

Dynamics of violaxanthin and lutein epoxide xanthophyll cycles in Lauraceae tree species under field conditions

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Summary Two xanthophyll cycles have been described in higher plants: the violaxanthin xanthophyll (V or VAZ) cycle, which is present in all species, and the taxonomically restricted lutein epoxide xanthophyll (Lx) cycle, which involves the light-induced de-epoxidation of Lx to lutein (L) and its epoxidation back to Lx in low light. Laboratory experiments indicate that the first reaction occurs quickly, but the second reaction is much slower. We investigated the Lx cycle under field conditions in several tree species of the Lauraceae family to determine its relationship with the ubiquitous V cycle. The field study was conducted in two natural laurel forests: one in the Canary Islands, where *Laurus azorica* (Seub.) Franco, *Ocotea foetens* (Aiton.) Benth, *Apollonias barbujana* (Cav.) Bornm. and *Persea indica* (L.) Spreng were studied; and one in the Basque Atlantic coast where *Laurus nobilis* L. was studied. The results were complemented by a taxonomic study. The presence of Lx was widespread among Lauraceae species, but its concentration varied even among closely related species. The V pool size correlated positively with growth irradiance, whereas the relationship between Lx pool size and growth irradiance varied with species. A functional Lx cycle was confirmed under field conditions only in *O. foetens* and *L. nobilis*. Furthermore, in *O. foetens*, a correlation between Lx de-epoxidation and photoinhibition suggested a protective role for this cycle. We conclude that, unlike the V cycle, which is normally correlated with irradiance, the operation and light dependence of the Lx cycle is species-dependent.

Keywords: de-epoxidation, laurel forest, *Laurus*, *Ocotea*, photoinhibition, photoprotection.

Introduction

Pigment composition of photosynthetic tissues is highly conservative among higher plants, with six major carotenoids present in almost all species: neoxanthin, lutein (L), β -carotene, violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) (Young et al. 1997). Carotenoids have an essential role in

light harvesting, thermal energy dissipation, triplet chlorophyll quenching, scavenging of singlet oxygen and membrane stabilization. In particular, carotenoids have a vital role in preventing damage to the photosynthetic apparatus, which they do by modulating the rate of thermal energy dissipation through the violaxanthin (V or VAZ) cycle, which involves the light-driven de-epoxidation of V into A and Z. Over-reduction of the electron transport chains occurs when excess light energy is absorbed. Under these conditions, the transfer of excitation energy from chlorophyll molecules to molecular oxygen or the leakage of electrons to oxygen leads to the formation of toxic active oxygen species (Elstner and Osswald 1994, Foyer and Noctor 2000). In forest canopies, the amount of V-cycle carotenoids (i.e., V, A and Z) per unit leaf area or per unit chlorophyll scales positively with long-term quantum flux density in the canopy (Logan et al. 1996, Niinemets et al. 1999, García-Plazaola and Becerril 2001, Hansen et al. 2002).

One exception to the general pattern of carotenoid pigments is in *Cuscuta reflexa* Roxb (Bungard et al. 1999), a parasitic plant, the leaves of which are depleted in neoxanthin but which possess lutein-5, 6-epoxide (Lx), which undergoes a light-driven de-epoxidation into lutein (L) in parallel with the V cycle. The conversion of Lx into L, and the re-epoxidation to Lx in the dark, constitutes the so-called Lx cycle. This cycle has been reported recently in other parasitic plants including mistletoes (Matsubara et al. 2001, 2003) and *Cassytha* (Close et al. 2006). It has also been observed in oaks (García-Plazaola et al. 2002, 2003, Llorens et al. 2002), leguminous trees (Watson et al. 2004, Matsubara et al. 2005) and other woody plants (García-Plazaola et al. 2004).

The parallel operation of the V and Lx cycles raises the question of whether both cycles, when present, are involved in photoprotection by modulating the rate of non-radiative dissipation (NRD). Although we lack direct evidence that L formation affects NRD, its potential role in photoprotection is supported by the observed correlation between the extent of Lx de-epoxidation and the rate of non-photochemical quenching (NPQ) (García-Plazaola et al. 2003). A photoprotective func-

tion is also supported by studies with *Arabidopsis* mutants showing that L, or any other xanthophyll with a β -cyclic de-epoxidized end-group structure, contributes to the development of NRD (Pogson et al. 1998). One difference between the V and Lx cycles is the rate of dark epoxidation, which is lower (or zero) in the Lx cycle (Matsubara et al. 2005). This has led to the suggestion that the Lx cycle represents a one-way (Lx to L) emergency mechanism of sustained energy dissipation after abrupt changes in the light environment (García-Plazaola et al. 2003, Matsubara et al. 2005) such as occurs, for example, following the formation of gaps in the forest canopy. Although operation of the full Lx cycle (light-induced de-epoxidation and dark recovery) has been demonstrated under controlled conditions (Snyder et al. 2005), this study is the first to demonstrate its occurrence in the field.

Previously, we showed that significant amounts of Lx are present in leaves of species of Fagaceae, Pinaceae, Magnoliaceae, Aceraceae, Fabaceae and Lauraceae (García-Plazaola et al. 2004). The Lauraceae includes 2500–3000 species and 50 genera with tropical and subtropical distribution. Phylogenetic studies (Chanderbali et al. 2001) show that the Lauraceae is divided into four major lineages: Cinamomeae, Laureae, Perseae and Cryptocareae. Our study focused on the laurel tree species *Persea indica* (L.) Spreng., *Laurus azorica* (Seub.) Franco, *Ocotea foetens* (Aiton.) Benth., *Apollonias barbujana* (Cav.) Bornm. and *Laurus nobilis* L. These species occur in forests that are dominated by laurels and which occur in subtropical regions where cloud and fog maintain high atmospheric humidity. In the Mediterranean region, laurel forests are relicts of the Tertiary Mediterranean flora that occupied southern Europe and northern Africa about 20 million years ago, but are now limited to small areas on the Atlantic coast (Santos 1990). The largest representative laurel forest is in the central and western Canary Islands at altitudes between 500 and 1400 m, where continuous northeast trade-winds raise atmospheric humidity on northern slopes. In general, laurel tree species are shade tolerant. Because laurel foliage has a high Lx pool, laurel forests appear to provide a good system for studying the role of the Lx cycle in photoprotection.

Previous field studies of the Lx cycle in laurel species (Matsubara et al. 2001, García-Plazaola et al. 2002) focused only on full sun and full shade leaves. In this study we sought to establish whether the Lx cycle operates in laurel tree species under a range of irradiances in the field and, if so, what role it plays in photoprotection.

Materials and methods

Experimental sites and species

The field study was carried out in the Urdaibai Biosphere Reserve (UBR), Bizkaia (Basque Country, Spain) (43°21' N, 2°40' W, 10 m a.s.l.) and in Anaga Natural Park (ANP), Tenerife (Canary Islands, Spain) (28°32' N, 16°7' W, 700 m a.s.l.). Plant material was collected in June 2004, January 2005 and July 2005 at UBR and in November 2004, February 2005, November 2005, April 2006 and May 2006 at ANP. The cli-

mate at Anaga is humid Mediterranean with a mean annual temperature of 14 °C, mean minimum of 10.7 °C and mean maximum of 17.7 °C and a mean annual precipitation of 733 mm. The climate at UBR is Atlantic with a mean annual temperature of 13.6 °C, mean minimum of 9.3 °C and mean maximum of 17.8 °C and a mean annual precipitation of 1195 mm. *Persea indica*, *Laurus azorica*, *Ocotea foetens* and *Apollonias barbujana* were studied at ANP and *L. nobilis* was studied at UBR. Foliage samples from other laurel species were collected at the University of the Basque Country campus in Leioa, Spain, at the University of La Laguna campus in Tenerife, Spain and at the Royal Kew Gardens (London, U.K.) (Table 1). Where available, sun and shade leaves were collected from each tree.

Experimental design and sampling

Leaf discs (5-mm diameter) were measured for fluorescence, and additional discs from the same leaves were frozen in liquid nitrogen and stored at –80 °C until required for pigment analysis. To measure maximum epoxidation of the Lx and V pools, branches from several adult trees were cut and stored in plastic bags for 16 h in the dark at room temperature (20–22 °C). To maximize the concentration of epoxidized xanthophylls, reduce the effects of diurnal variations in pigment composition and provide comparable conditions (García-Plazaola et al. 2000, Tausz et al. 2003), daily courses in pigment composition were analyzed in 5–7 leaves from the same adult trees. When sun and shade leaves were analyzed separately, sun leaves were defined as those facing south in the outer canopy at a height of at least 2 m, whereas shade leaves were collected from the inner, north-facing canopy.

Irradiance measurement

The light environment of each leaf was estimated by hemispherical photographs taken above each of the study branches with a Nikon Coolpix 4500 digital camera equipped with a Nikon Fisheye Converter. Photographs were analyzed with Gap Light Analyzer (GLA) Version 2.0 software. The proportion of monthly radiation below the canopy relative to that in the open (relative irradiance) was calculated using the same software.

Chlorophyll fluorescence

Chlorophyll a fluorescence was determined on leaves in the laboratory and in the field. Fluorescence was measured with a portable modulated fluorimeter (OS 5-FL, Opti-Sciences). Initial (F_o) and maximal (F_m) fluorescence were measured in dark-adapted leaves with a 0.8-s pulse of saturating light. The maximal photochemical efficiency of PSII was estimated as $F_v/F_m = (F_m - F_o)/F_m$.

Analytical methods

Photosynthetic pigments were extracted from leaf discs with acetone (100%) and analyzed by reverse-phase HPLC following the method of García-Plazaola and Becerril (1999) with the modifications described by García-Plazaola and Becerril

Table 1. Concentrations of violaxanthin (V) and lutein epoxide (Lx) in Lauraceae species (mean \pm SE, $n = 3-5$ replicates except for *Cinnamomum camphora* for which $n = 1$). Lineages: C, Cinnamomeae; CR, Cryptocariaceae; L, Laureae; O, Cinnamomeae-Ocotea complex; and P, Perseae. Plant material sources: ANP, Anaga National Park; RKG, Royal Kew Gardens; UBC, University of Basque Country campus; and ULC, University of La Laguna campus. Abbreviation: Chl, chlorophyll.

Species	Lineage	V (mmol mol ⁻¹ Chl)	Lx (mmol mol ⁻¹ Chl)	Lx/V	Source
<i>Umbellularia californica</i> (Hook & Arn.) Nutt. (sun)	O	76.3 \pm 10.4	48.0 \pm 6.4	0.63	RKG
<i>Umbellularia californica</i> (Hook & Arn.) Nutt. (shade)	O	28.5 \pm 1.4	43.9 \pm 1.2	1.54	RKG
<i>Ocotea foetens</i> (Aiton.) Benth. (sun)	O	33.2 \pm 5	10.8 \pm 1.1	0.33	ANP
<i>Ocotea foetens</i> (Aiton.) Benth. (shade)	O	33.0 \pm 1.1	6.3 \pm 0.2	0.19	ANP
<i>Laurus nobilis</i> L. (sun)	L	32.4 \pm 2.9	8.7 \pm 0.7	0.27	UBC
<i>Laurus nobilis</i> L. (shade)	L	33.2 \pm 1.2	38.8 \pm 2.7	1.17	UBC
<i>Laurus azorica</i> (Seub.) Franco (sun)	L	35.2 \pm 1.8	15.4 \pm 2.3	0.44	ANP
<i>Laurus azorica</i> (Seub.) Franco (shade)	L	34.8 \pm 1.9	10.2 \pm 1.9	0.29	ANP
<i>Litsea cubeba</i> (Lour.) Pers. (shade)	L	36.1 \pm 1.4	17.4 \pm 2.4	0.48	RKG
<i>Neolitsea sericea</i> (Bl.) Koidz. (shade)	L	43.5 \pm 1.3	18.4 \pm 1.0	0.42	UBC
<i>Sassafras randaiense</i> (Hayata) Rehder (shade)	L	47.6 \pm 1.1	2.7 \pm 0.4	0.06	RKG
<i>Persea americana</i> Mill. (shade)	P	32.4 \pm 2.6	16.2 \pm 2.6	0.50	ULC
<i>Apollonias barbuiana</i> (Cav.) Bornm. (sun)	P	44.8 \pm 2.3	3.9 \pm 1.0	0.09	ANP
<i>Apollonias barbuiana</i> (Cav.) Bornm. (shade)	P	34.5 \pm 2.0	9.6 \pm 1.6	0.28	ANP
<i>Persea palustris</i> (Raf.) Sarg. (sun)	P	62.8 \pm 9.8	15.8 \pm 4.6	0.25	RKG
<i>Persea palustris</i> (Raf.) Sarg. (shade)	P	34.4 \pm 1.2	24.0 \pm 5.2	0.70	RKG
<i>Persea indica</i> (L.) Spreng (sun)	P	49.2 \pm 1.2	1.3 \pm 0.1	0.03	ANP
<i>Persea indica</i> (L.) Spreng (shade)	P	34.5 \pm 2.0	2.5 \pm 1.3	0.07	ANP
<i>Cinnamomum japonicum</i> Siebold (shade)	C	38.8 \pm 0.3	38.8 \pm 4.8	1.00	RKG
<i>Cinnamomum daphnoides</i> Siebold & Zucc. (shade)	C	37.6 \pm 0.6	18.9 \pm 1.6	0.50	RKG
<i>Cinnamomum pseudopedunculatum</i> Hayata (shade)	C	32.6 \pm 3.3	13.2 \pm 0.3	0.40	RKG
<i>Cinnamomum tamala</i> T. Nees & Eberm. (shade)	C	38.2 \pm 3.0	14.5 \pm 2.6	0.40	RKG
<i>Cinnamomum camphora</i> (L.) Sieb. (shade)	C	37.6	3.7	0.10	UBC
<i>Beilschmiedia tawa</i> (Hook. f.) Kirk. (shade)	CR	55.1 \pm 1.7	17.13 \pm 0.5	0.31	RKG

(2001). Retention times and conversion factors for carotenoids were the same as described by García-Plazaola and Becerril (1999, 2001). The detection limit of lutein epoxide in our chromatographic system was about 100 μ mol mol⁻¹ chlorophyll.

Results

Pigment composition was analyzed in 17 Lauraceae species (Table 1). Lutein epoxide concentrations varied among species. In closely related species belonging to the same genus (e.g., *Cinnamomum* and *Persea*), Lx concentrations in shade leaves varied by as much as one order of magnitude. In three species, Lx concentration was almost negligible (*Sassafras randaiense*, *Cinnamomum camphora* and *Persea indica*), whereas in shade leaves of *Umbellularia californica*, *Laurus nobilis* and *C. japonicum*, the concentration of Lx was higher than that of V. The species with the lowest Lx concentrations belong to different lineages (Laureae, Cinnamomeae and Perseae) as do the species with the highest Lx concentrations (Cinnamomeae–*Ocotea* complex, Laureae and Cinnamomeae). In the other species studied, Lx concentration was more stable with Lx/V ratios in shade leaves ranging from 0.19 to 0.70. In general, sun leaves had higher Lx concentrations than shade leaves, except sun leaves of *Ocotea foetens* and *U. californica*. Both of these species are phylogenetically included in the so-called *Ocotea* complex.

To study the performance of the two xanthophyll cycles, pigment composition was studied in the field in intact leaves of five Lauraceae species characterized by high, medium and low Lx concentrations: *L. nobilis* (young and mature leaves), *O. foetens*, *Apollonias barbuiana*, *L. azorica* and *P. indica* (Figure 1). Leaves were sampled along the vertical light gradient in the canopy at noon and predawn to determine responses of the V and Lx pools to growth irradiance. In all species, the concentration of V before dawn was positively correlated with the relative growth irradiance of each leaf and was higher at predawn than at midday. This daily pattern, together with the corresponding formation of A + Z at noon, demonstrated the full operation of the V cycle in all species. Conversely, the response of Lx to the light environment varied widely among species. The concentration of lutein epoxide correlated positively with irradiance in *O. foetens*, negatively in *L. nobilis* and had no correlation in *L. azorica*, *P. indica* or *A. barbuiana*. Contrasting with V, the concentration of Lx did not increase significantly from noon until before dawn in *A. barbuiana*, *L. azorica* or *P. indica*, but did in *L. nobilis* and *O. foetens*. Analysis of young and mature leaves of *L. nobilis* revealed a similar pool of V but a higher pool of Lx in old leaves compared with young leaves. Across all species, there was no relationship between Lx concentration and the total carotenoid to chlorophyll ratio (data not shown). Contrasting with *L. nobilis*, V and Lx concentrations in *O. foetens* were positively correlated

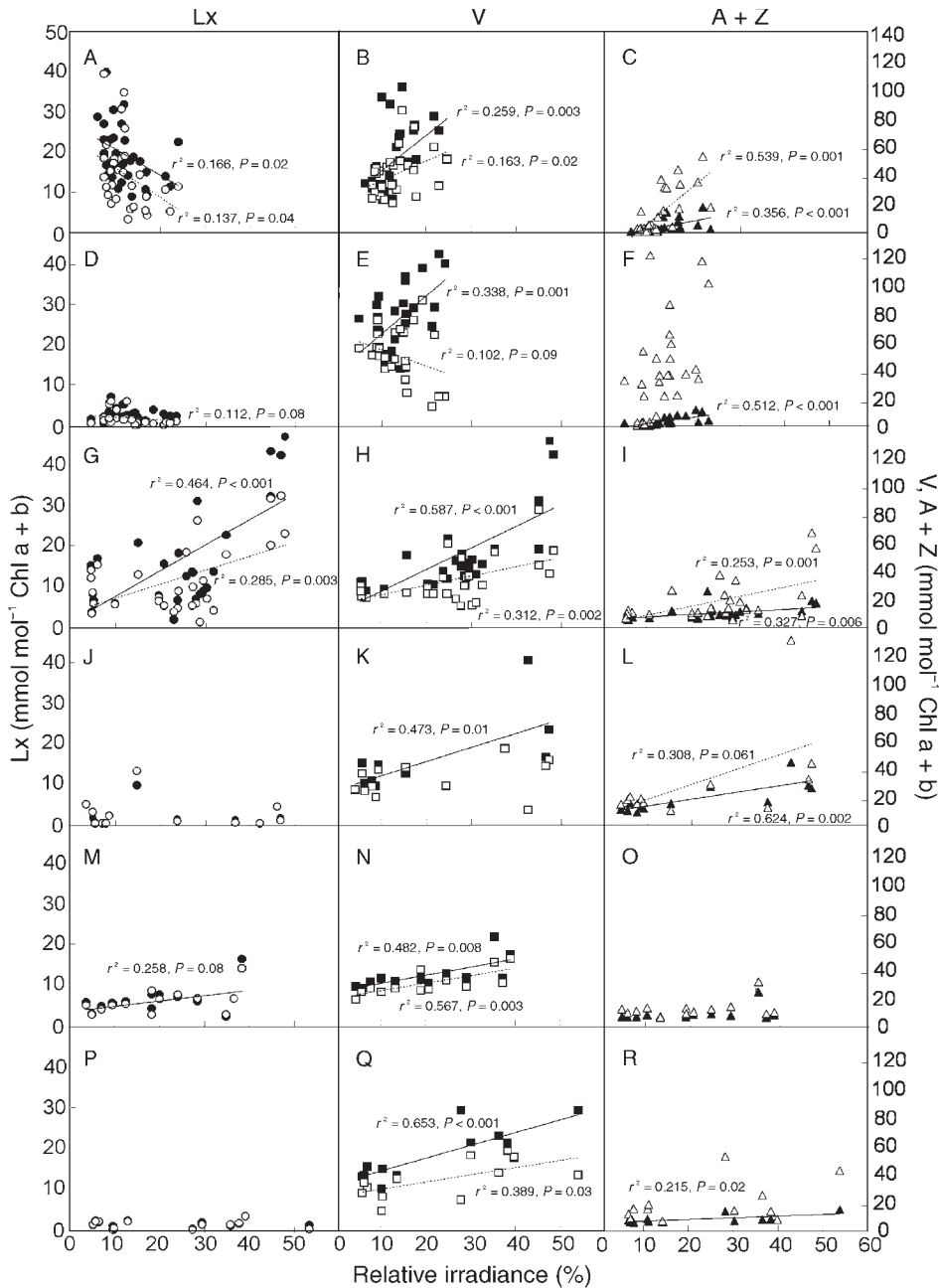


Figure 1. Relationship between the concentrations of lutein epoxide (Lx), violaxanthin (V) and antheraxanthin + zeaxanthin (A + Z) and relative growth irradiance in intact leaves from different trees growing in natural canopies of *Laurus nobilis* (old leaves, A–C; new leaves, D–F), *Ocotea foetens* (G–I), *Apollonia barbujana* (J–L), *Laurus azorica* (M–O) and *Persea indica* (P–R) measured at noon (open symbols) and predawn (closed symbols). Linear regressions (dotted line = noon, solid line = predawn) are shown when significant at $P < 0.1$.

(Figure 2A). Figure 2B shows the effects of irradiance on the daily conversions of V and Lx. In *O. foetens*, both activities correlated positively with irradiance, whereas in *L. nobilis* these activities were irradiance independent. All of these results point to a similar performance of V and Lx cycles in *O. foetens*.

To study the operation of the Lx cycle under field conditions in more detail, the daily cycle was followed in intact leaves of the two species for which night relaxation was observed (*O. foetens* and *L. nobilis*) (Figure 3). The experiment was first conducted in sun leaves, which contained the highest Lx pool, during the transition from an unusually warm and sunny period to a more typical cloudy and less stressful period. Irra-

diance changes were typical for a daily course in the laurel forest environment, with a brief period of clear sky around midday. During the first night, F_v/F_m recovered from the low values measured in the afternoon. This unusually low F_v/F_m may have been caused by the previous sunny period experienced by the trees. During the next morning, a slight decline in F_v/F_m was observed simultaneously with light-driven deoxidation of V and Lx. After midday, when it became cloudy and the air more humid, the pools of V and Lx increased continuously for the following 20 hours. The Lx pool was substantially higher at the end of the diurnal course than at the beginning. In parallel with the increase in V, F_v/F_m recovered. Furthermore, the relationship between the concentration

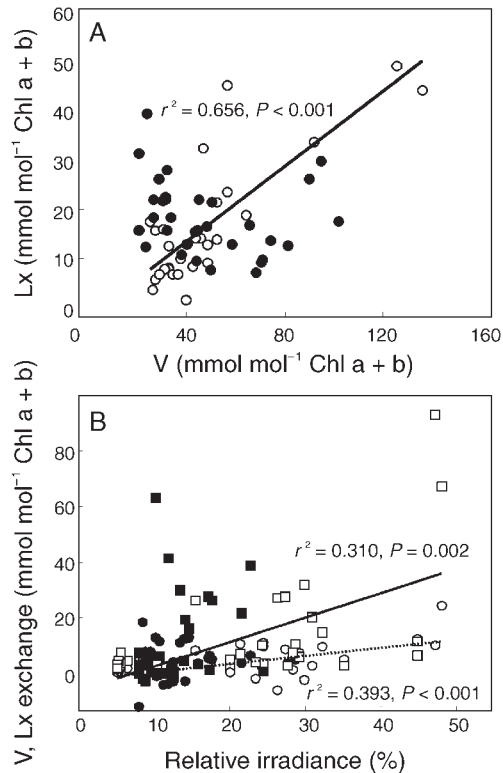


Figure 2. (A) Relationship between violaxanthin (V) and lutein epoxide (Lx) concentrations in leaves of *Laurus nobilis* (●) and *Ocotea foetens* (solid line, ○). (B) Relationship between daily changes in V (solid line, □, ■) and Lx (dotted line, ○, ●) concentrations in leaves collected from canopies of *L. nobilis* (closed symbols) and *O. foetens* (open symbols). For both panels, data have been redrawn from Figure 1. Linear regressions are shown when significant at $P < 0.1$.

of Lx and the F_v/F_m ratio in leaves of *O. foetens* was stronger than that of V and F_v/F_m (Figure 4), suggesting a potential role of the Lx cycle in long-term down-regulation of photosynthesis in this species. A similar diurnal course was followed in intact leaves of *L. nobilis* (Figure 3). The experiment was performed on semi-shade leaves on a sunny day in summer. Only brief sunflecks reached the studied leaves, leading to limited de-epoxidation. In parallel with the de-epoxidation of V at noon, a decrease in the maximal photochemical efficiency (F_v/F_m) occurred, and both parameters recovered during the rest of the day; however, an unexpected drop in F_v/F_m , probably because of the repeated sampling or application of saturating flashes, occurred during the night. No significant changes were detected in the Lx pool during the daily cycle, although a trend similar to V was observed.

Discussion

Recent papers have evaluated the presence and role of the Lx cycle in Viscaceae and Loranthaceae (Matsubara et al. 2003), *Acacia melanoxylon* R. Br. (Watson et al. 2004) *Inga* sp. (Matsubara et al. 2005), *Quercus* (García-Plazaola et al. 2002), several woody plants (García-Plazaola et al. 2004),

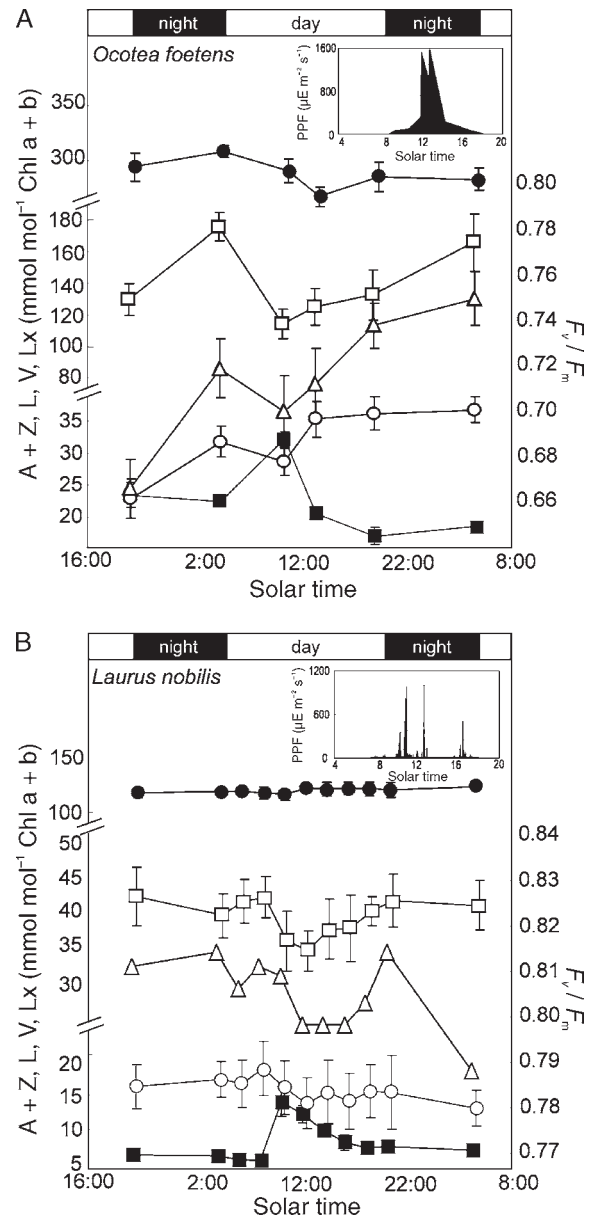


Figure 3. Daily courses of violaxanthin (V; □), lutein epoxide (Lx; ○), lutein (L; ●) and antheraxanthin + zeaxanthin (A + Z; ■) concentrations and F_v/F_m (Δ) in (A) *Ocotea foetens* growing at Anaga National Park (May 18, 2006) and (B) *Laurus nobilis* growing at Urdaibai Biosphere Reserve (July 21, 2005). Fluorescence measurements in *L. nobilis* were performed on a single leaf. Inner panels show daily changes in irradiance. Each value represents the mean \pm SE of 5–7 replicates collected from the same tree.

Cuscuta (Snyder et al. 2005) and in the hemi-parasitic *Cassytha* (Close et al. 2006). In the present work, we expand the list of species possessing Lx with a detailed study of the family Lauraceae. We found that the presence of Lx in chloroplasts was widespread among laurel species (Matsubara et al. 2003, García-Plazaola et al. 2004). However the concentration of Lx varied among species (2–44 mmol mol⁻¹ Chl) (Table 1). Compared with the Lx pool, the V pool was more stable, rang-

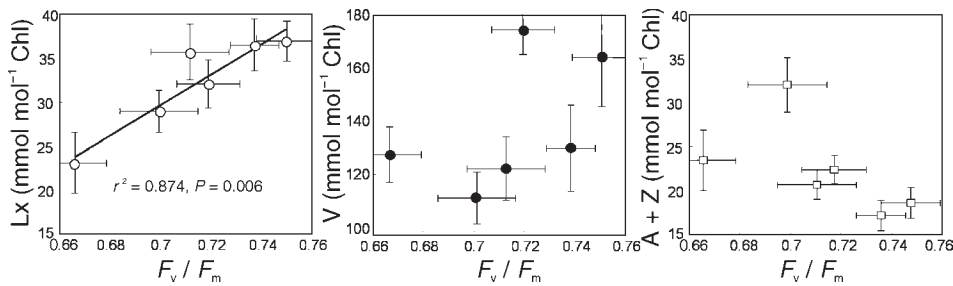


Figure 4. Relationships between the concentrations of lutein epoxide (Lx), violaxanthin (V) and antheraxanthin + zeaxanthin (A + Z) and F_v/F_m ratio in leaves of *Ocotea foetens*. Data have been redrawn from Figure 3. Linear regressions are shown when significant at $P < 0.1$. Each value represents the mean \pm SE of 5–7 replicates collected from the same tree.

ing from 28 to 55 mmol mol^{-1} Chl in shade leaves of the 17 species analyzed, and was similar to that of Lx in *Umbellularia californica*, *Laurus nobilis* and *Cinnamomum japonicum*. Species containing high or low amounts of Lx were found in the three major lineages of the Lauraceae (Cinamomeae, Laureae and Perseae), indicating that Lx concentration is not phylogenetically determined. This suggests that the role of Lx is not essential for chloroplast function and that it is not under strong selective pressure in these species. This result contrasts with those for other families, such as the Viscaceae and Loranthaceae (Matsubara et al. 2003), showing that Lx concentrations and phylogeny are strictly related.

Previous studies have shown that the concentration of Lx tends to be higher in shade leaves (Matsubara et al. 2001, 2005; García-Plazaola et al. 2004) and shaded tissues such as winter buds (García-Plazaola et al. 2004), cabbage heads (Kruk 2005) and inner tissues (Pancaldi et al. 1998) than in sun leaves and sun-exposed tissues. A similar pattern was observed in most of our study species for which sun and shade leaves were compared (Table 1), with the exception of *Ocotea foetens* and *U. californica*. Close et al. (2006) recently reported the presence of higher Lx in sun leaves than in shade leaves of a Lauraceae parasitic species (*Cassytha*). The high Lx concentration in sun leaves of *O. foetens* and *U. californica* implies that Lx is not de-epoxidated after exposure to light or that, once de-epoxidated, it is converted back to Lx in low light. The latter possibility, if true, indicates that *O. foetens* and *U. californica*—which belong to the same lineage (complex *Ocotea* within Cinamomeae group) (Chanderbali et al. 2001)—differ from other Lx species.

Most studies on the effect of light environment on Lx have been performed by comparing sun and shade leaves; however, both situations represent arbitrary points within a continuous light gradient. To characterize in more detail the influence of leaf growth irradiance on Lx concentration, we studied the pigment composition of several laurel species with different Lx concentrations in natural canopies in the field (Figure 1). As has been described in other canopies, predawn V concentration scaled positively with irradiance in all species (Niinemets et al. 1998, 1999, 2003), which is consistent with a higher demand for photoprotection in the outermost layers of the canopy. However, the concentration of Lx was unrelated to irradiance except in *O. foetens* and *L. nobilis*. In *O. foetens*, it increased with irradiance, whereas in *L. nobilis*, it decreased. In *O. foetens*, the Lx and V pools (Figure 2A) and their respective

daily rates of de-epoxidation correlated positively with irradiance (Figure 2B), indicating that both xanthophyll cycles have similar regulatory and photoprotective roles. In contrast, in *L. nobilis*, the V and Lx pools were not correlated ($r^2 = 0.067$), suggesting that the V and Lx cycles have complementary or substitutive roles.

When responses of V to irradiance at predawn and noon were compared (Figure 1), significant differences ($P < 0.01$) in the regression lines were observed in all species, indicating activation of the V cycle. This was confirmed by the reciprocal increase in its de-epoxidation products (A and Z). However, Lx de-epoxidation was observed only in *O. foetens* and *L. nobilis*. To our knowledge, this is the first report showing the activity of the Lx cycle under natural conditions. When the daily cycle was followed in intact leaves of both species in the field, transformations in Lx did not clearly match V changes. In the case of *L. nobilis*, despite significant changes in V concentration, no variation in Lx concentration was observed during the day. A detailed pigment study in sun and shade leaves of *Inga* sp. also failed to detect changes in Lx concentration, although pronounced variations in V concentration occurred (Matsubara et al. 2005). Conversely, in the daily cycle studied in *O. foetens*, the concentrations of Lx and V decreased until midday, then increased progressively for 20 h. This cycle was studied at the end of a period with high light and temperatures. Although noon weather was initially sunny, from 1400 h onward, it became cloudy and foggy. During this period, the increase in Lx concentration paralleled a recovery from photoinhibition, and F_v/F_m correlated more closely with Lx than with V or A + Z (Figure 4). Although not conclusive, this observation corroborates the proposed role of the Lx cycle in photoprotective sustained photoinhibition (García-Plazaola et al. 2003; Matsubara et al. 2005). It is also consistent with previous studies on trees from the Canarian laurel forest (González-Rodríguez et al. 2001; Tausz et al. 2004), showing a lack of correlation between V de-epoxidation and daily reductions in F_v/F_m in *L. azorica* and *Persea indica* compared with other species.

Some authors have proposed an accessory role of the Lx cycle (García-Plazaola et al. 2003) that could “lock in” dissipative mechanisms of photoacclimation after a marked change in the light environment (Matsubara et al. 2005), but others (Close et al. 2006) have shown that maximum engagement of the Lx cycle occurs under the most favorable conditions. The different patterns of Lx response to growth irradiance suggest

that the presence and performance of the Lx cycle is species-dependent. Considering present and previous data, four groups of tree species could be defined based on their Lx responses: (1) Lx responding positively to growth irradiance (represented by *O. foetens* and probably *U. californica*); (2) Lx responding negatively to growth irradiance (represented by *L. nobilis*); (3) low Lx concentration with small or no response to growth irradiance (represented by *P. indica*); and (4) absence of Lx. This pattern of response to irradiance in Lx contrasts with that of the V pool, which increases with the need for photoprotection. Because L is the main carotenoid in chloroplasts, differences in the affinity or accessibility of the epoxidase reaction for L seem to be the biochemical basis for the species-specific responses of Lx to growth irradiance, with L epoxidase activity decreasing from Group 1 to Group 4. In conclusion, we have demonstrated operation of the Lx cycle under field conditions, and have highlighted an unexpected diversity in the significance of this photoprotective mechanism. The results indicate the importance of field investigations to achieve a fuller knowledge of the ecophysiological function of the Lx cycle.

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