

## **PERSPECTIVES IN COMPARATIVE ECOPHYSIOLOGY OF SOME BRAZILIAN VEGETATION TYPES: LEAF $\text{CO}_2$ AND $\text{H}_2\text{O}$ GAS EXCHANGE, CHLOROPHYLL *a* FLUORESCENCE AND CARBON ISOTOPE DISCRIMINATION**

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### **Resumo:**

**“Perspectivas em ecofisiologia comparativa de algumas vegetações brasileiras: trocas gasosas de  $\text{CO}_2$  e  $\text{H}_2\text{O}$  em folhas, fluorescência da clorofila *a* e discriminação isotópica do carbono”**

A fotossíntese é responsável pela energia que a planta disporá para crescimento, defesa e reprodução. Logo, é fundamental para o metabolismo vegetal. Contudo, pouco se conhece sobre os efeitos dos fatores ambientais nas taxas de assimilação de carbono em vegetações brasileiras. Uma diminuição na assimilação líquida de  $\text{CO}_2$  pode estar relacionada a uma diminuição na difusão do  $\text{CO}_2$  para os sítios de carboxilação, devido ao fechamento estomático parcial ou total, e à inibição direta de processos fotossintéticos primários ou secundários nos cloroplastos. Uma vez que a redução fotossintética do carbono é uma das principais vias de utilização da energia luminosa, uma menor assimilação líquida de  $\text{CO}_2$  pode gerar um excedente de energia de excitação nos centros de reação dos fotossistemas e conseqüente diminuição na eficiência fotossintética de conversão da energia luminosa em energia química. Estudos de ecofisiologia comparativa de espécies nativas são imprescindíveis ao entendimento dos mecanismos que levam à alta diversidade da flora brasileira. Trocas gasosas de  $\text{CO}_2$  e  $\text{H}_2\text{O}$ , fluorescência da clorofila *a* e discriminação isotópica do carbono são ferramentas úteis na previsão da influência de fatores ambientais na performance vegetal *in situ*. Discuto aqui alguns dos resultados obtidos sobre a ecofisiologia comparativa de algumas vegetações brasileiras.

### **Abstract:**

Photosynthetic activity is responsible for the energy plants use for growth, defense and reproduction. Therefore, photosynthesis is fundamental to plant metabolism. However, the effects of environmental factors on net  $\text{CO}_2$  assimilation rates are as yet poorly known in Brazilian vegetation types. A decrease in net  $\text{CO}_2$  uptake may be related to a decrease in  $\text{CO}_2$  diffusion to the carboxylation sites due to partial or total stomata closure and to direct inhibition of primary and secondary photosynthetic processes in the chloroplasts. Photosynthetic carbon reduction is one of the most important pathways for the utilization of light energy. Hence, lower rates of  $\text{CO}_2$  uptake may cause an excess of excitation energy through the photosystems and consequently lead to a decline in the efficiency of photosynthetic energy conversion. The study of comparative ecophysiology of native species is essential for understanding the mechanisms that lead to the high diversity of the Brazilian flora.  $\text{CO}_2$  and  $\text{H}_2\text{O}$  gas exchange, chlorophyll *a* fluorescence and carbon isotope discrimination are powerful and predictive tools for addressing the influence of multiple environmental factors on plant performance *in situ*. Here I discuss the results of comparative plant physiological ecology studies in several Brazilian vegetation types.

## Introduction

Photosynthesis is a key process in plant metabolism. Harvesting of light energy and the exchange of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  at leaf level are directly related to nutrient acquisition and water uptake by the roots and the efficiency of the hydraulic transport system (Fig. 1). Fundamental biophysical and physiological mechanisms determine the chemical energy available for further leaf and root growth, for defense, and reproduction in plants (Bazzaz *et al.*, 1987). The simplified model presented in Figure 1 gives us an idea of how complex the study of the interrelations between above and belowground parts is within a whole-plant perspective. It is even more so when we deal with the responses of plants to multiple stress factors that characterize tropical environments.

As pointed out by Peters (1991), however, we should be aware to use operational concepts and to propose answerable questions to ecological problems. In this sense, the study of leaf  $\text{CO}_2$  and  $\text{H}_2\text{O}$  gas exchange has proved to be a powerful predictive approach to addressing the influence of environmental factors on plant performance in a given environment (Schulze & Caldwell, 1995). In this paper, I give an overview of the value of new methodologies that only recently have been used in Brazil. I also examine some perspectives in physiological plant ecology for specific Brazilian vegetation types.

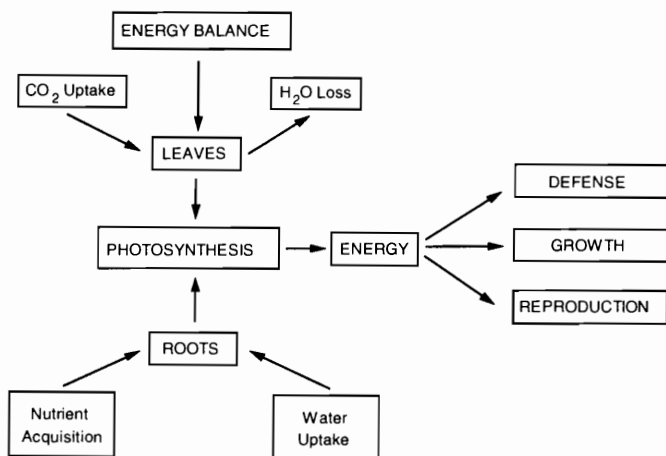


Figure 1. General physiological model of a plant. The availability of light,  $\text{CO}_2$ , water and minerals are the key factors for photosynthetic metabolism. The supply of chemical energy from photosynthesis is allocated to defense, growth and reproduction. Adapted from Solbrig *et al.* (1979).

## Environmental stress and leaf CO<sub>2</sub> and H<sub>2</sub>O gas exchange

Practically no studies have been done in Brazil on the effects of environmental factors on photosynthetic characteristics, and few species have been studied in field conditions. Most work has been done in the *cerrado* vegetation (a neotropical savanna see Eiten, 1972 and Coutinho, 1978).

In past decades, some authors (for a more general review see Goodland & Ferri, 1979) have postulated that *cerrado sensu stricto* plants are not subjected to a pronounced water deficit during the dry season. This hypothesis was corroborated by the presence of deep roots in many species and the observation with the torsion balance of no restriction of transpiration during the dry season. Thus, it was believed that many adult plants in the *cerrado* vegetation are able to take water from deep, moist layers in the soil profile and maintain water flow high enough to counterbalance the evaporative demand during the dry season. Moreover, Arens (1958) has proposed that the scleromorphic characteristics of *cerrado* vegetation are better explained by nutrient deficiency than water limitation. However, the Brazilian *cerrado* and other vegetation types characteristic of Brazil are actually subjected to multiple environmental stresses, such as fire, nutrient, water, wind, light, and others, which means that to seek for one causal factor may be misleading.

In the *cerrado*, Johnson *et al.* (1983) found during the dry season maximum values of net CO<sub>2</sub> uptake of about 15.1 and 4.2  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , in *Didymopanax macrocarpum* and *Ouratea hexasperma* respectively. In the middle of the dry season Netto & Hay (1986) observed maximum values for net CO<sub>2</sub> uptake of 10.2  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in *Caryocar brasiliense*. The ability of potted plants of *Copaifera langsdorffii* to maintain CO<sub>2</sub> uptake during water deficit was studied by Prado *et al.* (1994, 1995) who observed a decrease in stomatal conductance to water vapour when young plants of *C. langsdorffii* were subjected to higher leaf-to-air water vapour concentration difference ( $\Delta w$ ). Recently, the influence of the dry season on *in situ* leaf CO<sub>2</sub> and H<sub>2</sub>O gas exchange together with measurements of leaf water potential were studied more extensively in *cerrado* areas, where the occurrence of a dry season between May and September is characteristic (Mattos, 1992; Kanno, 1993; Mattos, 1996; Mattos *et al.*, 1997b).

Until recently, information was lacking on the water status of *cerrado* plants during the dry season. Transpiration rates do not give any information on the water status of plants. However, the water status of plants is closely related to leaf water potential. In particular, predawn leaf water potential ( $\Psi_{Lpd}$ ) is an appropriate parameter to describe the water status of the plant because it is related to the recovery capacity of leaf water potential during the night and the water content in the vicinity of the root system. Kanno (1993) and Mattos (1996) studied 7 species of *cerrado sensu stricto* vegetation in São Carlos (SP) and no value of predawn leaf water potential ( $\Psi_{Lpd}$ )

lower than  $-1.0$  MPa was observed, which apparently characterizes mild water stress. These results are in agreement with the hypothesis that no pronounced water deficit occurs during the dry season. However, even though the plants do not seem to be subjected to a pronounced water deficit during the dry season, Kanno (1993) and Mattos (1996), using a portable infrared gas analyzer, observed a decrease in the degree of stomatal conductance to water vapor ( $g_{\text{H}_2\text{O}}$ ) in several species during the dry season in a cerrado *sensu stricto* in São Carlos (SP).

Figure 2 shows a comparison between the wet and the dry season of daily courses of gas exchange and leaf water potential in adult plants of the evergreen *Rapanea umbellata* occurring in a cerrado *sensu stricto* (E.A. Mattos & J.A.P.V. Moraes, unpubl. data). As observed for other species in the same area, *R. umbellata* showed a decline in both leaf water potential and  $\text{CO}_2$  and  $\text{H}_2\text{O}$  gas exchange during the dry season. Despite very similar values of  $\Psi_{\text{Lpd}}$  in the dry season of 1993 and 1994, lower values of  $g_{\text{H}_2\text{O}}$  were observed in August 1993 when the difference in the water vapour concentration between leaf and air was very high (Table 1). These findings should be emphasized because, even though cerrado *sensu stricto* plants may not be subjected to a pronounced water deficit at the root system level, a decrease in  $g_{\text{H}_2\text{O}}$  may be necessary to maintain a favorable water status in an atmosphere of high evaporative demand. The results obtained for *R. umbellata* are used here as an example of one kind of response to the dry season in cerrado areas. It is clear, however, that there is a range of responses because of high species diversity. We are not yet able to fully understand the multiple responses to the interactive nature of stressful conditions in cerrado areas, but some questions are emerging with the study of leaf  $\text{CO}_2$  and  $\text{H}_2\text{O}$  gas exchange and I will try to highlight them here.

There is a close compromise between  $\text{CO}_2$  and  $\text{H}_2\text{O}$  exchange through stomata. The stomatal aperture that is necessary to maintain the diffusion of the  $\text{CO}_2$  in the air surrounding the leaf to the intercellular air spaces and then to the carboxylation sites in the chloroplasts is also responsible for the inevitable water loss to the atmosphere (Nobel, 1991; Jones, 1994). Figure 3 shows the relationship between  $\text{CO}_2$  uptake and water loss at different levels of stomatal conductance in *Nanuzia plicata*, a desiccation-tolerant plant, when subjected to water stress (E.A. Mattos *et al.*, unpubl. data). The data obtained on this species shows that transpiration loss may be more affected than  $\text{CO}_2$  uptake at moderate levels of stomatal conductance increasing the water use efficiency of  $\text{CO}_2$  assimilation, *i.e.*, the ratio of  $\text{CO}_2$  uptake to transpiration loss (Nobel, 1991). In some circumstances, however, there may be an advantage to controlling the water status of the leaf instead of maintaining maximal rates of  $\text{CO}_2$  uptake. The distinct midday depression of  $\text{CO}_2$  uptake observed in *R. umbellata* at the end of the 1994 dry season is an example of this situation (Fig. 2). Several investigations have shown depression of leaf gas exchange in plants growing in hot and arid environments, during periods when leaf temperatures and leaf-to-air vapour pressure deficit are greatest (Lange *et al.*, 1982; Roessler & Monson, 1985).

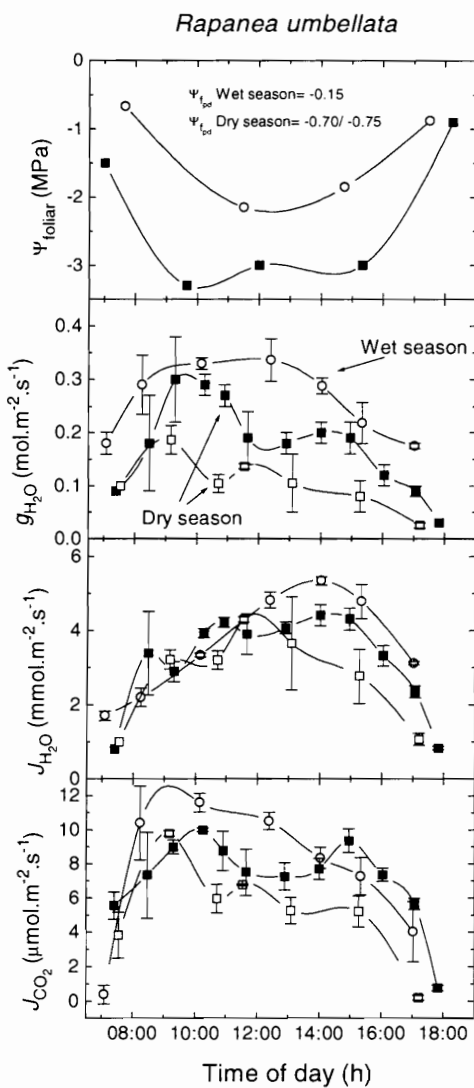


Figure 2. Comparison of wet and dry season diurnal changes in leaf water potential ( $\Psi_{\text{foliar}}$ ), stomatal conductance to water vapor ( $g_{\text{H}_2\text{O}}$ ), transpiration rates ( $J_{\text{H}_2\text{O}}$ ) and net  $\text{CO}_2$  uptake ( $J_{\text{CO}_2}$ ) in *Rapanea umbellata* occurring in a cerrado *sensu stricto* (São Carlos, SP). The predawn leaf water potential for both seasons is also presented ( $\Psi_{\text{Lpd}}$ ). The symbols represent average values of 3 leaves for water potential and 6 leaves for gas exchange measurements. Vertical bars indicate standard deviations.

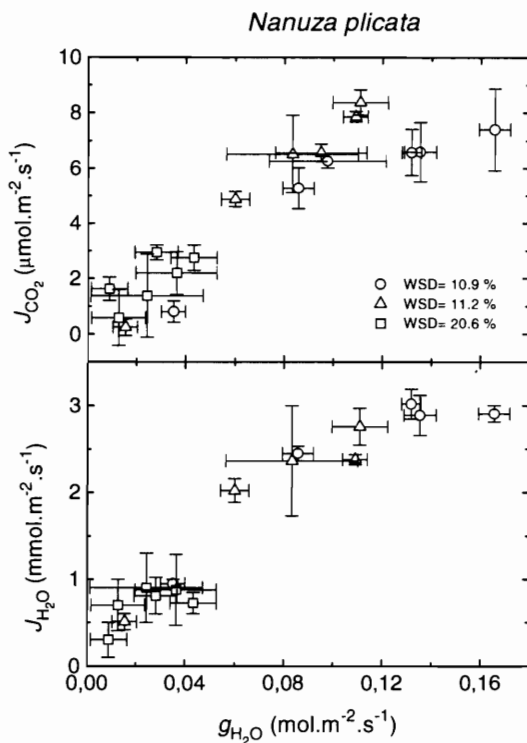


Figure 3. The relationship between net  $\text{CO}_2$  uptake ( $J_{\text{CO}_2}$ ) and transpiration rates ( $J_{\text{H}_2\text{O}}$ ) in relation to stomatal conductance to water vapor ( $g_{\text{H}_2\text{O}}$ ) in *Nanuzia plicata* under different water saturation deficits (WSD).

Table 1. Comparison of wet and dry season predawn leaf water potential ( $\Psi_{\text{Lpd}}$ ), minimum values during the day for relative humidity ( $\text{R.H.}_{\text{min}}$ ), maximal values of the difference in water vapor concentration from leaf to air ( $\Delta w$ ), average values for stomatal conductance to water vapor ( $g_{\text{avg}}$ ), integrated values during the day for transpiration rates ( $E_{\text{int}}$ ) and net  $\text{CO}_2$  assimilation rates ( $A_{\text{int}}$ ), average values for the intercellular to ambient  $\text{CO}_2$  concentration ratio ( $c_i/c_a$ ), diurnal average values of intrinsic water use efficiency ( $A/g_s$ ) and the ratio of irradiance to assimilation rates ( $I/A$ ) in *Rapanea umbellata* in a cerrado *sensu stricto* (São Carlos-SP).

Date	$\Psi_{\text{Lpd}}$ MPa	$\text{R.H.}_{\text{min}}$ %	$\Delta w_{\text{max}}$ $\text{mol.m}^{-3}$	$g_{\text{avg}}$ $\text{mol.m}^{-2}.\text{s}^{-1}$	$E_{\text{int}}$ $\text{mol.m}^{-2}.\text{day}^{-1}$	$A_{\text{int}}$ $\mu\text{mol.m}^{-2}.\text{day}^{-1}$	$c_i/c_a$	$A/g_s$ $\mu\text{mol.mol}^{-1}$	$I/A$ $\text{mol.mol}^{-1}$
26/4/94	-0.15	40	1.36	0.28	138.1	314.3	0.68	31.3	88
08/8/93	-0.70	19	2.51	0.13 (-54%)	102.0 (-26%)	195.5(-38%)	0.64	51.9 (+66%)	146 (+66%)
10/9/94	-0.75	37	1.21	0.25 (-11%)	129.0 (-7%)	282.8(-10%)	0.61	40.1 (+28%)	130 (+48%)

Despite the reduction in stomatal conductance during the dry season in *R. umbellata*, fairly high values of transpiration in both seasons were observed (Fig. 2). However, the differences in water vapour concentration from leaf to air ( $\Delta w$ ), generally, are much larger in the dry season (Table 1). These results suggest that the data obtained with the torsion balance may have led to erroneous interpretations of stomatal behaviour during the dry season for cerrado species. Since transpiration rate is given by stomatal conductance multiplied by  $\Delta w$ , the maintenance of approximately equal transpiration rates in both the dry and the wet season is possible only if there is a substantial reduction in stomatal aperture. High transpiration rates were also measured for species of the Venezuelan savannas and Sarmiento *et al.* (1985) hypothesized that plants growing in nutrient poor environments, like the savannas of Venezuela and the Brazilian cerrado, may be able to increase nutrient acquisition because of their capacity to maintain high transpiration rates.

Although the decrease of leaf water potential does not seem to be very pronounced for cerrado *sensu stricto* species in the dry season, the control of transpiration by decreasing stomatal conductance and, thus, maintaining a relatively high water potential may play an important role in the prevention of catastrophic xylem cavitation. Cavitation is the process by which xylem conduits become embolized (*i.e.*, air-filled) and lose their functionality in sap conduction (Magnani & Borghetti, 1995). Embolism is thought to arise in plants as a consequence, for instance, of air-seeding induced by water stress (Sperry & Tyree, 1988). The potential role of stomatal closure in the control of xylem embolism has been the object of recent research and there is a threshold water potential for cavitation, which varies both with stress pre-history of the plant and with the species (Jones & Sutherland, 1991; LoGullo *et al.*, 1995; Magnani & Borghetti, 1995).

The flow of water in the xylem is given by the Hagen-Poiseuille law, which describes the flow of a fluid in a cylinder as proportional to the fourth power of its radius and linearly dependent on the drop in hydrostatic pressure (Nobel, 1991). Thus, the high transpiration rates observed in cerrado species may be related to structural adaptations of the transport system and the presence of large xylem vessels. Vulnerability to cavitation is closely related to the conduit diameter and length, where vessels having the largest pores are the most vulnerable (Jones, 1994, LoGullo *et al.*, 1995). As pointed out by Jones & Sutherland (1991) "maintenance of a maximally efficient conducting system requires the stomata to close in an appropriate fashion as evaporative demand increases in order to prevent shoot water potentials falling below the threshold value at which cavitation occurs".

In addition to the probable role of stomata in maintaining the integrity of the water transport system, a decrease in stomatal conductance is also important in maintaining a leaf water potential favorable to leaf development during the dry season. E.A. Mattos & J.A.P.V. Moraes (unpubl. data) observed lower values of stomatal conductance in young leaves than in old leaves during the dry season.

The reduction in stomatal conductance during the dry season increased the stomatal limitation to CO<sub>2</sub> diffusion in *R. umbellata* as evidenced by the lower values of the ratio of intercellular to ambient concentration of CO<sub>2</sub> ( $c/c_a$ ) and hence, may have partially contributed to the lower values of net CO<sub>2</sub> uptake during the dry season (Table 1). As a consequence of lower net CO<sub>2</sub> assimilation, an increase in the ratio of irradiance to CO<sub>2</sub> assimilation ( $I/A$ ) was observed (Table 1). The increase in this ratio may indicate an excess of radiation energy that cannot be used to drive photosynthesis. It has recently been proposed that environmental stress, which may decrease net CO<sub>2</sub> assimilation rates, may predispose sun plants to some degree of light stress (Demmig-Adams & Adams, 1992; Osmond, 1994). Thus, photoinhibition of photosynthesis may occur when, because of stress or inherent limitations in the capacity to use high irradiances, leaves absorb more light energy than they can use in photosynthesis. The event of photoinhibition has been generally defined as a sustained decrease in the efficiency of photosynthetic energy conversion, *i.e.*, a decrease in the rate of CO<sub>2</sub> uptake or O<sub>2</sub> evolution at limiting photosynthetic photon flux densities (PPFD) or of the intrinsic efficiency of photosystem (PS) II as assessed by chlorophyll *a* fluorescence techniques, which I will describe in the next topic.

### Basic chlorophyll *a* fluorescence measurements

Photosynthetic activity is generally assumed to be very sensitive to environmental factors (Lawlor, 1995). The effect of adverse environmental conditions is generally observed as a decrease in net CO<sub>2</sub> assimilation rates (Osmond, 1994; Mattos, 1996). Lower rates of CO<sub>2</sub> uptake may be related to a decrease in CO<sub>2</sub> diffusion to the carboxylation sites due to partial or total closure of stomata and from direct inhibition of primary and secondary processes in the chloroplasts (Lawlor, 1995).

Green plants harvest a high proportion of incident light through the antenna pigments, and the excitation energy is transferred to the reaction centers of the two photosystems. There, the energy drives the primary photochemical reactions that initiate photosynthetic energy conversion (Krause & Weis, 1991). Part of the energy quanta absorbed by chlorophyll and not used to drive photosynthetic electron flow is emitted as fluorescence in the red. Fluorescence is part of competing pathways of de-excitation and emission characteristics can be used to study photosynthetic electron transport and associated physiological processes (Krause, 1988; Schreiber & Bilger, 1993). Fluorescence emission originates almost exclusively from PS II. Thus, fluorescence changes reflect primarily the state of PS II (Schreiber *et al.*, 1995).

Recently, with the development of new equipment, the detection of fluorescence emission characteristics in conditions of normal daylight became possible (Schreiber & Bilger, 1987). The differentiation of photochemical ( $q_p$ ) and total non-photochemical quenching ( $q_N$ ) by the saturation pulse method is now possible with the use of pulse amplitude modulation fluorometers (Schreiber *et al.*, 1995). Van Kooten & Snel (1990) proposed a standardization of fluorescence nomenclature which will be used here. After



a dark-adaptation period fluorescence emission is stimulated by a modulated measuring light of very low intensity that is not enough to drive any charge separation in the reaction centers of PS II. Thus, the minimal fluorescence yield,  $F_0$ , which occurs when all reaction centers are in the oxidized, or open, state is determined. By definition, at this moment  $q_p = 1$  and  $q_N = 0$ . After exposure to a brief light flash (saturation pulses) of an intensity and duration just sufficient to temporarily close all centers, which promotes total reduction of the pool of the primary electron acceptors and hence, elimination of the  $q_p$ , the maximal level of fluorescence yield in the dark,  $F_m$ , is determined. The  $F_v/F_m$  ratio (*i.e.*  $[F_m - F_0]/F_m$ ) represents a convenient measure of the potential quantum yield of PS II when all reaction centers are in the open state and  $q_N$  is equal to zero.

During initial illumination, the fluorescence yield,  $F$ , undergoes complex changes. However, and after steady state and with the use of saturation pulses photochemical and total non-photochemical quenching coefficients are determined as:  $q_p = (F' - F)/(F' - F_0)$  and  $q_N = 1 - [(F' - F_0) - (F_m - F_0)]/F_m$ , where  $F'$  is the maximal fluorescence yield in the light-adapted state after saturation pulses.  $F_0$  is the minimal fluorescence yield in the light-adapted state and is measured after the sample is darkened and a far-red light is turned on to ensure a reoxidation of the primary electron acceptors of PS II. Instead of  $q_p$ , the term  $1 - q_p$  is generally used, which is an estimative of the reduction state of the primary electron acceptor of the PS II,  $Q_A$ . The effective quantum yield of PS II during actinic light is given by  $\Delta F/F'$  or  $(F' - F)/F'$  (Genty *et al.*, 1989). The Stern-Volmer equation has also been used to describe non-photochemical quenching, *i.e.*,  $NPQ = (F_m - F')/F'$ . Apparent electron transport rates through PS II (ETR) are calculated as  $\Delta F/F'$  multiplied by the photosynthetic photon flux density (PPFD).

### **Chlorophyll *a* fluorescence as an indicator of photo system II activity**

Chlorophyll fluorescence serves as an intrinsic indicator of the photosynthetic reactions. PS II activity and net  $CO_2$  assimilation rates reflect the interdependence between light and dark reactions of photosynthesis as shown in figure 4. It is clear that any decrease in  $CO_2$  uptake will cause changes in the amount of energy used in photochemistry, and in turn, changes in the levels of the other de-excitation pathways which are responsible to some extent to maintaining the integrity of the photosynthetic apparatus. As PS II functioning is sensitive to a wide range of environmental stresses, chlorophyll fluorescence characteristics provide valuable information on stress effects in leaves (Schreiber *et al.*, 1995).

In accordance with the propositions of Osmond (1994) and Osmond & Grace (1995), figure 5 gives a general idea which may help us to integrate the effect of multiple stress on plant performance in a given environment. Environmental stress may decrease net  $CO_2$  assimilation rates in different ways and  $CO_2$  uptake is one of the most important pathways for using the light energy that reaches the photosynthetic apparatus (Lawlor,

1995). Thus, a decline in the use of excitation energy by photosynthetic carbon assimilation causes an increase of energy in excess to drive photosynthesis and probably leads to photoinhibition. The characterization of dynamic and chronic photoinhibition was proposed by Osmond (1994) according to the time required for reversible changes in decreased PS II antenna-based photosynthetic efficiency. Photoinhibition is a result of a continuum from photoprotection, in which excessive excitation energy is dissipated harmlessly as heat, to direct photodamage to the PS II reaction center, specifically damage to the functional integrity of the center, and subsequent destabilization by proteolysis of the D1 polypeptide (Osmond, 1994).

Leaves of various species have been shown to have a maximal PS II efficiency of 0.78-0.83, which means that the capture of light energy by the light-harvesting complex and its transfer to the reaction center and into the electron transport chain are not 100% efficient (Björkman & Demmig, 1987; Adams *et al.*, 1995).

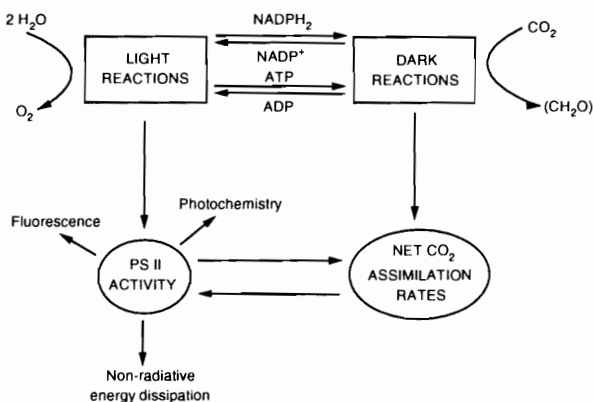


Figure 4. Photosystem II (PS II) and the de-excitation pathways as an intrinsic indicator of the interdependence between light and dark reactions of photosynthesis.

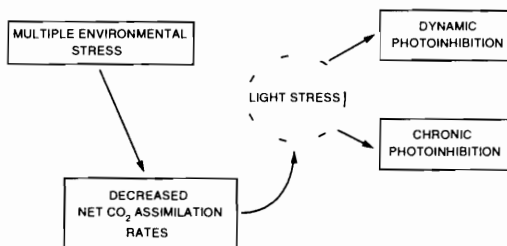


Figure 5. Light stress as a possibility to integrate the effects of multiple environmental factors on leaf  $\text{CO}_2$  assimilation rates.

Reversible decline in PS II efficiency, *i.e.*, potential quantum yield ( $F_V/F_M$ ), was related to the degree of stomatal conductance at midday in 3 woody species found on the Pão de Açúcar inselberg (Fig. 6 and Mattos *et al.*, 1997a). Values of  $F_V/F_M$  declined significantly during phase IV of CAM in semi-exposed and exposed plants of *Clusia hilariana* in the sandy coastal plains of Macaé (RJ), when lower internal  $CO_2$  levels probably prevailed (Franco *et al.*, 1996; Mattos *et al.*, 1997a). As already described for several species of *Clusia* by Franco *et al.* (1992),  $CO_2$  uptake in phase IV of CAM is very sensitive to water deficit, which might enhance the potential for photoinhibition. Thus, phase IV of CAM seems to be most prone to photoinhibition, as also reported for cacti in northern Venezuela (Adams *et al.*, 1989).

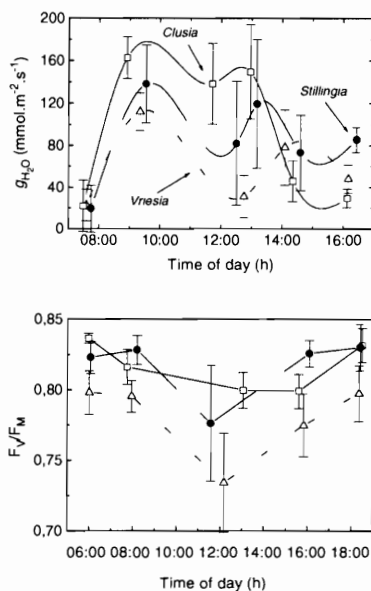


Figure 6. Diurnal time courses of stomatal conductance to water vapor ( $g_{H_2O}$ ) and potential quantum yield ( $F_V/F_M$ ) in *Clusia aff. parviflora*, *Stillingia dichotoma* and *Vriesia geniculata* on Pão de Açúcar outcrop, Rio de Janeiro, RJ. Vertical bars indicate standard deviations.

At the end of the dry season of 1994 the characteristics of fluorescence emission were studied for the first time in some cerrado *sensu stricto* species at Fazenda Água Limpa (Brasília, DF) by A.Ā. Franco *et al.* (unpubl. data). Although the degree of decline in  $F_V/F_M$  varied between the different species, it was almost completely reversible in most species at the end of the day (Fig. 7). This probably reflects a protective down-regulation of PS II which is mediated by zeaxanthin and prevents damage to the reaction centers under excess light energy (Demmig-Adams, 1990; Demmig-Adams & Adams, 1992; Demmig-Adams & Adams, 1996). However, some species showed sustained decrease in predawn  $F_V/F_M$  values, as observed in *Roupala montana*, which may indicate

chronic photoinhibition. The marked high values throughout the day of the desiccation-tolerant species *Vellozia squamata* are noteworthy. Similarly to several species in São Carlos (SP),  $\Psi_{Lpd}$  lower than  $-1.0$  MPa were not observed, which corroborates the idea of the occurrence of only moderate water stress during the dry season in adult plants from cerrado *sensu stricto* communities. However, it is clear that during the dry season some cerrado *sensu stricto* species may be subjected to light stress involving some degree of dynamic photoinhibition (Osmond & Grace, 1995).

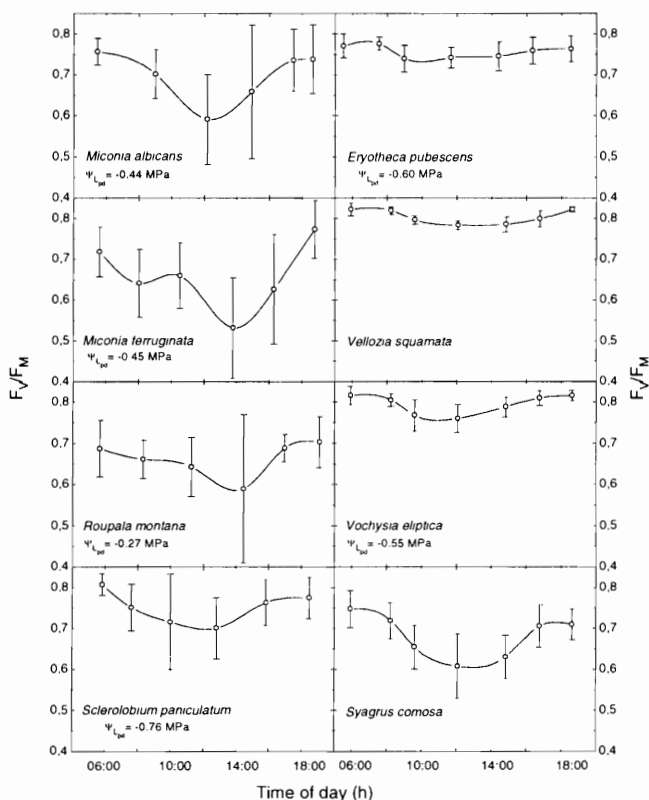


Figure 7. Diurnal time courses of potential quantum yield ( $F_v/F_m$ ) in several cerrado *sensu stricto* species studied at Fazenda Água Limpa (Brasília, DF) in August 1994. For some species predawn leaf water potential was also measured ( $\Psi_{Lpd}$ ). Vertical bars indicate standard deviations.

In addition to PS II efficiency measurements, the separation of photochemical and non-photochemical coefficients may help to characterize the interactions between different de-excitation pathways. Photosynthetic control is responsible for counterbalancing the light energy received by PS II with its capacity to use it. These processes allow a so-called down-regulation of photosynthesis.

During the wet season, cerrado species may be subjected to dry spells (*veranicos*) of different durations. Figure 8 shows the response of net CO<sub>2</sub> uptake rates ( $J_{CO_2}$ ) and chlorophyll quenching coefficients to a dry spell within the rainy season in *Miconia albicans*, a common woody evergreen species of the cerrado vegetation (E.A. Mattos *et al.*, unpubl. data). A marked "midday depression" of CO<sub>2</sub> uptake was observed. A 3-fold decrease was observed at around 13:00h in the maximum values of 10.3  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , obtained in the early morning. However, despite some increase in the reduction state of the primary electron acceptor of PS II ( $1-q_p$ ) during the midday depression of  $J_{CO_2}$ , it seems that the high capacity to rapidly increase total non-photochemical quenching ( $q_N$ ) may prevent irreversible photodamage to the reaction centers. It seems clear that the ability to divert to radiationless dissipation the energy in excess to drive photosynthesis, was remarkable in this species and deserves further investigation.

The use of chlorophyll *a* fluorescence in different Brazilian vegetation types is in its infancy and the preliminary results presented here are important to exemplify the power and usefulness of this new approach for plant physiological ecology research in Brazil. However, it is important to remind the readers about the complexity of the characteristics of chlorophyll *a* fluorescence emission (Govindjee, 1995). Hence, results should be interpreted with caution and better results will be obtained when other approaches, such as gas exchange, are used concomitantly.

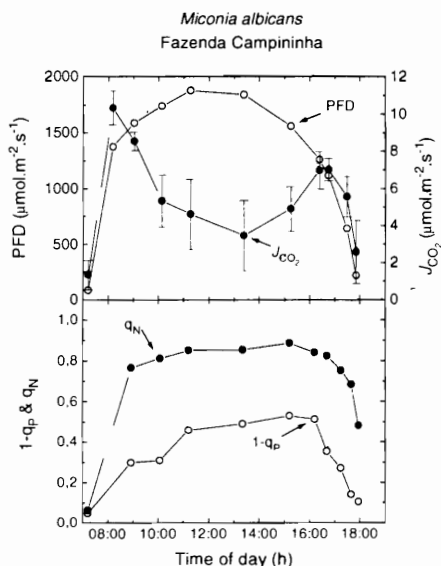


Figure 8. The effect of a dry spell within the rainy season on net CO<sub>2</sub> gas exchange, total non-photochemical quenching ( $q_N$ ) and the reduction state of the primary electron acceptor of photosystem II ( $1-q_p$ ) in leaves of *Miconia albicans*. Photon flux density is also given (PFD). Vertical bars indicate standard errors

## Carbon isotope ratio and photosynthetic metabolism

The natural abundance of stable isotopes has not been used extensively in physiological plant ecology in Brazil. In this section I will focus on carbon isotope composition ( $\delta^{13}\text{C}$ ) as an indicator of plant carbon metabolism. For a review of the use of other stable isotopes, such as nitrogen, oxygen, deuterium and sulfur, see Ehleringer & Dawson (1992), Handley & Raven (1992) and Trust & Fry (1992).

As von Caemmerer (1992) pointed out "plants discriminate against  $^{13}\text{CO}_2$  during  $\text{CO}_2$  fixation. The differential diffusivity of  $^{13}\text{CO}_2$  and  $^{12}\text{CO}_2$  during diffusion through stomata and fractionation associated with carboxylation enzymes are the major components contributing to the overall discrimination" (for reviews see O'Leary, 1981; Farquhar *et al.*, 1989). Thus, isotope fractionation occurring during photosynthetic carbon assimilation reflects both plant metabolism and environmental constraints (O'Leary, 1981). The natural abundance of  $^{13}\text{C}$  is expressed in units of  $\delta^{13}\text{C}$ , the parts per thousand difference between the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  in a sample relative to the PDB standard (Craig, 1957). The carbon isotope ratios of leaves can vary from -7 to  $-35\text{‰}$ , with  $\text{C}_4$  plants having values of -7 to  $-15\text{‰}$ , CAM plants -10 to  $-30\text{‰}$ , and  $\text{C}_3$  plants -20 to  $-35\text{‰}$  (Ehleringer, 1989; Griffiths, 1992).

Carbon isotope ratios have been extensively used to provide information on the distribution of  $\text{C}_3$  and  $\text{C}_4$  species in several ecosystems of the world (Throughton *et al.*, 1974; Ziegler *et al.*, 1981). Recently, Mozeto *et al.* (1996) presented data on the carbon isotope composition of 50 species of grasses distributed along a gradient of soil water availability in the area surrounding the Lobo Dam (Itirapina-SP). Both  $\text{C}_3$  and  $\text{C}_4$  species occurred in the wetlands whereas  $\text{C}_4$  species dominated in areas of *cerrado* vegetation.

E.A. Mattos *et al.* (unpubl. data) studied the carbon isotope composition of 10 species of *Rhipsalis* (Cactaceae). Although all species showed carbon isotope ratios characteristic of the CAM pattern (*i.e.*, from -12.8 to  $-18.2\text{‰}$ ) it was possible to distinguish differences with regard to habitat conditions and stem morphology. Species of *Rhipsalis* are found in a diversity of environments such as exposed rocky outcrops of southeastern Brazil or underneath the tree canopies of the Atlantic rain forest, as epiphytes. In spite of the differences in environmental conditions of these different habitats all species seem to be subjected to some degree of water stress because of the characteristics of the substrate.

Rocky outcrops are characteristic of southeastern Brazil. Annual rainfall in these areas does not characterize a dry climate. However, plants in these habitats grow on shallow soils or attached to bare rock. As a result, these plants have great potential to be subjected to water deficits. Meirelles *et al.* (1997) found a great number of desiccation-tolerant plants in these areas. Leaf samples for  $\delta^{13}\text{C}$  determination from two of these species, *Pleurostima purpurea* and *Barbacenia fragans* (Velloziaceae), were collected on the Pão-de-Açúcar granitic-gneiss outcrop (Rio de Janeiro-RJ) and Morro

do Camelo sandstone relictual relief (Analândia-SP), respectively. Both species showed a  $\delta^{13}\text{C}$  value of approximately  $-28.5\text{‰}$ . However, *P. purpurea* growing in stands of very small size (ca. 20 cm. length) have a  $\delta^{13}\text{C}$  of  $-25.6\text{‰}$ , which may suggest greater water use efficiency.

The  $\delta^{13}\text{C}$  values can be used to calculate isotopic discrimination ( $\Delta$ ) as described by Farquhar *et al.* (1982) and Farquhar & Richards (1984). In  $\text{C}_3$  plants, leaf  $\Delta$ -values are related to leaf gas exchange processes according to the model proposed by Farquhar & Richards (1984):

$$\Delta = a + (b - a) \frac{c_i}{c_a} \quad (1)$$

where  $a$  and  $b$  are fractionation constants due to diffusion through the stomata (4.4‰) and net  $\text{CO}_2$  fixation by RuBP (27‰), respectively,  $c_a$  is the ambient  $\text{CO}_2$  concentration and  $c_i$  is the intercellular  $\text{CO}_2$  concentration.  $c_i/c_a$  ratio differs between plants because of variation in both stomatal conductance and photosynthetic demand for  $\text{CO}_2$ . A decrease in stomatal conductance determines an increase in diffusional fractionation and a lowering of  $c_i$  through stomatal closure (Evans *et al.*, 1986; Farquhar *et al.*, 1989). Some studies showed that leaf  $\Delta$  is a reliable estimate of  $c_i/c_a$  during the lifetime of that leaf for  $\text{C}_3$  species (Farquhar *et al.*, 1989; Ehleringer, 1995). As shown in Table I a reduction in  $c_i/c_a$  ratio was observed in *R. umbellata* during the dry season (Mattos, 1996). As mentioned above, a lower  $c_i/c_a$  ratio may result from an increase in stomatal limitation to  $\text{CO}_2$  diffusion or from an increase in  $\text{CO}_2$  uptake, both promoting a reduction in  $c_i$ .

A comparison of dry and the wet season carbon isotope discrimination ( $\Delta$ ) may provide information on gas exchange activity for cerrado plants. For 13 species of a cerrado *sensu stricto*, Mattos *et al.* (1997b) obtained median values of  $\Delta$  significantly lower in the dry season than the wet season. The difference between dry and wet season was higher than  $2\text{‰}$  in 9 of 13 species examined. Medina & Francisco (1994) also observed lower values of  $\Delta$  during the dry season in *Curatella americana* and *Godmania macrocarpa* in a savanna of Venezuela. As observed by Mooney *et al.* (1989) a decrease in leaf  $\Delta$  values during the dry season may be related to an increase in stomatal limitation due to water stress. Mattos (1992), Kanno (1993) and Mattos (1996) observed a general decrease in  $\text{CO}_2$  assimilation rates for several cerrado tree species during the dry season in São Carlos (SP). Moreover, the gas exchange data obtained with *R. umbellata* and other species by Mattos (1996) indicate that a decrease in the  $c_i/c_a$  ratio during the dry season should be expected as a result of lower stomatal conductance.

It is interesting to note that different approaches, *i.e.* gas exchange measured by infra-red gas analyzer and carbon isotope ratios, do not support the established idea of no stomatal restriction during the dry season for cerrado plants. The use of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  gas exchange measurements together with determinations of stable isotopes in

Brazilian vegetation types may provide important insights and test hypotheses related to carbon gain, water and nutrient use efficiency. Thus, we should expect an increase in the use of this approach in next years in Brazil.

### Concluding remarks

In the near future, it will be an enormous challenge for Brazilian plant physiological ecology research to deal with the high diversity of our flora. We should be able to propose and test new hypotheses and to construct a theoretical framework to achieve our goal of understanding and predicting the responses of plants to the interactive nature of stressful conditions in a tropical environment. Although new research groups are emerging, the number of published papers is still too meagre for us to claim the ability to predict the responses of Brazilian vegetation types to the increasing level of modification caused by human activities. Our understanding of the role of the diversity of leaf carbon, water and nutrient relations within an energy balance context of most plants from different Brazilian vegetation types is almost nonexistent.

Despite the pioneering work of Arens, Coutinho, Ferri, Labouriau, Rawitscher and others in past decades, physiological plant ecologists in Brazil are only beginning to collect significant data that will be important to providing the basis for a more predictive approach to environmental problems. Cooperative studies and exchange programs that try to integrate different laboratories and provide different approaches to the same problem will undoubtedly have a major role to play in furthering our comprehension of Brazilian vegetation. More than this, however, we should be open to scientific criticism and hope that the creative genius of scientific discovery can cope with the complexity of the different Brazilian vegetation types.

### Acknowledgements

I am grateful to A.C. Franco (UnB), S.T. Meirelles (USP) and U. Lüttge (TU-Darmstadt) for the encouragement and helpful discussions during the last years. I would like to thank my colleagues of the Universidade Federal de São Carlos, where much of this work was done. CNPq, CAPES and FAPESP (grants no. 96/1966-9 and 96/1967-5) are also gratefully acknowledged. Special thanks are reserved for F.R. Scarano who gave me the opportunity to write this viewpoint. Although I have never talked with Prof. Dr. Luiz Gouvêa Labouriau, I would like to dedicate, *in memoriam*, this paper to him and his inspiring ideas found in several published papers on the cerrado vegetation.

### References

- ADAMS, W.W. III; B. DEMMIG-ADAMS; A.S. VERHOEVEN & D.H. BARKER. 1995. Photoinhibition during winter stress: involvement of sustained xanthophyll cycle-dependent energy dissipation. *Australian Journal of Plant Physiology*, **22**: 261-276.



- ADAMS, W.W. III; M. DIAS & K. WINTER. 1989. Diurnal changes in photochemical efficiency, the reduction state of Q, radiationless energy dissipation, and non-photochemical fluorescence quenching in cacti exposed to natural sunlight in northern Venezuela. *Oecologia*, **80**: 553-561.
- ARENS, K. 1958. O cerrado como vegetação oligotrófica. *Boletim da Faculdade de Filosofia, Ciências e Letras da USP 224. Botânica*, **15**: 59-77.
- BAZZAZ, F.A.; N.R. CHIARIELLO; P.D. COLEY & L.F. PITELKA. 1987. Allocating resources to reproduction and defense. *BioScience*, **37**(1): 58-67.
- BJÖRKMAN, O. & B. DEMMIG. 1987. Photon yield of O<sub>2</sub> evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origin. *Planta*, **170**: 489-504.
- COUTINHO, L.M. 1978. O conceito de cerrado. *Revista Brasileira de Botânica*, **1**: 17-23.
- CRAIG, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometry analysis of carbon dioxide. *Geochimica Cosmochimica Acta*, **12**: 133-149.
- DEMMIG-ADAMS, B. 1990. Carotenoids and photoprotection in plants. A role for the xanthophyll zeaxanthin. *Biochimica Biophysica Acta*, **1020**: 1-24.
- DEMMIG-ADAMS, B. & W.W. ADAMS III. 1992. Photoprotection and other responses of plants to high light stress. *Annual Review of Plant Physiology and Plant Molecular Biology*, **43**: 599-626.
- DEMMIG-ADAMS, B. & W.W. ADAMS III. 1996. Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. *Planta*, **198**: 460-470.
- EHLERINGER, J.R. 1989. Carbon isotope ratios and physiological processes in aridland plants. pp. 41-54. In: Rundel, P.W.; J.R. Ehleringer, & K.A. Nagy (eds.), *Stable Isotopes in Ecological Research*. Springer, Berlin.
- EHLERINGER, J.R. 1995. Variations in gas exchange characteristics among desert plants. pp. 361-392. In: Schulze, E.-D. & M.M. Caldwell(eds.), *Ecophysiology of Photosynthesis*. Springer, Berlin.
- EHLERINGER, J.R. & T.E. DAWSON. 1992. Water uptake by plants: perspectives from stable isotope composition. *Plant, Cell and Environment*, **15**: 1073-1082.

- EITEN, G. 1972. The cerrado vegetation of Brazil. *Botanical Review*, **38**(2): 201-341.
- EVANS, J.R.; T.D. SHARKEY; J.A. BERRY & G.D. FARQUHAR. 1986. Carbon isotope discrimination measured concurrently with gas exchange to investigate CO<sub>2</sub> diffusion in leaves of higher plants. *Australian Journal of Plant Physiology*, **13**: 281-292.
- FARQUHAR, G.D.; J.R. EHLERINGER & K.T. HUBICK. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology*, **40**: 503-537.
- FARQUHAR, G.D.; M.H. O'LEARY & J.A. BERRY. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9**: 121-137.
- FARQUHAR, G.D. & R.A. RICHARDS. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology*, **11**: 539-552.
- FRANCO, A.C.; E. BALL & U. LÜTTGE. 1992. Differential effects of drought and light levels on accumulation of citric acid and malic acids during CAM in *Clusia*. *Plant, Cell and Environment*, **15**: 821-829.
- FRANCO, A.C.; A. HAAG-KERWER; B. HERZOG; T.E.E. GRAMS; E. BALL; E.A. MATTOS; F.R. SCARANO; S. BARRETO; M.A. GARCIA; A. MANTOVANI & U. LÜTTGE. 1996. The effect of light levels on daily patterns of chlorophyll fluorescence and organic acid accumulation in the tropical CAM tree *Clusia hilariana*. *Trees*, **10**: 349-355.
- GENTY, B.; J.M. BRIANTAIS & N.R. BAKER. 1989: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica Biophysica Acta*, **990**: 87-92.
- GOODLAND, R. & FERRI, M.G. 1979. *Ecologia do Cerrado*. Editora Itatiaia, Belo Horizonte.
- GOVINDJEE. 1995. Sixty-three years since Kautsky: chlorophyll *a* fluorescence. *Australian Journal of Plant Physiology*, **22**: 131-160.
- GRIFFITHS, H. 1992. Carbon isotope discrimination and the integration of carbon assimilation pathways in terrestrial CAM plants. *Plant, Cell and Environment*, **15**: 1051-1062.
- HANDLEY, L.L. & J.A. RAVEN. 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant, Cell and Environment*, **15**: 965-985.

- JOHNSON, C.; A.C. FRANCO & L.S. CALDAS. 1983. Fotossíntese e resistência foliar em espécies nativas do cerrado: metodologia e resultados preliminares. *Revista Brasileira de Botânica*, **6**(2): 91-97.
- JONES, H.G. 1994. *Plants and Microclimate: a quantitative approach to environmental plant physiology*. Second edition, Cambridge University Press, NY.
- JONES, H.G. & R.A. SUTHERLAND. 1991. Stomatal control of xylem embolism. *Plant, Cell and Environment*, **14**: 607-612.
- KANNO, S.S. 1993. *Curso diário sazonal das trocas gasosas e potencial hídrico foliar em três espécies lenhosas do cerrado: Anacardium nanum S.T. Hill, Andira humilis Marth ex. Benth e Eriotheca gracilipes*. M.Sc. Dissertation. Universidade Federal de São Carlos, São Carlos, SP, Brazil.
- KRAUSE, G.H. 1988. Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiologia Plantarum*, **74**: 566-574.
- KRAUSE, G.H. & E. WEIS 1991. Chlorophyll fluorescence and photosynthesis: The basics. *Annual Review of Plant Physiology and Plant Molecular Biology*, **42**: 313-349.
- LANGE, O.L.; J.D. TENHUNEN & M. BRAUN. 1982. Midday stomatal closure in Mediterranean type sclerophylls under habitat conditions in an environmental chamber. *Flora*, **172**: 138-160.
- LAWLOR, D.W. 1995. The effects of water deficit on photosynthesis. pp.129-160. In: Smirnoff, N. (ed.), *Environmental and Plant Metabolism: flexibility and acclimation*. Bios Scientific, Oxford.
- LoGULLO, M.A.; S. SALLEO; E.C. PIACERI & R. ROSSO. 1995. Relations in vulnerability to xylem embolism and xylem conduit dimensions in young trees of *Quercus cerris*. *Plant, Cell and Environment*, **18**: 661-669.
- MAGNANI, F. & M. BORGHETTI. 1995. Interpretation of seasonal changes of xylem embolism and plant hydraulic resistance in *Fagus sylvatica*. *Plant, Cell and Environment*, **18**: 689-696.
- MATTOS, E.A. 1992. *Trocas gasosas em folhas de três espécies arbóreas do cerrado da Fazenda Canchim, São Carlos (SP): a disponibilidade hídrica durante a estiagem de inverno limita a abertura estomática?* M.Sc. Dissertation. Universidade Federal de São Carlos, São Carlos, SP, Brazil.

- MATTOS, E.A. 1996. *Ecofisiologia comparativa de espécies arbóreas de um cerrado "sensu stricto" durante estação seca e chuvosa em São Carlos (SP): Trocas gasosas, potencial hídrico, peso específico foliar e discriminação isotópica do carbono*. D.Sc. Thesis. Universidade Federal de São Carlos, São Carlos, SP, Brazil.
- MATTOS, E.A.; T.E.E. GRAMS; E. BALL; A.C. FRANCO; A. HAAG-KERWER; B. HERZOG; F.R. SCARANO & U. LÜTTGE. 1997a. Diurnal patterns of chlorophyll *a* fluorescence and stomatal conductance in species of two types of coastal tree vegetation in southeastern Brazil. *Trees*, **11**: 363-369.
- MATTOS, E.A.; F. REINERT & J.A.P.V. MORAES. 1997b. Comparison of carbon isotope discrimination and CO<sub>2</sub> and H<sub>2</sub>O gas exchange between the dry and the wet season in leaves of several *cerrado* woody species. *Revista Brasileira de Fisiologia Vegetal*, **9**(2): 77-82.
- MEDINA, E. & M. FRANCISCO. 1994. Photosynthesis and water relations of savanna species differing in leaf phenology. *Tree Physiology*, **14**: 1367-1381.
- MEIRELLES, S.T.; E.A. MATTOS & A.C. SILVA. 1997. Potential desiccation tolerant vascular plants from southeastern Brazil. *Polish Journal of Environmental Studies* **6**(4): 17-21
- MOONEY, H.A.; S.H. BULLOCK & J.R. EHLERINGER. 1989. Carbon isotope ratios of plants of a tropical dry forest in Mexico. *Functional Ecology*, **3**: 137-142.
- MOZETO, A.A.; F.M.B. NOGUEIRA; M.H.A.O. SOUZA & R.L. VITÓRIA. 1996. C<sub>3</sub> and C<sub>4</sub> grasses distribution along soil moisture gradient in surrounding areas of the Lobo Dam (São Paulo, Brazil). *Anais da Academia Brasileira de Ciências*, **68**(1): 113-121.
- NETTO, A.B.P. & J.D. HAY. 1986. Fotossíntese em *Caryocar brasiliense* no cerrado. *Revista Brasileira de Botânica*, **9**: 259-262.
- NOBEL, P.S. 1991. *Physicochemical plant physiology*. Academic Press, San Diego.
- O'LEARY, M.H. 1981. Carbon isotope fractionation in plants. *Phytochemistry*, **20**: 553-567.
- OSMOND, C.B. 1994. What is photoinhibition? Some insights from comparison of shade and sun plants. pp 1-24. In: Baker, N.R. & J.R. Bowyer (eds.), *Photoinhibition of Photosynthesis: from molecular mechanisms to the field*. Bios Scientific, Oxford.
- OSMOND; C.B. & S.C. GRACE. 1995. Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and dark reactions of photosynthesis? *Journal of Experimental Botany*, **46**: 1351-1362.

- PETERS, R.H. 1991. *A Critique for Ecology*. Cambridge University Press, New York.
- PRADO, C.H.B.A.; J.A.P.V. MORAES & E.A. MATTOS. 1994. Gas exchange and leaf water status in potted plants of *Copaifera langsdorffii*. 1. Responses to water stress. *Photosynthetica*, **30**(2): 207-213.
- PRADO, C.H.B.A.; J.A.P.V. MORAES & E.A. MATTOS. 1995. Gas exchange and leaf water status in potted plants of *Copaifera langsdorffii*. 2. Probable influence of low air humidity. *Photosynthetica*, **31**(1): 31-36.
- ROESSLER, P.G. & R.K. MONSON. 1985. Midday depression in net photosynthesis and stomatal conductance in *Yucca glauca*. Relative contributions of leaf temperature and leaf-to-air water vapor concentration difference. *Oecologia*, **67**: 380-387.
- SARMIENTO, G.; G. GOLDSTEIN & F. MEINZER. 1985. Adaptive strategies of woody species in neotropical savannas. *Biological Review*, **60**: 315-355.
- SCHREIBER, U. & W. BILGER. 1987. Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. pp.27-53. In: Tenhunen, J.D.; F.M. Catarino; O.L. Lange & W.D. Oechel (eds.), *Plant Response to Stress*. Springer, Berlin.
- SCHREIBER, U. & W. BILGER. 1993. Progress in chlorophyll fluorescence research: major developments during the last years in retrospect. *Progress in Botany*, **54**: 151-173.
- SCHREIBER, U.; W. BILGER & C. NEUBAUER. 1995. Chlorophyll fluorescence as a noninvasive indicator for rapid assessment of *in vivo* photosynthesis. pp.49-70. In: Schulze, E.-D. & M.M. Caldwell (eds.), *Ecophysiology of Photosynthesis*. Springer, Berlin.
- SCHULZE, E.-D & M.M. CALDWELL (eds.). 1995. *Ecophysiology of Photosynthesis*. Springer, Berlin.
- SPERRY, J.S. & M.T. TYREE. 1988. Mechanism of water stress-induced xylem embolism. *Plant Physiology*, **88**: 581-587.
- SOLBRIG, O.T.; JAIN, S.; JOHNSON, G.B. & RAVEN, P.H. (eds.). 1979. *Topics in Plant Population Biology*. Columbia University Press, New York.
- THROUGHTON, J.H.; K.A. CARD & C.H. HENDY. 1974. Photosynthetic pathways and carbon isotope discrimination by plants. *Carnegie Institute Washington Year Book*, **73**: 768-780.

- TRUST, B.A. & B. FRY. 1992. Stable sulphur isotopes in plants: a review. *Plant, Cell and Environment*, **15**: 1105-1110.
- van KOOTEN, O. & J.F.H. SNEL. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research*, **25**: 147-150.
- von CAEMMERER, S. 1992. Carbon isotope discrimination in C<sub>3</sub>-C<sub>4</sub> intermediates. *Plant, Cell and Environment*, **15**: 1063-1072.
- ZIEGLER, H.; K.H. BATANOUNY; N. SANKHLA; O.P. VYAS & W. STICHLER. 1981. The photosynthetic pathways types of some desert plants from India, Saudi Arabia, Egypt, and Iraq. *Oecologia*, **48**: 93-99.

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