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Leaf characteristics and diurnal variation of chlorophyll fluorescence in leaves of the ‘Bana’ vegetation of the Amazon region

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Abstract

In six dominant species of the Amazonian ‘Bana’ vegetation, leaf blade characteristics, pigment composition, and chlorophyll (Chl) fluorescence parameters were measured in young and mature leaves under field conditions. Leaf $\delta^{13}C$ was comparable in the six species, which suggested that both expanding and expanded leaves contained organic matter fixed under similar intercellular and ambient CO_2 concentration (C_i/C_a). High leaf C/N and negative $\delta^{15}N$ values found in this habitat were consistent with the extreme soil N-deficiency. Analysis of Chl and carotenoids showed that expanding leaves had an incomplete development of photosynthetic antenna when compared to adult leaves. Dynamic inactivation of photosystem 2 (PS2) at midday was observed at both leaf ages as F_v/F_m decreased compared to predawn values. Adult leaves reached overnight F_v/F_m ratios typical of healthy leaves. Overnight recovery of F_v/F_m in expanding leaves was incomplete. F_0 remained unchanged from midday to predawn and F_v tended to increase from midday to predawn. The recovery from midday depression observed in adult leaves suggested an acclimatory down-regulation associated with photo-protection and non-damage of PS2.

Additional key words: acclimation; down regulation; leaf C/N; nitrogen deficiency; photo-protection; sclerophyllous leaves; species differences; $\delta^{13}C$; $\delta^{15}N$.

Introduction

Fluorescence induction kinetics provides insight into the utilization of excitation energy by photosystem 2 (PS2), as well the changes induced in the latter by different forms of stress (Krause and Weis 1984, Long *et al.* 1994, Šesták and Šiffel 1997, Roháček 2002, Lichtenthaler *et al.* 2005). Under high irradiance stress, plants absorb excess photon energy, inducing photoinhibition due to a decline of the potential efficiency of PS2 (Demmig-Adams and Adams 1992). The photoinhibition may be dynamic and is readily reversible within hours (Long *et al.* 1994). This has been recognized as a photo-protective mechanism leading to non-photochemical quenching of excess photons (Krause and Weis 1984, Demmig-Adams and Adams 1992). In contrast, chronic photoinhibition involves damage of PS2 integrity due to the proteolysis of the D1 protein. In this case, the irradiation-induced inactivation overrides the capacity for subsequent repair in the short-term (Greer *et al.* 1986).

The ‘Banas' are typical landscape elements present on sandy podzolized soils of the upper Rio Negro basin in the Amazonas State (Venezuela); they occupy relatively high mounds along an elevation gradient (Herrera 1977). Soils drain after a few rainless days, but the water table is never below 0.4 m from the surface (Bonger *et al.* 1985). These communities are composed of low-stature trees with scleromorphic leaves, which have a remarkable ability to restrict leaf water loss at the time of maximal irradiance at midday (Sobrado 1977, Sobrado and Medina 1980). Consequently, midday carbon gain is also restricted by about 40 % as compared to mid-morning values, whereas photon flux density increases by about 30 % at midday compared with mid-morning values (Medina and Cuevas 2000). Restriction of midday leaf gas exchange allows these species the maintenance of a high water status (Sobrado 1977). Therefore, at the time of high irradiation, leaves could be prone to suffer photoinhibition of PS2, which may affect differentially leaves in dissimilar developmental stages. This could be

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due to the fact that differences in chlorophyll (Chl) fluorescence characteristics related to leaf age can be larger than those induced by environmental effects (Šesták and Šiffel 1997). To assess this hypothesis, leaf blade characteristics and pigment composition of young expanding and adult leaves were determined in six 'Bana' species, and fluorescence parameters were measured under field conditions.

Materials and methods

Study site and plants: The study was carried out at a 'Bana' located in the upper Rio Negro region in Venezuela, 11 km from the town of San Carlos de Rio Negro (1°54'N, 67°03´W). The site has a typical equatorial climate with total annual rainfall of 3.8 m; every month features over 0.1 m. The mean annual temperature is 26 °C. 'Banas' occupy podzolized sandy soils which are extremely oligotrophic (Herrera *et al.* 1978). Wallace (1889) described these zones as dry, sandy soils with very few insects. A more detailed description of the site and the vegetation can be found in Sobrado and Medina (1980) and Bonger *et al.* (1984). Sampling and measurements were completed during clear rainless days in February 2007. The six dominant species of this site, selected following Sobrado (1977), are: *Catostemma sancarlosianum* Steyerm, *Heteropterys* sp., *Macairea rufescens* DC, *Pachira sordida* (RE Schult.) WS. Alverson, *Retiniphyllum concolor* (Spruce *ex* Benth.) Mull. Arg., and *Remijia morilloi* Steyerm. Expanding and adult leaves were selected by their colour, texture, and position within terminal fully exposed branches. Leaves were tagged in at least three individual trees for each of the six species.

Leaf fluorescence: In the tagged leaves, Chl fluorescence (F) parameters (initial, F_0 and maximum, F_m) were measured at midday and again at predawn in the mid part of the leaves, avoiding major veins. Variable fluorescence (F_v) was calculated by subtracting F_0 from F_m. Measurements were taken on the adaxial leaf side, which is sun exposed and highly susceptible to suffer midday photoinhibition (Lichtenthaler *et al.* 2005). Chl F was measured after leaves were dark-adapted for 15 min. Preliminary tests showed that there were not significant differences in F parameters of leaves dark-adapted between 10 and 30 min. A Chl fluorometer (model *OS-30p*, *OptiSciences*, Hudson, USA) provided with white leaf-clips to avoid overheating leaf tissue upon dark-adaptation (Weng 2006) was used. This instrument uses an "actinic light" source and dual mode pulse modulated detection system. After F measurements were completed, leaves were harvested in two parallel sets. In one batch leaf characteristics were determined; the second batch of leaves was kept frozen for pigment composition determination.

Leaf characteristics: Fresh mass (FM) and leaf area were measured. Dry mass (DM) was determined after samples were oven-dried at 60 ºC to constant mass. The results were used for calculation of leaf water content (W_c) as the difference between FM and DM and expressed as percentage of FM. The ratio of leaf DM to leaf area (S_w) was also calculated. Samples of dry leaves were ground for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) measurements. Isotope analysis was performed at the Stable Isotope Research Facility for Ecological Research (SIRFER), University of Utah (Salt Lake City, USA). A detailed description of procedures for isotope determinations has been presented by Ehleringer and Osmond (1989).

Chl and carotenoids (Car): The determinations were performed following the procedures of Lichtenthaler and Wellburn (1983).

Statistical analysis: Comparison between young expanding leaves and adult leaves was carried out for each individual species by using an independent *t*-test. The leaf F measurements for each species at both leaf ages were compared by using an ANOVA followed by an LSD test (Sokal and Rohlf 1969).

Results and discussion

Leaf characteristics: In all six species studied, young expanding leaf tissue had significantly higher water content as compared to adult leaves (Table 1). Conversely, S_w tended to increase with leaf age, as previously detected in these species (Sobrado and Medina 1980). A high leaf C/N ratio was observed in all species, except in expanding leaves of *Heteropterys* sp. and *P. sordida* (Table 1). Furthermore, in four of the species, C/N was not different in expanding and adult leaves. Mean values for all species were 51.6±7.1 and 55.6±4.4 kg kg^{-1} in expanding and adult leaves, respectively. There is a close link between C and N metabolism in higher plants: N-containing metabolites are required to allow C to be utilized for growth (Fritz *et al.* 2006). The photosynthetic process provides the C skeletons, NADPH, and ATP required for assimilation of inorganic N. In this study, leaf C/N ratios were comparatively higher than those found in mature leaves of other species, but agreed well with those found in senescent leaves or lignified wood tissue (Pate and Atkins 1983, Kao *et al.* 2002, Koike *et al.* 2003, Grechi *et al.* 2007). High C/N in the 'Bana' species would reflect the low availability of N

Table 1. Percentage of water on fresh tissue (W_c), dry mass per unit leaf area (S_w), and ratio of carbon to nitrogen (C/N) in expanding (E) and adult (A) leaves of six 'Bana' species. Means (standard error) of measurements performed in three trees per species. Significant differences between leaf ages for each species are indicated at $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$; non-significant differences are denoted as ns.

Species	W_c [%]		S_w [g m^{-2}]		C/N [kg kg^{-1}]	
	E	A	E	A	E	A
Catostemma sancarlosianum	68 (1)**	61 (1)	81 (1)***	152 (4)	52 (3)^{ns}	44 (2)
Heteropterys sp.	65 (2)**	52 (2)	91 (7)***	170 (6)	16 (2)**	34 (6)
Macairea rufescens	70 (1)*	63 (2)	184 (7)^{ns}	202 (7)	70 (9)^{ns}	60 (2)
Pachira sordida	66 (3)*	56 (2)	195 (8)***	294 (3)	33 (2)**	72 (3)
Retiniphyllum concolor	65 (1)*	57 (2)	180 (7)^{ns}	186 (14)	56 (6)^{ns}	47 (1)
Remijia morilloi	63 (1)***	56 (1)	239 (9)*	292 (15)	90 (2)^{ns}	85 (14)

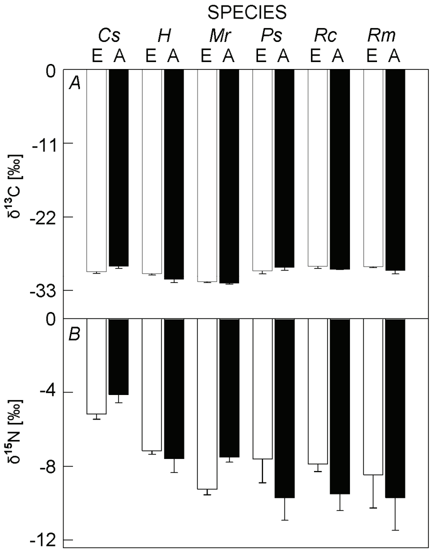

Fig. 1. (*A*) Carbon (δ^{13}C) and (*B*) nitrogen (δ^{15}N) isotopic composition in expanding (E; *white bars*) and adult (A; *black bars*) leaves of six 'Bana' species: *C. sancarlosianum* (*Cs*), *Heteropterys* sp. (*H*), *M. rufescens* (*Mr*), *P. sordida* (*Ps*), *R. concolor* (*Rc*), and *R. morilloi* (*Rm*). Means (standard error) of samples taken in three trees per species. No significant differences were observed between leaf ages for each species.

required for growth in this environment. The δ^{13}C was variable among species, but both leaf ages within species had similar values (Fig. 1*A*). This suggests that both expanding and expanded leaves were constituted by organic matter fixed under similar internal to ambient CO_2 concentration C_i/C_a. The mean value of leaf δ^{13}C was –30.2±0.2 and –30.3±0.3 ‰ in expanding and adult leaves, respectively. These δ^{13}C values of the 'Bana' species were similar to those found, between –29 and –31 ‰, in canopy leaves of other forest types adjacent to the 'Bana' (Medina and Minchin 1980). Therefore, despite short sporadic droughts experienced at the 'Bana' site, leaf δ^{13}C does not provide any indication of relatively greater long-term water use efficiency as compared to other types of forest species in the area. Values of leaf δ^{15}N are more negative with the increasing soil-N limitation (Martinelli *et al.* 1999). In the present case, δ^{15}N was negative in all species at both leaf ages (Fig. 1*B*). The δ^{15}N value averaged –7.2±0.4 and –7.9±0.6 ‰ in expanding and adult leaves, respectively. High leaf C/N and low δ^{15}N found in this habitat is consistent with the extreme soil-N deficiency in this region. Comparable leaf δ^{15}N values have been found in other tropical species also thriving in N-impoverished white sands in Brazil (Martinelli *et al.* 1999).

Pigment composition: Chl (*a*+*b*) content was significantly lower in expanding leaves than in adult leaves (Fig. 2*A*). Expanding leaves contained 33 to 46 % of the amount present in the adult ones. Throughout the leaf life span, Chl synthesis prevails during leaf development and a relative balance between synthesis and degradation is present at leaf maturity (Šesták and Šiffel 1997). The ratio of Chl *a/b* was significantly higher in adult than expanding leaves in all species studied (Fig. 2*B*). This is a typical pattern found during leaf development in previous studies (Dickmann 1971, Šesták 1969, 1985). Lower Chl *a/b* in expanding compared to adult leaves suggested that expanding leaves had incomplete development of antenna. Consequently, Chl *a/b* has been positively related with the ratio of PS2 cores to light-harvesting Chl-protein complex (Fritschi and Ray 2007). Thus, higher Chl *a/b* (>3) in adult leaves would be related to a larger photochemical capacity of PS2 as compared to expanding leaves. Values of Chl *a/b* above three are considered typical for adult, healthy, sunny leaves (Šesták 1985, Hagg *et al.* 1992). The content of Car followed the trend of the Chl (*a*+*b*) content, showing an increase with leaf age (Fig. 2*C*). Therefore, changes in photosynthetic pigments from expanding to adult leaves agreed with those found in developing leaf tissue of other species (Šesták 1985).

A higher investment of N in Chls as indicated by the Chl (*a*+*b*)/N ratio was observed in adult when compared to expanding leaves (Fig. 2*D*). I assume that expanding leaves invest a larger part of N in secondary compounds to avoid herbivore damage. This would be costly in N-poor environment such as the 'Bana', where soil N is limiting and leaf N is reabsorbed by the plant during leaf senescence (Sobrado and Medina 1980). However,

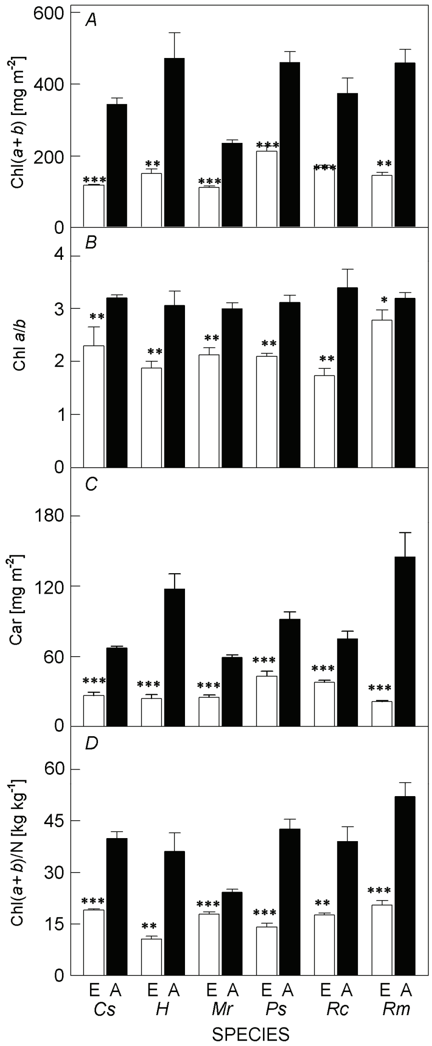

Fig. 2. (*A*) Chlorophyll (Chl) *a*+*b*, (*B*) Chl *a*/*b*, (*C*) carotenoids (Car), and Chl (*a*+*b*)/leaf nitrogen as measured in expanding (E; *white bars*) and adult (A; *black bars*) leaves of six 'Bana' species: *C. sancarlosianum* (*Cs*), *Heteropterys* sp. (*H*), *M. rufescens* (*Mr*), *P. sordida Ps*), *R. concolor* (*Rc*), and *R. morilloi* (*Rm*). Means (standard error) of measurements performed in three trees per species. Significant differences between leaf ages for each species are indicated at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

secondary N compounds are very labile in the plant, which favours production in high quantities and subsequent reuse for photosynthesis (Gulmon and Mooney 1983). Leaf toughening is the main factor in decreasing herbivore damage of mature leaves in tropical rain forests (Kursar and Coley 2003).

Diurnal variation of F parameters: Midday depression of variable-to-maximum fluorescence yield ratio (F_v/F_m) was observed at both leaf ages in all six species (Fig. 3*A*). The ratio tended to recover overnight as shown by higher predawn F_v/F_m values. Expanding leaves of *C. sancarlosianum* showed slight but non-significant changes in F_v/F_m. The mean predawn F_v/F_m was 0.69±0.01 and 0.74±0.01 in expanding and adult leaves, respectively. Typical F_v/F_m values for mature healthy tissue are between 0.74 and 0.85 (Litchtenthaler *et al.* 2005). In expanding leaves, predawn F_v/F_m was below this range. Similar results have been found in young leaves of other tropical species and have been interpreted as a regulatory mechanism leading to damage of the D1 protein (Krause *et al.* 1995, Sobrado 1996). However, insufficient F_v/F_m overnight recovery in expanding leaves of the 'Bana' species may be the result of their lower photochemical capacity due to undeveloped antenna as shown by their low Chl (*a*+*b*) and Chl *a*/*b* (Fig. 2*B*). Therefore, expanding as well as adult leaves at the 'Bana' site experience dynamic changes in the maximum quantum yield of PS2 from midday to predawn, which suggests down-regulation (Kitajima and Butler 1975, Demmig-

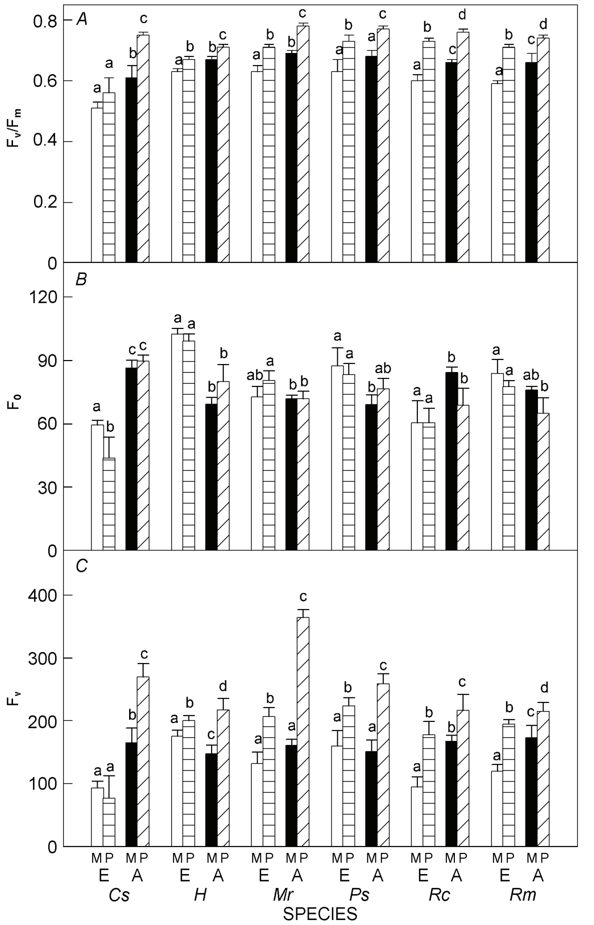

Fig. 3. Fluorescence parameters (F_v/F_m, F_0, and F_v) measured in expanding leaves (E; *white and horizontal-stripped bars*) and adult leaves (A; *black and oblique-stripped bars*) measured at midday (M; *white and black bars*) and again at predawn (P; *stripped bars*) in six 'Bana' species: *C. sancarlosianum* (*Cs*), *Heteropterys* sp. (*H*), *M. rufescens* (*Mr*), *P. sordida* (*Ps*), *R. concolor* (*Rc*), and *R. morilloi* (*Rm*). Means (standard error) of measurements performed in three trees per species. For each species *different letters* indicate statistical difference at $p<0.05$.

Adams and Adams 1992). This fast recovery may not require D1 protein synthesis and may be related to xantophyll-cycle activity (Krause *et al.* 1995). In *C. sancarlosianum,* some degree of chronic photoinhibition could take place which requires longer recovery periods to complete *de novo* synthesis of the D1 protein (Aro *et al.* 1993).

Diurnal changes of F_v/F_m were paralleled by those in F_m and F_v. The mean midday F_m values were 207±7 and 234±12 in expanding and adult leaves, and increased at predawn up to about 250±7 and 343±15, respectively. Differences in F_m at predawn between both ages were related to higher Chl (*a+b*) in adult leaves. Higher Chl (*a+b*) enhances the possibility of radiant energy absorption (Šantrůček *et al.* 1992). F_v increased significantly from midday to predawn at all leaf ages except in expanding leaves of *C. sancarlosianum* (Fig. 3*C*). Overall, values of F_v were significantly lower in expanding than in adult leaves. The F_0 was highly variable, and no consistent trend between leaf ages within each species was found. However, except in *C. sancarlosianum*, F_0 did not change significantly from midday to predawn for any age in the six species (Fig. 3*B*). A significant decline of F_0 from midday to predawn in *C. sancarlosianum* further suggested some degree of chronic photoinhibition. In the other species and leaf ages, the decreased midday F_v/F_m was the consequence of a lower F_v without any change in F_0. This further suggests down-regulation due to the activation of a protective process to avoid PS2 damage (Kitajima and Butler 1975, Guo *et al.* 2006). The F_v/F_0 is a very sensitive indicator of the maximum efficiency of a photochemical process in PS2, as well as of the potential photosynthetic activity (Roháček 2002, Lichtenthaler *et al.* 2005). However, simultaneous changes in F_0 and F_v were not observed in the present study, and the trend of F_v/F_0 was similar to that of F_v/F_m. Thus, the mean midday F_v/F_0 ratios were about 1.65±0.07 and 2.12±0.09 in expanding and adult leaves, and increased at predawn up to about 2.53±0.01 and 3.44±0.08, respectively. An increase of F_v/F_0 from midday to predawn is another indication of the possible activation of photo-protective mechanisms at midday. This would represent an acclimatory down-regulation without damage of PS2. Further studies should elucidate the underlying midday photo-protection mechanisms in this habitat.

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