gaps (Thiele *et al.* 1998) that recovery from photoinhibition during a first phase of about 1 h was closely related to reconversion of Zx via Ax to Vx in the xanthophyll cycle. This suggested that the photoinhibition of PSII in nature is to a large extent caused by a persistent binding of Zx to light-harvesting Chl *a* and *b* binding protein complexes and represents a photoprotective down-regulation of PSII that facilitates thermal dissipation of excitation energy in addition to the fast-relaxing  $\Delta\text{pH}$ -dependent quenching ('qE mechanism'). The latter is induced by the build-up of a high *trans*-thylakoid proton gradient and is strongly facilitated by the formation of Zx and, possibly, of Ax (Demmig-Adams & Adams 1992b; Pfündel & Bilger 1994; Horton, Ruban & Walters 1996; Gilmore 1997). As expected, exposure to direct sunlight induced the formation of high levels of Zx + Ax from Vx via the xanthophyll cycle (Fig. 3). Surprisingly, pioneer and late-succession species differed neither in the size of their pools of xanthophyll cycle pigments nor in the degree of de-epoxidation in high light (Figs 2 & 3). All species tested exhibited a similar increase in the pool size of xanthophyll cycle pigments (based on Chl) and, accordingly, accumulated similar amounts of Zx + Ax in high light, depending on the size of the simulated gap.

The amounts of extractable UV-B-absorbing compounds (expressed per unit leaf area) increased with gap size (Fig. 7). These substances consist mostly of flavonoids and related compounds that are thought to be concentrated in the adaxial leaf epidermis (Teramura & Sullivan 1994;



**Figure 7.** Relative amount of extractable UV-B-absorbing substances (measured at the peak of absorbance between 270 and 280 nm) in leaves of (a–c) three pioneer species and (d–f) three late-succession species acclimated to simulated gaps of different sizes. Data are given in relative absorbance units referred to leaf area and represent means  $\pm$  SD for three samples from different leaves (n.d., not determined).



Teramura & Ziska 1996). There were differences between species in the content of these UV-B-protecting substances: in wide gaps, higher levels were found in leaves of *F. insipida* and *Q. asterolepis* than in *C. obtusifolia*, *O. pyramidale* and *D. standleyi*, but significant differences between pioneer and late-succession species were not found (Fig. 7). The data indicate that the tree species studied were capable of adjusting UV-B protection according to the severity of direct sunlight exposure, irrespective of successional status.

The higher degree of photoinhibition observed in the leaves of late-succession plants than in those of pioneer species indicates that the former experience higher light stress under comparable light conditions. This may be based on several factors:

- 1 Pioneer species of tropical forests are well known to have much higher capacities of photosynthetic CO<sub>2</sub> assimilation than shade-tolerant species (e.g. Kitajima 1994; Poorter 1999). This should diminish the fraction of excessive light during sun exposure. As higher rates of photosynthesis are associated with increased transpiration, lower leaf temperatures might have contributed to reduced photoinhibition in the pioneer plants.
- 2 The pioneer plants studied here exhibited a significant increase in the levels of  $\beta$ -Car with gap size, while  $\alpha$ -Car levels declined; higher  $\beta$ -Car levels were found in the leaves of the pioneers than in those of late-succession plants developed in the wide gaps (Fig. 5). Since shade leaves are known to possess roughly equal amounts of  $\alpha$ - and  $\beta$ -Car and sun leaves very low  $\alpha/\beta$ -Car ratios (see Introduction), one may speculate that due to its larger conjugated  $\pi$ -electron system,  $\beta$ -Car might contribute efficiently to photoprotection, whereas  $\alpha$ -Car functions as a light-collecting pigment. A strong decline in the  $\alpha/\beta$ -Car ratio was observed when shade-grown seedlings of *Anacardium excelsum*, an intermediate between pioneer and late-succession trees, were acclimated to 1 h daily sun exposure in the presence and absence of ambient UV-B for 7 d (Krause *et al.* 1999a).
- 3 The leaves of the pioneer species responded to the prolonged sun exposure in wider gaps with a significant increase in Chl *a/b* ratios, which was not observed in the late-succession plants (Fig. 6). In the wide gaps, the leaves of pioneers, but not those of late-succession plants, exhibited Chl *a/b* ratios above 3.0. Increased Chl *a/b* ratios indicate a lowered amount of peripheral Chl *a* and *b* binding, light-harvesting complexes, resulting in a decreased absorption cross-section of PSII and, consequently, diminished light stress under a given PAR. However, it should be noted that photoinhibition in seedling leaves of late-successional species may not be representative of the light responses of sun-exposed leaves in mature canopies. Reduced photoinhibition in canopy sun leaves probably results from increased photosynthetic capacities concomitant with decreased specific leaf areas.

The physiological significance of higher lutein levels and the tendency for higher neoxanthin contents in the leaves of

late-succession plants in comparison with pioneers (Table 1) is difficult to evaluate at present. Increases in lutein levels occurring in response to light stress have been reported before (Krause, Carouge & Garden 1999b; Korolova, Krause & Brüggemann 2000; see also Introduction). Photoprotective functions of lutein have been discussed in conjunction with studies of *Chlamydomonas reinhardtii* and *Arabidopsis thaliana* mutants lacking Zx and/or lutein (Pogson *et al.* 1996, 1998; Niyogi, Björkman & Grossman 1997; Niyogi 2000).

In conclusion, the present study shows that seedlings of both pioneer and late-succession tree species of a moist tropical lowland forest are capable of similar acclimation to high-light stress under direct solar irradiation with respect to pool sizes, the turnover of xanthophyll cycle pigments and the content of UV-B protectants. Increased levels of  $\beta$ -Car and high Chl *a/b* ratios may provide additional photoprotection to the pioneer plants and contribute, alongside known physiological and morphological traits (Kitajima 1994; Poorter 1999), to the superior performance of pioneer species in high light.

## ACKNOWLEDGMENTS

The authors thank Aurelio Virgo, Ingrid Prikulis and Nicola Bergmann-Karaboue for assistance in data processing, and the staff of the Municipality of Panama City for assistance in running the experiment at Summit Gardens. The study was supported by the Deutsche Forschungsgemeinschaft (SFB 189), the Smithsonian Tropical Research Institute and the Andrew W. Mellon Foundation.

## REFERENCES

- Anderson J.M., Chow W.S. & Goodchild D.J. (1988) Thylakoid membrane organisation in sun/shade acclimation. *Australian Journal of Plant Physiology* **15**, 11–26.
- Aro E.-M., Virgin I. & Andersson B. (1993) Photoinhibition of photosystem II. Inactivation, protein damage and turnover. *Biochimica et Biophysica Acta* **1143**, 113–134.
- Barker D.H., Logan B.A., Adams W.W. III & Demmig-Adams B. (1998) Photochemistry and xanthophyll cycle-dependent energy dissipation in differently oriented cladodes of *Opuntia stricta* during the winter. *Australian Journal of Plant Physiology* **25**, 95–104.
- Barth C. & Krause G.H. (1999) Inhibition of photosystems I and II in chilling-sensitive and chilling-tolerant plants under light and low-temperature stress. *Zeitschrift für Naturforschung* **54**, 645–657.
- Barth C., Krause G.H. & Winter K. (2001) Responses of photosystem I compared with photosystem II to high-light stress in tropical shade and sun leaves. *Plant, Cell and Environment* **24**, 163–176.
- Brugnoli E., Scartazza A., De Tullio M.C., Monteverdi M.C., Lauteri M. & Augusti A. (1998) Zeaxanthin and non-photochemical quenching in sun and shade leaves of C<sub>3</sub> and C<sub>4</sub> plants. *Physiologia Plantarum* **104**, 727–734.
- Croat T.B. (1978) *Flora of Barro Colorado Island*. Stanford University Press, Stanford, CA.

- Dalling J.W., Lovelock C.E. & Hubbell S.P. (1999) Growth responses of seedlings of two neotropical pioneer species to simulated forest gap environments. *Journal of Tropical Ecology* **15**, 827–839.
- Dalling J.W., Winter K., Nason J.D., Hubbell S.P., Murawski D.A. & Hamrick J.L. (2001) The unusual life-history of *Alseis blackiana*: a shade-persistent pioneer tree? *Ecology* **82**, 933–945.
- Demmig-Adams B. & Adams W.W. III (1996) Chlorophyll and carotenoid composition in leaves of *Euonymus kiautschovicus* acclimated to different degrees of light stress in the field. *Australian Journal of Plant Physiology* **23**, 649–659.
- Demmig-Adams B. (1998) Survey of thermal energy dissipation and pigment composition in sun and shade leaves. *Plant Cell Physiology* **39**, 474–482.
- Demmig-Adams B. & Adams W.W. III (1992a) Carotenoid composition in sun and shade leaves of plants with different life forms. *Plant, Cell and Environment* **15**, 411–419.
- Demmig-Adams B. & Adams W.W. III (1992b) Photoprotection and other responses of plants to high light stress. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 599–626.
- Färber A., Young A.J., Ruban A.V., Horton P. & Jahns P. (1997) Dynamics of xanthophyll-cycle activity in different antenna subcomplexes in the photosynthetic membranes of higher plants. *Plant Physiology* **115**, 1609–1618.
- Giersch C. & Krause G.H. (1991) A simple model relating photoinhibitory fluorescence quenching in chloroplasts to a population of altered photosystem II reaction centres. *Photosynthesis Research* **30**, 115–121.
- Gilmore A.M. (1997) Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. *Physiologia Plantarum* **99**, 197–209.
- Horton P., Ruban A.V. & Walters R.G. (1996) Regulation of light harvesting in green plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 655–684.
- Hubbell S.P., Foster R.B., O'Brien S.T., Harms K.E., Condit R., Wechsler B., Wright S.J. & Loo de Lao S. (1999) Light-gap disturbances, recruitment limitation and tree diversity in a neotropical forest. *Science* **283**, 554–557.
- Kitajima K. (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* **98**, 419–428.
- Königer M., Harris G.C., Virgo A. & Winter K. (1995) Xanthophyll cycle pigments and photosynthetic capacity in tropical forest species: a comparative field study on canopy gap and understorey plants. *Oecologia* **104**, 280–290.
- Koroleva O.Y., Krause G.H. & Brüggemann W. (2000) Effects of long-term chilling under excessive light on xanthophyll cycle activity and non-photochemical fluorescence quenching in *Lycopersicon* genotypes. *Journal of Plant Physiology* **156**, 341–349.
- Krause G.H., Schmude C., Garden H., Koroleva O.Y. & Winter K. (1999a) Effects of solar ultraviolet radiation on the potential efficiency of photosystem II in leaves of tropical plants. *Plant Physiology* **121**, 1349–1358.
- Krause G.H., Carouge N. & Garden H. (1999b) Long-term effects of temperature shift on xanthophyll cycle and photoinhibition in spinach (*Spinacia oleracea*). *Australian Journal of Plant Physiology* **26**, 125–134.
- Krause G.H., Virgo A. & Winter K. (1995) High susceptibility to photoinhibition of young leaves of tropical forest trees. *Planta* **197**, 583–591.
- Krause G.H. & Weis E. (1991) Chlorophyll fluorescence: the basics. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 313–349.
- Krause G.H. & Winter K. (1996) Photoinhibition of photosynthesis in plants growing in natural tropical forest gaps. A chlorophyll fluorescence study. *Botanica Acta* **109**, 456–462.
- Leitsch J., Schnettger B., Critchley C. & Krause G.H. (1994) Two mechanisms of recovery from photoinhibition *in vivo*: reactivation of photosystem II related and unrelated to D1-protein turnover. *Planta* **194**, 15–21.
- Niyogi K.K. (2000) Safety valves for photosynthesis. *Current Opinions in Plant Biology* **3**, 455–460.
- Niyogi K.K., Björkman O. & Grossman A.R. (1997) The roles of specific xanthophylls in photoprotection. *Proceedings of the National Academy of Sciences USA* **94**, 14162–14167.
- Öquist G., Anderson J.M., McCaffery S. & Chow W.S. (1992) Mechanistic differences in photoinhibition of sun and shade plants. *Planta* **188**, 422–431.
- Pfündel E. & Bilger W. (1994) Regulation and possible function of the violaxanthin cycle. *Photosynthesis Research* **42**, 89–109.
- Pogson B., McDonald K., Truong M., Britton G. & DellaPenna D. (1996) *Arabidopsis* carotenoid mutants demonstrate that lutein is not essential for photosynthesis in higher plants. *Plant Cell* **8**, 1627–1639.
- Pogson B.J., Niyogi K.K., Björkman O. & DellaPenna D. (1998) Altered xanthophyll compositions adversely affect chlorophyll accumulation and non-photochemical quenching in *Arabidopsis* mutants. *Proceedings of the National Academy of Sciences USA* **95**, 13324–13329.
- Poorter L. (1999) Growth responses of 15 rain-forest tree species to a light gradient: the relative importance of morphological and physiological traits. *Functional Ecology* **13**, 396–410.
- Teramura A.H. & Sullivan J.H. (1994) Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. *Photosynthesis Research* **39**, 463–473.
- Teramura A.H. & Ziska L. (1996) Ultraviolet-B radiation and photosynthesis. In *Photosynthesis and the Environment* (ed. N. R. Baker), pp. 435–450. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Thayer S.S. & Björkman O. (1990) Leaf xanthophyll content and composition in sun and shade determined by HPLC. *Photosynthesis Research* **23**, 331–343.
- Thiele A., Krause G.H. & Winter K. (1998) *In situ* study of photoinhibition of photosynthesis and xanthophyll cycle activity in plants growing in natural gaps of the tropical forest. *Australian Journal of Plant Physiology* **25**, 189–195.
- Thiele A., Schirwitz K., Winter K. & Krause G.H. (1996) Increased xanthophyll cycle activity and reduced D1 protein inactivation related to photoinhibition in two plant systems acclimated to excess light. *Plant Science* **115**, 237–250.
- Thiele A., Winter K. & Krause G.H. (1997) Low inactivation of D1 protein of photosystem II in young canopy leaves of *Anacardium excelsum* under high-light stress. *Journal of Plant Physiology* **151**, 286–292.
- Walters R.G. & Horton P. (1999) Structural and functional heterogeneity in the major light-harvesting complexes of higher plants. *Photosynthesis Research* **61**, 77–89.
- Watling J.R., Robinson S.A., Woodrow I.E. & Osmond C.B. (1997) Responses of rainforest understorey plants to excess light during sunflecks. *Australian Journal of Plant Physiology* **24**, 17–25.

Received 28 April 2001; received in revised form 6 August 2001; accepted for publication 6 August 2001



This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.