

# Photostress, photoprotection, and water soluble antioxidants in the canopies of five Canarian laurel forest tree species during a diurnal course in the field

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## Summary

The Canarian laurel forest ecosystem is composed of several co-dominant evergreen tree species including *Ilex perado*, *I. canariensis*, *Myrica faya*, *Laurus azorica*, and *Persea indica*. With leaves of these trees the diurnal course of stress parameters (chlorophyll fluorescence Fv/Fm, pigments, ascorbate, glutathione, gas exchange, water relations) was investigated during a mildly stressful summer day. Sun leaves generally had lower photochemical efficiencies (morning Fv/Fm in sun leaves were below 0.80 and above 0.80 in shade leaves), less chlorophyll, a larger xanthophyll cycle pool per unit chlorophyll, and more glutathione and ascorbate.

Minimal relative water contents of more than 85% indicated that dehydration was not a stress factor. Stomatal conductances decreased from 150 to 200 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in the morning to about 50 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> during the day in all species, but this did not limit CO<sub>2</sub> uptake.

De-epoxidation of xanthophylls only occurred in sun leaves of *I. canariensis* (to more than 50%) and *M. faya* (more than 60%). Decreases in Fv/Fm were only found in sun leaves of *P. indica* (from ca. 0.80 in the morning to a minimum of 0.70) and, as a trend, also in *L. azorica* (from ca. 0.75 to ca. 0.65). *I. perado* showed neither of those responses. *P. indica* and *L. azorica* exhibited the highest photosynthesis rates of about 10 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> compared to 8 in the other species. The photoprotection strategy of *P. indica* and *L. azorica* admitted slow recovery from photoinhibition, did not activate protective energy dissipation through xanthophylls, and allowed highest production under these typical conditions.

Key words: antioxidants, chlorophyll fluorescence, gas exchange, photoinhibition, xanthophyll cycle.

## Introduction

The Canarian laurel forest ecosystem mainly occurs in the very humid section of the Northern slopes in medium elevations of the Canary Islands where cloud belts govern the water regime. Laurel forest trees are typically evergreen, broadleaved species of different systematic origin. In contrast to typical Mediterranean sclerophyllous forests drought periods are mitigated by the

high frequency of fogs in the Canarian laurel forests. The air humidity is typically very high and the forest has the appearance of a cloud forest (Höllermann 1981).

Laurel forest trees may have to cope with quickly changing conditions due to sunny periods when the irradiation reaches 2000 μmol m<sup>-2</sup> s<sup>-1</sup> and the high light energy imposes photo-oxidative stress on the leaves (Polle & Rennenberg 1994). When light energy is absorbed in excess of the amount that can be used for

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carbon fixation the photosystems become over-excited and the electron transport chains may become over-reduced. Transfer of excitation energy from excited chlorophyll molecules to molecular oxygen or the leakage of electrons to oxygen lead to the formation of highly toxic active oxygen species (AOS, Elstner & Osswald 1994; Foyer 1997; Foyer & Noctor 2000). To avoid damage to the photosynthetic tissues under such changing conditions flexible protection mechanisms are required. The harmless dissipation of excitation energy as heat can be conducted by protective pigments, the carotenoids (Young 1991). The xanthophyll cycle, comprising the carotenoids violaxanthin, antheraxanthin, and zeaxanthin, is involved in the adaptation of the energy conversion efficiency in the photosynthetic membranes (Demmig-Adams & Adams 1994; Müller et al. 2001). Violaxanthin which contains two epoxide groups can be reversibly and quickly (within minutes) converted into zeaxanthin with two de-epoxidized epoxide groups with the intermediate antheraxanthin. Zeaxanthin contributed to heat dissipation of light energy whereas violaxanthin does not. A higher de-epoxidation state of this cycle (more on the side of zeaxanthin at the expense of violaxanthin) is related to a stronger protection of the photosynthetic apparatus and a lower photochemical efficiency. Other carotenoids, such as lutein, may also contribute to energy dissipation or, such as  $\beta$ -carotene, may quench singlet oxygen which is produced from molecular oxygen through excitation energy transfer, directly in the pigment bed. Alternatively, excess light energy can also be used in the photorespiratory pathway or in the Mehler peroxidase reaction leading to a water-water cycle which may protect the photosynthesis from damage (Asada 1999; Niyogi 2000). However, in all these cases AOS are formed and have to be kept under control. The detoxification of AOS is done by the antioxidant defense system, comprising defense enzymes and low molecular antioxidants such as glutathione (GSH) and ascorbate (Noctor & Foyer 1998). If the protective capacities of the photosynthetic apparatus are not sufficient, damages to the photosystems may occur.

In the dense canopy structure of the Canarian laurel forest marked differences between sun and shade foliage exist with respect to environmental conditions, structural properties and physiological responses (Morales et al. 1996a, b, c; Jiménez et al. 1999; González-Rodríguez et al. 2001a; 2002a, b). In other sclerophyllous tree species, sun and shade leaves are also different with respect to their defence capacities and adaptations during the daily course (Faria et al. 1996; García-Plazaola et al. 1997).

The physiological performance of forest trees under the typical potential stress situations in their ecosystem is indicative of their competitive behavior. The capacity

to resist photodamages was found to contribute strongly to the ecological role of species in broadleaved deciduous (Kitao et al. 2000) and evergreen forest ecosystems (García-Plazaola et al. 2000).

Photosynthesis has been characterized in detail on the laurel forest species (González-Rodríguez et al. 2001a; 2002a, b). In a previous paper, different responses of xanthophylls and tocopherols along a high irradiation diurnal course have been observed in these species. In short, *Myrica faya*, a species which is growing at degraded sites as well as in the closed canopy laurel forest, seemed to be more robust against photooxidative stress than the other two species (González-Rodríguez et al. 2001b). Since thylakoid bound systems (xanthophylls and tocopherols) are only one part of the antioxidative defense system, the question arises if water soluble redox systems fit in that hypothesis. Furthermore, under the typical conditions in the laurel forest, responses to high-light periods alternating with cloudy phases are probably of more importance than responses to longer term high light stress on completely clear days.

The present study therefore aims at the comparative characterization of physiological traits of five common tree species of the Canarian laurel forest under field conditions. Responses of gas exchange, water relations, biochemical photoprotective and antioxidant systems together with chlorophyll fluorescence parameters indicative of the photochemical efficiency, were measured in the sun and shade canopy during a summer day with long sunny periods alternating with shading by occasional clouds. In particular in the closed canopy structure of the Canarian laurel forest with several co-dominating tree species, the differences in the fine tuning of physiological responses and protection strategies toward the environmental conditions may be of major importance for the overall physiological performance and contribute to competitive differences among species.

## Material and methods

### Field site and plant materials

The investigations were carried out at an experimental site at Agua García mountain, Tenerife, Canary Islands (28° 27' 32" N and 16° 24' 20" W). The climate at this site is humid mediterranean with average annual temperature of 14°C, average minimum of 10.7°C, and an average maximum of 17.7°C, average relative humidity of 80%, and an annual precipitation of 733 mm. The forest is situated at 820 m a.s.l. on a slight NNE orientated slope (8–12°). Meteorological data during the investigation period are presented in Figure 1.

A wooden scaffolding tower gave access to the top of the canopy. Dominant, about 40 years old and 20 m high individ-

uals of five tree species were investigated: *Persea indica* (L.) C. K. Spreng. and *Laurus azorica* (Seub.) Franco (both Lauraceae), *Myrica faya* Aiton (Myricaceae), *Ilex canariensis* Poir. and *Ilex perado* Aiton subsp. *platyphylla* (Webb & Berthel.) Tutin (both Aquifoliaceae). Sampling was conducted in the sun and shade (about 2 m below) canopy. The sampled trees were representative for the stand structure which is described in detail by Morales et al. (1996b, c).

## Environmental data

Environmental data were read every 30 min using a PAR sensor (Li-Cor, Lincoln, USA) and a thermo-hygrometer positioned at the tower platform (Figure 1). Leaf temperatures were measured using a contact thermocouple (Thandar Ltd., UK) and did not exceed 20°C (data not shown).

## Sampling times

Sampling for biochemical measurements, photosynthetic and water relation parameters was conducted four times in the sun canopy and two times in the shade canopy after at least 40 min of almost unchanged cloudless conditions (arrows in Fig-

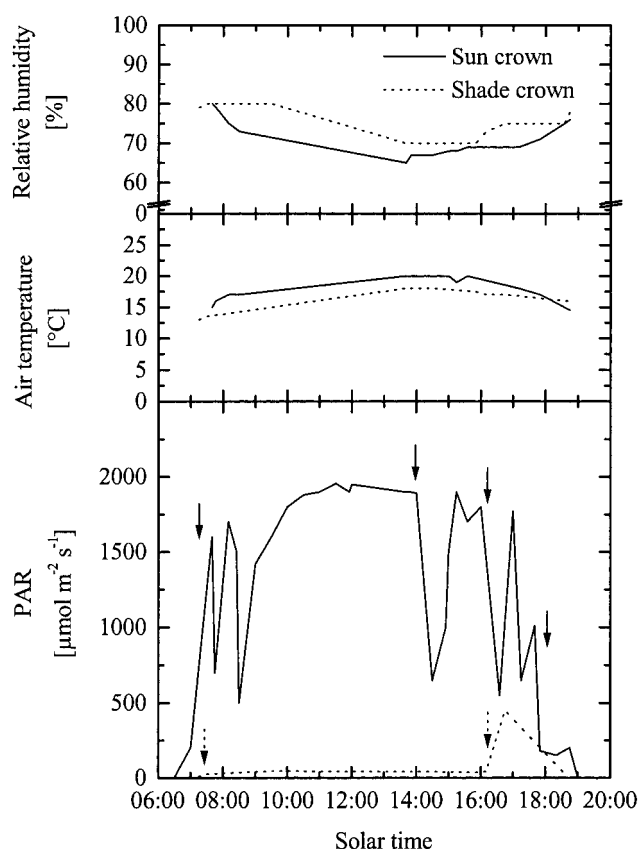


Fig. 1. Environmental conditions during the measurement period. Arrows indicate the sampling times for biochemical analysis and chlorophyll fluorescence measurements y.

ure 1). Morning data were sampled about 30 min after sunrise. As a control, branches were darkened overnight using aluminum foil and samples taken from those darkened branches for biochemical analysis and for fluorescence determinations. Significant differences to the morning values were not found (data not shown).

## Water relations

Leaf water potentials were measured in the morning and at midday with a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA) in four species. The petiole morphology did not allow the measurement of *I. perado* leaves with the instrument used.

Relative water contents (RWC) were determined on leaves collected into pre-weighed plastic bags which were sealed. Leaf fresh mass (fw) was determined and the weight at water saturation (sw) was determined after leaving the leaves for 24 hours in water saturated atmosphere immersing their petioles in water. Leaf dry mass (dw) was determined after oven drying the leaves at 80°C for 48 h. RWC was calculated as the proportion of water content in situ (fw-dw) to the maximal water content at saturation (sw-dw) expressed in %.

## Gas exchange

Leaves of the youngest fully developed age class were measured under natural site conditions with a portable Infrared Gas Analyser (LCA4, ADC Analytical Development Company, UK) repeatedly during the daily course. Gas exchange rates were calculated according to Caemmerer & Farquhar (1981).

## Chlorophyll a fluorescence

Chlorophyll a fluorescence was determined using a portable fluorimeter (Plant Efficiency Analyser PEA, Hansatech, UK). Measurements were made after the leaf part was dark-adapted for 30 min using leaf clips. The maximal apparent photochemical efficiency of photosystem II (PS II) was calculated as the ratio of variable to maximal fluorescence (Fv/Fm). The values are regarded as representative for the time when the dark adaptation clip was set.

## Pigments and antioxidants

Pigment and antioxidant analyses were carried out essentially as described in Tausz et al. (2003). In short: Whole leaves were cut at the petiole and immediately (within seconds) frozen in liquid nitrogen. The leaves were lyophilized, ground in a dismembrator under liquid nitrogen and the leaf powder was stored humidity proof at -25°C until analysis.

Pigments were determined using an HPLC gradient-method permitting separation of all important chloroplast pigments in one step: Column Spherisorb S5 ODS2 250 × 4.6 mm with precolumn S5 ODS2 50 × 4.6 mm. Solvent A: acetonitrile.

trile : methanol : water = 100 : 10 : 5 (v/v/v), solvent B: acetone : ethylacetate = 2 : 1 (v/v), linear gradient from 10% solvent B to 70% solvent B in 18 min, run time 30 min, flow 1 ml min<sup>-1</sup>, photometric detection at 440 nm. The leaf dry powder was shaken in ice-cold acetone and centrifuged at 2°C and 10000 x g. The supernatants of three re-extractions were combined and adjusted to a fixed final volume. Extracts were injected (20 µl) using a cooled autosampler.

The determination of total glutathione was conducted using a gradient HPLC-analysis (Column Spherisorb S5 ODS2 250 × 4.6 mm with precolumn S5 ODS2 50 × 4.6 mm). Lyophilized leaf powder was extracted in 0.1 M HCl and incubated with 200 mM CHES-buffer [2-8N-cyclohexylaminoethanesulfonic acid] and DTT (dithiothreitol) to reduce thiol-groups. The SH-groups were labelled by monobromobimane. Recovery rates of added GSH standards were checked for each species.

Concentrations of total ascorbic acid were measured as follows: Extracts of lyophilized leaf powder in metaphosphoric acid (1.5%) were reduced using DTT and separation was carried out on an isocratic HPLC system (Column Spherisorb S5 ODS2 250 × 4.6 mm with precolumn S5 ODS2 50 × 4.6 mm) using hexadecylammoniumbromide as ion pairing agent in aqueous methanol (30% vol) as solvent. Detection was performed at 248 nm. Recovery rates of added ascorbate standards were checked for each species.

## Statistical analysis

Statistical analyses were completed using the Statistica (StatSoft, USA) software package. The data were tested first for significant diurnal variations by Kruskal-Wallis non-parametric analysis of variance. If no diurnal variations were observed, data were pooled and species effects were evaluated on the sun foliage using Kruskal-Wallis test with Conover's cross-comparisons (Bortz et al. 1990). Differences between sun and shade leaves within the same species were evaluated by Mann-Whitney's U-test. P < 0.05 was regarded significant. In Tables and Figures median values and median deviations or quartile ranges are presented.

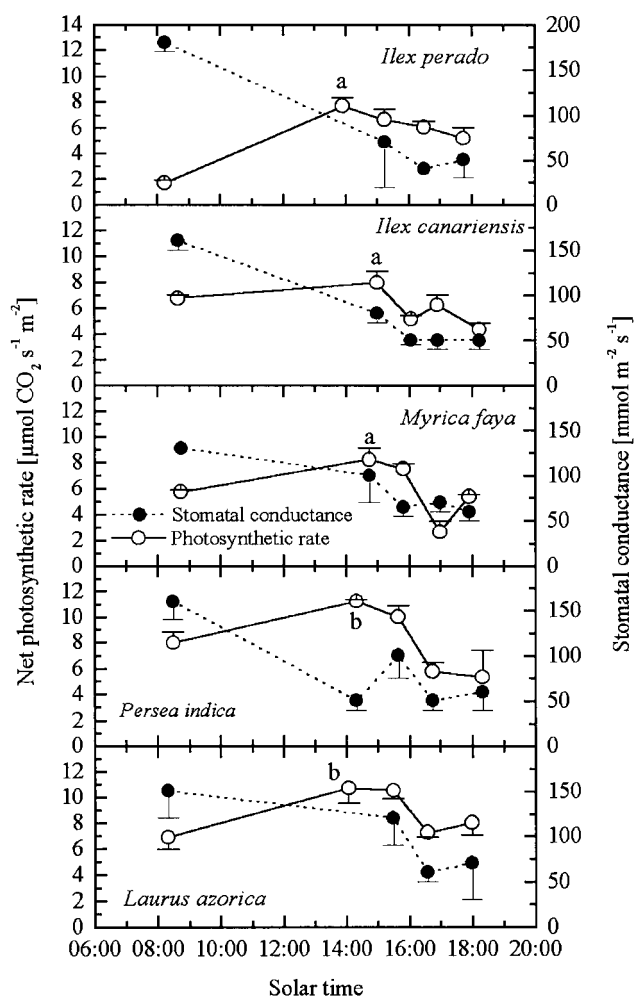


Fig. 2. Gas exchange rates in the sun canopy of five laurel forest tree species along the daily course. Data are medians and median deviations of 4 to 6 independent leaves. Different letters indicate significant differences of maximal rates between species.

Table 1. Water relations in the sun and shade canopy of laurel forest tree species.

Species		Leaf water potential [MPa]		RWC [%]	
		maximum	minimum	maximum	minimum
<i>Ilex canariensis</i>	sun	-0.45 ± 0.05	-0.90 ± 0.10*	98.9 ± 0.7	94.7 ± 0.6
	shade	-0.35 ± 0.05	-0.55 ± 0.08	96.3 ± 4.0	93.3 ± 3.8
<i>Myrica faya</i>	sun	-0.40 ± 0.05	-0.90 ± 0.05*	94.1 ± 4.0	86.6 ± 2.0*
	shade	-0.40 ± 0.05	-0.55 ± 0.08	92.4 ± 7.0	92.3 ± 3.0
<i>Persea indica</i>	sun	-0.50 ± 0.05	-0.85 ± 0.15*	99.3 ± 4.1	96.3 ± 3.1
	shade	-0.43 ± 0.03	-0.50 ± 0.08	99.0 ± 1.0	97.6 ± 1.2
<i>Laurus azorica</i>	sun	-0.40 ± 0.05	-0.90 ± 0.15*	98.9 ± 0.5	93.0 ± 0.3*
	shade	-0.45 ± 0.08	-0.40 ± 0.05	98.0 ± 0.9	94.3 ± 2.9

Maxima (morning) and minima (early afternoon) of the diurnal course are shown. Data are medians and half total spans of at least three independent measurements. Asterisks indicate significant differences between maximum and minimum.

## Results

### Water relations

Water relations were comparable in all investigated species: Sun foliage showed a decrease of water potential during the day whereas shade leaves maintained constant, less negative water potentials during the day (Table 1). The RWC stayed high in all investigated species and leaf types, the minimum values were in no case lower than 80% (Table 1).

### Gas exchange

Stomatal conductances decreased in all investigated species from about 150 mmol m<sup>-2</sup> s<sup>-1</sup> in the morning to around 50 mmol m<sup>-2</sup> s<sup>-1</sup> during the day. Irrespective of the decline in stomatal conductance, photosynthetic rates were highest around midday when PPFD was high (Figure 2). *P. indica* and *L. azorica* had maximum CO<sub>2</sub> fixation rates of more than 10 μmol m<sup>-2</sup> s<sup>-1</sup>, and *M. faya* and the two *Ilex* species reached only 8 μmol m<sup>-2</sup> s<sup>-1</sup> (Figure 2).

### Chloroplast pigments

Pigment contents of sun and shade leaves differed in all species. In most cases, these differences were statistically significant (Table 2). Total chlorophyll concentra-

tions per g leaf dry mass were higher in shade leaves compared to sun leaves. The xanthophyll cycle pool per unit chlorophyll was significantly greater in sun leaves of all investigated species whereas other pigments including β-carotene only showed a trend toward higher concentrations in sun leaves in some species (Table 2).

Chlorophyll contents in the sun foliage were significantly lower in the two *Ilex* species compared to *P. indica*. α-Carotene was absent in *M. faya* leaves and very low in *I. canariensis*. The V + A + Z pool per unit chlorophyll also varied significantly among the species with the lowest values in *M. faya* leaves (Table 2).

Significant time course variations during the measurement period were only found for the xanthophyll de-epoxidation in *I. canariensis* and *M. faya* (Figure 3).

### The xanthophyll cycle and the photochemical efficiency measured as Fv/Fm

Shade leaves had higher Fv/Fm values than sun leaves even in the morning (except in *P. indica*) and depressions during the day were not observed in the shade foliage. Along the diurnal course, Fv/Fm only varied significantly in sun leaves of *P. indica* (and, as a not significant trend, correspondingly in *L. azorica*), but not in the other investigated species. In *P. indica* sun leaves Fv/Fm decreased during the day and was fully recovered the next morning (Figure 4).

Table 2. Antioxidants and pigments in the sun and shade canopy of five tree species of the laurel forest.

Species		Asc [μmol g <sup>-1</sup> dw]	GSH [nmol g <sup>-1</sup> dw]	Chl [μmol g <sup>-1</sup> dw]	Carotenoids [nmol μmol <sup>-1</sup> chlorophyll]				
					Neoxanthin	Lutein	V + A + Z	α-Carotene	β-Carotene
<i>Ilex canariensis</i>	sun	13.6 ± 2.8a	546 ± 228a	3.53 ± 0.28a	32 ± 3	159 ± 14	78 ± 17ab	1 ± 1a	81 ± 15
	shade	8.1 ± 3.0	157 ± 68*	6.38 ± 1.01*	38 ± 7	141 ± 14	41 ± 27*	2 ± 1	71 ± 23
<i>Ilex perado</i>	sun	17.9 ± 2.3a	519 ± 164a	3.67 ± 0.48a	32 ± 3	132 ± 7	87 ± 9 a	13 ± 2b	76 ± 7
	shade	17.5 ± 2.8	37 ± 22*	6.68 ± 0.79*	36 ± 3	115 ± 7	44 ± 6*	15 ± 2	50 ± 11*
<i>Myrica faya</i>	sun	31.1 ± 2.4b	422 ± 139a	4.14 ± 0.41ab	35 ± 2	146 ± 6	46 ± 9b	–	77 ± 5
	shade	16.2 ± 4.8*	197 ± 41*	7.32 ± 0.45*	36 ± 2	126 ± 6*	25 ± 10*	–	67 ± 10
<i>Persea indica</i>	sun	29.7 ± 3.4b	1286 ± 189b	6.37 ± 0.51b	37 ± 3	156 ± 5	69 ± 14ab	20 ± 2b	78 ± 6
	shade	28.9 ± 4.0	819 ± 173*	7.40 ± 1.08	41 ± 6	144 ± 19	32 ± 6*	22 ± 3	73 ± 9
<i>Laurus azorica</i>	sun	15.1 ± 2.2a	477 ± 123a	4.65 ± 0.39ab	37 ± 3	150 ± 13	71 ± 8ab	17 ± 5b	78 ± 11
	shade	7.5 ± 1.9	254 ± 80	8.43 ± 0.45*	41 ± 3	135 ± 4	34 ± 4*	31 ± 5	56 ± 5*

Medians half quartile ranges for n = 6 (shade canopy) and n = 12 (sun canopy) samples. Letters denote significant differences of sun canopy data among species (P < 0.05, n = 12). Asterisks indicate significant differences between sun and shade canopy within the same species (P < 0.05). Asc = total ascorbate, GSH = total glutathione, Chl = chlorophyll a + b, V + A + Z = violaxanthin + antheraxanthin + zeaxanthin.

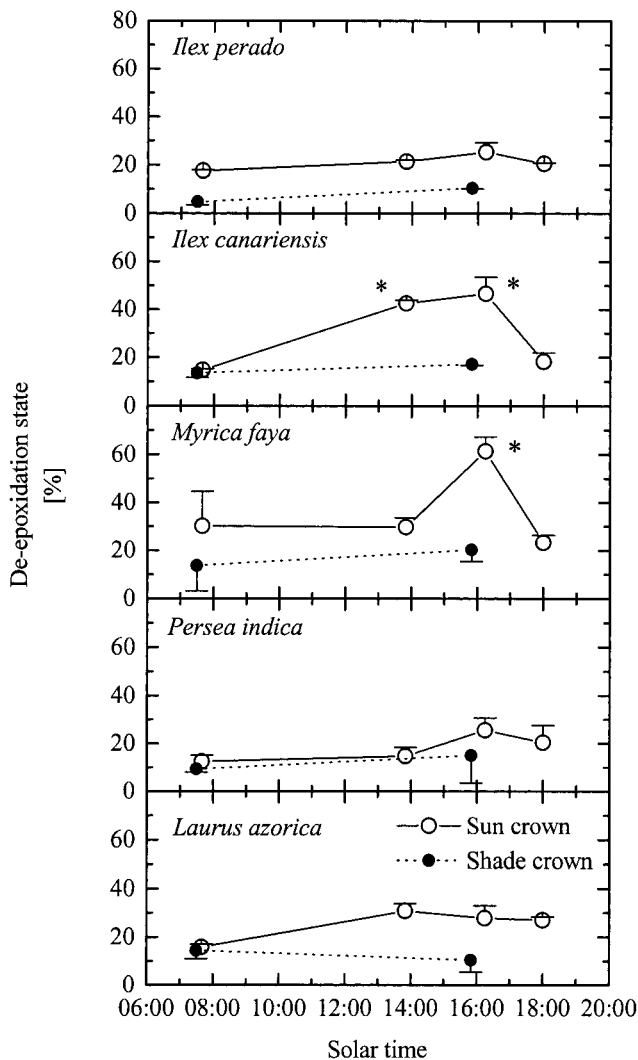


Fig. 3. The de-epoxidation state of the xanthophyll cycle calculated as  $(Z+0.5*A)/(V+A+Z)$  in leaves of the sun and shade canopy of five laurel forest tree species. Z = zeaxanthin, A = antheraxanthin, V = violaxanthin. Data are medians and median deviations of 3 to 4 independent samples. Asterisks indicate significant differences to morning values.

The lower PS II efficiency measured as  $F_v/F_m$  was not directly connected to zeaxanthin formation, since in the morning the de-epoxidation state of the xanthophyll cycle was not significantly different between sun and shade leaves and equal to leaves darkened with aluminum foil during the night (data not shown). Significant de-epoxidation of xanthophylls during the day was only observed in the sun canopies of *M. faya* and *I. canariensis*, species which did not show a diurnal variation in  $F_v/F_m$ . Under shade canopy conditions neither de-epoxidation of xanthophylls nor diurnal changes in  $F_v/F_m$  were observed (Figure 3, Figure 4).

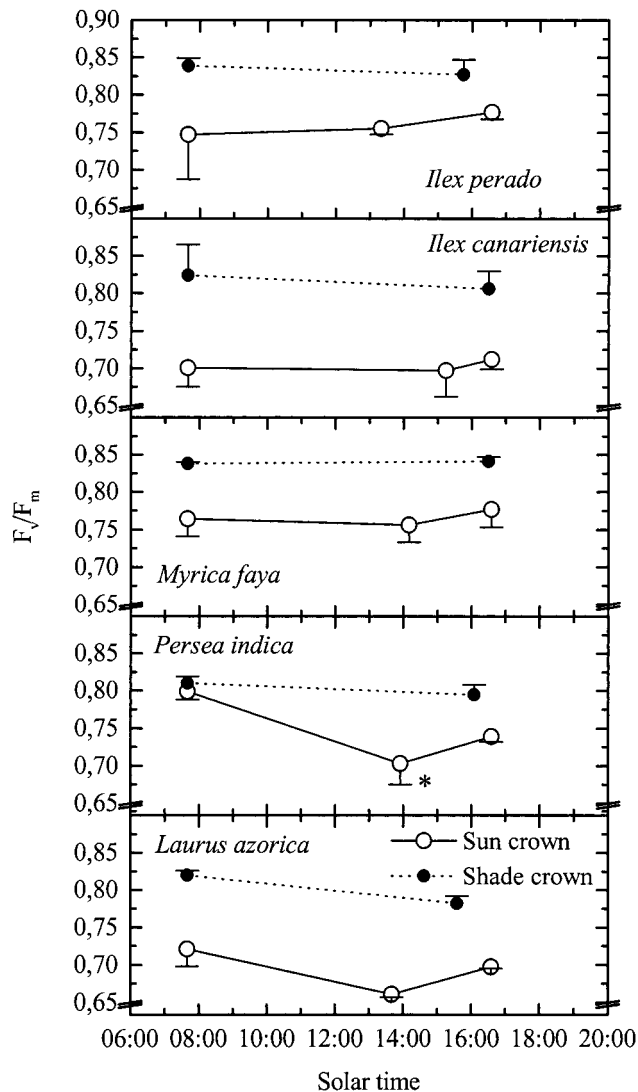


Fig. 4. Chlorophyll fluorescence measurements in the sun and shade canopy of five laurel forest tree species along a daily course. Data are medians and median deviations of 3 to 4 independent leaves. Asterisks indicate significant differences to morning values.

### Ascorbate and glutathione

Ascorbate concentrations were generally higher in the sun leaves than in shade leaves. Diurnal changes of ascorbate concentrations were not observed. *P. indica* and *M. faya* had the highest ascorbate concentrations and *I. canariensis* and *L. azorica* the lowest (Table 2). Glutathione concentrations were higher in the sun crown as compared to the shade crown in all five tree species (Table 2). *P. indica* leaves contained significantly more GSH than the foliage of the other species, both

in sun and in shade leaves (Table 2). A significant diurnal variation of glutathione concentrations was not observed.

## Discussion

The responses of stomatal conductances with maxima of 150 to 200 mmol m<sup>-2</sup> s<sup>-1</sup> in the morning and a decline at midday without recovery until the evening are well comparable to other evergreen broadleaved trees at natural summer conditions, such as the Mediterranean sclerophyllous species *Quercus suber* (Faria et al. 1996). The stomatal closure was similar in all species investigated in the present study. In contrast to those more drought exposed *Q. suber* trees (Faria et al. 1996) the laurel forest species had maximal photosynthesis rates around midday which shows that the CO<sub>2</sub> fixation was not limited by the stomatal conductance under the present conditions. In coincidence with other results (González-Rodríguez et al. 2001 a; 2002 a, b), maximum photosynthetic rates were reached by *P. indica* and *L. azorica* with about 10 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, and all other species reached a maximal carbon fixation rate of about 8 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. These data are well comparable to various data of evergreen trees (Larcher 1995). The rates obtained on this day are in the upper limit of values registered during a year of measurements on *L. azorica* (González-Rodríguez et al. 2001 a), *P. indica* (González-Rodríguez et al. 2002 b), and *M. faya* (González-Rodríguez et al. 2002 a) in natural conditions, indicating that no serious limitations of the CO<sub>2</sub> uptake were experienced by those trees during the measurement period.

The high morning leaf water potentials point toward a good water supply and the maximal decrease was also moderate compared to Mediterranean sclerophyllous tree species under summer conditions, but with good water supply (a decrease in *Q. suber* leaves from -0.4 at predawn to less than -2.2 MPa at midday is reported by Faria et al. 1996). Under different conditions, also *L. azorica* may experience a stronger decline in sun foliage leaf water potentials to about -1.5 MPa at midday (Morales et al. 1996 a). The RWC remained clearly above 80% in all cases. Investigations on laurel forest species (Jiménez et al. 1999) and other plants (Kaiser 1987; Cornic & Massacci 1996) showed that dehydration does not adversely affect the photosynthetic apparatus above a RWC of 70%. Hence, the present data may be regarded representative for the laurel forest trees where water shortage is mitigated by high humidity and sporadic stress conditions may result from short term high irradiation periods rather than from low water availability.

Species dependent differences in thylakoid pigmentation were confined to the total chlorophyll contents,

the xanthophyll cycle pool size, and the α-carotene contents. α-Carotene is not ubiquitous in higher plants (Thayer & Björkman 1990). Among the investigated species only *M. faya* lacked α-carotene completely (as previously reported in Morales et al. 1997), whereas *I. canariensis* contained only small, but measurable amounts. *I. perado*, *P. indica* and *L. azorica* contained comparable concentrations of this pigment. The xanthophyll cycle pool was smallest in *M. faya*. Compared to sun leaves of other evergreen trees, such as *Citrus* sp. (more than 200 nmol μmol<sup>-1</sup> chl<sup>-1</sup> reported in Thayer & Björkman 1990) or Mediterranean sclerophylls as *Q. suber* (Faria et al. 1996) all species investigated in the present study had relatively small xanthophyll cycle pools. This may indicate a potentially lower photoprotective capacity of the laurel forest species compared to those trees. However, in sun leaves of the Mediterranean sclerophyllous species *Laurus nobilis* (Brugnoli et al. 1998), *Rhamnus alaternus*, *Arbutus unedo*, and *Quercus ilex* (García-Plazaola et al. 2000) comparable pool sizes of the xanthophyll cycle (about 100 μmol mol<sup>-1</sup> chl) are reported. The comparative relation of the pigment pools to the photo-protective capacities among different groups of species may be difficult and, furthermore, the xanthophyll cycle pool may vary due to longer-term acclimation to different radiation conditions and seasonal change (Demmig-Adams & Adams 1996; García-Plazaola et al. 2000). For three species, *M. faya*, *P. indica* and *L. azorica*, slightly different pigment concentrations were measured in previous studies, but the relative ranking of the species did not change (Morales et al. 1997; González-Rodríguez et al. 2001 b).

The differences in pigmentation between sun and shade leaves within the same species coincide with previous observations for evergreen tree species (Thayer & Björkman 1990; Faria et al. 1996). Chlorophyll contents on a leaf dry weight basis were higher in most species (not significant only in *P. indica*), and the xanthophyll cycle pool per unit chlorophyll was significantly lower in shade leaves corresponding to a lower need for photoprotection in the shade canopy. There were no consistent differences in neoxanthin, lutein, and carotenes per unit chlorophyll between sun and shade leaves. Significant diurnal changes were absent for pigment pools in all species and in both leaf types. Apparently, the environmental conditions during the investigated day did not induce pigment degradation due to excessive photo-oxidative stress.

Sun leaves had higher GSH concentrations and, in some species, also higher ascorbate concentrations than the shade foliage. This corresponds to data of many other evergreen species (Polle & Rennenberg 1994; Grace & Logan 1996) and can be related to the higher need for antioxidant protection in the sun canopy (Pol-

le & Rennenberg 1994) or, alternatively, to the higher assimilatory rates providing the precursors for the antioxidant synthesis.

Depressions in Fv/Fm are often closely correlated to zeaxanthin formation and thus ascribed to the increase in energy dissipation by the xanthophyll cycle (Thiele & Krause 1994, but compare Niyogi 2000; Müller et al. 2001). However, in the present data there is a clear discrepancy between Fv/Fm and xanthophyll de-epoxidation. In sun leaves of *P. indica*, for instance, Fv/Fm varied significantly during the day whereas the xanthophyll cycle de-epoxidation state did not (Figure 3 and 4). On the other hand, *M. faya* and *I. canariensis* showed a significant zeaxanthin formation in the sun foliage whereas no corresponding diurnal changes in Fv/Fm were found (Figure 3 and 4). Comparable differences were also reported for sun and shade foliage of *Quercus suber*, where both leaf types showed xanthophyll de-epoxidation, but only in sun leaves a transient midday decrease in Fv/Fm was observed (Faria et al. 1996). Brugnoli et al. (1998) also reported discrepancies between xanthophyll de-epoxidation and non-photochemical fluorescence quenching. The following explanation is suggested: The determination of Fv/Fm in the present study as well as in the *Q. suber* study (Faria et al. 1996) involved a 30 min dark adaptation in closed leaf clips. Hence, only those changes in the PS II efficiency that require longer recovery periods than 30 min are measured. The xanthophyll cycle dependent decrease of the PS II efficiency might relax upon darkening in a comparable time span (Jahns & Miede 1996) and the fast recovering component of photoinhibition (Thiele et al. 1996) is probably not shown in the Fv/Fm data. In the present data set, diminishing Fv/Fm reflect more long-term adaptations responsible for a decrease in PS II efficiency or a photodamage requiring a longer recovery period than 30 min. Under this point of view the de-epoxidation status data (Figure 3) and the Fv/Fm data (Figure 4) complement each other reflecting different effects.

The Fv/Fm data of the investigated species showed two types of longer-term differences: (1) Sun leaves had in nearly all cases constitutively lower PS II efficiencies than shade leaves, the early morning included. This reflects the structural and long-term (with a recovery time of at least more than one night) adaptations in the photosynthetic membranes with a modified structure of the antennae system (Lichtenthaler et al. 1981). (2) Under the present environmental conditions, Fv/Fm data varied only in sun leaves of *P. indica* significantly and, as a trend, also in *L. azorica* along the diurnal course showing a decreasing PS II efficiency in the sun followed by a recovery towards the evening and a further increased morning value. This change might reflect a photoinactivation of reaction centers requiring their

de-novo synthesis and hence, a recovery time of more than 30 min. In a previous study, a high irradiation day was found to cause a more persistent decline in Fv/Fm in *P. indica* (recovering only overnight), whereas *M. faya* quickly recovered from photoinhibition (González-Rodríguez et al. 2001 b).

It is noteworthy that such a decrease in Fv/Fm during the day was only observed in such trees that exhibited only little de-epoxidation response of the xanthophyll cycle. Apparently, under the given environmental conditions different protection strategies were used by the investigated species: One, pursued by *M. faya* and *I. canariensis* involves an increased energy dissipation rate by zeaxanthin formation which successfully prevents the slow recovery component of photoinhibition. The de-epoxidation status of the xanthophyll cycle changes, but Fv/Fm remains constant, since it reflects only longer-term changes requiring a recovery time of more than 30 min. The second strategy, pursued by *P. indica* and, less pronounced *L. azorica*, does not activate energy dissipation under the present conditions, but allows possibly more AOS formation and also a slowly reversible decline in PS II efficiency which requires repair processes to recover during the evening and the night. This hypothesis is corroborated by previously described differences in diurnal relations between xanthophyll cycle de-epoxidation state and Fv/Fm changes in *M. faya*, *P. indica*, and *L. azorica* (González-Rodríguez et al. 2001 b). Third, as in sun leaves of *I. perado*, a structural long-term adaptation of photosystems appears sufficient to maintain a constant (comparably low) PS II efficiency during the day without the activation of zeaxanthin dependent energy dissipation.

The variations in photochemical efficiency by energy dissipation systems are only one aspect of photoprotection (Asada 1999). If plants allow the absorbance of excess energy without energy dissipation directly in the pigment bed, they have to cope with the accumulating toxic products of oxygen activation, either resulting from oxygen reduction in the photosynthetic apparatus or from photorespiration (Foyer 1997; Asada 1999; Foyer & Noctor 2000). In the present study, concentrations of the major antioxidants GSH and ascorbate were highest in *P. indica* leaves, which may indicate the highest capacity of AOS scavenging in sun leaves of this species. According to the hypothesis developed above, this would relate to the higher probability of AOS formation in the leaves of this species. On the other hand, *M. faya* also contained high ascorbate levels in the sun foliage compared to both *I.* species and to *L. azorica*, potentially strengthening the photoprotection in *M. faya* leaves, since ascorbate is required for the xanthophyll conversions.

In summary, the protection strategy of *M. faya* may be considered as the most robust granting maximal pro-

tection. From all the investigated species *M. faya* advances most vigorously into forest degradation stages outside the closed laurel forest canopy, and it is a constant member of heath-dominated shrublands on the Canary Islands. This may be enabled by the efficient protection mechanisms of this species against over-energetization of leaves, even if this occurs at the expense of photosynthetic efficiency. In coincidence with former studies (González-Rodríguez et al. 2001a; 2002a, b), the corresponding photosynthetic rates were not the highest. On the opposite side is the strategy of *P. indica*. According to year-round gas-exchange measurements (González-Rodríguez et al. 2002b) it allows high production under the prevailing environmental conditions in the laurel forest, but at the expense of more repair requirements. It is important that these differences in coping with potential photostress are observed on a mildly stressful day with some cloudy periods in the afternoon that may allow a relaxation of the photostress. Under these conditions *P. indica* and *L. azorica* were able to maintain the highest photosynthetic CO<sub>2</sub> fixation rate and repair the slower recovering photoinhibition apparent as PS II efficiency decline during the night. This seems to contribute to a slight advantage to *P. indica* and *L. azorica* with respect to photosynthetic production (González-Rodríguez et al. 2001a; 2002a, b) and, thus, competition under these typical laurel forest conditions. As shown in the paper of González-Rodríguez et al. (2001b), under strong stress conditions (cloudless days) this type of strategy may be more risky when a repair of photodestructive damages may consume more reserves or is even impaired. It might well be the case that for such reasons in particular *P. indica* and *I. perado* (without any diurnal changes in de-epoxidation state and Fv/Fm ratios) are more confined than the other species to the laurel forest interior. The present results do not exclude, however, that under more severe stress conditions all species may activate a maximum of energy dissipation and/or at the same time may experience a slowly reversible decline in photosynthetic efficiency. In this case, species related differences would not to be observed and Fv/Fm and the de-epoxidation state of the xanthophyll cycle would coincide, because both effects depend on the illumination conditions (compare results in Morales et al. 1997).

Nevertheless, the strategic differences in coping with light stress are of potential importance in the physiological fine tuning of the plant response to the environment and can contribute to variable mutual advantages and disadvantages in competition among species. Under the typical conditions of the laurel forest ecosystem represented in the present study, the investigated *P. indica* and *L. azorica* trees may have considerable advantages which may be related to their dominating role in the closed canopy forest.

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## References

- Asada, K. (1999): The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. – *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**: 601–640.
- Bortz, J.; Lienert, G. A. & Boenke, K. (1990): Verteilungsfreie Methoden in der Biostatistik. – Springer Verlag, Berlin.
- Brugnoli, E.; Scartazza, A.; De Tullio, M. C.; Monte-verdi, 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. – *Physiol. Plant.* **104**: 727–734.
- Caemmerer, S. v. & Farquhar, G. D. (1981): Some relationships between the biochemistry of photosynthesis and gas exchange of leaves. – *Planta* **153**: 376–387.
- Cornic, G. & Massacci, A. (1996): Leaf photosynthesis under drought stress. In: Baker, N. R. (ed.): *Photosynthesis and the environment*. pp. 347–366. – Kluwer Acad. Publ., Dordrecht.
- Demmig-Adams, B. & Adams, III. W. W. (1994): Light stress and photoprotection related to the xanthophyll cycle. In: Foyer, C. & Mullineaux, P. M. (eds.): *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants*. pp. 105–126. – CRC Press, Boca Raton.
- Demmig-Adams, B. & Adams, III. W. W. (1996): Chlorophyll and carotenoid composition in leaves of *Euonymus kiautschovicus* acclimated to different degrees of light stress in the field. – *Austr. J. Plant Physiol.* **23**: 649–659.
- Elstner, E. F. & Osswald, W. (1994): Mechanisms of oxygen activation during plant stress. – *Proc. Roy. Soc. Edinburgh* **102B**: 131–154.
- Faria, T.; García-Plazaola, J. I.; Abadía, A.; Cerasoli, S.; Pereira, J. S. & Chaves, M. M. (1996): Diurnal changes in photoprotective mechanisms in leaves of cork oak (*Quercus suber*) during summer. – *Tree Physiol.* **16**: 115–123.
- Foyer, C. (1997): Oxygen metabolism and electron transport in photosynthesis. In: Scandalios, G. (ed.): *Oxidative Stress and the Molecular Biology of Antioxidant Defences*. pp. 587–621. – Cold Spring Harbor Laboratory Press, U. K.
- Foyer, C. & Noctor, G. (2000): Oxygen processing in photosynthesis: regulation and signalling. – *New Phytol.* **146**: 359–388.

- García-Plazaola, J. I.; Faria, T.; Abadía, J.; Abadía, A.; Chaves, M. M. & Pereira, J. S. (1997): Seasonal changes in xanthophyll composition and photosynthesis of cork oak (*Quercus suber* L.) leaves under mediterranean climate. – *J. Exp. Bot.* **48**: 1667–1674.
- García-Plazaola, J. I.; Hernández, A. & Becerril, J. M. (2000): Photoprotective responses to winter stress in evergreen Mediterranean ecosystems. – *Plant Biol.* **2**: 530–535.
- González-Rodríguez, A. M.; Morales, D. & Jiménez, M. S. (2001a): Gas exchange characteristics of a Canarian laurel forest tree species (*Laurus azorica*) in relation to environmental conditions and leaf canopy position. – *Tree Physiol.* **21**: 1039–1045.
- González-Rodríguez, A. M.; Tausz, M.; Wonisch, A.; Jiménez, M. S.; Grill, D. & Morales, D. (2001b): The significance of xanthophylls and tocopherols in photooxidative stress and photoprotection of three Canarian laurel forest tree species on a high radiation day. – *J. Plant Physiol.* **158**: 1547–1554.
- González-Rodríguez, A. M.; Morales, D. & Jiménez, M. S. (2002a): Leaf gas exchange characteristics in relation to leaf canopy position of *Myrica faya* in its native environment (Tenerife, Canary Islands). – *Plant Biol.* **4**: 576–583.
- González-Rodríguez, A. M.; Morales, D. & Jiménez, M. S. (2002b): Leaf gas exchange characteristics of a Canarian laurel forest tree species *Persea indica* (L.) K. Spreng. under natural conditions. – *J. Plant Physiol.* **159**: 695–704.
- Grace, S. C. & Logan, B. A. (1996): Acclimation of foliar antioxidant systems to growth irradiance in three broadleaved evergreen species. – *Plant Physiol.* **112**: 1631–1640.
- Höllermann, P. (1981): Microenvironmental studies in the laurel forest of the Canary Islands. – *Mountain Res. Developm.* **1**: 193–207.
- Jahns, P. & Miede, B. (1996): Kinetic correlation of recovery from photoinhibition and zeaxanthin epoxidation. – *Planta* **198**: 202–210.
- Jiménez, M. S.; González-Rodríguez, A. M. & Morales, D. (1999): Effect of dehydration on the photosynthetic apparatus of sun and shade leaves of laurel forest trees. – *Zeitschr. Naturforsch.* **54c**: 704–710.
- Kaiser, W. M. (1987): Effects of water deficit on the photosynthetic capacity. – *Physiol. Plant.* **71**: 142–149.
- Kitao, M.; Lei, T. T.; Koike, T.; Tobita, H. & Maruyama, Y. (2000): Susceptibility to photoinhibition of three deciduous broadleaf tree species with different successional traits raised under various light regimes. – *Plant Cell Environ.* **23**: 81–89.
- Larcher, W. (1995): *Physiological Plant Ecology*. – Springer, Berlin–Heidelberg.
- Lichtenthaler, H. K.; Buschmann, C.; Döll, M.; Fietz, H. J.; Bach, T.; Kozel, U.; Meier, D. & Rahmsdorf, U. (1981): Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. – *Photosynth. Res.* **2**: 115–141.
- Morales, D.; González-Rodríguez, A. M.; Čermak, J. & Jiménez, M. S. (1996a): Laurel forests in Tenerife, Canary Islands: The vertical profiles of leaf characteristics. – *Phyton Ann. Rei Bot.* **36**: 251–263.
- Morales, D.; Jiménez, M. S.; González-Rodríguez, A. M. & Čermak, J. (1996b): Laurel forests in Tenerife, Canary Islands: I. The site, stand structure and stand leaf area distribution. – *Trees, Structure and Function* **11**: 41–46.
- Morales, D.; Jiménez, M. S.; González-Rodríguez, A. M. & Čermak, J. (1996c): Laurel forests in Tenerife, Canary Islands: II. Leaf distribution patterns in individual trees. – *Trees, Structure and Function* **11**: 47–52.
- Morales, D.; González-Rodríguez, A. M.; Tausz, M.; Grill, D. & Jiménez, M. S. (1997): Oxygen stress and pigment composition in Canarian laurel forest trees. – *Phyton Ann. Rei Bot.* **37**: 181–186.
- Müller, P.; Xiao-Ping, L. & Niyogi, K. K. (2001): Non-photochemical quenching. A response to excess light energy. – *Plant Physiol.* **125**: 1558–1566.
- Niyogi, K. K. (2000): Safety valves for photosynthesis. – *Current Opinion Plant Biol.* **3**: 455–460.
- Noctor, G. & Foyer, C. (1998): Ascorbate and glutathione: Keeping active oxygen under control. – *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49**: 249–279.
- Polle, A. & Rennenberg, H. (1994): Photooxidative stress in trees. In: Foyer, C. & Mullineaux, P. M. (eds.): *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants*. pp. 199–218. – CRC Press, Boca Raton.
- Tausz, M.; Wonisch, A.; Grill, D.; Morales, D. & Jiménez, M. S. (2003): Measuring antioxidants in tree species in the natural environment. From sampling to data evaluation. – *J. Exp. Bot.* **54**: 1505–1510.
- Thayer, S. S. & Björkman, O. (1990): Leaf xanthophyll content and composition in sun and shade determined by HPLC. – *Photosynth. Res.* **23**: 331–341.
- Thiele, A. & Krause, G. H. (1994): Xanthophyll cycle and thermal energy dissipation in photosystem II: relationship between zeaxanthin formation, energy-dependent fluorescence quenching and photoinhibition. – *J. Plant Physiol.* **144**: 324–332.
- 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.
- Young, A. J. (1991): The photoprotective role of carotenoids in higher plants. – *Physiol. Plant.* **83**: 702–708.