DOI: 10.1007/s11099-012-0018-6 PHOTOSYNTHETICA 50 (1): 95-108, 2012

Fluorescence imaging of light acclimation of brazilian atlantic forest tree species

L. DOS ANJOS+, M.A. OLIVA, and K.N. KUKI

Departamento de Biologia Vegetal, Universidade Federal de Viçosa (DBV/UFV), Av. PH Rolfs, s/n, Viçosa, MG 36571-000, Brazil

Abstract

In the pursuit of knowledge on the biological behavior of Brazilian Atlantic Forest tree species, this study evaluated the susceptibility of the light-demanding species, *Schinus terebinthifolia* Raddi., *Pseudobombax grandiflorum* (Cav.) A. Robyns and *Joannesia princeps* Vell., and of the shade-tolerant species, *Hymenaea courbaril* L. var. stilbocarpa and *Lecythis pisonis* Camb, to photoinhibition and acclimation capacity. These species were first cultivated under two irradiance conditions, I_{20} (20% direct sunlight radiation) and I_{100} (all-sky or direct sunlight) and then transferred from I_{20} to I_{100}. The effects of the sudden increase in light radiation intensity on photosynthetic activity were then evaluated through chlorophyll (Chl) fluorescence imaging, HPLC xanthophylls analysis, and cell membrane lipid peroxidation measurements. Light-demanding species were found to present a higher photochemical efficiency and higher acclimation capacity under high light irradiance than shade-tolerant species. The higher photoinhibition tolerance observed in light-demanding species was associated to their higher capacity for photochemical dissipation and dissipation of excess excitation energy *via* the xanthophyll cycle, leading to a lower ROS generation. The obtained results suggested that a knowledge of acclimation capacity, by means of Chl fluorescence imaging yields, is a useful indicator of species successional grouping.

Additional key words: chlorophyll fluorescence, light stress, photochemical efficiency, thermal dissipation, xanthophyll cycle.

Introduction

The Brazilian Atlantic Forest is the world's fifth terrestrial ecosystem. It presents the most significant reservoir of species diversity and endemism but nonetheless, it is under significant human threat (Mittermeier *et al.* 1999). It is hence worth emphasizing the high priority in conserving the remaining fragments of this ecosystem, as well as recovering the degraded areas which do not present enough resilience for self-regeneration (Mittermeier *et al*. 1999, Carpanezzi 2005). Although efforts have been made, the success of many Atlantic Forest cover recovery programs has been impaired due to the lack of proper knowledge on the plant species biological behavior (Kageyama and Gandara 2005, Barbosa 2006).

In tropical forests, light is known to be one of the necessary environmental factors capable for growth, survival and competitive interactions in the plant community (Houter and Pons 2005, Valladares and Niinemets 2008). Notwithstanding, gap openings in tropical forest canopy are a frequent event that always results in physical environmental changes primarily marked by a sudden increase in direct light on the inferior forest stratus (Walters 2005). On one hand, this new environmental condition may create an opportunity for germination and

Received 8 June 2011, *accepted* 29 November 2011.
+Corresponding author; phone: (54) (31) 3899 1667, fax: (54) (31) 3899 2148; e-mail: leticiadosanjos@gmail.com
Abbreviations: A – antheraxanthin; DEPS – de-epoxidation state; F – transient fluorescence; F_0 – dark fluorescence yield; F_m – maximum fluorescence yield after dark adaptation; F_m' – maximum fluorescence in the light-adapted state; F_v/F_m – maximum quantum yield of PSII; HPLC – high performance liquid chromatography; LHCs – light-harvesting complexes; MDA – malondialdehyde; V – violaxanthin; Y(II) – effective quantum yield of PSII; Y(NO) – quantum yield of nonregulated nonphotochemical energy dissipation in PSII; Y(NPQ) – quantum yield of regulated nonphotochemical energy dissipation in PSII; Z – zeaxanthin.
Acknowledgements: The authors would like to thank Dr. Fábio Murillo DaMatta for providing the xanthophyll pigments and Dr. Raimundo Santos Barros for his permission to use their HLPC device. Our sincere thanks also go to the following institutions: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPEMIG (Fundação de Amparo à Pesquisa de Minas Gerais) for their invaluable financial support, and finally to Vale do Rio Doce Company for providing the seedlings used.

establishment of a new generation of tree species. But, on the other hand, the sudden increase of light radiation may also result in an oxidative stress to understory plants known as photoinhibition (Gandolfi 2003, Houter and Pons 2005).

Ultraviolet light and strong blue light primarily inactivate the oxygen-evolving complex (OEC) through the release of manganese (Mn) from the PSII complex. Inactivation of the OEC is known to prolong the lifetime of $P680^+$, the oxidized form of P680, and hence accumulated $P680^+$ damages the reaction center of PSII. Photodamaged PSII is repaired through the replacement of PSII proteins, in particular, the D1 protein, by newly synthesized proteins (Takahashi and Murata 2008). Furthermore, under conditions of excess light for photosynthesis, the rate of repair is depressed owing to inhibition of the *de novo* synthesis of the D1 protein at the protein translation step. Excess photosynthetic light and other environmental stresses can directly limit the Calvin cycle activity through the aforementioned processes or indirectly through stomatal closure thus leading to the production of ROS. It is likely that the generation of ROS under these conditions be primarily responsible for the inhibition of the *de novo* synthesis of D1 protein and PSII repair cycle (Takahashi and Badger 2011). This can, in turn, decrease the photochemical efficiency and photosynthetic activity, and growth and productivity (Chen *et al*. 2011). Photodamage and inhibition of PSII repair are prevented by avoiding light absorption by the manganese cluster of the OEC and effectively consuming, or dissipating, the light energy absorbed by photosynthetic pigments, respectively. Plants can dissipate excess light energy absorbed by the light-harvesting complexes (LHCs) of PSII as harmless longer wavelength heat energy. This is done through a mechanism associated to the conversion of violaxanthin to zeaxanthin *via* antheraxanthin, by the catalyst violaxanthin de-epoxidase, and through the protonation of the PSII protein subunit PsbS in LHCs. When light is no longer in excess, zeaxanthin is epoxidized back to violaxanthin. Both of these component reactions are enhanced by low lumenal pH accompanied by the generation of a ΔpH through linear- and cyclic-electron flows in the light (Niyogy 1999). The changes in the levels of antheraxanthin and zeaxanthin in illuminated leaves are strongly correlated with the quantum yield of nonphotochemical quenching [Y(NPQ)] which is a measure of the fraction of energy dissipated as heat (Demmig-Adams and Adams 1996). Moreover, plants may undergo a series of biochemical, physiological and structural alterations in their photosynthetic apparatus in order to increase their efficiency of light energy utilization, by modulation of antenna size, alteration of the ratio between the number of PSI and PSII units and inactivation of a subpopulation of PSII reaction centers. This process is known as light acclimation and is often a result of regulating the complex light-dependent gene expression occurring at transcriptional, translational and post-translational levels. This process takes days or even weeks, and at the level of the thylakoid membrane, it involves significant compositional and structural alterations (Valladares and Niinemets 2008, Ruban 2009).

It is believed that light-demanding species are able to acclimate more rapidly and more efficiently to any sudden increase in light radiation than shade-tolerant species. Hence, the susceptibility to photoinhibition and the acclimation capacity to changes in light regimes are directly related to species ecological groups (Delagrange *et al*. 2004, Ribeiro *et al*. 2005, Valladares and Niinemets 2008). Thus, the degree of susceptibility to photoinhibition and the acclimation capacity under changes in light regime may be considered the main determinant factor in the competition for space between plant species thus influencing species population composition, structure and dynamics (Walters 2005).

In a very similar way, in vegetation covers of degraded areas, the degree of susceptibility to photoinhibition and the capacity of light acclimation may also play a significant role on young plant performance in the field (Kitao *et al.* 2006). Therefore, a sound knowledge of species light tolerance and the correct management of species in field are important considerations for the successful achievement of any recovery program.

However, increasing evidences have shown recently that both species groups are able to acclimate to sudden increases in light regimes in a similar manner, giving rise to the hypothesis that the degree of adjustments in response to new environmental conditions may not necessarily be related to species successional groups (Rozendaal *et al.* 2006, Souza *et al.* 2009).

The measurement of Chl fluorescence is a noninvasive and practical method widely applied in detecting initial stresses in plants and to support inferences of processes, such as plant acclimation to light variations in their growth environment (Einhorn *et al.* 2004, Lichtenthaler *et al*. 2005). However, most published papers deal with measurements carried out on single leaf spots, which means that in each measurement taken, the fluorescence of a small area of the leaf is captured. The obtained information in such cases is punctual and does not allow inferences from the stress pattern. This type of information flaws in its generalizations and may compromise the comprehension of the physiological behavior of plant species (Lichtenthaler *et al*. 2007a).

On the other hand, the Chl fluorescence imaging technique has been suggested to be a promising novel method for detecting initial plant stress. The potential of this method lies in the possibility of comparing the heterogeneity of the photosynthetic activity throughout the leaf blade added to the fact that images always provide helpful information to assist data interpretation and comprehension (Govindjee and Nedbal 2000, Oxborough 2004, Calatayud *et al.* 2006).

Although Chl fluorescence imaging has been applied

in many plant studies to detect photoinhibition of photosynthesis (Gray *et al*. 2003, Calatayud *et al.* 2006, Hogewoning and Harbinson 2007, Lichtenthaler *et al*. 2007a,b Guidi and Degl'Innocenti 2008), a detailed review of the literature found nothing on fluorescence imaging applied to the study of high-light acclimation on tropical tree species of different successional groups.

The aim of the present study is to evaluate the effects of a sudden increase of light radiation on photosynthetic activity and the acclimation capacity of Brazilian Atlantic Forest tree species of different successional groups in an effort to answer the following questions: (*1*) do the acclimation capacity and the susceptibility to photoinhibition differ between light-demanding species and shade-tolerant species?; (*2*) in case the above question is affirmative, do these differences imply in a lower thermal dissipation efficiency of nonphotochemical quenching and in a higher susceptibility of the photosynthetic apparatus to oxidative damages of plants considered more susceptible to photoinhibition?

Materials and methods

Study area, plant material and experimental design: The study was conducted in the Plant Growth Unit of the Plant Biology Department of the Federal University of Viçosa (20°45'25.64''S and 42°52'23.91''W), Brazil, between the period of April, 2009 and July, 2010. Seedlings were provided by the Quadrilátero Ferrífero Conservation and Research Center Nursery of a mining company located in Sabará, Minas Gerais State. The species used were chosen based on their success in recovery programs carried out by the Research Centre. The species ecological classification was performed based on literature (Lorenzzi 2000, Carvalho 2006) and on field empirical experience.

Five Atlantic Forest tree species of different successional groups were chosen. The species *Schinus terebinthifolia* Raddi. (Anacardiaceae), *Pseudobombax grandiflorum* (Cav.) A. Robyns (Malvaceae) and *Joannesia princeps* Vell. (Euphorbiaceae) belong to the light-demanding group. These species are commonly found in pioneer and secondary vegetation and in a variety of vegetation types such as evergreen ombrophile forests, semidecidual forests, *cerrado* (Brazilian savanna), sand-banks and mangroves, as in the case of *S. terebinthifolia.* All three species are known to be rapid-growth species with a high leaf production and rapidly decomposed leaves, and fruits that are very attractive to animals thus helping in seeds dispersal (Barros and Brandi 1975, Carneiro *et al.* 1996, Lorenzzi 2000, Carvalho 2006). On the other hand, the species *Hymenaea courbaril* L. var. *stilbocarpa* (Hayne) Y. T. Lee *et* Langenh (Leguminosae: Caesalpinioideae) and *Lecythis pisonis* Camb (Lecythidaceae) belong to the shade-tolerant group commonly found in late successional vegetations and are restricted to certain vegetation types such as evergreen ombrophile forests and semidecidual forests. Both species have wood of high economic value, and fruits and seeds attractive to animals (Lorenzi 2000, Carvalho 2006). These characteristics make these species very recommended for degraded area recovery.

Young plants of approximately two months of age were transplanted to plastic pots containing five liters of substrate. The substrate used was a mixture of soil, sand, and humus (1:1:1) with its acidity and fertility corrected accordingly to Ribeiro (1999). Initially, pots containing samples were subjected to two treatments, one consisting of the all-sky (I_{100}) (full sun situation under 100% of solar radiation) and the other of an artificial shade setting provided by a shade cloth with 20% of incident solar radiation (I_{20}). Incident photosynthetic radiation was monitored by a *Line Quantum Sensor LI-191* (*LI-COR Inc*., Nebraska, USA). The environmental conditions of I_{100} and I_{20} are depicted in Table 1. Throughout the experiment, plants were kept well watered and fertilized with nitrogen and phosphorus. Six months after being kept at shade (I_{20}), four plants of each species were transferred to all-sky (I_{100}). The randomnized experiment was designed with four repetitions per species and three treatments: I_{20} (20% of full incident solar radiation), I_{100} (all-sky) and $I_{20} \rightarrow I_{100}$ (transfer from low- to high light).

The acclimation process and the effects caused by the sudden increase in light radiation on the photosynthetic activity of the "transferred plants" were monitored during a 30-d period through fluorescence imaging. The contribution of thermal dissipation of nonphotochemical quenching and the damages caused on the photosynthetic apparatus by oxidative stress were confirmed by HPLC xanthophylls analysis and cell membrane lipid peroxidation.

An evaluation of the "transferred plants" was made on day 0 before transfer, and on days 1, 3, 7, 14, and 30 after transfer while the "shade control plants" and of "sun control plants" were evaluated on days 0 and 30. On every plant, the evaluations were conducted on the same leaves from the distal third of the plant, which were fully expanded, were not shaded by other leaves, presented the most leaf typical angle of the plant and did not show herbivory or any signs of pathogen attack.

Table 1. Mean daily active photosynthetic radiation (R_{PAR}), mean daily air temperature (T) and mean daily air relative humidity (RH) obtained in I_{100} treatment (all-sky) and in I_{20} treatment (20% of incident solar radiation). Data are means ± SE (n=4).

Treatments	R_{PAR} [μmol m^{-2} s^{-1}]	T [°C]	RH [%]
I_{20}	137.2 ± 21.9	24.2 ± 0.3	76.7 ± 1.3
1_{100}	757.8 ± 88.6	25.5 ± 0.4	72.8 ± 1.0

Chl fluorescence imaging: The fluorescence images were obtained using a modulated fluorometer *Imaging-PAM* (*Heinz Walz*, Effeltrich, Germany) Standard version, equipped with a special leaf clip *IMAG-USH* (Universal Sample Holder) and a CCD camera of 640 × 480 pixels resolution. Initially, nondetached leaves were previously maintained in darkness for 30 min. Then placed at a fixed distance from the camera and made to receive modulated light pulses of wavelength between 650 nm (red) and 780 nm (close to infrared) for the determination of leaf absorbance. Soon after, the dark fluorescence yield (F_0) was obtained using a modulated beam of low frequency (1 Hz) and low intensity [0.5 µmol(photon) m^{-2} s^{-1}]. A saturation light pulse [24,000 µmol(photon) m^{-2} s^{-1}] supplied for 800 ms was used to determine the maximum fluorescence yield after dark adaptation (F_m). Finally, fluorescence induction was started with actinic light of 470 nm [600 µmol(photon) m^{-2} s^{-1}] for 90 s and superimposed on a saturation pulse to determine the maximum fluorescence in the light-adapted state (F_m') and the transient fluorescence images after light adaptation (F). Analysis of the fluorescence parameters was performed using the *ImaginWin* software and by considering the right part of the leaf blade, except the midrib and the veins. The values of F_0, F_m, F, and F_m' were considered to calculate the maximum quantum yield of PSII accordingly to Kitajima and Butler (1975) given by $F_v/F_m = (F_m–F_0)/F_m$, the effective quantum yield of PSII given by $Y(II) = (F_m'–F)/F_m'$, the quantum yield of regulated nonphotochemical energy dissipation in PSII given accordingly to Gently *et al.* (1989) by $Y(NPQ) = (F/F_m') – (F/F_m)$ and the quantum yield of nonregulated nonphotochemical energy dissipation in PSII given accordingly to Hendrickson *et al.* (2004) and Klughammer and Schreiber (2008) by $Y(NO) = F/F_m$. The Y(II) corresponds to the fraction of energy that is photochemically converted in PSII. Y(NPQ) corresponds to the fraction of energy dissipated in form of heat *via* the regulated photoprotective NPQ-mechanism while Y(NO) reflects the fraction of energy that is passively dissipated in form of heat and fluorescence, mainly due to closed PSII reaction centers. Herein, we chose to adopt the concept of complementary PSII quantum yields [Y(II), Y(NPQ) and Y(NO)] for being quite practical for the analysis of the photosynthetic performance of plants based on the saturation pulse method, and for its validity not only for the lake model but also for the puddle model as well as its not requiring any knowledge of F_0'.

HPLC xanthophyll analysis: Quantification of xanthophylls was carried out on a reversed-phase high performance liquid chromatography *HPLC* (*Hewlett Packard, series 1050*, USA) using an end-capped C_{18}, 5 µm *Spherisorb ODS-2* column (250 × 4.6 mm). Pigment extraction was conducted accordingly to Ramalho *et al*. (1997) and Johnson *et al.* (1993), and the identification was done based on the pigment absorption spectrum and retention time. The quantification of each xanthophyll was performed by comparing the area under chromatograms obtained for a wavelength of 440 nm and the area of chromatograms of the xanthophyll pattern obtained for the same wave length. Xanthophyll content was expressed as [mmol(xanthophyll) mol^{-1}(total Chl)]. Each xanthophyll concentration, violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z) was expressed as a percentage of the total xanthophyll. The de-epoxidation state (DEPS) involving compounds of the xanthophyll cycle (V, A, and Z) was calculated accordingly to Ramalho *et al.* (1997), where DEPS = (Z+A)/(Z+A+V).

Cell membrane lipid peroxidation was evaluated by quantifying the malondialdehyde (MDA) content by employing the thiobarbituric acid (TBA) reaction as described by Hodges *et al.* (1999). Approximately 0.250 g of leaf tissue was homogenized with 1.5 ml of a 0.1% p/v trichloroacetic acid (TCA) and centrifuged at 10,000 × *g* and 4°C for 10 min. After centrifugation, 500 µl of supernatant was mixed with 1.5 ml 0.5% TBA in 20% TCA and incubated in boiling water (90°C) for 20 min and subsequently cooled immediately in ice to stop reaction, and centrifuged at 3,000 × *g* for 4 min. The absorbance at 532 nm and at 600 nm was determined and the nonspecific absorption at 600 nm was subtracted from the absorption at 532 nm. Finally, the MDA was determined by using a 157 nM^{-1} cm^{-1} absorption coefficient of extinction and expressed in percentage of initial content.

Statistical analysis: The experiment was randomly designed and the results subjected to a one-way *ANOVA* in order to test significance differences between days in "transferred plants" for each species and to a split-plot *ANOVA* to compare significance differences between light treatment at the beginning and at the end of the experiment for each species. *Post hoc* average comparisons were performed using *Tukey*'s test at 5% significance level. Analyses were carried out using the software *Statistica 9.0* (*StatSoft Inc*. 2010).

Results

Chl imaging fluorescence: Except for *J. princeps*, the "sun control plants" kept under I_{100} treatment were found to show lower F_v/F_m values than the "shade control plants" kept under I_{20} treatment as can be clearly perceived from the fluorescence images of *H. courbaril* and *L. pisonis* (Fig. 1), the species which showed the most pronounced differences in the F_v/F_m parameter among the "control plants". Before transfer, the F_v/F_m values of the "transferred plants" of all species obtained on day 0 were found to be similar that of the "shade control plants" (Fig. 2). However, after transfer, these values for the "transferred plants" showed a significant

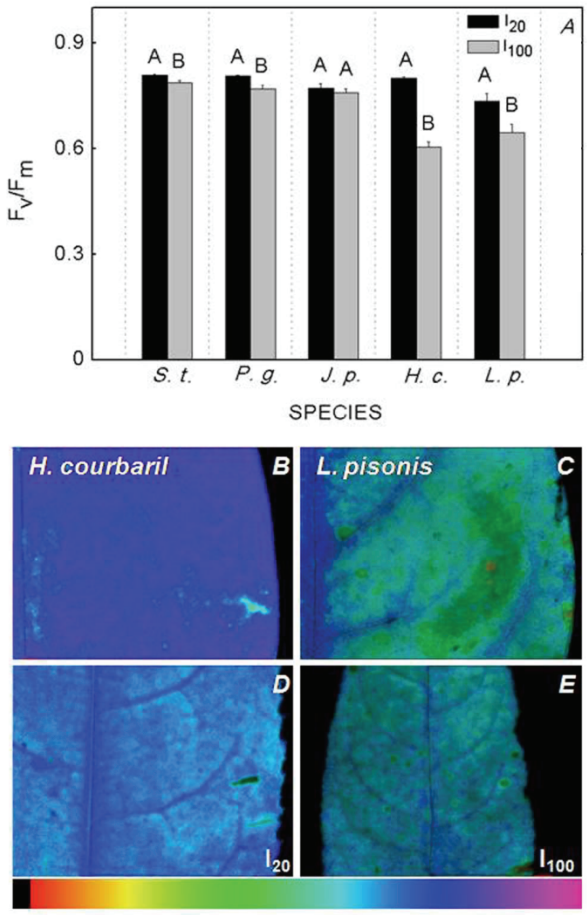

Fig. 1. Maximum quantum yield of PSII (F_v/F_m) obtained for the "shade control plants" (I_{20}) and "sun control plants" (I_{100}) of *S. terebinthifolia* (*S.t.*, *A*), *P. grandiflorum* (*P.g.*, *B*), *J. princeps* (*J.p.*, *C*), *H. courbaril* (*H.c.*, *D*), and *L. pisonis* (*L.p.*, *E*), and chlorophyll fluorescence images obtained for the "shade and sun control plants" on the first day after transfer of *H. courbaril* (*F–G*) and *L. pisonis* (*H–I*). *Different letters* indicate significant differences between means ($P<0.05$; $n = 5$, mean ± SE). The color scales below the fluorescence images correspond to values ranging from 0 to 1, or black to purple, respectively.

decrease in the fluorescence parameter as can be easily confirmed from the images of *S. terebinthifolia* and *L. pisonis*. Moreover, images of the Chl fluorescence relative to the decrease in fluorescence signal were observed to present an uneven and unspecific distribution pattern throughout the leaf blade.

Except for *J. princeps* which presented a major decrease in F_v/F_m day 14, most species were found to show the highest decrease in F_v/F_m on day 7 after transfer. The decline in the amplitude of F_v/F_m caused by the sudden increase in light irradiance was found to follow the order: *S. terebinthifolia*<*P. grandiflorum* < *J. princeps* < *H. courbaril* < *L. pisonis* (Fig. 2). Then after, "transferred plants" of all species showed a gradual recovery until the 30th day of acclimation and F_v/F_m values similar to those obtained on day 0 before transfer. The highest F_v/F_m recoveries were observed in the species *L. pisonis* and *J. princeps* 37.3% and 28.0%, respectively) while a less pronounced recovery was observed in the species *H. courbaril* (10 %).

The Y(II) value relative to the "sun control plants" of all species was lower than that of "shade control plants" (Fig. 3). The "transferred plants" showed a sharp reduction in Y(II) soon after the first day of transfer. The species *P. grandiflorum, L. pisonis* and *J. princeps* were found to show the highest decrease in Y(II) after transfer (88.7%, 80.7%, and 76.9%, respectively) while *S. terebinthifolia* and *H. courbaril* suffered less pronounced reduction (56.5% and 38.9%, respectively). The highest recovery of the effective quantum yield of PSII was observed in the species *S. terebinthifolia* (34.6%) while the lowest recovery was verified for *J. princeps* (2.2%) as can be confirmed from the fluorescence images of Fig. 3. On day 30, the images of all "transferred plants" were similar to those obtained for the "sun control plants".

An increase in Y(NPQ) was found to be followed by a reduction in Y(II) for the "transferred plants". Here, most of species showed increases of Y(NPQ) between 98.5% and 137.5% after transfer, except for *H. courbaril* which presented an increase of 12.3%. These results were supported by the fluorescence images (Fig. 4).

Except for *L. pisonis* which presented a significant decrease followed by a recovery in Y(NO) on day 7 after transfer, most species did not show any significant difference in this parameter as shown by the images presented in Fig. 5.

HPLC xanthophylls analysis: An increase in DEPS was observed on the shade-tolerant species *L. pisonis* as well as in the light-demanding species *P. grandiflorum* (Fig. 6*A*). *L. pisonis* was observed to show an increase in the de-epoxidase state of 204% on day 3 while *P. grandiflorum* showed an increase of 133% on day 3 after transfer. In addition, the increase in DEPS was followed by an increase in the concentration of the total xanthophyll (V, A and Z) in both species, particularly in *L. pisonis* (Fig. 6*B*).

Within the range of high DEPS, the concentration of Z+A showed an increase of 95% and 88% of total xanthophyll content in *L. pisonis* and *P. grandiflorum* species, respectively (Fig. 7*A*,*B*). The maximum accumulation of the final de-epoxidation product, (Z), was observed on day 7 in both species, with an increase in 10.6 folds in *L. pisonis* and 5.9 folds in *P. grandiflorum* relative to the concentration of Z obtained in leaves of same plants on day 0 before transfer. At the end of 30 days of acclimation, the amount of Z accumulated after exposure to high irradiance proved to be almost completely converted back into A and V.

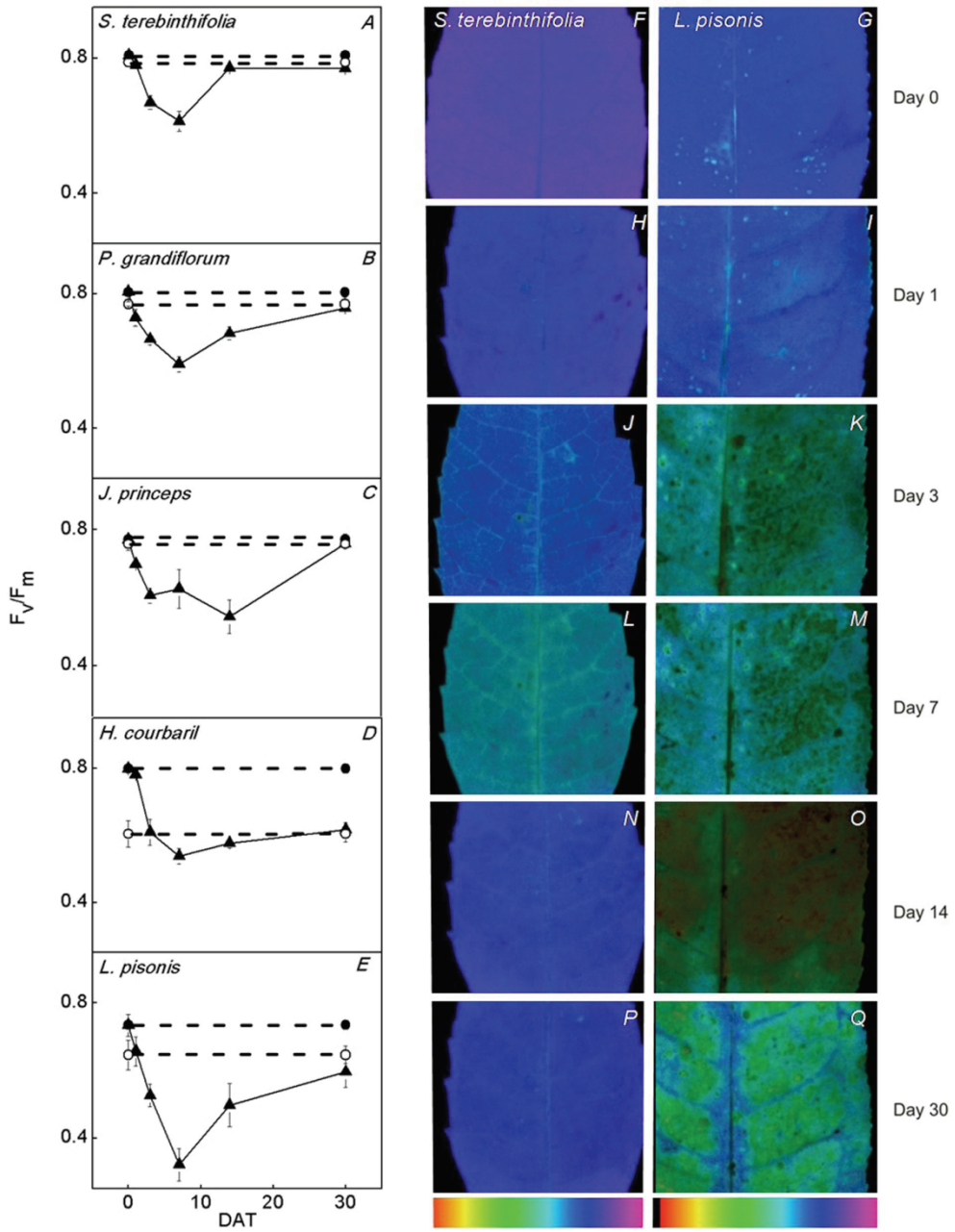

Fig. 2. Changes in chlorophyll fluorescence of maximum quantum yield of PSII (F_v/F_m) values, obtained for the "shade control plants" (I_{20} – ●), "sun control plants" (I_{100} – ○) and for the "transferred plants" (I_{20} → I_{100} – ▲) of *S. terebinthifolia* (*A*), *P. grandiflorum* (*B*), *J. princeps* (*C*), *H. courbaril* (*D*), *and L. pisonis* (*E*). Evaluation of the "transferred plants" was made on day 0 before transfer and on days 1, 3, 7, 14, and 30 after transfer while the "shade control plants" and the "sun control plants" were evaluated on days 0 and 30. ($P<0.05$; $n = 5$, mean ± SE). Chlorophyll fluorescence imaging of F_v/F_m obtained for the "transferred plants" of *S. terebinthifolia* (*F,H,J,L,N,P*) and of *L. pisonis* (*G,I,K,M,O,Q*). The false color code depicted at the bottom of images ranges from 0.00 (black) to 1.00 (pink), respectively.

Cell membrane lipid peroxidation: Lipid peroxidation was found to be higher on day 7 after transfer when *P. grandiflorum* showed an increase in MDA of 113% and *L. pisonis* of 129% compared to that on day 0 before transfer (Fig. 8). *P. grandiflorum* showed the highest levels of lipid peroxidation during the whole experiment, although the higher relative increase in MDA after the exposure of "transferred plants" to high irradiance was observed in *L. pisonis*. After seven days of acclimation, the MDA in both species returned to levels similar to those obtained on the same plants before transfer.

Discussion

The images of Chl fluorescence of all species studied showed low fluorescence signal around the midrib and veins (*e.g*. Fig. 2*Q*) hence pointing to low photochemical activity in these areas, particularly under low irradiance. This seems to be a leaf inherent characteristic since it had been verified in other species under different study conditions, such as CO_2 assimilation, source-drain and water-deficiency (Bros *et al.* 1996, Meng *et al*. 2001, Calatayud *et al*. 2006).

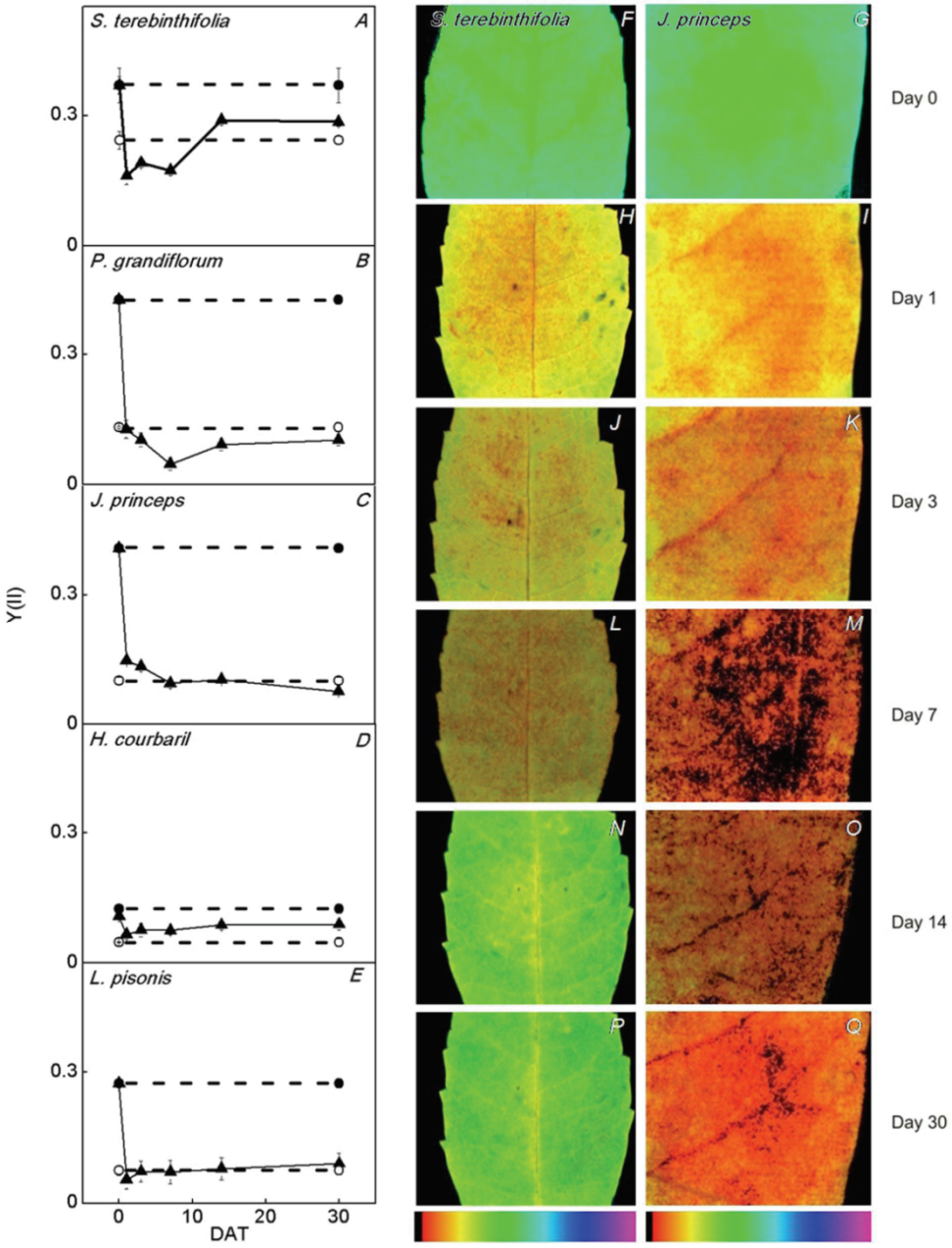

Fig. 3. Changes in chlorophyll fluorescence of effective quantum yield of PSII [Y(II)] values, obtained for the "shade control plants" (I_{20} – ●), "sun control plants" (I_{100} – ○) and for the "transferred plants" ($I_{20} \rightarrow I_{100}$ – ▲) of *S. terebinthifolia* (*A*), *P. grandiflorum* (*B*), *J. princeps* (*C*), *H. courbaril* (*D*), *and L. pisonis* (*E*). Evaluation of the "transferred plants" was made on day 0 before transfer and on days 1, 3, 7, 14, and 30 after transfer while the "shade control plants" and the "sun control plants" were evaluated on days 0 and 30. ($P<0.05$; $n = 5$, mean ± SE). Chlorophyll fluorescence imaging of Y(II) obtained for the "transferred plants" of *S. terebinthifolia* (*F,H,J,L,N,P*) and of *J. princeps* (*G,I,K,M,O,Q*). The false color code depicted at the bottom of images ranges from 0.00 (black) to 1.00 (pink), respectively.

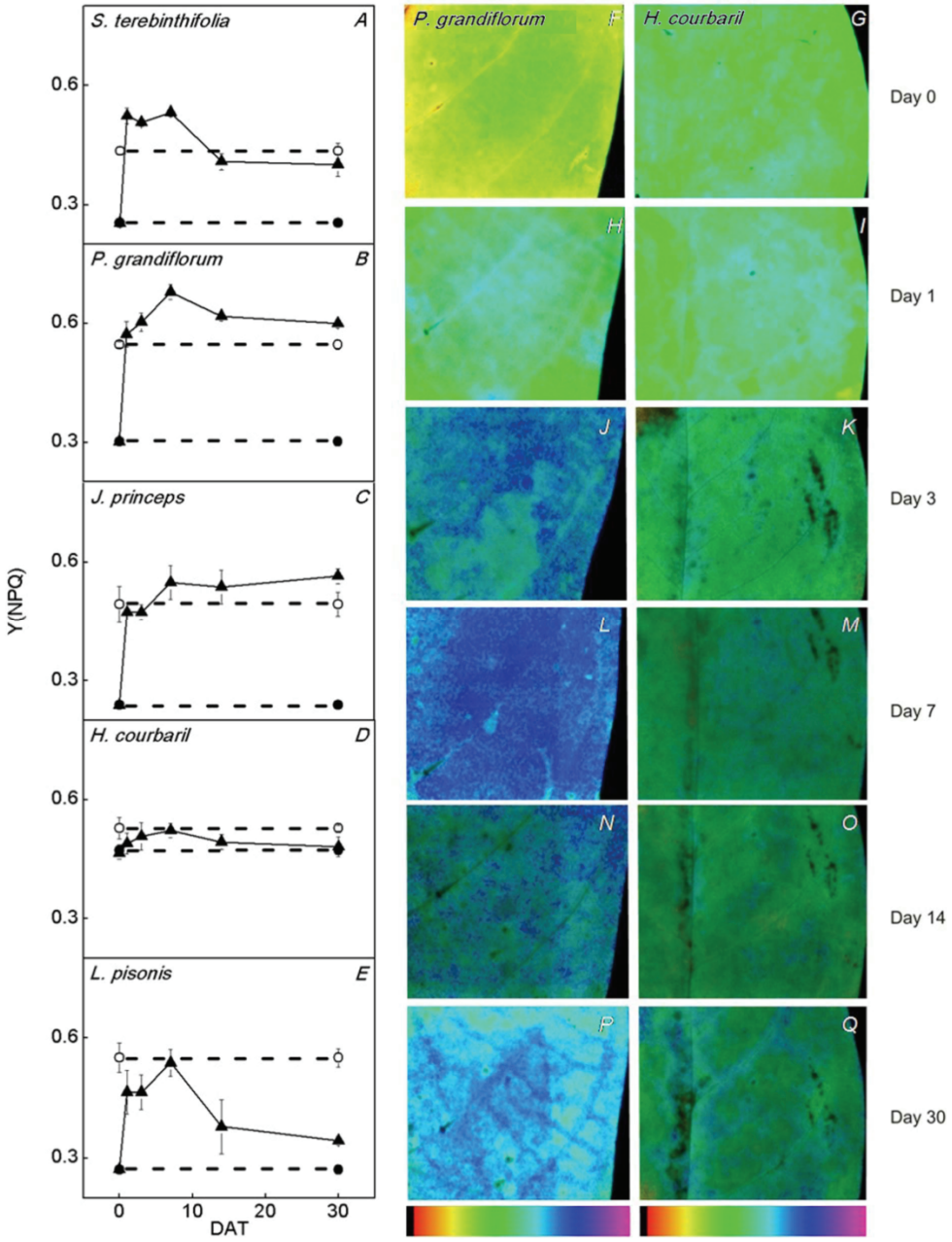

Fig. 4. Quantum yield of regulated nonphotochemical energy dissipation in PSII [Y(NPQ)] obtained for the "transferred plants" ($I_{20} \rightarrow I_{100}$) of *S. terebinthifolia* (*A*), *P. grandiflorum* (*B*), *J. princeps* (*C*), *H. courbaril* (*D*), and *L. pisonis* (*E*), and chlorophyll fluorescence images obtained for the "transferred plants" of *P. grandiflorum* (*F, H, J, L, N, P*) and *H. courbaril* (*G, I, K, M, O, Q*). Differences between means ($P<0.05$; $n = 5$, mean ± SE). The color scales below the fluorescence images correspond to values ranging from 0 to 1, or black to purple, respectively.

Besides, as a consequence of the sudden increase in light irradiance, the fluorescence signal and the photochemical activity were observed to decrease heterogeneously in an unspecific pattern along the leaf surface. This may be attributed to leaf characteristics such as differences in pigment bed and in stomatal function (Terashima 1992, Poole *et al*. 1996). These differences should make some leaf segments more susceptible to light stress, justifying the heterogeneous pattern of florescence images captured during the photoinhibition process (*e.g*. Fig. 3*M*). Spatial differences in fluorescence signal were also found in sun- and shade leaves of *Acer pseudoplatanus* L., *Tilia cordata* Mill. and *Abies alba* Mill., as a consequence of light stress (Lichtenthaler *et al*. 2007a). These results support the idea that light stress must be, in fact, characterized by a heterogeneous and random response along the leaf area.

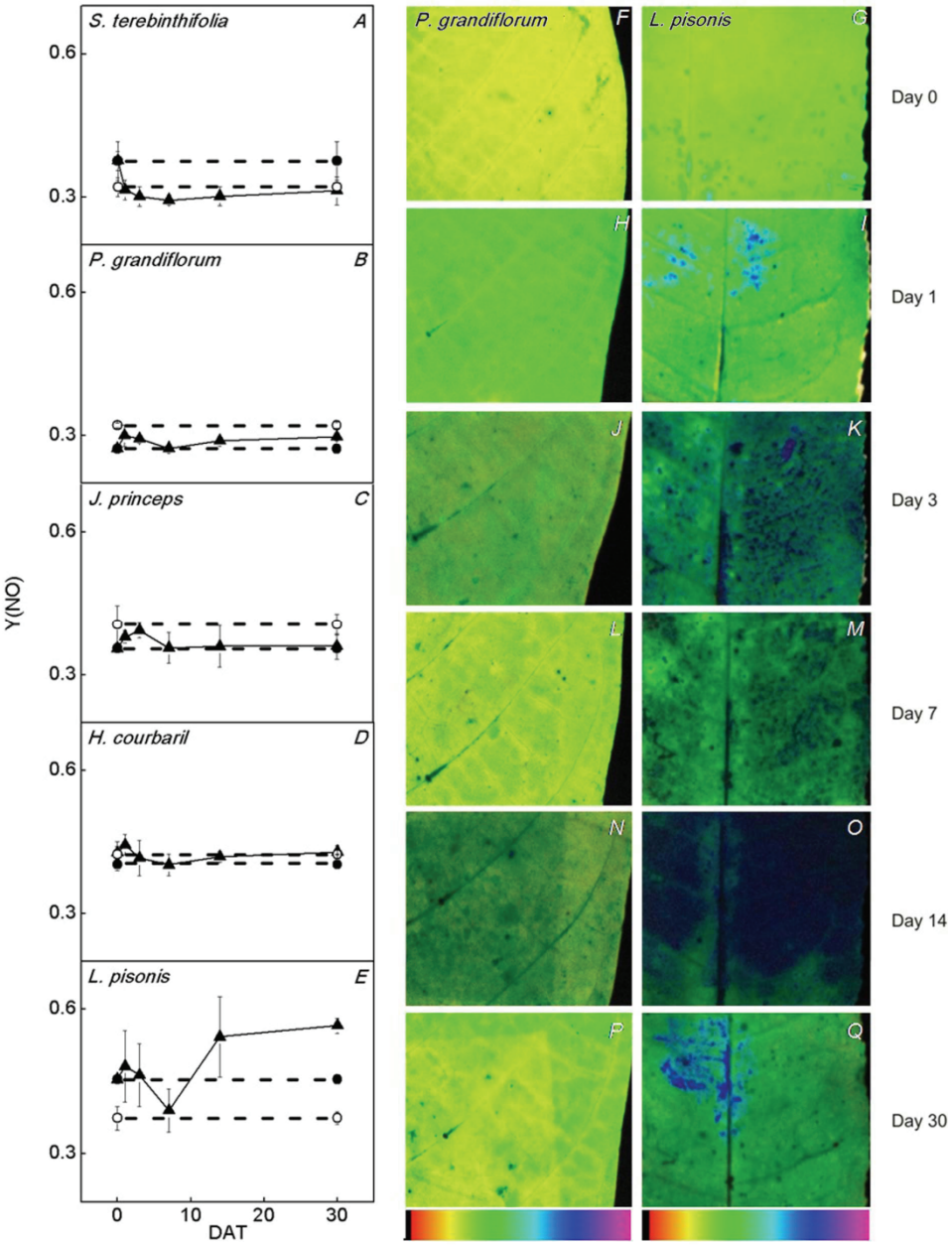

Fig. 5. Quantum yield of nonregulated nonphotochemical energy dissipation in PSII [Y(NO)] obtained for the "transferred plants" (I_{20} → I_{100}) of *S. terebinthifolia* (*A*), *P. grandiflorum* (*B*), *J. princeps* (*C*), *H. courbaril* (*D*), and *L. pisonis* (*E*), and chlorophyll fluorescence images obtained for the "transferred plants" of *P. grandiflorum* (*F, H, J, L, N, P*) and *L. pisonis* (*G, I, K, M, O, Q*). Differences between means ($P<0.05$; $n = 5$, mean ± SE). The color scales below the fluorescence images correspond to values ranging from 0 to 1, or black to purple, respectively.

The progressive decrease in F_v/F_m shown on the images of all "transferred plants", starting on the first day after transfer, was found to persist until the seventh day for the majority of species. In the case of *J. princeps*, this decrease persisted until the fourteenth day. The observed decreases ranged from 24.1%, for *S. terebinthifolia*, to 56.1%, for *L. pisonis*, and reached values as low as 0.32 (Fig. 2). The F_v/F_m is a fluorescence parameter that permits inferences on the maximum efficiency at which light absorbed by PSII is used for the reduction of the Q_A pool (Baker 2008). This parameter is a sensitive indicator of photosynthetic performance (Baker and Rosenqvist 2004). Accordingly to Björkman and Demmig (1987), the value of F_v/F_m of healthy plants should be around of 0.83 and any decrease of this parameter under stress conditions is indicative of the photoinhibition of photosynthesis. In this work, all plants were found to present an F_v/F_m value below 0.83 after the exposure to

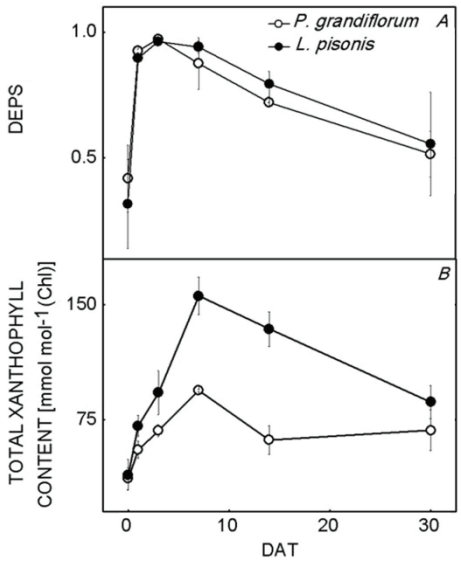

Fig. 6. Changes in the de–epoxidase state of the xanthophyll cycle (DEPS) (*A*) and in the total xanthophylls content (*B*) of the "transferred plants" ($I_{20} \rightarrow I_{100}$) of *P. grandiflorum* and *L. pisonis* species ($n = 4$, mean ± SE).

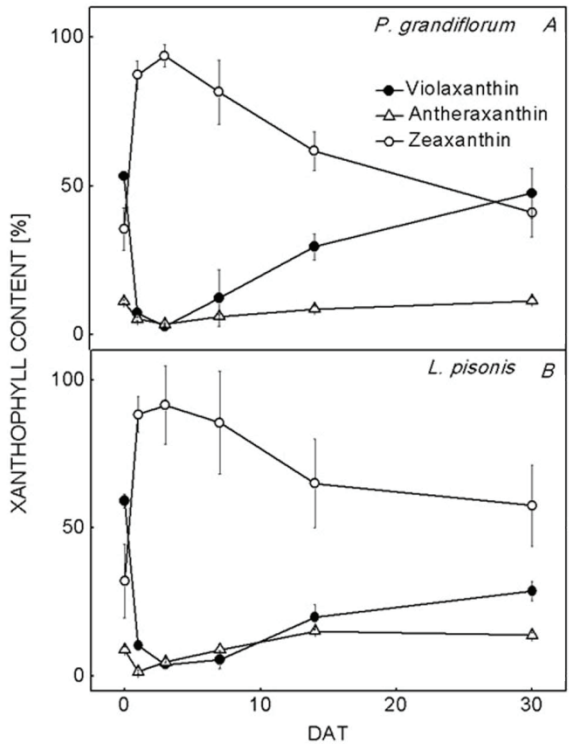

Fig. 7. Changes in violaxanthin, antheraxanthin and zeaxanthin concentration of the "transferred plants" ($I_{20} \rightarrow I_{100}$) of *P. grandiflorum* (*A*) and *L. pisonis* (*B*) species ($n = 4$, mean ± SE).

high irradiance. Hence, the persistent decrease in F_v/F_m observed in "transferred plants" indicates the occurrence of chronic photoinhibition and an irreversible loss of the PSII function, probably caused by the formation of ROS,

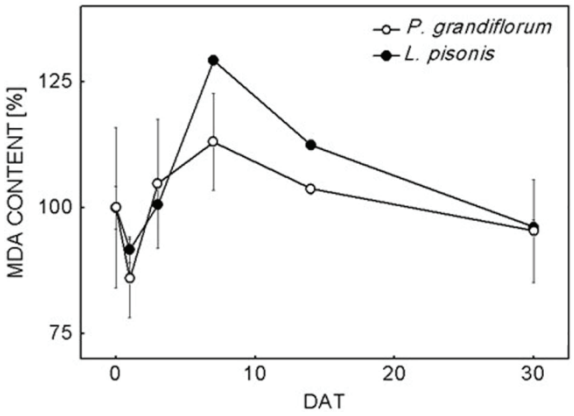

Fig. 8 Changes in malonaldehyde (MDA) content of the "transferred plants" ($I_{20} \rightarrow I_{100}$) of *P. grandiflorum* (*A*) and *L. pisonis* (*B*) ($n = 4$, mean ± SE).

the breakdown of lipid membrane and protein modification, induced by the excess light energy absorbed. The generation of ROS can be indicated by an increase in lipid peroxidation verified in *P. grandiflorum* and *L. pisonis* in this study (Fig. 8). The decrease in F_v/F_m and the increase in lipid peroxidation in response to an increase in light intensity observed in this study was found to be in good agreement with results obtained in other studies (Burritt and Mackenzie 2003, Ali *et al.* 2005, Kitao *et al*. 2006, Guo *et al.* 2006, Tobita *et al.* 2010).

The higher recovery in F_v/F_m observed in "transferred plants" of the light-demanding species *S. terebinthifolia*, *P. grandiflorum*, and *J. princeps* 30 days after their transfer revealed that these species were able to adjust their photosynthetic apparatus to the new light condition. This recovery may be attributed to an increase of functional reaction centers, probably due to *de novo* synthesis of D1 protein (Tobita *et al.* 2010), besides the contribution of photoprotection mechanisms, verified by the increase in fluorescence signal of Y(NPQ). A detailed study carried out on *L. pisonis* and *P. grandiflorum* species showed that this increase in Y(NPQ) is followed by an increase in the de-epoxidation rate of xanthophyll cycle and by the synthesis of new xanthophylls (Figs. 6, 7). An increase in A + Z and nonphotochemical quenching have been thought to be an energy dissipation mechanism that protects the photosynthetic apparatus against excess light (Demmig-Adams and Adams 1992). Thus, the results observed in this study suggest that the xanthophyll cycle plays an important role in mitigating photoinhibition during the light acclimation process by dissipating excess energy on plant exposure to high-light stress. The relationship between the F_v/F_m recovery and the increase in nonphotochemical quenching observed in the present study was found to be in good agreement with results obtained for *Spinacia oleracea* L., *Anacardium excelsum* Bert. & Barb., *Castilla elastic* Sessé and *Garcinia spp.* (Leitsch *et al.* 1994, Thiele *et al*. 1996, Guo *et al*. 2006). Nonetheless, the light-demanding

species *P. grandiflorum* presented a more efficient thermal dissipation compared to the shade tolerant species *L. pisonis*. As expected, *P. grandiflorum* should present a larger pool of xanthophylls. However, the HPLC analysis confirmed a lower xanthophyll content for this species.

On the other hand, the highest and persistent decreases in F_v/F_m observed in the shade-tolerant species *H. courbaril* and *L. pisonis* indicate these species underwent a more severe photoinhibition as shown by the yellow–greenish color of the fluorescence signal captured from the "transferred plants" of *L. pisonis* (Fig. 2). The higher increase in lipid peroxidation verified in *L. pisonis* plants under transfer to high light is a clear indication of membrane damage. Therefore, the results suggest a loss in photosynthetic activity due to the ROS induced lipid peroxidation and protein photodamage. Moreover, although *L. pisonis* had presented a larger xanthophyll pool it was found to undergo a more sever photooxidative process, including Chl bleaching (not shown) and commitment of its heat dissipation capacity, responsible for avoiding ROS-mediated inhibition of the *de novo* synthesis of the D1 protein. Often, physiological alterations needed to maintain the photosynthetic efficiency under the new environmental condition cannot occur in mature and completely expanded leaves in which the photosynthetic apparatus is already completely formed. In these cases, the alterations may only occur in the new developing leaves (Naidu and DeLucia 1997, Walters 2005). However, even the "sun control plants" of the *H. courbaril* and *L. pisonis* species, which remained seven months under high light condition, showed a low F_v/F_m (*see* Fig. 1) thus revealing small acclimation capacity of these species to high light irradiance.

After transfer, all species were found to show a reduction in Y(II), similar to that obtained for the "sun control plants". The Y(II) parameter provides an estimate of the effective quantum PSII efficiency indicating the proportion of light energy absorbed by the PSII associated Chl that is in fact directed to reduce the first stable electron acceptor (Q_A) and to induce the photochemical process (Genty *et al.* 1989). The long-term exposure of plants to higher light intensity is associated to the lack of photon energy dissipation capacity through photochemical process. Results obtained in this study indicate a less pronounced decrease in photochemical quenching in light-demanding species. The decrease in Y(II) shows that a percentage of the absorbed quanta is not converted into chemical energy by photochemical charge separation in PSII reaction centers (Calatayud *et al*. 2006). Conversely, the remaining quanta are dissipated through the nonphotochemical quenching, as reveled mainly by the increase in Y(NPQ). The nonphotochemical quenching involves two dissipation components, one corresponding to the quantum yield of nonphotochemical quenching resulting from regulated thermal dissipation represented by Y(NPQ) which corresponds to the fraction of energy dissipated in form of heat *via* a regulated photoprotective NPQ-mechanism, which refers to the regulated dissipation of excess energy in the antenna system *via* the xanthophyll cycle (Hendrickson *et al.* 2004, Klughammer and Schreiber 2008). The protector rule of this cycle occurs through excitation energy transfer from Chl to zeaxanthin molecules which rapidly build up from the de–epoxidation of violaxanthin *via* intermediate antheraxanthin and by an allosterically protein modification regulated by the proton gradient through the thylakoid membrane (Horton *et al.* 2005). Thermal dissipation through xanthophyll cycle may correspond up to 80% of the total thermal dissipation of nonphotochemical quenching and up to 90% of excitation energy that should be directed to photochemical quenching (Alves *et al.* 2002). Therefore, high values of Y(NPQ) are a strong indicator of a high plant photoprotective capacity. In some cases, like in the case of *S. terebinthifolia* after transfer, the Y(NPQ) may even cause a reduction in Y(NO), the second component of nonphotochemical quenching. Y(NO), on the other hand, represents the quantum yield of nonphotochemical excitation quenching from nonregulated primary constitutive losses which correspond to fluorescence emission and to the sum of nonregulated heat dissipation, such as the energy lost through resonance transfer between Chl molecules in light-harvesting complexes (LHCs) and that dissipated by constitutive PSII antenna carotenoids (luteins and β–carotene) (Hendrickson *et al*. 2004, Klughammer and Schreiber 2008). According to Kramer *et al*. 2004, Y(NO) also includes long-term quenching caused by chronic photoinhibition or other processes including ^{3}carotenoids and $^3Chl^*$ responsible for oxidative detriments in the core of PSII reaction centers associated to D1 protein damage and the persistent zeaxanthin and antheraxanthin retention in the LHCs (Demming-Adams *et al*. 2006). Therefore, at saturating light intensity, high Y(NO) and low Y(NPQ) values reflect a suboptimal capacity of photoprotective reactions which eventually will lead to photodamage (Klughammer and Schreiber 2008).

This was the case of *L. pisonis* which showed a substantial increase in Y(NO) after exposure of plants to high light irradiance. The photoinhibition was confirmed by the pronounced reduction of F_v/F_m, low Y(II) values and by the concomitant maximum accumulation of Z. Recent studies have suggested that PSII photoinhibition is associated, in a larger scale, to the persistence binding of zeaxanthin in LHCs than to the inactivation of D1 protein (Ebbert *et al*. 2001, Demming-Adams *et al.* 2006). Therefore, the maximum accumulation of Z along with the major depression of F_v/F_m verified in this study indicates the persistent presence of this xanthophyll in LHCs and the contribution of this mechanism to the chronic depression of the photosynthetic capacity. The relationship between F_v/F_m depression and the sustained zeaxanthin retention in antennas observed in this study was found to be in very

good agreement with results from other studies (Ebbert *et al*. 2001, Cheng 2003, Demming-Adams *et al.* 2006). Although the mechanisms involved in the sustained zeaxanthin retention are still not very clear, studies have shown that it is not a ΔpH-dependent mechanism and is probably associated to the upregulation of other light-harvesting proteins and the PSII core modification by sustained phosphorylation (Heddad and Adamska 2000, Ebbert *et al*. 2005, Demming-Adams *et al.* 2006). High values of Y(NO) were also observed in "transferred plants" of *H. courbaril*. This species was observed to show the lowest F_v/F_m recovery besides low Y(II) and Y(NPQ) values. Furthermore, *H. courbaril* showed low variation amplitude values in its fluorescence parameters for both the "transferred plants" and "control plants". These results are indicative of a low photochemical and nonphotochemical mechanism regulation capacity for absorbed light energy dissipation thus revealing a high susceptibility to photoinhibition of the shade-tolerant species *H. courbaril*, too.

Nonetheless, the photoinhibition verified in *H. courbaril* and in *L. pisonis* may not only represent damages in the photosynthetic apparatus, but also an alternative strategy of these species to consume the excess of energy absorbed and to overcome the light stress caused by the long-term exposure to high light irradiance. Based on this, photoinhibition can be considered a protective adaptation in cases where the thermal dissipation mechanism, *via* the xanthophyll cycle, proves insufficient (Öquist *et al.* 1992b, Einhorn *et al*. 2004). Some species, tend to keep nonfunctional PSII damaged by excess light energy instead of replacing it with new functional reaction centers (Öquist *et al*. 1992a). This behavior is generally verified in shade-tolerant species as a reflection of a more conservative usage strategy of resources. The rapid replacement of damaged reaction centers involves the synthesis of new proteins at a higher energy cost. These energy expenditures may not represent a good strategy to light-demanding plants. Nonetheless, to shade tolerant species that have low photosynthetic rates, the retention of nonfunctional PSII may represent a better choice for energy conservation (Öquist *et al*. 1992a).

In conclusion thereof, the results obtained through fluorescence imaging support the idea that photoinhibition and light acclimation processes are characterized by a heterogeneous and random response pattern along the leaf surface. Furthermore, the results support the initial hypotheses, indicating that light-demanding species are less susceptible to photoinhibition and possess a higher light acclimation capacity. Also, the higher photoinhibition tolerance observed in light-demanding species may be associated to their higher capacity for photochemical dissipation and dissipation of excess excitation energy via the xanthophyll cycle thus leading to lower ROS generation. Lastly, the obtained results suggest that a knowledge of acclimation capacity, by means of Chl fluorescence imaging yields, is a useful indicator of species successional grouping.

References

Ali, M.B., Hahn, E., Paek, K.: Effects of light intensities on antioxidant enzymes and malondialdehyde content during short-term acclimatization on micropropagated Phalaenopsis plantlet. – Environ. Exp. Bot. **54**: 109-120, 2005.

Alves, P.L.C.A., Magalhães, A.C.N., Barja, P.R.: The phenomenon of photoinhibition of photosynthesis and its importance in reforestation. – Bot. Rev. **68**: 193-208, 2002.

Baker, N.R., Rosenqvist, E.: Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. – J. Exp. Bot. **55**: 1607-1621, 2004.

Baker, N.R.: Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. – Annu. Rev. Plant Biol. **59**: 89-113, 2008.

Barbosa, L.M.: [Forest restoration of degraded areas in São Paulo State: history, current situation and projects. – In: Barbosa L.M. (ed.): Manual for Restoration of Degraded Areas of São Paulo State - Riparian Forests of Inland São Paulo State.] Pp. 4-25. Instituto de Botânica, São Paulo 2006. [In Portuguese.]

Barros, N.F., Brandi, R.M.: [Influence of three forest species over soil fertility of pasture in Viçosa, Minas Gerais State.] – Brasil Florestal **6**: 24-29, 1975. [In Portuguese.]

Björkman, O., Demmig, B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77k among vascular plants of diverse origins. – Planta **170**: 489-504, 1987.

Bros, E., Meyer, S., Genty, B.: Heterogeneity of leaf CO_2 assimilation during photosynthetic induction. – Plant Cell Environ. **19**: 1349-1358, 1996.

Burritt, D.J., Mackenzie, S.: Antioxidant Metabolism during Acclimation of *Begonia* x *erythrophylla* to High Light Levels. – Ann. Bot. **91**: 783-794, 2003.

Calatayud, A., Roca, D., Martínez, P.F.: Spatial-temporial variations in rose leaves under water stress conditions studies by chlorophyll fluorescence imaging. – Plant Physiol. Biochem. **44**: 564-573, 2006.

Carneiro, M.A.C., Siqueira, J.O., Davide, A.C., Gomes, L.J., Curi, N., do Vale, F.R.: Mycorrhizal fungi and superphosphate and the growth of tropical woody species. – Sci. Forest. **50**: 21-36, 1996.

Carpanezzi, A.A.: [Grounds for rehabilitation of ecosystems forest. – In: Galvão, A.P.M., Porfírio-da-Silva V. (ed.): Forest Restoration - Fundamentals and Case Studies.] Pp. 27-46. Embrapa, Colombo-PR 2005. [In Portuguese.]

Carvalho, P.E.R.: [Brazilian tree species.] Vol 1 and 2. Embrapa, Colombo-PR, 2006. [In Portuguese.]

Chen, X., Li, W., Lu, Q., Wen, X., Li, H., Kuang, T., Li, Z., Lu, C.: The xanthophyll cycle and antioxidative defense system are enhanced in the wheat hybrid subjected to high light stress. – J. Plant Physiol. **168**: 1828-1836, 2011.

Cheng, L.: Xanthophyll cycle pool size and composition in relation to the nitrogen content of apple leaves. – J. Exp. Bot. **54**: 385-393, 2003.

Delagrange, S., Messier, C., Lechowicz, M.J., Dizengremel, P.: Physiological, morphological and allocational plasticity in understory deciduous trees: importance of plant size and light availability. – Tree Physiol. **22**: 775-84, 2004.

Demming-Adams, B.; Adams, W.W., III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **43**: 599-626, 1992.

Demming-Adams, B.; Adams, W.W., III: The role of xanthophyll cycle carotenoids in the protection of photosynthesis. – Trends Plant Sci **1**: 21-26, 1996.

Demmig-Adams, B., Ebbert, V., Zarter, C.R., Adams, W.W., III.: Characteristics and species-dependent employment of flexible versus sustained thermal dissipation and photoinhibition. – In: Demming-Adams, B.; Adams W.W., III, Matoo, K. (ed.): Advances in Photosynthesis and Respiration - Photoprotection, Photoinhibition, Gene Regulation and Environment. Vol. 21. Pp. 39-48. Springer, Dordrecht 2006.

Ebbert, V., Demmig-Adams B., Adams, W.W., III., Mueh, K.E., Staehelin, L.A.**:** Correlation between persistent forms of zeaxanthin-dependent energy dissipation and thylakoid protein phosphorylation. – Photosynth. Res. **67**: 63-78, 2001.

Ebbert, V., Adams W.W., III, Mattoo, A.K., Sokolenko A., Demmig-Adams B.: Upregulation of a PSII core protein phosphatase inhibitor and sustained D1 phosphorylation in zeaxanthin-retaining, photoinhibited needles of overwintering Douglas fir. – Plant Cell Environ**. 28**: 232-240*,* 2005.

Einhorn, K.S., Rosenqvist, E., Leverenz, J.W.: Photoinhibition in seedlings of *Fraxinus* and *Fagus* under natural light conditions: implications for forest regeneration? – Oecologia **140**: 241-251, 2004.

Gandolfi, S.: [Light regime in semidecidual forests and its possible consequences. – In: Alfredo, Jún. (ed.): Brazilian Ecosystems: Management and Conservation.] Pp. 305-311. Expressão Gráfica e Editora, Fortaleza-Ceará 2003. [In Portuguese.]

Genty, B., Briantais, J., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Govindjee, Nedbal, L.: The chlorophyll fluorescence imaging and its application in plant science and technology – 3rd Regional Photosynthetic Workshop – Photosynthetica **38**: 481-482, 2000.

Gray, G.R., Hope, B.J., Qin, X., Taylor, B.G., Whitehead, C.L.: The characterization of photoinhibition and recovery during cold acclimation in Arabidopsis using chlorophyll fluorescence imaging. – Physiol. Plant. **119**: 365-375, 2003.

Guidi L., Degl'Innocenti E.: Ozone effects on high light-induced photoinhibition in *Phaseolus vulgaris*. – Plant Sci. **174**: 590-596, 2008.

Guo, X.R., Cao, K.F., Xu, Z.F.: Acclimation to irradiance in seedlings of three tropical rain forest *Garcinia* species after simulated gap formation. – Photosynthetica **44**: 193-201, 2006.

Heddad, M., Adamska, I.: Light stress-regulated two-helix proteins in *Arabidopsis thaliana* related to the chlorophyll *a*/*b*-binding gene family. – Proc. Natl. Acad. Sci. U.S.A. **97**: 3741-3746, 2000.

Hendrickson, L., Furbank, R.T., Chow, W.S.: A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. – Photosynth. Res. **82**: 73-81, 2004.

Hodges, D.M., Delong, J.M., Forney, C., Prange, R.K.: Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. – Planta **207**: 604-611, 1999.

Hogewoning, S.W., Harbinson, J.: Insights on the development, kinetics, and variation of photoinhibition using chlorophyll fluorescence imaging of a chilled, variegated leaf. – J. Exp. Bot. **58**: 453-463, 2007.

Horton, P., Wentworth, M., Ruban, A.: Control of the light harvesting function of chloroplast membranes: the LHCII-agreegation model for non-photochemical quenching. – FEBS Lett. **579**: 4201-4206, 2005.

Houter, N.C., Pons, T.L.: Gap size effects on photoinhibition in understorey saplings in tropical rainforest. – Plant Ecol. **179**: 43-51, 2005.

Johnson, G.N., Scholes, J.D., Horton, P., Young, A.J.: Relationship between carotenoid composition and growth habit in British plant species. – Plant Cell Environ. **16**: 681-686, 1993.

Kageyama, P.Y., Gandara, F.B.: [Grounds for rehabilitation of ecosystems forest.] – In: Galvão A.P.M., Porfírio-da-Silva V. (ed.): Forest Restoration - Fundamentals and Case Studies. Pp. 47-58. Embrapa, Colombo-PR 2005. [In Portuguese.]

Kitajima, M., Butler, W.L.: Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. – Biochim. Biophys. Acta **376**: 105-115, 1975.

Kitao, M., Yoneda, R., Tobita, H., Matsumoto, Y., Maruyama, Y., Arifin, A., Azani, M., Muhamad, M.N.: Susceptibility to photoinhibition in seedlings of six tropical fruit tree species native to Malaysia following transplantation to a degraded land. – Trees **20**: 601-610, 2006.

Klughammer, C., Schreiber, U.: Complementary FSII quantum yield calculated from simple fluorescence parameters measured by PAM fluorometry and the saturation pulse method. – PAM Application Notes **1**: 27-35, 2008.

Kramer, D.M., Johnson, G., Kiirats, O., Edwards, G.E.: New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. – Photosynth. Res. **79**: 209-218, 2004.

Leitsch, J., Schnettger, B., Critchley, C., Krause, G.H.: Two mechanisms of recovery from photoinhibition in vivo: reactivation of photosystem II related and unrelated to D1-protein turnover. – Planta **194**: 15-21, 1994.

Lichtenthaler, H.K., Langsdorf, G., Lenk, S., Buschmann, C.: Chlorophyll fluorescence imaging of photosynthetic activity with the flash-lamp fluorescence imaging system. – Photosynthetica **43**: 355-369, 2005.

Lichtenthaler, H.K., Ac, A., Marek, M.V., Kalina, J., Urban, O.: Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. – Plant Physiol. Biochem. **45**: 577-588, 2007a.

Lichtenthaler, H.K., Babani, F., Langsdorf, G.: Chlorophyll fluorescence imaging of photosynthetic activity in sun and shade leaves of trees. – Photosynth. Res. **93**: 235-244, 2007b.

Lorenzi, H.: [Brazilian Trees: Manual of Identification and Growing of Native Tree Plants of Brazil.] – Instituto Plantarun de Estudos da Flora Ltda, Nova Odessa – São Paulo 2000. [In Portuguese.]

Meng, Q., Siebke, K., Lippert, P., Baur, B., Mukherjee, U., Weis, E.: Sink-source transition in tobacco leaves visualized using chlorophyll fluorescence imaging. – New Phytol. **151**: 585-595, 2001.

Mittermeier, R.A., Myers, N., Gil, P.R., Mittermeier, C.G.: HOTSPOTS – Earth's biologically richest and most endangered terrestrial ecoregions. – Conservation International, the Mexican conservation organization Agrupación Sierra Madre and the cement company CEMEX, Mexico City 1999.

Naidu, S.L., DeLucia, E.H.: Growth, allocation and water relations of shade-grown *Quercus rubra* L. saplings exposed to a late-season canopy gap. – Ann. Bot**. 80**: 335-344, 1997.

Öquist, G., Anderson, J.M., McCaffery, S., Chow, W.S.: Mechanistic differences in photoinhibition of sun and shade plants. – Planta **188**: 422-431, 1992a.

Öquist, G., Chow, W.S., Anderson J.M.: Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. – Planta **186**: 450-460, 1992b.

Oxborough, K.: Imaging of chlorophyll *a* fluorescence: theoretical and practical aspects of an emerging technique for the monitoring of photosynthesis performance. – J. Exp. Bot. **55**: 1195-1205, 2004.

Poole, I., Weyers, J.D.B., Lawson, T., Raven, J.A.: Variations in stomatal density and index: implications for palaeoclimatic reconstructions. – Plant Cell Environ. **19**: 705-712, 1996.

Ramalho, J.C., Pons, T.L., Groeneveld, H.W., Nunes, M.A.: Photosynthetic responses of *Coffea arabica* leaves to a short-term high light exposure in relation to N availability. – Physiol. Plant **101**: 229-239, 1997.

Ribeiro, A.C.: [Recommendation for liming and fertility of soil for seedling, pits and flowerbeds. – In: Ribeiro A.C., Guimarães P.T.G., Álvares V.H. (ed.): Commissions of Soil Fertility of Minas Gerais State - Recommendations for the use of lime and fertilizers in Minas Gerais State.] – 5th Ed. Aproximação. Departamento de Solos da Universidade Federal de Viçosa, Viçosa-MG 1999. [In Portuguese.]

Ribeiro, R.V., Souza, G.M., Oliveira, R.F., Machado, E.C.: Photosynthetic responses of tropical tree species from different successional groups under contrasting irradiance conditions. – Ver. Bras. Bot. **28**: 149-161, 2005.

Rozendaal, D.M.A., Hurtado, V.H., Pooter, L.: Plasticity in leaf traits of 38 tropical tree species in response to light: relationships with demand and adult stature. – Funct. Ecol. **20**: 207-216, 2006.

Ruban, A. V.: Plants in light. - Communicative & Integrative Biology **2**: 1, 2009.

Souza, G.M., Ribeiro, R.V., Prado, C.H.B.A., Damineli, D.S.C., Sato, A.M., Oliveira, M.S.: Using network connectance and autonomy analyses to uncover patterns of photosynthetic responses in tropical woody species. – Ecol. Complexity **6**: 15-26, 2009.

Takahashi, S., Badger, M. R.: Photoprotection in plants: a new light on photosystem II damage. – Trends Plant Sci. **16**: 1-1, 2011.

Takahashi, S., Murata, N.: How do environmental stresses accelerate photoinhibition? - Trends Plant Sci. **13**: 178-182, 2008.

Terashima, I.: Anatomy of non-uniform leaf photosynthesis. – Photosynth. Res. **31**: 195-212, 1992.

Thiele, A., Schirwitz, K., Winter, K., Krause, G.H.: Increase xanthophylls cycle activity and reduced D1 protein inactivation related to photoinhibition in two plant systems acclimated to excess light. – J. Plant Sci. **155**: 237-250, 1996.

Tobita, H., Utsugi, H., Kitao, M., Kayama, M., Uemura, A., Kitaoka, S., Maruyama, Y.: Variation in photoinhibition among *Sasa senanensis*, *Quercus mongolica*, and *Acer mono* in understory of a deciduous broad-leaved Forest exposed to canopy gaps caused by typhoons. – Trees **24**: 307-319, 2010.

Valladares, F., Niinemets, U.: Shade tolerance, a key plant feature of complex nature and consequences. – Annu. Rev. Ecol. Evol. System. **39**: 237-257, 2008.

Walters, R.G.: Towards an understanding of photosynthetic acclimation. – J. Exp. Bot. **56**: 435-447, 2005.