Leaf and photosynthetic characteristics of pioneer and forest species in tropical montane habitats

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Abstract

Gas exchange and chlorophyll *a* fluorescence measurements of expanding and adult leaves of four plant species were compared under field conditions. The pioneer species (PS) tended to have thinner leaves with lower nitrogen content and higher stomatal density compared to forest species (FS). Expanding leaves featured lower photosynthetic pigment contents and gas exchange capacity than adult leaves consistent with an immature photosynthetic apparatus. At the time of maximum irradiance, sun-exposed leaves of both PS and FS showed alteration of initial, variable, and maximum fluorescence as well as their ratios indicating photoinhibition. However, leaves recovered to some extent at predawn, suggesting the activation of photoprotective mechanisms. Sun-exposed leaves had comparable responses to high irradiance.

Additional key words: *Chletra*; chlorophyll *a* fluorescence; *Croton*; *Ficus*; intercellular CO_2 concentration; leaf age; *Oyedea*; photoinhibition; photoprotection; stomatal conductance.

Introduction

Deforestation has substantial impact on complex forests, which are rapidly transformed into simpler succession communities with bare soils and few scattered pioneer trees. Pioneer species (PS) possess ecological adaptations allowing them to tolerate habitats with environmental extremes and to exploit their resources successfully, compared to forest species (FS; Bazzaz 1979). PS seem to be opportunistic, with both higher leaf water loss and carbon gain than FS (Larcher 1969, Bazzaz 1979, Hölscher *et al*. 2006). Consequently, short- and long-term water use efficiency (WUE) of PS is lower compared to FS (Huc *et al.* 1994, Sobrado 2003). Furthermore, a trade-off between water transport and leaf WUE has been suggested: PS are more efficient in conducting water to the leaves but have low control over stomatal conductance (g_s), when compared to FS (Sobrado 2003).

The dissipation of solar energy differs between closed canopy forest environments and deforested areas with scattered trees. In forest areas, heat transfer processes occur at the canopy level, where transpiration is the major component of heat loss (Monteith and Unsworth 1990). In deforested areas, the soil receives a large proportion of solar radiation, where convection is the main heat loss process (Monteith and Unsworth 1990). Latent heat loss for transpiration is more effective than sensible heat loss for convection. This difference results in pronounced diurnal fluctuations in light and temperature in forested *versus* deforested areas which may impact the FS and PS species associated with them, respectively (Bazzaz and Mezga 1973, Parrish and Bazzaz 1982). Information about eco-physiological characteristics of tropical forest comparing succession in deforested areas is insufficient to understand the integrated functioning of plants in these habitats (Mooney *et al.* 1980). Exposed leaves of PS seem to have reflectance, transmittance, and absorptance properties comparable to those of FS (Poorter *et al.* 2000). Therefore, under high irradiance and temperature, PS could be more susceptible to absorb excessive photon energy compared to FS. This would lead to a decline of photosynthetic efficiency as a result of photoinhibition (Demmig-Adams and Adams 1992).

This study was designed to provide insight into the gas exchange and chlorophyll (Chl) *a* fluorescence responses to high irradiance of fully sun-exposed leaves

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of both plant groups in order to obtain information for the eco-physiological understanding of both plant groups. Additionally, the effect of sunlight on fluorescence parameters may affect differently leaves of contrasting ages (Šesták and Šiffel 1997). Consequently, measurements were performed in expanding (E) and adult (A) leaves of PS and FS occupying contrasting habitats in a tropical montane zone.

Materials and methods

Study site: The study area is a tropical area with both forest and deforested areas located at 10°20′N, 66°50′W (near Caracas, Venezuela) and 1 200 m of altitude, with annual rainfall between 800 and 1 000 mm (Baruch *et al.* 1989, Sobrado 2003). Deforested areas have mostly bare soil with sparse tree PS, and forest areas have a closed canopy with well structured tree strata. These montane forests as well as similar ones on the hills surrounding the city of Caracas were used to shelter coffee plantations maintaining the original tree species to shade the coffee plants (Steyermark and Huber 1978). We selected two early PS occupying deforested sites and two FS occupying the outer canopy in the forest.

Plants: The species selected were the two evergreen PS *Chletra lanata* Mart. & Gal. (Chletraceae) and *Oyedea verbesinoides* D.C. (Compositae), which are between 2−6 m tall; occasionally, *C. lanata* may reach 8 m (Steyermark and Huber 1978). The two FS were the drought-deciduous species *Croton xantochloros* Cruizat (Euphorbiaceae) and the evergreen *Ficus mathewsii* Miq (Moraceae), whose heights can vary between 8−25 m and 6−8 m, respectively (Steyermark and Huber 1978). The measurements were conducted during the rainy season (June−September 2007) in 3−4 tagged trees per species, selecting individuals that reach the upper canopy. Fully sun-exposed E and A leaves were used for *in situ* measurements and for subsequent analysis.

Leaf gas exchange: Midday leaf gas exchange was measured in 15−20 leaves from each of the tagged trees by using a portable gas analyser system model *LCA-2* (*Analytical Development Co.*, Hoddesdon, Herts, UK). The leaf chamber has a built-in solar radiation meter which allows to determine the radiation incident on the leaf. Thus, gas exchange was measured at an irradiance of 1 463±93 μmol m^{-2} s^{-1}, air and leaf temperatures between 29.0±0.2 and 30.1±0.2 °C, and ambient CO_2 concentration (C_a) of 348±1 μmol mol^{-1}. The parameters determined were net photosynthetic rates (P_N), g_s, and intercellular CO_2 concentration (C_i), following Caemmerer and Farquhar (1981).

Leaf Chl *a* fluorescence: In the same leaves used for gas exchange measurements, Chl *a* fluorescence (F) parameters (initial, F_0; maximum, F_m; and variable, F_v) were measured at midday and again the following day at predawn, in the mid-part of the leaves, avoiding major veins. In order to detect differential susceptibility to midday photoinhibition, measurements were performed on both adaxial and abaxial leaf sides (Lichtenthaler *et al.* 2005). Chl *a* fluorescence was measured after leaves were dark-adapted for 15 min. A Chl fluorometer (model *OS-30p*, *OptiSciences*, Hudson, USA) provided with white leaf-clips to avoid overheating leaf tissue upon dark-adaptation was used (Weng 2006).

Leaf characteristics, anatomy, and photosynthetic pigments: After gas exchange and fluorescence measurements, the leaves were harvested for subsequent analysis, forming three sub-samples per tree per leaf age. A first sub-sample was used for fresh mass (FM) and leaf area measurement. Dry mass (DM) was determined after the samples were oven-dried at 60 ºC to constant mass. The results were used for calculation of leaf water content (W_c) as water mass per leaf area and of DM per leaf area (S_a). Samples of dry leaves were ground and N content was determined by Kjeldahl analysis. In the second sub-sample of samples, leaf blade cross sections parallel to the midrib were prepared from E and A leaves. Tissue from the leaf blade centre was dehydrated progressively in an ethanol series (70−100 %) and infiltrated with warm paraffin (56−58 °C). Leaf slices of 2−3 μm were obtained with a rotation microtome *(Leitz*, Wetzlar, Germany). The slices were de-paraffinized, re-hydrated, and stained with safranin/fast green (1 % aqueous safranin and 0.5 % fast green in 95 % ethanol), mounted in glycerol, and photographed in order to measure leaf thickness and to count the number of epidermal spongy and palisade layers. To count stomata, leaf pieces (1×1 cm) were treated with bleach (NaOCl) to eliminate the mesophyll tissue and separate the epidermises. When mesophyll remains were eliminated, the pieces were stained with toluidine blue and mounted in glycerol. The third leaf sub-sample was used for the determinations of Chl *a* and *b* and carotenoids (xanthophylls+carotenes) following the procedures of Lichtenthaler and Wellburn (1983).

Statistical analysis: All measurements were conducted with independent replicates taken randomly. For each parameter, the normal distribution and homogeneity of the data was determined for the Kolmogorov-Smirnov and Levene test, respectively, and subsequently one-way ANOVA was used. Afterwards, statistical differences among means were determined with a posteriori LSD or Duncan test when variances were homogenous or non-homogeneous, respectively. Significance level was set at $p \leq 0.05$. Statistical analysis was performed using *SPSS 10.0 for Microsoft Windows* (*SPSS*, Chicago, IL, USA).

Results and discussion

Leaf characteristics, anatomy, and photosynthetic pigment composition: Leaf mass per area (S_w) was comparable among species except in the case of *F. mathewsii*, which had significantly higher values at both ages (Table 1). The values of S_w and leaf thickness were not strictly correlated, indicating differences in leaf tissue density among species (Witkowski and Lamont 1991). W_c in A leaves was less than in E leaves in PS, whereas it did not tend to change with leaf age in FS (Table 1). Nitrogen content was similar or slightly higher in E as compared to A leaves (Table 1). The leaf N in PS averaged 10.3±0.4 and was 21.1±1.2 g kg^{-1} in FS. Leaf transverse sections showed typical features of C_3 plants (Fig. 1). Similar anatomical arrangements of palisade and spongy tissues as well as layer number were observed in FS and PS. The leaf blade of *F. mathewsii* is slightly succulent (Fig. 1*D*) and has a multiseriate epidermis typical of the genus *Ficus*, formed by periclinal and anticlinal divisions of the outer layer of the leaf meristem (Esau 1965, Beardsell and Norden 2004). Leaf thickness increased significantly in A leaves compared to E leaves, except in *C. xantochloros* (Table 1). All the species bore stomata only on their abaxial surface, and stomatal density (SD) tended to be higher in PS than in FS. In A leaves, stomata density averaged 654±61 and 217±9 in PS and FS, respectively. Therefore, PS tended to have thinner leaves with lower N content and higher SD than FS. Chl *a*+*b* content was significantly lower in E than A leaves of the four species (Fig. 2*A*). Values of PS overlapped those in FS. The Chl *a*/*b* ratio was 2.7±0.1 and 3.1±0.1 in E and A leaves of PS, whereas in FS it was 2.9±0.4 and 3.0±0.1, respectively. Lower Chl *a*+*b* content and Chl *a*/*b* ratio in E leaves suggests a less developed antenna system and fewer photosynthetic units. Carotenoids (Car) tended to be significantly higher in A than E leaves except in *C. lanata*, but comparable across species. This suggests that sun leaves of PS and FS may have similar capability for excess photon energy dissipation by the xanthophyll-cycle activity. In PS, the ratio Chl/Car was 6.1±0.4 and 6.4±0.3 in E and A leaves, while in FS it was 6.3±0.9 and 7.1±0.8, respectively. Therefore, across species pigment composition of PS and FS was similar. The changes of photosynthetic pigments from the stage of immature E to mature A leaves were consistent with the prevalence of Chl synthesis upon degradation during leaf expansion (Šesták 1969, 1985, Šesták and Šiffel 1997).

Table 1. Leaf blade characteristics: dry mass per unit leaf area (S_w), thickness, water (W_c) and nitrogen (N) contents, stomata density, and number of layers of palisade and spongy tissue. Measurements were taken in expanding (E) and adult (A) leaves. Means ± SE of 3−4 tagged trees per species. Means followed by different letters were statistically different at $p<0.05$.

	PS				FS			
	Chletra		*Oyedea*		*Croton*		*Ficus*	
	E	A	E	A	E	A	E	A
S_w [g m^{-2}]	96±7^{c}	105±4^{c}	93±10^{c}	109±3^{c}	86±10^{c}	97±32^{c}	166±3^{b}	291±7^{a}
Thickness [μm]	168±3^{e}	190±4^{d}	126±4^{f}	182±4^{e}	203±4^{c}	202±3^{c}	253±8^{b}	309±6^{a}
W_c [g m^{-2}]	336±8^{d}	260±23^{c}	323±63^{d}	251±18^{c}	303±7b	271±13^{bc}	387±56^{a}	409±32^{a}
N [g kg^{-1}]	11.3±1.4^{c}	9.5±0.2^{c}	10.8±0.4^{c}	9.6±0.7^{c}	24.8±0.9^{a}	25.7±0.5^{a}	18.4±0.7^{b}	15.7±0.1^{b}
Stomata [mm^{-2}]	955±56^{a}	916±44^{a}	429±58^{b}	331±24^{c}	177±15^{e}	184±12^{e}	381±13^{b}	242±15^{d}
Palisade [no.]	1−2	2−3	1−2	2	1	1	2	2
Spongy [no.]	3−4	4−5	3−4	4−5	3−4	4	4−5	4−6

Leaf gas exchange: P_N was significantly higher in A than E leaves of all the species (Fig. 3*A*). This is consistent with an immature photosynthetic apparatus in E leaves, also suggested by the pattern of photosynthetic pigments (Fig. 2). Similarly, g_s followed this pattern except in *F. mathewsii*, where age differences were not detected (Fig. 3*B*). However, the C_i/C_a ratio did not change with leaf age in PS, but declined in A leaves of FS compared to E leaves (Fig. 3*C*). Furthermore, A leaves of FS operated at lower C_i/C_a than those in PS, which is consistent with their high WUE (Huc *et al.* 1994, Sobrado 2003).

Leaf Chl *a* fluorescence: At the time of maximum irradiance at midday, sun-exposed leaves showed changes of F_0, F_v, and F_m and their ratios, measured at adaxial and abaxial leaf surfaces (Fig. 4). Efficiency and stability of photosystem 2 (PS2) was detected by means of diurnal changes in the ratio F_v/F_m. Higher F_v/F_m values at predawn were found in E and A leaves compared to those measured at midday. This indicated overnight recovery of PS2 efficiency to some extent. A higher F_v/F_m in A leaves at predawn indicated larger overnight recovery of PS2 efficiency compared to E leaves. Typical F_v/F_m values for mature healthy tissue are 0.74−0.85 (Litchtenthaler *et al.* 2005). In E leaves, F_v/F_m was below this range at midday as well as at predawn, which may be the result of their lower photochemical capacity due to undeveloped antenna as shown by their low Chl content. The only

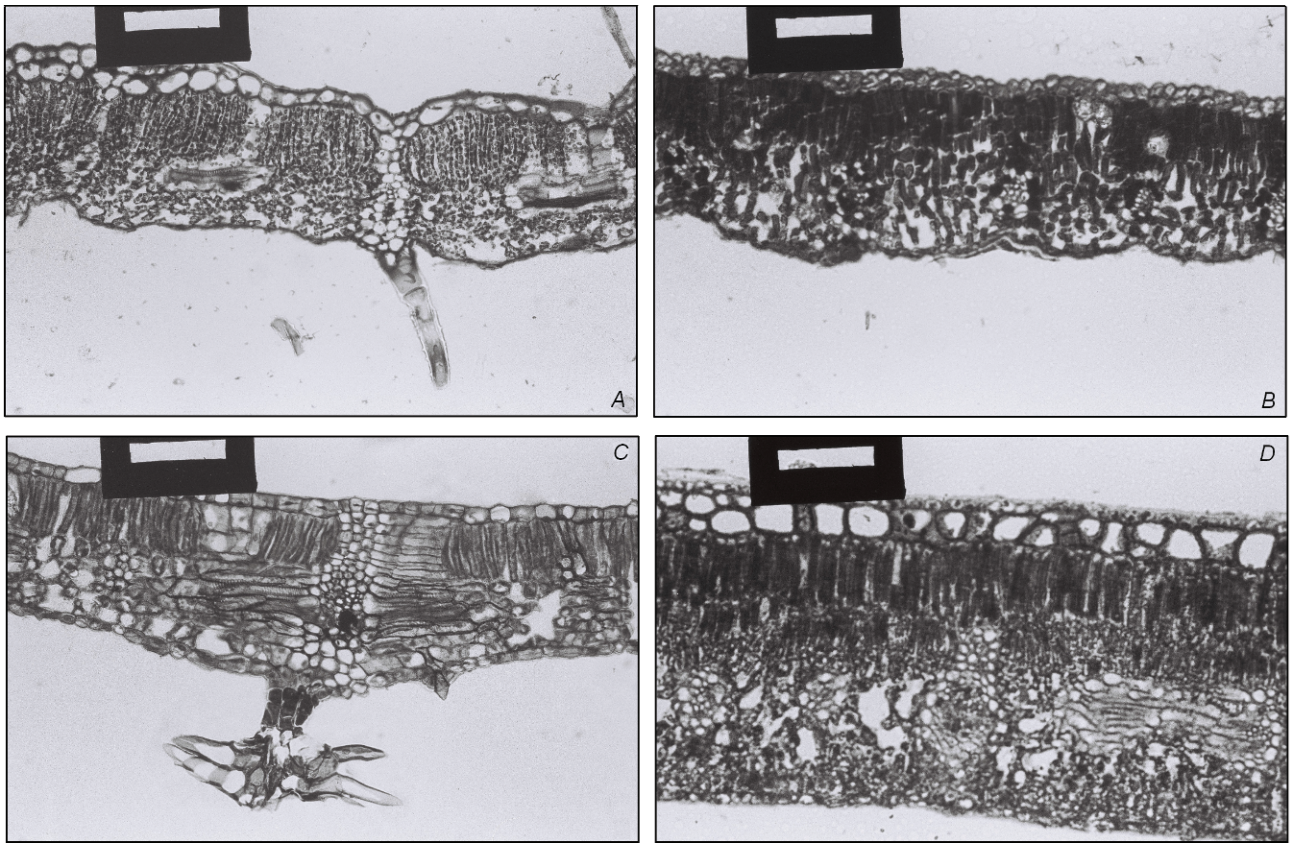

Fig. 1. Typical cross-sections of adult leaves from two pioneer species: *C. lanata* (*A*) and *O. verbesinoides* (*B*) and two forest species: *C. xantochloros* (*C*) and *F. mathewsii* (*D*). *Bars* represent 100 μm.

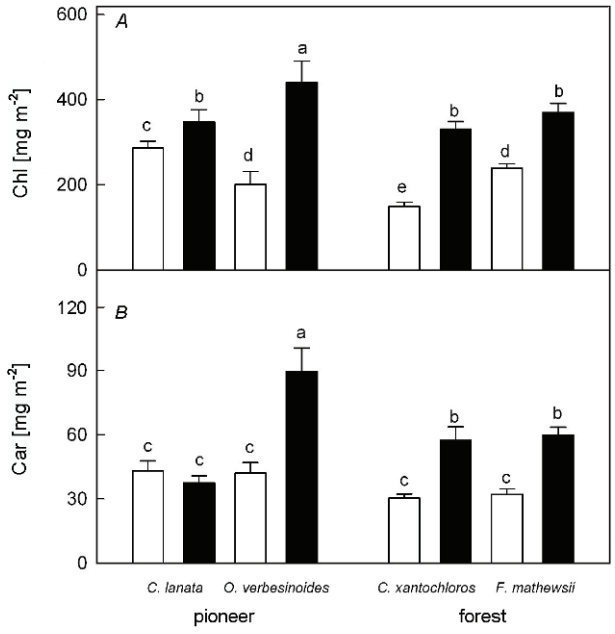

Fig. 2. Contents of chlorophylls, Chl (*A*) and carotenoids, Car (*B*) in expanding (*white bars*) and adult (*black bars*) leaves. Means±SE of 3−4 tagged trees per species. Across species and leaf ages, means followed by different letters were statistically different at $p<0.05$.

exception to this was found in the adaxial side of E leaves of *O. verbesinoides* with predawn F_v/F_m value of 0.76±0.01. By contrast, A leaves had predawn F_v/F_m within the typical range of non-stressed tissue (Fig. 4*A*). Midday depression in F_v/F_m reveals the diurnal decline in PS2 efficiency as a result of photoinhibition (Demmig-Adams and Adams 1992). Chl *a* fluorescence measured at the adaxial leaf side can be somewhat lower than that determined at the abaxial side (Lichtenthaler *et al.* 2005). Among species, differences in midday depression of PS2 between abaxial and adaxial surfaces did not point out towards a conclusive tendency for the adaxial side to be more vulnerable to photoinhibition at either age. In E leaves of *C. lanata* and *C. xantochloros* the abaxial surface showed significantly higher photoinhibition vulnerability compared to adaxial surface, whereas in *F. mathewsii* the reverse tendency was found at both leaf ages (Fig. 4). The differential photoinhibition between adaxial and abaxial sides may be related to leaf thickness in *F. mathewsii* (Table 1). However, differences in optical properties of abaxial and adaxial sides could not be discarded in this species. In *F. elastica* and F. *benjamina* the abaxial surface has larger scattering coefficient and reflectance than the adaxial side, which leads to a slightly higher absorption coefficient at the upper leaf side (Cordón and Lagorio 2007). The differential fluorescence

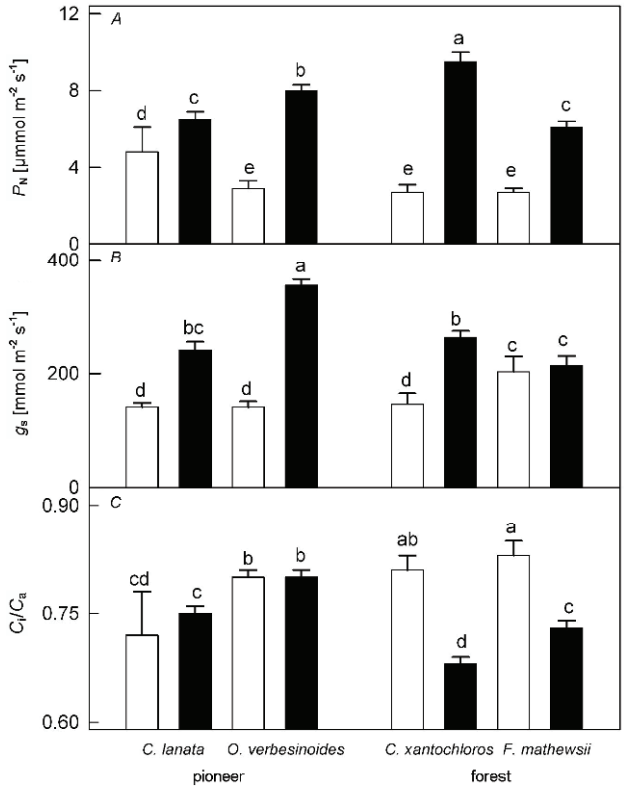

Fig 3. Net photosynthetic rate, P_N (*A*), stomatal conductance, g_s (*B*), and internal to ambient CO_2 ratio, C_i/C_a (*C*) measured in expanding (*white bars*) and adult (*black bars*) leaves. Means±SE of 3−4 tagged trees per species. Across species and leaf ages, means followed by different letters were statistically different at $p<0.05$.

characteristics of abaxial and adaxial leaf sides found in *F. mathewsii* leaves are in agreement with findings in *F. elastica* (Lichtenthaler *et al.* 2005). However, it remains to be established if it is typical of the genus *Ficus* to have leaves with abaxial and adaxial surfaces with contrasting optical properties and/or fluorescence characteristics.

Overall, the midday F_v/F_m ratio was about 88 and 93 % of that at predawn in E and A leaves, respectively. This trend is comparable to that found in studies on tropical species (Krause *et al.* 1995, Sobrado 1996, 2008). When photoinhibition is slight, F_v/F_m changes very little, whereas F_v/F_0 exhibits a large and significant decline (Lichtenthaler *et al.* 2005). Here, average midday F_v/F_0 was about 73 and 79 % of that at predawn in E and A leaves, respectively (Fig. 4*B*). Therefore, F_v/F_0 is more sensitive to changes in fluorescence parameters when midday depression of PS2 is low, as shown in the present case, where F_v and F_0 changed simultaneously in midday and predawn measurements. Generally, F_v/F_0 shows higher amplitude under stress conditions, since changes in F_0 and/or F_v are immediately reflected in their ratio (Roháček 2002, Lichtenthaler *et al.* 2005, Wilhelmová *et al.* 2005, Ranjbarfordoei *et al.* 2006). Photoinhibition includes both photoprotection mechanisms and photodamage that reduces the efficiency of photosynthetic energy conversion as the result of high irradiance (Chow 1994). Decrease in PS2 efficiency measured by low F_v/F_m and F_v/F_0 ratios at midday, with subsequent recovery at predawn in adults, may be a result of photoprotective thermal energy dissipation associated

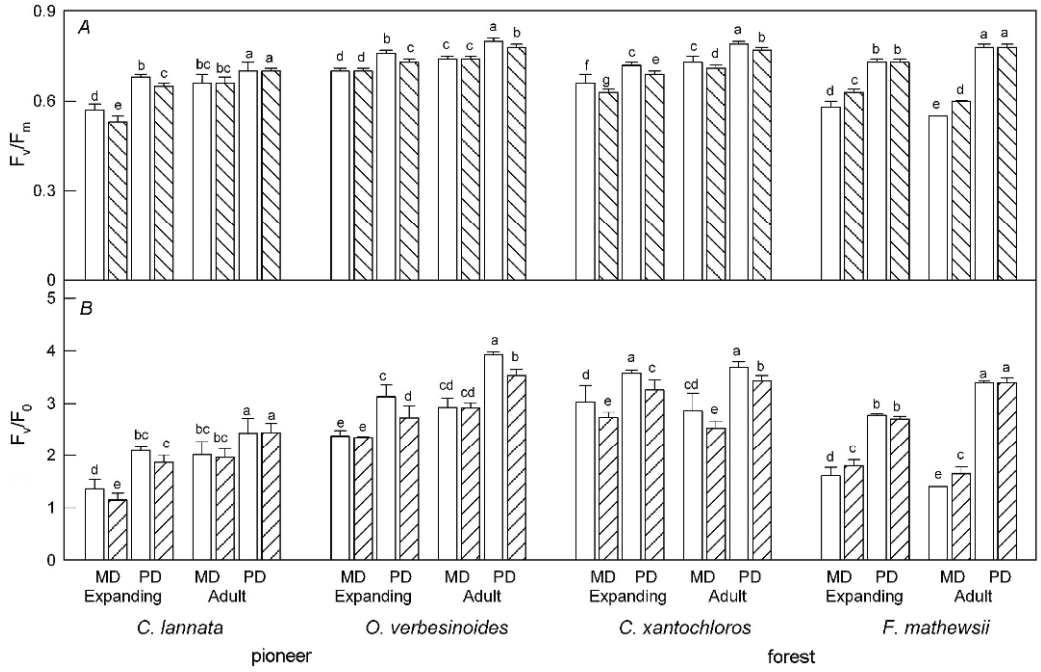

Fig. 4. Ratios of variable to maximum fluorescence, F_v/F_m (*A*) and of variable to initial fluorescence, F_v/F_0 (*B*) measured at midday (MD) and again at predawn (PD) on the adaxial (*unhatched*) and abaxial (*hatched*) faces in expanding and adult leaves. Means±SE of 3−4 tagged trees per species. For each individual species, different letters indicate statistical difference at $p<0.05$.

with the xanthophyll cycle (Kitajima and Butler 1975, Demmig-Adams and Adams 1992, Long *et al.* 1994, Krause *et al.* 1995). Conversely, an insufficient overnight recovery in E leaves could be attributed to the fact that the photosynthetic apparatus was still developing.

Conclusions: Despite the difference in habitats occupied by PS and FS, sun-exposed leaves had comparable responses to high irradiance. The four species studied underwent a slight dynamic inactivation of PS2 at mid-day, which depended on the degree of leaf development. At leaf level, these results suggest similar vulnerability to midday photoinhibition in sun-exposed leaves of PS and FS, as the result of photoprotection. However, on the whole tree we would expect that in the case of PS with an open canopy, all leaves may be exposed to full sunlight and undergo photoinhibition. By contrast, forest tree canopies are more complex, with leaf layers fully sun-exposed and others at different degrees of shade, depending on their position. Thus, shade leaves of FS may never undergo photoinhibition.

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