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Lutein epoxide cycle, light harvesting and photoprotection in species of the tropical tree genus *Inga*

SHIZUE MATSUBARA[1], G. HEINRICH KRAUSE[2,3], MARTIN SELTMANN[1,2], AURELIO VIRGO[3], THOMAS A. KURSAR[3,4], PETER JAHNS[2] & KLAUS WINTER[3]

[1]*Phytosphere Institute (ICG-3), Research Centre Jülich, 52425 Jülich, Germany;* [2]*Institute of Plant Biochemistry, Heinrich-Heine University Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany;* [3]*Smithsonian Tropical Research Institute, Apartado Postal 0843-03092, Panama, Republic of Panama; and* [4]*Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA*

ABSTRACT

Dynamics and possible function of the lutein epoxide (Lx) cycle, that is, the reversible conversion of Lx to lutein (L) in the light-harvesting antennae, were investigated in leaves of tropical tree species. Photosynthetic pigments were quantified in nine *Inga* species and species from three other genera. In *Inga*, Lx levels were high in shade leaves (mostly above 20 mmol mol^{-1} chlorophyll) and low in sun leaves. In *Virola surinamensis*, both sun and shade leaves exhibited very high Lx contents (about 60 mmol mol^{-1} chlorophyll). In *Inga marginata* grown under high irradiance, Lx slowly accumulated within several days upon transfer to deep shade. When shade leaves of *I. marginata* were briefly exposed to the sunlight, both violaxanthin and Lx were quickly de-epoxidized. Subsequently, overnight recovery occurred only for violaxanthin, not for Lx. In such leaves, containing reduced levels of Lx and increased levels of L, chlorophyll fluorescence induction showed significantly slower reduction of the photosystem II electron acceptor, Q_A, and faster formation as well as a higher level of non-photochemical quenching. The results indicate that slow Lx accumulation in *Inga* leaves may improve light harvesting under limiting light, while quick de-epoxidation of Lx to L in response to excess light may enhance photoprotection.

Key-words: carotene; lutein; non-photochemical quenching; photoacclimation; shade leaves; sun leaves; xanthophyll.

INTRODUCTION

A remarkable feature of photosynthetic light-harvesting systems in plants is the ability to modulate the light-harvesting efficiency depending on the balance between the light energy absorbed and utilized. For photosystem II (PSII), dynamic adjustment of light-harvesting efficiency can be monitored by means of chlorophyll *a* (Chl *a*) fluorescence (Baker & Rosenqvist 2004; Krause & Jahns 2004; Schreiber 2004). When photosynthesis is light limited, PSII light-use efficiency is high. When the supply of light energy becomes excessive, thermal energy dissipation is induced in the light-harvesting antenna complexes to alleviate photooxidative stress (Demmig-Adams & Adams 1992; Niyogi 1999). Rapid and flexible adjustment of these two processes, light harvesting and dissipation, allows land plants to operate successfully under heterogeneous and highly fluctuating light environments (Külheim, Ågren & Jansson 2002).

Pigment composition of higher-plant antenna complexes is highly conserved. In addition to Chl *a* and Chl *b*, three xanthophylls, lutein (L), violaxanthin (V) and neoxanthin (N), are usually found, while two further xanthophylls, zeaxanthin (Z) and antheraxanthin (A), accumulate under excessive photosynthetic photon flux density (PPFD) (Yamamoto & Bassi 1996). All these xanthophyll pigments, as well as their precursors α- and β-carotene (α- and β-car, respectively) that are mostly bound in the core complexes of PSII and photosystem I, are synthesized in the two branches of the carotenoid biosynthetic pathway: β,β-carotenoids (β-car, Z, A, V and N), having two β-rings at the ends of the phytoene chain, are synthesized in one branch and β,ε-carotenoids (α-car and L) with β- and ε-ring at the ends of the phytoene chain in the other branch (Cunningham & Gantt 2001; Hirschberg 2001).

Light-induced accumulation of Z is due mainly to the activity of violaxanthin de-epoxidase (VDE) that catalyses the de-epoxidation reactions from V to Z via A under low lumenal pH (Yamamoto, Nakayama & Chichester 1962; Hager 1969). The reverse reaction from Z back to V is catalysed by zeaxanthin epoxidase (ZE), whose activity is most evident under dim light (Yamamoto *et al.* 1962; Siefermann-Harms 1977). This light-dependent interconversion between V, A and Z in the so-called xanthophyll cycle (or V cycle) is a key component in the regulation of thermal energy dissipation and photoprotection in the light-harvesting antenna complexes (Demmig-Adams & Adams 1992; Horton, Ruban & Walters 1996; Niyogi 1999; Gilmore 2001; Morosinotto *et al.* 2003; Krause & Jahns 2004). In many plants, such xanthophyll cycling does not involve L, the major β,ε-xanthophyll in the light-harvesting antennae, even though it has a β-ring that can theoretically be

Correspondence: S. Matsubara. Fax: +49 2461 61 2492; e-mail: s.matsubara@fz-juelich.de

epoxidized by ZE to produce lutein epoxide (Lx). Typically, no more than a trace of Lx is detected in leaf pigment extracts of many higher plant species (Young 1993).

Nonetheless, in the photosynthetic tissue of certain taxa, epoxidation of L to Lx and light-induced de-epoxidation of Lx to L do occur, that is, the Lx–L cycle (or Lx cycle) operates in parallel with the V cycle, probably catalysed by the same enzymes, VDE and ZE (García-Plazaola, Matsubara & Osmond 2007). In many of these plants, the pool size of Lx is typically high under shaded conditions. Upon high-light exposure, Lx is converted to L, as is V to A and Z, but dark recovery of Lx is slower than that of V (Matsubara, Gilmore & Osmond 2001; Snyder, Clark & Bungard 2005; Esteban *et al.* 2007). Probably owing to the slow dark recovery of Lx, in sun leaves of *Inga sapindoides* Willd., a tropical legume tree grown under semi-controlled conditions at the Biosphere 2 Center in Arizona (Leigh *et al.* 1999; Osmond *et al.* 2004), both Lx and L levels underwent little diurnal change, while the V-cycle pigments responded strongly in the expected manner to changing PPFD throughout the day (Matsubara *et al.* 2005). It is not known whether such characteristics of the Lx cycle in shade and sun leaves, upon shade–sun exposure and during daily fluctuations in PPFD, apply to plants *in situ* and whether they are a general trait among *Inga* species.

In the study presented here, we therefore explored the presence and dynamics of the Lx cycle, together with measurements of PSII activity, light harvesting and net CO_2 assimilation, in sun and shade leaves of a range of neotropical forest tree species in Panama, with the main focus on species of *Inga*. It was demonstrated that in *Inga* leaves, Lx indeed accumulates very slowly under shade environments in the absence of intermittent periods of high irradiance. The results provide new insights into mechanistic aspects of the Lx cycle and its possible role in regulating light harvesting and shade acclimation in leaves of tropical forest trees.

MATERIALS AND METHODS

Leaf samples for the pigment survey were collected in Central Panama (9°N, 80°W) at the end of the rainy season in November–December 2005. A diurnal time-course experiment was conducted in Gamboa, Panama, during the dry season in March 2006. Experiments using potted seedlings were performed in January–February 2006 at the Smithsonian Tropical Research Institute Santa Cruz Experimental Field Facility in Gamboa. Additional experiments were conducted under controlled conditions in a climate chamber at Phytosphere Institute, Research Centre Jülich, Germany, by using potted plants cultivated in a greenhouse. Photosynthetic pigments were analysed at the Institute of Plant Biochemistry, Heinrich-Heine University Düsseldorf, Germany.

Plant material

The species used in the pigment survey are listed in Table 1. Leaves were collected in the morning (between 0830 and 1140 h, local time) and dark adapted for 4 h before sample discs (0.95 cm^2) for pigment analysis were taken. Leaf discs were frozen in liquid nitrogen and stored at −70 or −80 °C until pigment extraction, except during transportation from Panama to Germany on dry ice (<48 h).

A diurnal time-course experiment was conducted on a large tree of *Inga spectabilis* (Vahl) Willd. growing near the Santa Cruz Experimental Field Facility, and was also used in the pigment survey. Seedlings of *Inga marginata* Willd. were cultivated in soil in 15 L pots (50 cm high) at the Santa Cruz Experimental Field Facility under two different light regimes: under a synthetic, glass roof with the maximal PPFD typically ≤1800 μmol photons m^{-2} s^{-1} (sun-acclimated plants) and under a shade cloth (neutral shade) with the maximal PPFD ≤150 μmol photons m^{-2} s^{-1} (shade-acclimated plants). These plants were used in the pigment

Table 1. List of study sites and tree species

Site	Family	Genus	Species	Authority	Comment
San Lorenzo National Park Moist primary lowland forest on the Caribbean slope	Fabaceae	*Inga*	*edulis*	Mart.	Trees of ca. 8 m in height
			vera (ssp. *vera*)	Willd.	Seedlings on the forest floor
	Myristicaceae	*Virola*	*surinamensis*	(Rol. ex Rottb.) Warb.	Large tree
Barro Colorado Island Moist, seasonally dry lowland forest in the Panama Canal area	Fabaceae	*Inga*	*laurina*	(Sw.) Willd.	Small trees in the forest understorey
			pezizifera	Benth.	
			quaternata	Poepp. and Endl.	
			sapindoides	Willd.	
Gamboa, Santa Cruz Experimental Field Facility	Bombacaceae	*Pseudobombax*	*septenatum*	(Jacq.) Dugand	>3-year-old potted plants
	Clusiaceae	*Calophyllum*	*longifolium*	Willd.	>3-year-old potted plants
Plants growing at the forest edge (*Inga goldmanii* and *Virola sebifera*), near *(Inga spectabilis)* or at the Experimental Field Facility (potted plants)	Fabaceae	*Inga*	*goldmanii*	Pittier	Young tree of ca. 3 m in height
			marginata	Willd.	Potted seedlings
			spectabilis	(Vahl) Willd.	Large tree
	Myristicaceae	*Virola*	*sebifera*	Aubl.	Young tree of ca. 1 m in height

survey and for the experiments with shade or sunlight treatment. Seedlings of *I. marginata* cultivated under a shade cloth (the maximal PPFD typically ≤50 μmol photons m^{-2} s^{-1}) in a greenhouse at Phytosphere Institute, Research Centre Jülich, were used for analysis of non-photochemical fluorescence quenching.

Diurnal time-course experiment

Photosynthetic photon flux density incident on leaves was measured with a quantum sensor (LI-189B; Li-Cor, Lincoln, NE, USA). Maximal PSII efficiency [$F_v/F_m = (F_m - F_o)/F_m$; fluorescence nomenclature according to van Kooten & Snel 1990] was determined by measuring Chl *a* fluorescence (Mini-PAM; Walz, Effeltrich, Germany) on sun and shade leaves of *I. spectabilis* after 10-min dark adaptation using leaf clips. Leaf discs (0.95 cm^2) for pigment analysis were taken from the same leaves as for F_v/F_m measurements, but without dark adaptation. Leaf discs were frozen immediately and stored as described earlier.

Experiment on long-term Lx recovery

Sun-acclimated seedlings of *I. marginata* were transferred to deep shade (PPFD ≤ 20 μmol photons m^{-2} s^{-1}). Changes in F_v/F_m and pigment composition were monitored for 4 d starting from the day of transfer (day 0). Measurements of Chl *a* fluorescence were performed on fully expanded, mature leaves of the same seedlings at around dawn every morning by using a Mini-PAM. Leaf discs (0.64 cm^2) for pigment analysis were taken and frozen in liquid nitrogen.

Induction of Lx de-epoxidation in shade-acclimated seedlings

Shade-acclimated seedlings of *I. marginata* were exposed to the sunlight of 500–800 μmol photons m^{-2} s^{-1} for 30–40 min in the morning (day 1) to induce de-epoxidation. Subsequently, these plants were transferred to deep shade (PPFD ≤ 20 μmol photons m^{-2} s^{-1}) until the next morning (day 2) for full epoxidation of the V cycle. Leaf disc sampling (0.64 cm^2) and F_v/F_m measurements (Mini-PAM) were conducted at dawn and at the end of the sunlight treatment on day 1 and at dawn on day 2. These plants were used for experiments of fast fluorescence induction and gas-exchange analyses described as follows.

Analysis of fast fluorescence induction

At dawn on days 1 and 2 (above), leaves were taken from the seedlings, wrapped in wet tissues and kept in the dark. Fast fluorescence induction was measured with a PEA instrument (Hansatech, King's Lynn, UK) in the laboratory. After each measurement, a leaf disc (0.64 cm^2) was taken for pigment analysis and immediately frozen in liquid nitrogen.

Fluorescence was measured for 2 s with red light (centred at 650 nm) of 3500 μmol photons m^{-2} s^{-1} on days 1 and 2. All data were normalized to the maximal fluorescence intensity P which corresponds to F_m in dark-adapted samples. Normalized induction curves of different plants were then averaged for days 1 and 2. In addition, the same analysis was performed on day 1 by using different excitation light intensities (3500, 3000 and 2100 μmol photons m^{-2} s^{-1}). The slope of the linear regression line in the so-called photochemical phase (Strasser, Srivastava & Govindjee 1995) was calculated for each sample using the data points between 0.2 and 0.3 ms. The regression coefficient was $r^2 = 0.999$ for all samples.

Induction and relaxation of non-photochemical quenching

Seedlings of *I. marginata* were grown under a shade cloth in a greenhouse (PPFD ≤ 50 μmol photons m^{-2} s^{-1}). Three discs (0.385 cm^2) per leaf were taken from four dark-adapted plants on the first day of the experiment (day 1). One of the discs was vacuum infiltrated with 5 mM Hepes buffer (pH 7.0) and another one with 10 mM dithiothreitol (DTT) in 5 mM Hepes (pH 7.0) to inhibit light-induced de-epoxidation in the two xanthophyll cycles (García-Plazaola *et al.* 2003). The maximal PSII efficiency (F_v/F_m) was measured by a Handy PEA instrument (Hansatech) in all samples placed on a moist tissue. The impact of vacuum infiltration treatment was assessed in the samples treated with Hepes. Immediately after the F_v/F_m measurements, the control samples (without vacuum infiltration) were frozen in liquid nitrogen for pigment analysis. For the samples treated with DTT, F_v/F_m measurements were followed by non-photochemical quenching (NPQ) measurements ($NPQ = 1 - F_m'/F_m$). A moderate light intensity of red light (300 μmol photons m^{-2} s^{-1}) was used for NPQ induction to minimize photoinhibition. Formation of NPQ (for 5 min) and subsequent dark relaxation (for 4.5 min) were monitored by a series of 0.8 s saturation pulses (3500 μmol photons m^{-2} s^{-1}) released every 30 s. After the measurement, the samples were frozen in liquid nitrogen for pigment analysis.

Following the measurements on day 1, the seedlings were exposed to white light of 800 μmol photons m^{-2} s^{-1} at 25 °C for 30 min in a climate chamber to induce de-epoxidation in the two xanthophyll cycles. At the end of the light exposure, another disc was removed from the same leaves to assess the extent of de-epoxidation. Subsequently, the plants were brought back into the greenhouse and allowed to recover under the growth condition. By the next morning (day 2), the V cycle was almost fully epoxidized again, while the pigments in the Lx cycle had changed little after the high-PPFD treatment on the previous day. The same set of measurements as on day 1 was conducted on day 2 by using discs taken from the same leaves as used on day 1.

Gas exchange measurements

Light-response curves of CO_2 exchange were measured in shade-acclimated seedlings on day 1 (before exposure to increased irradiance) and on day 2. At least 1 h before the gas

exchange measurements on day 2, plants were transferred from deep shade (PPFD ≤ 20 μmol photons m^{-2} s^{-1}) to moderate shade (PPFD ≤ 150 μmol photons m^{-2} s^{-1}; growth light regime). For comparison, measurements were conducted for sun-acclimated seedlings. To avoid high-light stress, the sun-acclimated seedlings were also transferred to moderate shade > 1 h prior to the gas exchange measurements.

All gas exchange measurements were performed with a LI-6400 (Li-Cor) equipped with a blue + red LED light source (6400-02B LED; Li-Cor) at ambient CO_2 concentration (about 390 ppm) in the morning. Air flow rate through the leaf chamber was maintained at 250 and 500 μmol s^{-1} during the experiment for shade- and sun-acclimated seedlings, respectively. The apparent quantum yield of CO_2 assimilation and the light compensation point were estimated from the initial slope of the light-response curve; in this study, the linear regression line at ≤20 μmol photons m^{-2} s^{-1} was used as the initial slope. The regression coefficient was: $r^2 = 0.992$ for shade, day 1; $r^2 = 0.982$ for shade, day 2; $r^2 = 0.949$ for sun.

Pigment analysis

Photosynthetic pigments were extracted from leaf discs according to Krause *et al.* (2006). Chlorophylls and carotenoids were quantified by a method modified from Färber *et al.* (1997) as described by Krause *et al.* (2003). For Lx, the extinction coefficient of A was used. Later calibration showed that the coefficient of Lx differed from that of A by <1%.

RESULTS

Carotenoid composition in shade and sun leaves

In contrast to the rather moderate variations in N levels (0–15% lower in sun than in shade leaves), the pool size of the V-cycle pigments (V + A + Z) exhibited marked sun–shade responses, being 1.5–5 times larger in sun than in shade leaves (Table 2). Young, pale-green leaves of *I. marginata* (shade) and *Inga edulis* (sun) contained 60–70% more of the V-cycle pigments (based on Chl $a + b$) than mature leaves growing under the same light environments (cf. Krause, Virgo & Winter 1995; García-Plazaola *et al.* 2007). Accumulation of Lx was detected in all species analysed, albeit to different extents. Shade leaves of *Inga* species, with an exception of young leaves of *I. marginata*, contained high levels of Lx, whereas sun leaves were characterized by much lower Lx levels. Very high amounts of Lx were found in leaves of *Virola surinamensis* (ca. 60 mmol mol^{-1} Chl $a + b$), in which neither Lx nor L differed significantly between sun and shade leaves, even though Chl *a* to Chl *b* ratios (Chl *a*/*b*) and V + A + Z indicated typical sun–shade characteristics. As has been previously reported for other neotropical forest species (Krause *et al.* 2001, 2003, 2004), all plants examined in this survey contained large amounts of α-car in addition to β-car in shade leaves. Hence, the ratio of α-car to β-car (α/β-car) was substantially higher in shade than in sun leaves.

Figure 1 depicts the balance between pigments synthesized via the β,ε-branch (α-car, L and Lx) and β,β-branch (β-car, Z, A, V and N) of the carotenoid biosynthetic pathway in sun and shade leaves of different *Inga* species. When the ratio α/β-car in sun and shade leaves was plotted versus the ratio of the total pigments synthesized in the β,ε- and β,β-branch (ratio β,ε/β,β-carotenoids), a linear correlation was found with a slope very close to 1. Likewise, the ratio of β,ε- to β,β-xanthophylls was also linearly correlated with the ratio of the total pigment levels in the two branches; the slope in this case was <1.

Diurnal xanthophyll turnover in leaves of *I. spectabilis*

In sun leaves of *I. spectabilis* (Fig. 2), the V-cycle pigments exhibited marked diurnal variations. Rapid de-epoxidation of V to A + Z in the morning was followed by gradual epoxidation of A + Z back to V when PPFD was reduced by clouds at midday and in the afternoon. On the contrary, the changes in the Lx-cycle pigments were negligible throughout the day, with the levels of Lx being much lower and those of L higher than found in sun leaves of the same tree at the end of the rainy season (Table 2). Shade leaves contained much higher levels of Lx and lower levels of L and V-cycle pigments compared with sun leaves (Fig. 2b,c). The xanthophyll levels in shade leaves remained nearly unchanged, reflecting the constantly low PPFD for these inner canopy leaves. The diurnal patterns in the maximal PSII efficiency (F_v/F_m) of both sun and shade leaves were in good agreement with the operation of the V cycle (Fig. 2d). In sun leaves, lowest F_v/F_m was measured at the time of the highest de-epoxidation. As was the case with the xanthophyll composition, shade leaves displayed no significant change in F_v/F_m.

Long-term recovery of Lx in the shade

Despite the lack of Lx recovery on a daily basis (Fig. 2), pronounced Lx accumulation found in shade leaves of nine *Inga* species (Table 2) implies that epoxidation of L to Lx does happen in these leaves. To monitor longer-term recovery kinetics of Lx in *Inga*, we transferred sun-acclimated seedlings of *I. marginata* (grown under PPFD ≤ 1800 μmol photons m^{-2} s^{-1}) to deep shade (≤20 μmol photons m^{-2} s^{-1}) and examined the changes in pigment composition over 4 d (Fig. 3). Leaf discs for pigment analysis were collected from the plants immediately after the measurements of predawn F_v/F_m on each day, starting from the day of transfer (day 0).

Under the deeply shaded condition, Lx and L content underwent gradual changes (Fig. 3a), although not in a stoichiometric manner (after 4 d about +15 and −40 mmol-mol^{-1} Chl for Lx and L, respectively). These changes in Lx and L did not affect predawn F_v/F_m (Fig. 3b). The data demonstrate that epoxidation of L to Lx does take place in *Inga* leaves in shaded environments, that is, in the absence

Table 2. Comparison of the pigment compositions in sun and shade leaves of different species growing in Panama

Species		Chl *a*/*b*	N	V	A	Z	V + A + Z	Lx	L	Lx + L	α-car	β-car	α/β-car
Inga marginata	Shade	3.1 (0.1)	38.7 (0.6)	16.8 (2.6)	0.2 (0.3)	nd	17.0 (2.8)	25.8 (4.2)	108.1 (4.9)	133.9 (5.1)	47.3 (6.3)	28.3 (3.6)	1.7 (0.4)
	Sun*	3.7 (0.1)	34.5 (2.2)	38.6 (13.1)	27.0 (6.3)	34.3 (23.6)	99.8 (23.1)	2.3 (1.5)	154.2 (19.2)	156.5 (18.8)	13.5 (3.2)	75.1 (5.1)	0.2 (0.0)
	Shade†	2.8 (0.1)	22.1 (0.6)	27.9 (2.5)	nd	nd	27.9 (2.5)	3.3 (1.3)	99.0 (3.9)	102.3 (2.9)	33.3 (2.9)	26.2 (1.7)	1.3 (0.1)
Inga edulis	Shade	3.2 (0.1)	37.3 (1.3)	24.4 (3.3)	0.4 (0.4)	nd	24.9 (3.7)	23.7 (4.2)	112.7 (12.1)	136.4 (16.3)	39.5 (5.6)	44.3 (5.7)	0.9 (0.2)
	Sun	3.8 (0.2)	27.0 (4.0)	31.3 (7.0)	2.3 (1.6)	2.0 (2.9)	35.6 (6.8)	8.7 (2.3)	90.4 (7.9)	99.1 (9.6)	31.9 (0.5)	59.5 (6.8)	0.5 (0.1)
	Sun*†	3.4 (0.2)	22.7 (5.7)	33.3 (12.0)	10.6 (6.9)	16.5 (20.9)	60.5 (16.1)	7.4 (2.0)	148.2 (6.7)	155.6 (8.6)	14.3 (0.8)	66.4 (6.0)	0.2 (0.0)
Inga spectabilis	Shade	3.1 (0.1)	37.1 (0.7)	25.7 (1.2)	0.9 (0.6)	0.2 (0.4)	26.8 (0.5)	27.5 (4.3)	99.0 (3.6)	126.5 (8.3)	38.8 (1.6)	42.7 (3.3)	0.9 (0.1)
	Sun	3.9 (0.1)	31.7 (0.7)	40.5 (3.5)	1.8 (1.2)	1.4 (0.5)	43.7 (4.1)	18.7 (5.1)	99.7 (9.4)	118.4 (13.1)	22.7 (4.5)	72.1 (5.4)	0.3 (0.1)
Inga goldmanii	Shade	3.0 (0.1)	38.1 (1.4)	15.4 (0.7)	0.7 (0.1)	nd	16.2 (0.8)	25.5 (8.7)	102.5 (6.5)	128.1 (14.8)	43.6 (2.8)	31.7 (0.9)	1.4 (0.1)
Inga laurina	Shade	3.1 (0.1)	38.6 (2.6)	15.3 (1.4)	0.1 (0.2)	nd	15.5 (1.3)	38.5 (8.1)	103.3 (4.3)	141.8 (11.7)	48.8 (2.4)	29.5 (2.1)	1.7 (0.1)
Inga pezizifera	Shade	2.9 (0.1)	39.3 (1.3)	18.8 (6.4)	0.0 (0.1)	nd	18.9 (6.5)	38.5 (27.4)	101.1 (3.2)	139.7 (26.5)	40.0 (5.3)	34.8 (1.9)	1.1 (0.2)
Inga quaternata	Shade	3.1 (0.1)	37.1 (1.6)	16.0 (1.1)	0.4 (0.3)	nd	16.5 (1.4)	18.3 (6.7)	113.0 (8.1)	131.4 (3.2)	39.9 (4.8)	37.2 (3.4)	1.1 (0.2)
Inga sapindoides	Shade	3.1 (0.1)	37.0 (1.6)	22.3 (4.3)	nd	nd	22.3 (4.3)	28.4 (15.3)	93.8 (14.2)	122.3 (18.3)	44.8 (3.2)	30.9 (1.4)	1.5 (0.1)
Inga vera	Shade	3.1 (0.1)	36.3 (1.2)	21.1 (1.9)	nd	nd	21.1 (1.9)	29.6 (7.3)	96.0 (5.7)	125.7 (8.0)	34.0 (2.3)	42.9 (3.4)	0.8 (0.1)
Calophyllum longifolium	Shade	3.0 (0.1)	36.1 (1.1)	17.7 (1.3)	0.1 (0.2)	nd	17.8 (1.2)	7.3 (2.9)	128.1 (4.4)	135.5 (4.0)	20.4 (2.7)	57.2 (4.4)	0.4 (0.1)
	Sun	3.7 (0.1)	36.9 (1.4)	76.7 (10.7)	12.5 (7.7)	9.9 (6.4)	99.1 (23.3)	2.1 (0.6)	148.5 (25.9)	150.6 (26.1)	5.7 (0.7)	87.1 (4.1)	0.1 (0.0)
Pseudobombax septenatum	Shade	2.8 (0.0)	35.5 (0.8)	20.7 (1.7)	nd	nd	20.7 (1.7)	6.0 (0.9)	115.9 (6.0)	121.9 (5.5)	33.3 (5.6)	36.3 (6.0)	0.9 (0.3)
	Sun	4.2 (0.2)	34.4 (1.0)	45.1 (5.3)	7.3 (3.4)	14.4 (7.9)	66.7 (11.1)	1.7 (0.7)	126.5 (1.1)	128.2 (0.9)	8.5 (2.5)	92.1 (8.6)	0.1 (0.0)
Virola surinamensis	Shade	3.1 (0.1)	38.2 (0.8)	15.7 (1.2)	0.3 (0.6)	nd	21.7 (1.0)	62.4 (10.2)	102.6 (7.7)	164.5 (19.9)	43.5 (4.0)	35.5 (8.2)	1.2 (0.4)
	Sun	3.9 (0.1)	33.0 (0.8)	40.8 (4.7)	8.0 (4.4)	4.4 (1.7)	53.3 (8.1)	62.1 (9.5)	93.8 (9.4)	155.9 (5.9)	16.4 (3.9)	79.6 (2.8)	0.2 (0.1)
Virola sebifera	Shade	3.1 (0.1)	37.8 (0.4)	20.7 (0.5)	0.8 (0.1)	nd	21.5 (0.5)	25.7 (3.8)	106.5 (2.4)	132.2 (1.6)	56.2 (2.0)	25.9 (1.1)	2.2 (0.1)

*Leaves were not fully dark adapted.
†Young leaves with ca. 30% (*I. marginata*) or 40% (*I. edulis*) Chl $a + b$ (per unit leaf area) of the mature leaves growing under similar conditions.
Contents of carotenoids are given on a chlorophyll basis (mmol mol^{-1} Chl).
Means (±SD) are presented; $n = 3–5$.
nd, not detectable.

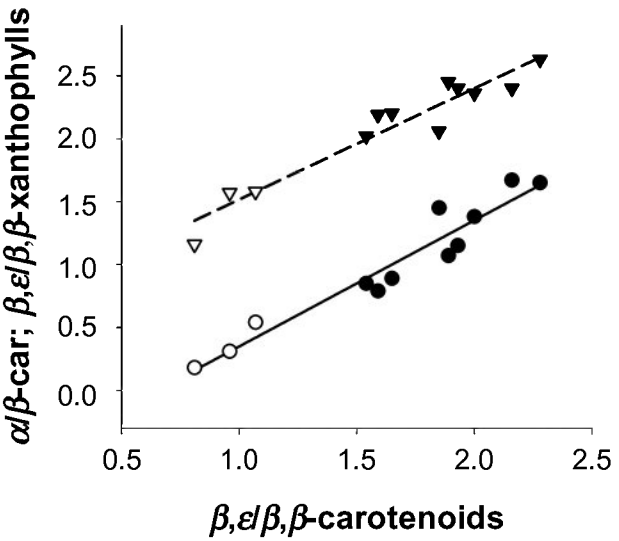


Figure 1. Relationships between carotenoids synthesized via the β,ε- and β,β-carotenoid pathways in sun and shade leaves (open and closed symbols, respectively) of different *Inga* species. Data from young leaves of *Inga marginata* and *Inga edulis* were not included. Circles, ratio α/β-car; triangles, ratio β,ε/β,β-xanthophylls. The slopes of the two regression lines are: 1.002 (±0.087) for α/β-car and 0.883 (±0.083) for β,ε/β,β-xanthophylls (±SE, $P < 0.0001$). The fits for the regression lines are $r^2 = 0.924$ for α/β-car and $r^2 = 0.912$ for β,ε/β,β-xanthophylls.

of light-induced de-epoxidation. During the 4 d shade acclimation, there were only minor alterations in β,β-xanthophylls (Fig. 3a) and no significant changes in Chl *a*/*b* ratios (data not shown) and total Chl content (Fig. 3b).

Effects of Lx de-epoxidation in shade leaves

The effects of slowly reversible de-epoxidation in the Lx cycle were investigated in shade-acclimated seedlings of *I. marginata* (grown under PPFD ≤ 150 μmol photons m^{-2} s^{-1}). Plants were exposed to increased PPFD (500–800 μmol photons m^{-2} s^{-1}) for 30–40 min in the morning (day 1) to induce de-epoxidation; both Lx and V showed a ca. 50% decrease (Fig. 4a) which was accompanied by an increase in L and A + Z (Fig. 4b). Subsequently, the plants were allowed to recover in deep shade (PPFD ≤ 20 μmol photons m^{-2} s^{-1}). On the next morning (predawn, day 2), the V-cycle pigments were fully epoxidized, while the levels of Lx and L were indistinguishable from those seen following the short, high-PPFD treatment on the previous day. There was no significant change in the predawn F_v/F_m values between days 1 and 2.

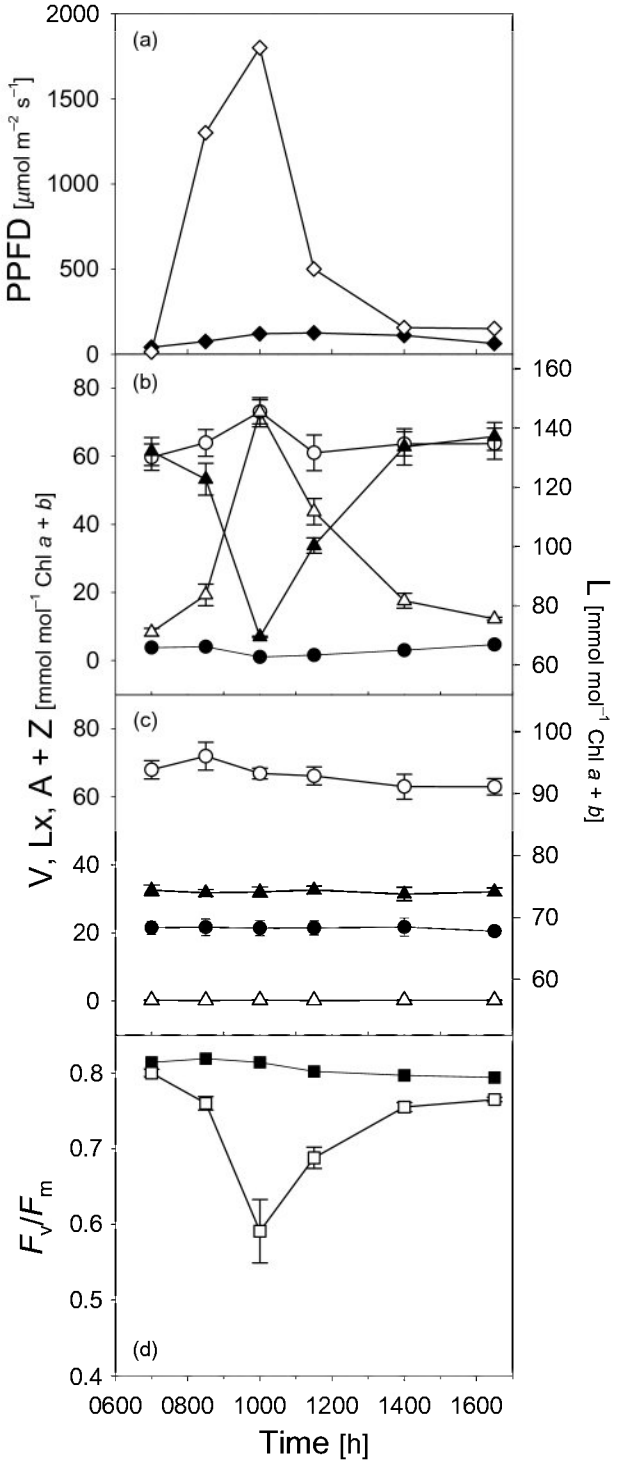
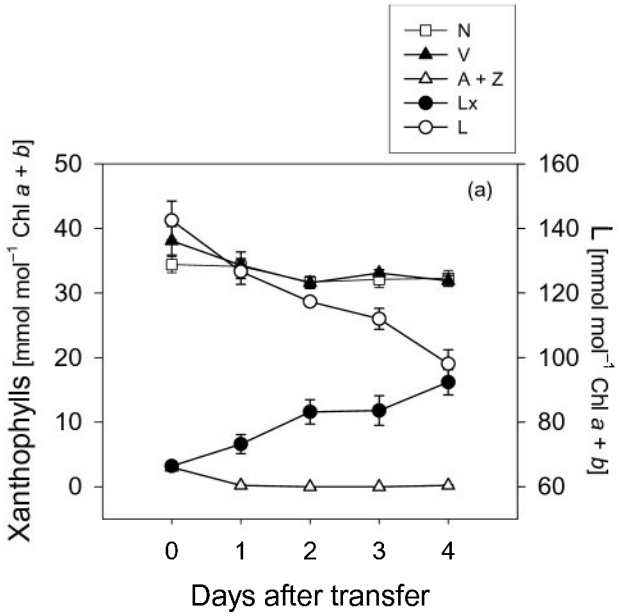


Figure 2. Diurnal changes in the xanthophyll levels and the maximal photosystem II efficiency in sun and shade leaves of *Inga spectabilis*. (a) Incident light intensities measured on sun (open diamond) and shade leaves (closed diamond). Only one measurement was done for sun and shade at each time point. Xanthophyll contents in (b) sun and (c) shade leaves are given on a chlorophyll basis. Open circle, lutein (L); closed circle, lutein epoxide; open triangle, antheraxanthin + zeaxanthin (A + Z); closed triangle, violaxanthin (V). (d) Maximal PSII efficiency (F_v/F_m) in sun and shade leaves (open and closed symbols, respectively). Leaf discs for pigment assay were removed from the same leaves as used for the fluorescence measurements. Error bars in panels b, c and d (shown when larger than symbols) denote SE ($n = 5$).



(b)

	Day 0	Day 1	Day 2	Day 3	Day 4
Chl *a* + *b*	336 (48)	340 (42)	355 (52)	386 (41)	404 (32)
F_v/F_m	0.78 (0.01)	0.79 (0.01)	0.80 (0.01)	0.80 (0.01)	0.79 (0.01)

Figure 3. Sun-to-shade acclimation of *Inga marginata* seedlings. (a) Changes in the xanthophyll composition during the shade-acclimation treatment. Sun-acclimated seedlings of *I. marginata* [grown under photosynthetic photon flux density (PPFD) ≤ 1800 photons μmol m^{-2} s^{-1}] were transferred to deep shade (PPFD ≤ 20 μmol m^{-2} s^{-1}) on day 0. Error bars (shown when larger than symbols) denote SE (n = 4–5). (b) Chlorophyll content (Chl $a + b$; μmol m^{-2}) and the maximal photosystem II efficiency (F_v/F_m) (±SE). Leaf discs for pigment assay were taken from the same leaves as used for the fluorescence measurements.

However, conspicuous changes were apparent in the analysis of fast fluorescence induction measured in the same leaves on day 1 (prior to exposure to increased PPFD) and on day 2 (Fig. 5a). The fluorescence rise in the first phase of the so-called OJIP curve (Govindjee 1995; Strasser *et al.* 1995) was slower on day 2 than on day 1. All data from days 1 to 2 are normalized to the maximal fluorescence intensity (P, corresponding to F_m). Neither O nor P level significantly differed between the two days, with or without normalization. As can be seen in the difference curve (days 1–2; dashed red line) at the bottom of Fig. 5a, the change in fluorescence induction appeared in a biphasic manner, that is, the main effect in the O-J phase was followed by a minor effect in the J-I phase. In the O-J phase, the slope of the linear regression line between 0.2 and 0.3 ms (Fig. 5a, inset) indicated a 22% slower fluorescence rise for day 2 compared with day 1 (from 0.700 to 0.544; $P < 0.0001$; Fig. 5c).

The fluorescence rise in the O-J phase, termed 'photochemical phase', represents the momentary maximum rate of reduction of the electron acceptor Q_A in PSII and is known to be influenced by excitation light intensity (Strasser *et al.* 1995; Vredenberg & Bulychev 2003; Schansker, Tóth & Strasser 2006). In a separate experiment, lowering the excitation light intensity from 3500 to 3000 or 2100 μmol photons m^{-2} s^{-1} resulted in a 19 or 51% reduction in the slope of the O-J phase, respectively (from 0.701 to 0.570 or 0.344; Fig. 5b,c). Unlike the comparison between days 1 and 2, the difference between 3500 and 3000 μmol photons m^{-2} s^{-1} was essentially only in the photochemical phase (Fig. 5b, dashed green line). When the light intensity was lowered to 2100 μmol photons m^{-2} s^{-1}, fluorescence rise was somewhat delayed in the J-I phase as well (Fig. 5b, dashed blue line), illustrating an effect of light intensity in this phase (Schansker *et al.* 2006).

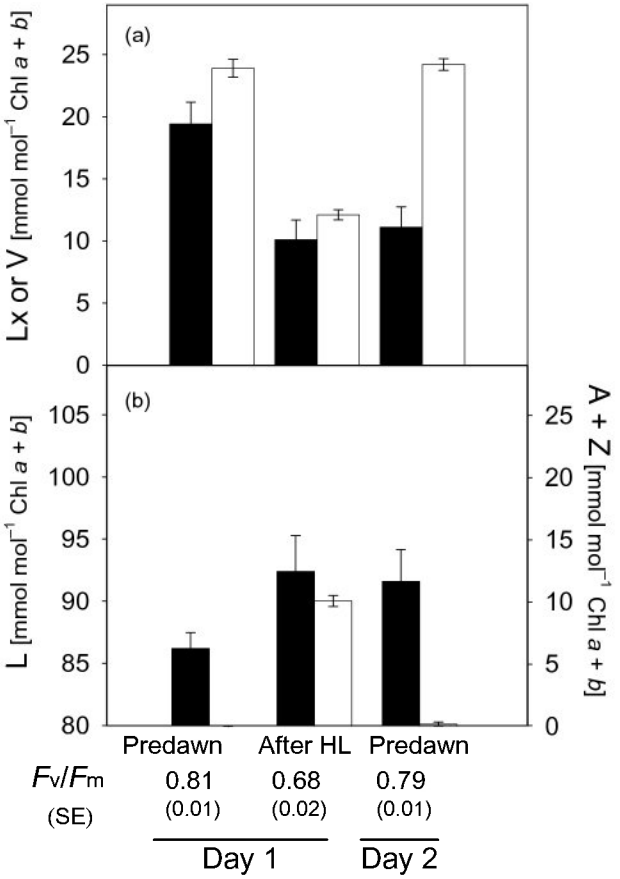
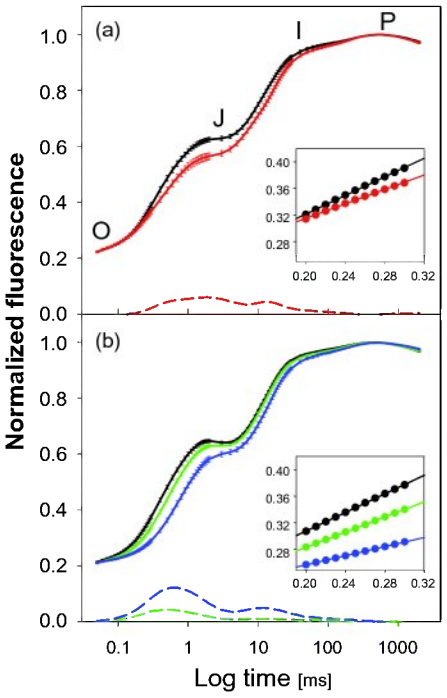


Figure 4. Responses of the two xanthophyll cycles in shade-acclimated seedlings of *Inga marginata*. (a) Amounts of lutein epoxide (Lx, closed bars) and violaxanthin (V, open bars). (b) Amounts of lutein (L, closed bars) and the sum of antheraxanthin and zeaxanthin (A + Z, open bars). Error bars denote SE (n = 10). Seedlings were grown under photosynthetic photon flux density (PPFD) < 150 μmol photons m^{-2} s^{-1}. After the first measurement and sampling on day 1, the seedlings were exposed to the sunlight of 500–800 μmol photons m^{-2} s^{-1} for 30–40 min (HL). Subsequently, the plants were transferred to deep shade (PPFD < 20 μmol photons m^{-2} s^{-1}) and allowed to recover until the next morning (day 2). The maximal photosystem II efficiency (F_v/F_m) is given at the bottom of panel b (±SE; n = 10).



(c)

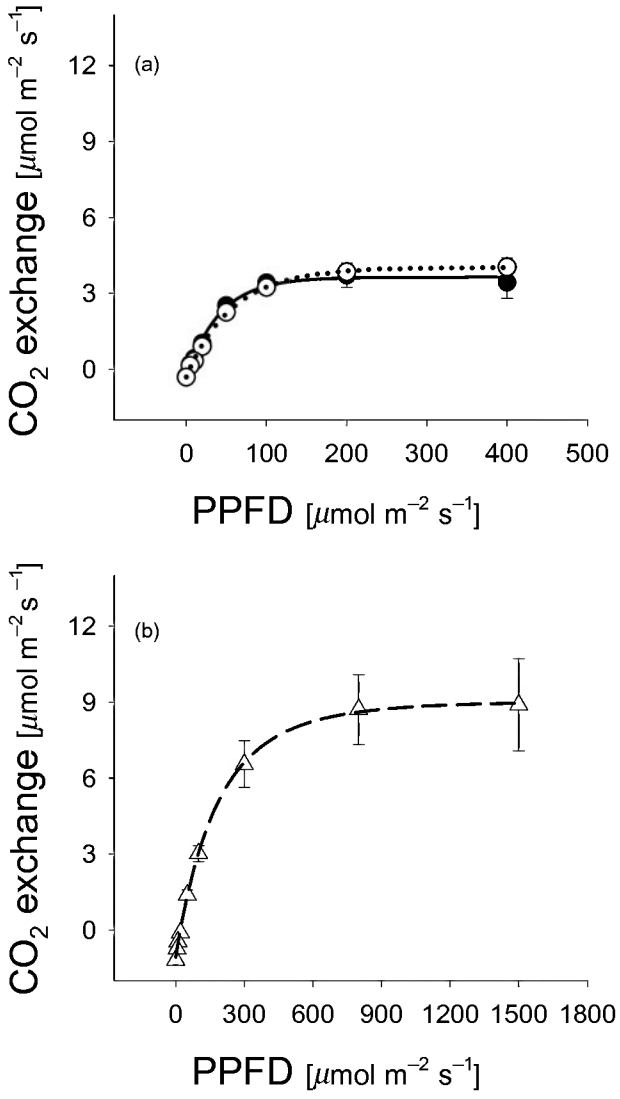
	High-light exposure		Excitation light intensity		
	Day 1	**Day 2**	**3500**	**3000**	**2100**
F_v/F_m	0.778 (0.001)	0.773 (0.004)	0.790 (0.002)	0.786 (0.003)	0.790 (0.003)
Slope of linear regression	0.700 (0.005)	0.544 (0.006)	0.701 (0.003)	0.570 (0.001)	0.344 (0.001)

Figure 5. Kinetics of fast fluorescence induction (OJIP curve) in shade-acclimated seedlings of *Inga marginata*. Fast fluorescence induction was measured in dark-adapted leaves. (a) Measurements were performed on day 1 prior to treatment with increased photosynthetic photon flux density (PPFD) (black line) and day 2 (red line). The sunlight treatment was as described for Fig. 4. The excitation light intensity used in this experiment was 3500 μmol photons m^{-2} s^{-1}. (b) Three different intensities of excitation light (3500, 3000 and 2100 μmol photons m^{-2} s^{-1} for black, green and blue lines, respectively) were applied to dark-adapted control leaves (shade leaves not treated with increased PPFD). All data were normalized to the maximal fluorescence intensity. The dashed lines at the bottom of each panel show the difference (a) between days 1 and 2 (dashed red line) and (b) between 3500 and 3000 (dashed green line) or between 3500 and 2100 μmol photons m^{-2} s^{-1} (dashed blue line). The linear regression lines calculated from the data between 0.2 and 0.3 ms are shown in the insets ($r^2 = 0.999$). Symbols in the insets represent (a) day 1 (black) and day 2 (red) or (b) 3500 (black), 3000 (green) and 2100 μmol photons m^{-2} s^{-1} (blue), respectively. (c) Slopes of the regression lines (±SE; $P < 0.0001$) and the maximal photosystem II efficiency (F_v/F_m). Error bars of the induction curves denote SE ($n = 4$).

Because the analysis of fast fluorescence induction revealed different kinetics of fluorescence rise in the light-dependent phase of the OJIP curve between days 1 and 2, the light response of net CO_2 exchange rate was examined (Fig. 6). The apparent quantum yield of CO_2 fixation, calculated as the initial slope of the light-response curve, did not differ significantly between days 1 and 2, although values were by 6.6–15.6% higher on day 1 in three out of four leaves (from four individual plants) studied. The light compensation point (Fig. 6c) was lower on day 1 than on day 2 ($P = 0.03$; one-tailed paired *t*-test). By comparison, sun-grown seedlings of *I. marginata* exhibited much higher light-saturated maximal CO_2 exchange rates (Fig. 6b) and light compensation points (Fig. 6c), indicating a large capacity of these plants to acclimate to sun-exposed conditions. The apparent quantum yield of CO_2 exchange was lower in sun than in shade plants (Fig. 6c).

In order to examine the effect of slowly reversible Lx→L conversion on light-induced energy dissipation, NPQ measurements were performed on days 1 and 2 (Fig. 7). In this experiment using shade-grown (<50 μmol photons m^{-2} s^{-1}) seedlings of *I. marginata*, exposure of the plants to 800 μmol photons m^{-2} s^{-1} for 30 min on day 1 caused ca. 30% de-epoxidation in the V cycle and a ca. 30% reduction in the Lx content (data not shown). The V pool recovered almost fully by the next morning, while Lx and L remained nearly unchanged, as was seen in the experiment shown in Fig. 4. The NPQ was induced by illuminating the samples with a moderate intensity of red light (300 μmol photons m^{-2} s^{-1}) for 5 min, and relaxation was monitored for the following 4.5 min in the dark. As the experiment was focused on the effect of the sustained de-epoxidation in the Lx cycle, de-epoxidation of V and Lx during the 5 min illumination was prevented by treating the leaf discs with 10 mM DTT prior to the fluorescence measurements. This concentration of DTT was found to be sufficient to inhibit de-epoxidation in the two xanthophyll cycles in leaves of *I. marginata* under our experimental conditions (data not shown).

The vacuum infiltration with 5 mM Hepes (pH 7.0) resulted in a decrease in F_v/F_m that was similar in the presence or absence of 10 mM DTT (Fig. 7a). There was no significant difference in F_v/F_m between days 1 and 2 for control as well as for Hepes- or DTT-treated samples. The effect of Lx→L conversion on the NPQ formation was then investigated in the DTT-treated samples on days 1 and 2 (Fig. 7b). A much faster NPQ induction was observed in the leaf disc samples on day 2, which also reached a higher maximal NPQ level within 5 min (Fig. 7b). Most of the NPQ disappeared quickly upon darkening on both days 1 and 2, with a slightly higher level of residual NPQ remaining in the day 2 samples at the end of the dark period (0.05 and 0.09 on days 1 and 2, respectively). When the maximal NPQ level attained in the samples during the 5 min illumination was compared with the content of different pigments in the two xanthophyll cycles, it was correlated with the pools of Lx and L, with a correlation being negative for the former and positive for the latter (Fig. 8a,b). In the nearly completely epoxidized V-cycle pool, on the other hand, neither V nor A + Z was correlated with the maximal NPQ in these DTT-treated samples (Fig. 8c,d).



(c)

	Apparent quantum yield	Light compensation point
Shade		
Day 1	0.062 (0.004)	3.2 (0.6)
Day 2	0.058 (0.002)	4.3 (0.5)
Sun	0.048 (0.003)	21.7 (2.0)

Figure 6. Photosynthetic light-response curves in sun- and shade-acclimated seedlings of *Inga marginata*. (a) Light-response curves of net CO_2 exchange rate in shade-acclimated seedlings on day 1, prior to treatment with increased irradiance (closed circles) and on day 2 (open circles). For treatment on day 1, see legend to Fig. 4. Error bars (shown when larger than symbols) denote SE ($n = 4$). (b) Light-response curves of CO_2 exchange in sun-acclimated seedlings (open triangles). (c) The apparent quantum yields of CO_2 assimilation (calculated as the initial slope of the light-response curve, from 0 to 20 μmol photons m^{-2} s^{-1}) and the light compensation points (μmol photons m^{-2} s^{-1}) ($\pm$SE; $n = 4$). Correlation coefficients of linear regression were between 0.97 and 0.99 for shade, and 0.95 for sun leaves. The difference in the light compensation point between shade-acclimated seedlings on days 1 and 2 was statistically significant ($P = 0.03$; one-tailed paired t-test).

DISCUSSION

Lutein epoxide cycle and shade acclimation

Leaves of all tree species investigated contained Lx. The highest amount was found in sun and shade leaves of *V. surinamensis* (Table 2), with the Lx level in sun leaves (about 60 mmol mol^{-1} Chl) being the highest reported for sun-acclimated leaves so far. The similarity of Lx content in sun and shade leaves of *V. surinamensis* contrasts with the pattern in other species of the present (Table 2) and previous studies (Matsubara *et al.* 2001, 2002, 2005), showing higher Lx contents in shade than in sun leaves, but resembles the situation found in *Umbellularia californica* (Lauraceae), having >40 mmol Lx mol^{-1} Chl in both sun and shade leaves (Esteban *et al.* 2007). The function of the Lx cycle in the genus *Virola* will be the subject of further investigations.

In a previous study with *I. sapindoides* (Matsubara *et al.* 2005), constantly low Lx contents in sun leaves have been attributed to slow L→Lx epoxidation impeding overnight recovery of Lx, presumably a consequence of low affinity of the epoxidase enzyme to L (Matsubara *et al.* 2003) and strong binding of L to the light-harvesting antenna complexes after Lx→L photoconversion. As demonstrated here, slow Lx recovery seems to be a common feature among *Inga* species. Substantial Lx recovery occurred neither in the diurnal time-course experiment with *I. spectabilis* in the field (Fig. 2), nor after 1 d dark adaptation of shade-grown *I. marginata* following brief exposure to increased irradiance (Fig. 4).

In *Cuscuta reflexa*, slow Lx recovery, requiring >24 h, occurs without a concomitant decrease in L (Snyder *et al.* 2005). The authors proposed that the Lx accumulation in this plant reflects *de novo* synthesis of Lx via L in the β,ε-carotenoid pathway. In contrast, the gradual accumulation of Lx in sun-grown *I. marginata* over the 4 d shade acclimation period was accompanied by a marked decrease in L both on a Chl basis (Fig. 3a) and on a leaf area basis (data not shown). Thus, the slow Lx recovery in *Inga* may involve the existing pool of L in the light-harvesting antennae. The data in Fig. 3 clearly demonstrate that accumulation of Lx in these plants happens only when VDE is inactive over days, whereas the dark period in a normal day–night cycle allows a full recovery of V. Because Lx is quickly converted to L under illumination (Fig. 4), continuously shaded environments seem to be a prerequisite for high Lx accumulation in *Inga* leaves.

In the experiment of Fig. 3, the sum of L and Lx slowly decreased because of the more-than-proportional reduction in L while the levels of all β,β-xanthophylls (N, V, A, Z) remained largely unchanged (Fig. 3). This marked decrease in L suggests selective degradation of this pigment, in

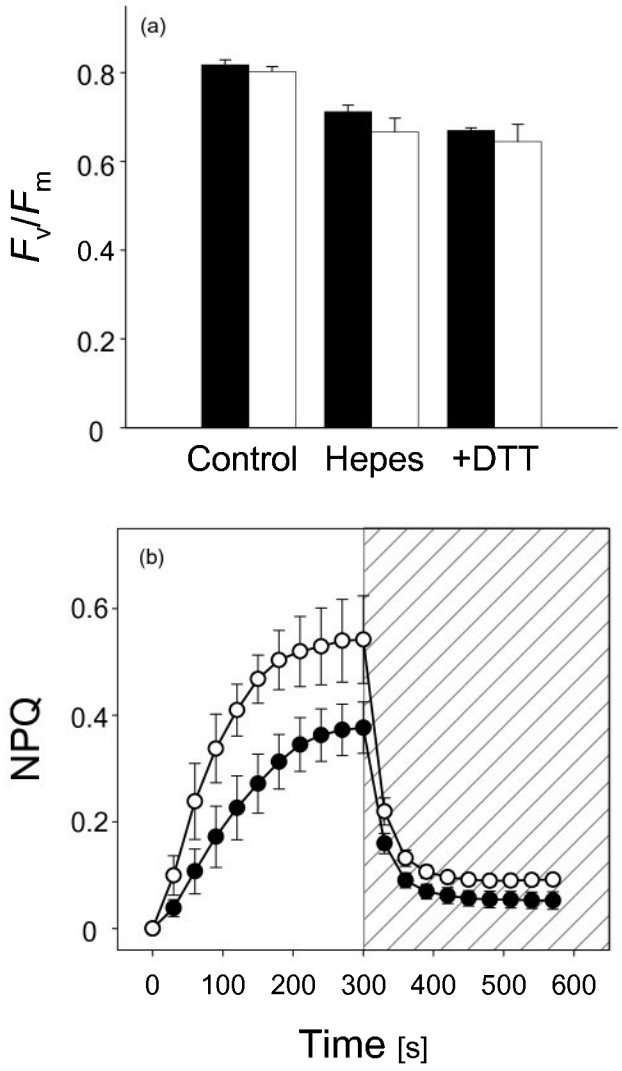


Figure 7. Changes in the maximal photosystem II efficiency (F_v/F_m) and non-photochemical quenching (NPQ) in shade-acclimated seedlings of *Inga marginata* (grown under photosynthetic photon flux density (PPFD) < 50 μmol photons m^{-2} s^{-1}) on day 1 (closed bars and symbols) and day 2 (open bars and symbols). (a) Comparison of F_v/F_m measured in dark-adapted leaf discs with or without (control) vacuum infiltration with 5 mM Hepes (Hepes) or 10 mM dithiothreitol (DTT) in 5 mM Hepes (+DTT) (±SE; $n = 4$). (b) Light-induced NPQ formation and dark relaxation in leaf discs treated with DTT (±SE; $n = 4$). The NPQ induction was measured for 5 min under a moderate intensity of red light (300 μmol photons m^{-2} s^{-1}) to minimize photoinhibition. The hatched background indicates a dark period. After the measurements on day 1, the plants were exposed to 800 μmol photons m^{-2} s^{-1} at 25 °C for 30 min in a climate chamber. The plants were then transferred back to the growth condition and allowed to recover until the measurements on the next morning (day 2).

addition to its epoxidation to Lx, following sun-to-shade transition. As Chl $a + b$ content does not indicate downsizing of the light-harvesting antennae during the shade acclimation (Fig. 3b), we hypothesize that the fraction of L degraded upon shading may correspond to L molecules either free in the lipid membrane phase or bound in peripheral binding sites of light-harvesting antenna complexes. Nevertheless, the striking photoacclimatory shift between the β,ε- and β,β-carotenoid pathways in sun and shade leaves of different *Inga* species (Fig. 1) strongly suggests that longer-term shade acclimation eventually leads to a relative increase in the β,ε-carotenoids, presumably by shade-induced up-regulation of the ε-cyclase activity relative to the β-cyclase activity (Pogson *et al.* 1996; Hirschberg 2001). The slow Lx accumulation (Fig. 3a) may represent a first step of shade acclimation in the photosynthetic pigments of *Inga* plants, followed by the adjustment in the carotenoid biosynthetic pathways to bring about typical α/β-car ratios and pool sizes of V-cycle pigments (Table 2).

Lutein epoxide cycle and light harvesting

Despite altered Lx and L levels obtained in the experiments shown in Figs 3–5 and 7, F_v/F_m measured in dark-adapted *I. marginata* leaves remained nearly unchanged, indicating that the Lx cycle does not significantly affect the maximal PSII efficiency. This was observed by the fluorescence measurements using strong saturation pulses of either white light (PAM system, Figs 3 & 4) or red light (PEA system, Figs 5 & 7).

An intriguing symptom revealed by the measurements of fast fluorescence induction in dark-adapted leaves following the Lx→L conversion is the deceleration of fluorescence rise in the photochemical phase (Fig. 5), which is evident in the slope of the linear regression line in the O-J phase (Fig. 5c). As demonstrated in Fig. 5b, the slope of fluorescence induction in this phase increases with increasing excitation light intensity (Strasser *et al.* 1995; Vredenberg & Bulychev 2003; Schansker *et al.* 2006). Thus, the steeper slope of the photochemical phase found in leaves on day 1 compared with day 2 could imply that more excitation energy was transferred from the light-harvesting antennae to the reaction centre of PSII on day 1, when more Lx was present.

Different energy transfer efficiencies in the light-harvesting antenna complexes containing either more Lx or more L could explain the variation in the slope of fluorescence rise. In fact, it has been demonstrated recently that light-harvesting efficiency increases in recombinant Lhcb5 when it is reconstituted with Lx instead of L (Matsubara *et al.* 2007). In that *in vitro* study, absorption and fluorescence excitation spectra indicated that Lx can augment energy transfer efficiency from xanthophylls to Chl *a* as well as between Chl *a* molecules in recombinant Lhcb5. As the red light of the PEA instrument used for the measurements of fast fluorescence induction does not directly excite carotenoids (including Lx), we assume that the effect of the Lx→L conversion detected in the experiment in Fig. 5 reflects the difference between Lx and L in facilitating excitation energy transfer between Chl *a* molecules.

Even though the change in the slope of fluorescence rise between 0.2 and 0.3 ms accompanying the Lx→L

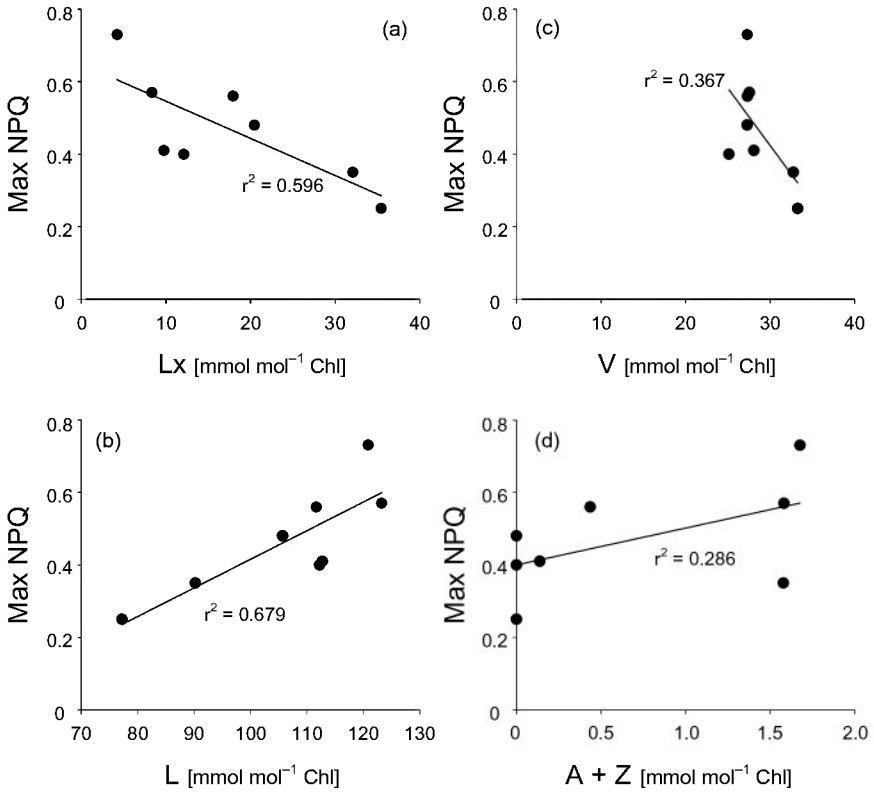


Figure 8. Correlation between the maximal non-photochemical quenching (NPQ) level reached within 5 min in leaf discs pretreated with dithiothreitol (DTT) and the amounts of different xanthophyll-cycle pigments found in the same samples at the end of the experiment. (a) Lx, (b) L, (c) V and (d) A + Z. Each symbol represents the xanthophyll amount and the maximal NPQ level measured in a single leaf disc. Data of days 1 and 2 from the experiment in Fig. 7 are pooled in this figure. The linear regression lines were obtained by using all eight data points (4 × day 1; 4 × day 2) from four individual leaves. For the experimental procedure, see legend to Fig. 7.

conversion was similar to the difference between 3500 and 3000 μmol photons m^{-2} s^{-1} (Fig. 5c), fluorescence induction recorded on day 2 was further decelerated during the J-I phase to become more similar to the pattern measured with 2100 μmol photons m^{-2} s^{-1} in the J-I-P phase (Fig. 5a,b). We cannot exclude that effects on the PSII reaction centre (Vredenberg & Bulychev 2003; Zhu *et al.* 2005; Lazár 2006) have occurred simultaneously with the pigment alteration in light-harvesting antenna complexes, and contributed to the changes in the fluorescence induction curve, although not affecting F_v/F_m. However, in view of *in vitro* data (Matsubara *et al.* 2007) and the strong alteration in Lx and L levels found in the samples (Fig. 4), the most plausible explanation for the phenomenon depicted in Fig. 5 is improvement of light harvesting in the presence of Lx. The electric voltage across thylakoid membranes, measured as absorbance change at 515 nm and reaching the maximum at the position of J (Schreiber & Neubauer 1990), may also play a role in the differential effects of Lx and L on fast fluorescence induction. Analysis of Chl *a* fluorescence lifetimes may provide information on the processes in PSII that accompany the Lx→L conversion and result in altered Q_A reduction kinetics.

Improved light harvesting in the presence of Lx should result in an increased quantum yield of CO_2 assimilation. Measurements of apparent quantum yield were not fully conclusive (Fig. 6) even though values were higher on day 1 (with larger amounts of Lx) in three out of four leaves. Furthermore, unchanged rates of dark respiration on days 1 and 2 (data not shown), together with a lower light compensation point on day 1, are consistent with increased quantum yield on day 1. Future studies under non-photorespiratory conditions may provide improved resolution of differences in quantum yield between leaves differing in Lx content. In sun-acclimated leaves, where highly efficient light harvesting is less important and Lx levels are very low, characteristics of photosynthesis were strikingly different from those of shade leaves (Fig. 6b,c), demonstrating a high photoacclimatory capacity. Pronounced photosynthetic sun–shade acclimation has been reported for seedlings of other neotropical tree species, with the capacity to acclimate to strong light being larger in pioneer than late-successional species (Krause *et al.* 2001, 2003, 2004).

Lutein epoxide cycle and photoprotection

Light harvesting in shade leaves is one possible function of the Lx cycle; photoprotection is another. For the V cycle, retention of Z by sustained de-epoxidation can result in a marked reduction in F_v/F_m, indicating the formation of strongly dissipative antenna complexes (Adams *et al.* 1995; Ottander, Campbell & Öquist 1995; Niyogi, Grossman & Björkman 1998; Gilmore & Ball 2000; Dall'Osto, Caffarri & Bassi 2005). However, there have been studies in which retention of Z did not cause sustained energy dissipation, such as in leaves of *Alocasia brisbanensis* (Logan *et al.* 1997) or *Yucca* species (Baker *et al.* 2002). In these plants, energy dissipation seemed to be primarily ΔpH dependent,

and the presence of Z alone did not sustain dissipation in the dark (or in the shade).

Analogously, the transgenic *Arabidopsis* lutOE plants, having elevated amounts of L at the expense of V because of the over-expression of ε-cyclase, exhibited acceleration of light-induced (ΔpH-dependent) energy dissipation rather than sustained energy dissipation (Pogson & Rissler 2000). For Lx-cycle species, it has been suggested that de-epoxidation in the Lx cycle may contribute to energy dissipation, in much the same way as de-epoxidation in the V cycle, based on the correlation between NPQ and the extent of de-epoxidation in the two xanthophyll cycles found in leaves of oak trees (García-Plazaola *et al.* 2003). In the study with oak leaves, both xanthophyll cycles were operating in parallel, and therefore, the effects of the two cycles could not be separated. Our data from the DTT-treated leaves of *I. marginata*, however, strongly suggest that de-epoxidation in the Lx cycle can enhance the ΔpH-dependent, rapidly reversible component of NPQ (Figs 7 & 8).

In the experiment with lutOE plants, in which de-epoxidation of V to Z was not inhibited during the NPQ measurements, the NPQ-promoting effect of L was found only in the kinetics of fluorescence quenching, not in its maximal level (Pogson & Rissler 2000). However, the DTT-treated leaves of *I. marginata* exhibited faster formation as well as a higher level of NPQ on day 2 compared with day 1 (Fig. 7b). Hence, we propose that part of the L pool in the Lx cycle, especially the photoconverted fraction bound at the internal and peripheral xanthophyll binding sites of the light harvesting antenna complexes (Matsubara *et al.* 2007), may be involved in the regulation of light harvesting by modulating thermal energy dissipation. With photoconverted L molecules retained overnight, shade leaves could engage in faster and stronger energy dissipation when suddenly exposed to excessive light, which is rapidly reversible in the dark (Fig. 7b) or under low PPFD. As xanthophyll conversion in the Lx cycle does not significantly influence F_v/F_m values (Figs 3–5 & 7), L formed from Lx clearly does not cause sustained energy dissipation. Thus, species having both Lx and V cycles offer an interesting system to investigate roles and impact of L and Z in the regulation of ΔpH-dependent energy dissipation and other photoprotective processes, such as scavenging of reactive oxygen species and quenching of triplet excited Chls (Havaux & Niyogi 1999; Dall'Osto *et al.* 2006; Johnson *et al.* 2007).

Lutein epoxide cycle in *Inga* species in their natural habitat

The genus *Inga* consists of around 300 species of medium-sized trees in the neotropics. About one-half of them are probably capable of fast growth under high-light conditions in forest gaps. Nevertheless, they are not true pioneers because their fleshy seeds require shade and high humidity for germination (Pennington 1997, 1998). The success of *Inga* thus depends on survivorship in the shade during the seedling stage and the ability to exploit sunlight in tree-fall gaps for rapid growth, to eventually become subcanopy and canopy components of the mature forest. Young leaves of *Inga* species are characterized by marked chemical defence expression (Lokvam, Coley & Kursar 2004; Lokvam *et al.* 2007), which may reduce loss of leaf area and seedling mortality caused by herbivores and pathogens.

In the shaded forest understorey, a high capacity of existing leaves to capture and utilize light energy is of critical importance for survival (Björkman 1981). The distichous phyllotaxis and horizontal growth of branches, which are highly conserved throughout the genus *Inga* (Pennington 1998), allow efficient light interception while minimizing leaf overlap (Leigh 1999). In addition to such architectural adaptation, improvement of light harvesting by optimizing photosynthetic pigment composition would contribute to a positive energy budget.

The ability to accumulate Lx in shade leaves of *Inga* species, together with the formation of large antenna complexes (Anderson, Chow & Goodchild 1988), may be a trait that supports seedling growth and survival in the forest understorey. When leaves of these seedlings are suddenly exposed to direct sunlight by occurrence of sunflecks or in tree-fall gaps, de-epoxidation quickly converts Lx to L in parallel with V to Z (Fig. 4) to reduce the efficiency of light energy transfer (Fig. 5), induce rapidly reversible thermal energy dissipation (Fig. 7) and enhance photoprotection. The slowly reversible Lx→L conversion results in overnight retention of L, but without causing unnecessary loss of carbon fixation under low PPFD by slowly reversible NPQ, which exacerbates the leaf carbon budget under shaded environments (Zhu *et al.* 2004). When a high PPFD condition persists, new leaves grow that are sun acclimated regarding pigment biosynthesis (Figs 1 & 2), CO_2 fixation and respiration (Fig. 6). Such saplings may be overtopped and shaded by fast-growing gap species and by growth of the surrounding large trees. Because *Inga* leaves can survive for several years, it is important that leaves have the capacity to adapt from sun to shade (Fig. 3) and vice versa. Reorganization of the light-harvesting antennae through operation of the Lx cycle may contribute to such photoacclimatory plasticity and confer an advantage to *Inga* plants experiencing contrasting light environments.

In conclusion, our study suggests a regulatory role of the Lx cycle in light harvesting and photoprotection *in vivo*, reports commonly high Lx levels in shade leaves of a range of *Inga* species *in situ* and is consistent with previous studies showing extremely low rates of L→Lx epoxidation in *Inga*.

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