

Spondias tuberosa trees grown in tropical, wet environments are more susceptible to drought than those grown in arid environments

Árboles de *Spondias tuberosa* que crecen en ambientes húmedos de las zonas tropicales son más susceptibles a la sequía que los cultivados en ambientes áridos



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Fruits of *Spondias tuberosa*.
Photo: K. Rego Mendes

ABSTRACT

In this study, we investigated the different responses of *Spondias tuberosa* (umbu) trees, which grow in two different ecological life zones in northeast Brazil: tropical wet and tropical arid ecosystems. We evaluated the responses of plants grown under humid and dry conditions by measuring the photosynthesis, water status, fluorescence parameters, carbon isotopes and antioxidant system activity. The higher net photosynthesis values were recorded contemporaneously with the lower VPD values. The highest internal-to-ambient CO₂ concentration and the absence of typical changes in the fluorescence parameters suggested an onset of a nonstomatal limitation in the photosynthesis. Our results showed that umbu plants can adjust their antioxidant activity during the dry season as a defensive strategy against the deleterious effects of water stress. This evidence is supported by the observed modifications in the pigment concentrations, increased accumulation of hydrogen peroxide and malondialdehyde, high levels of electrolyte leakage, increased antioxidant activity, and decreased carbon isotope discrimination in the umbu trees during the dry season. Supported by multivariate analysis of variance, significantly effect of interaction between categorical “months of collect and location” predicts a strong “dry season effect” on our dataset. Taken together, our data show that umbu trees grown in a wet tropical environment are more susceptible to drought, as compared with their tropical arid counterparts.

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Additional keywords: Caatinga, carbon isotope composition, global climate change, nonstomatal limitations, Umbuzeiro, water stress.

RESUMEN

En este trabajo se investigaron las respuestas de árboles de *Spondias tuberosa* (umbu), que crecen en dos zonas ecológicas del nordeste de Brasil: húmeda tropical y árida tropical. Se evaluaron las respuestas de las plantas con base en fotosíntesis, estado hídrico, parámetros de fluorescencia, isótopos de carbono y actividad del sistema antioxidante. Los altos valores de las tasas de fotosíntesis fueron correlacionados con bajos valores del DPV; los altos niveles de concentración interna de CO₂ y la ausencia de cambios típicos en los parámetros de fluorescencia sugieren la aparición de una limitación no estomática de la fotosíntesis. Los resultados indicaron que las plantas de umbu pueden ajustar su actividad antioxidante durante la estación seca como una estrategia de defensa ante los efectos perjudiciales de un estrés por sequía. Esta afirmación está soportada por las modificaciones observadas en la concentración de pigmentos, incrementos en la acumulación de peróxido de hidrógeno y malondialdehído, altos niveles de electrolitos libres, incremento de la actividad antioxidante y decrecimientos en la discriminación isotópica del carbono en la localidad árida. El análisis de varianza multivariado mostró efectos significativos para la interacción “mes de colecta y localidad”, lo cual fue fuertemente predecible en la localidad. El análisis integral de los datos demostró que los árboles de umbu que crecieron en un ambiente húmedo tropical son más susceptibles a la sequía, comparados con su contraparte de ambientes áridos tropicales.

Palabras clave adicionales: Caatinga, composición isotópica del carbono, cambio climático, limitaciones no estomáticas, umbuzeiro, estrés hídrico.

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INTRODUCTION

Water stress is an important factor that affects plant growth and yield, especially in the hot and seasonally wet-dry climates in the semiarid regions of the world (Ondrasek, 2014).

A combination of drought and excess light decreases the efficiency of photosynthesis and may also lead to leaf overheating, especially when the transpirational leaf cooling is reduced due to water deficits (Rizhsky *et al.*, 2004; Rizhsky *et al.*, 2002; Valladares and Pearcy, 1997).

Tropical plants growing under water stress exhibit modifications in photosynthetic processes,

including disruptions in the stomatal control of the leaf gas exchange (Arcoverde *et al.*, 2011; Lawlor and Tezara, 2009; Lima Filho, 2007; Pompelli *et al.*, 2010a; Santos *et al.*, 2013), alterations in chlorophyll fluorescence kinetics (Faraloni *et al.*, 2011; Maxwell and Johnson, 2000), damage to photosynthetic membranes (Krieger-Liszkay, 2005), changes in the carbohydrate status (Chimenti *et al.*, 2002; Silva *et al.*, 2010), and others. The water stress-induced limitation of net photosynthesis (P_N) may cause damage to the plant with exposure to excess irradiance; if this energy is not safely dissipated, it may cause an over-reduction of the reaction centers and increased

production of reactive oxygen species (ROS) in the chloroplasts, leading to photosystem PSII damage (Smirnoff, 1995).

Knowledge of species physiology *in situ* under arid/semiarid conditions may contribute significantly to our understanding of how to optimize productivity in trees under drought conditions in natural environments. Data regarding species physiology under arid conditions may also indicate how these species can be used in the regeneration of important regions undergoing desertification. In this context, we examined *Spondias tuberosa* Arruda (“umbuzeiro” or “umbu plant”), which is a small native tree that grows naturally among Caatinga vegetation throughout arid, semiarid and wet environments in northeastern Brazil, where it is adapted to survive under water stress conditions (Lins Neto *et al.*, 2012). This tree is considered to be a species with great economic, social and ecological importance to the arid/semiarid northeast, Brazil (Lins Neto *et al.*, 2010). At the end of the dry season, the umbuzeiro partially shed their leaves to avoid transpiration, reducing the plant’s growth rate, leaf initiation and leaf expansion during the drought, until the beginning of the first rains (Cavalcanti *et al.* 1996). During this period, the survival of the species is assured by a specialized root system bearing tubers (or xylopodia), whose function is to store water, soluble sugars, starch, minerals, and other solutes (Lins Neto *et al.*, 2010). Old plants growing in their native fields can have more than 1,000 xylopodia, and their fresh weight can exceed 2,000 kg. These resources are used during the dry season to maintain normal plant metabolism (Cavalcanti and Resende, 2006; Lima Filho, 2001; Lima Filho, 2004).

There are few reports describing the physiological behavior of plants under arid conditions and their interactions with the environment (Campos *et al.*, 2012; Cattivelli *et al.*, 2008; Dombroski *et al.*, 2011; Lima Filho, 2004). For example, Lima Filho (2004) observed that the environmental difference between the dry and rainy seasons

is the primary factor that influences the course of gas exchange in umbu trees under semiarid conditions. Nevertheless, Lima Filho (2001) suggested that, under dry conditions, the diurnal water balance of the *S. tuberosa* would be maintained at the expense of water stored in the tubers and by restricted transpiration. In addition, Lima Filho (2007) also noted that the gas exchange of umbu plants with fewer tubers was more strongly affected than the gas exchange of plants with more tubers under water-stressed soil conditions. These investigators suggested that the root tubers may act as an important source of water under stress conditions. However, the aforementioned studies (Lima Filho, 2001; Lima Filho, 2004; Lima Filho, 2007) only describe the gas exchange of umbu trees under semiarid conditions and the importance of tubers in helping plants avoid stress.

In this study, we hypothesized that variations in the seasonal rainfall regime distinctly affect the ecophysiological plant performance in contrasting environments (wet and arid). It may affect the photoprotection and carbon gain and may also limit growth in *S. tuberosa* trees, mostly in plants grown in tropical wet regions, because the root mass fraction of well-watered trees is often reduced, as compared with that of water-stressed trees reducing the photosynthetic capacity (Poorter *et al.*, 2012). Therefore, the main objective of this study was to evaluate the effects of the seasonal rainfall regime alterations on the plant physiological behavior for the gas exchange and photoprotection of *S. tuberosa* trees growing under wet and arid conditions in two regions of Bahia State, northeast, Brazil.

MATERIALS AND METHODS

Site description and experimental design

This experiment was conducted with 2.0 to 3.0-m tall, 9-year-old *Spondias tuberosa* Arruda (Anacardiaceae) trees that were grown on commer-

cial plantations following appropriate cultural practices, except water supplementation (Araújo, 2007). Two different commercial plantations located in Cruz das Almas (12°35' S; 39°15' W; 126 m a.s.l.) and Andaraí (12°48' S; 41°19' W; 395 m a.s.l.) in northeast Brazil were evaluated. These two cities are at least 300 km away from each other and located in different ecological life zones, which are tropical wet - Cruz das Almas and tropical arid - Andaraí (Tanajura *et al.*, 2010). As presented in figure 1, two seasons were observed during the study period (June 2010 to May 2011): a dry season (precipitation less than 50 mm) and a rainy season. During the study period, the accumulated precipitation in Cruz das Almas and Andaraí was 1397±60 mm and 692±48 mm, respectively. Uniform and healthy *S. tuberosa* trees were selected from populations at the study sites. On the plantations, the tree density is 156 plants/ha with a spacing of 8 m x 8 m. Annually, 75 g of a slow-release fertilizer (containing 15% N, 9% P, 12% K, 1% Mg, 2.3% S, 0.05% Cu, 0.45% Fe, 0.06% Mn and 0.02% Mo) are applied per plant. The fertilizer was applied at a 10-cm depth from the soil surface. In both environments (tropical wet and tropical arid), the *S. tuberosa* plants partially shed their leaves at the end of the dry season. The data were collected from trees grown under field conditions during the humid (July 2010 and April 2011) and dry (September and November 2010) seasons. The air temperature and rainfall during the experiment period were obtained from an automatic weather station installed within 5 km of the experiment sites. The soil water balance and actual evapotranspiration data were obtained from AgriTempo (2013).

Gas-exchange and fluorescence measurements

The gas exchange and chlorophyll fluorescence parameters were measured with an infrared gas analyzer (Li-6400, Li-Cor, Lincoln, NE) coupled with a leaf chamber fluorometer (LCF; Li-6400-40, Li-Cor, Lincoln, NE) on two fully expanded leaves per plant and the saplings in each season

(n=10). These parameters were measured *in situ* under clear-sky conditions. The leaf chamber was configured to 1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ saturating light from LED source and ambient CO_2 concentration (380 $\mu\text{mol mol}^{-1}$). The data were collected at ambient conditions of temperature and relative humidity. To assess the effect of time on g_s and net photosynthesis (P_N), we collected data at five different times (08:00, 10:00, 12:00, 14:00 and 16:00 h; solar time), using the same leaves throughout the day on each occasion.

The chlorophyll fluorescence was measured after 30-min with dark-adapted leaves and all of the parameters are estimated according to Genty *et al.* (1989) and Maxwell and Johnson (2000, 2008).

Water potential measurements

The leaf-water potential (Ψ_w) was measured using a Scholander-type pressure chamber (mod 3005F01; Soil Moisture Equipment Corp, Santa Barbara, CA). The measurements were taken in one leaf per plant at predawn (n=10).

Carbon isotope composition

From each experiment tree (n=5), one expanding leaf was collected *in situ* and it was oven-dried for 72 h at 70°C. The isotope ratio, which was expressed as $\delta^{13}\text{C}$, was calculated as

$$\delta^{13}\text{C}(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000,$$

where R_{sample} is the $^{13}\text{C}/^{12}\text{C}$ ratio in the sample and R_{standard} is the $^{13}\text{C}/^{12}\text{C}$ ratio in the standard (PDB-Pee Dee Belemnite).

Biochemical analysis

Leaf discs (1.4 cm in diameter) were collected *in situ* at 09:00-10:00 a.m. under clear-sky conditions, rapidly frozen in liquid nitrogen, and stored at -20°C until analysis.

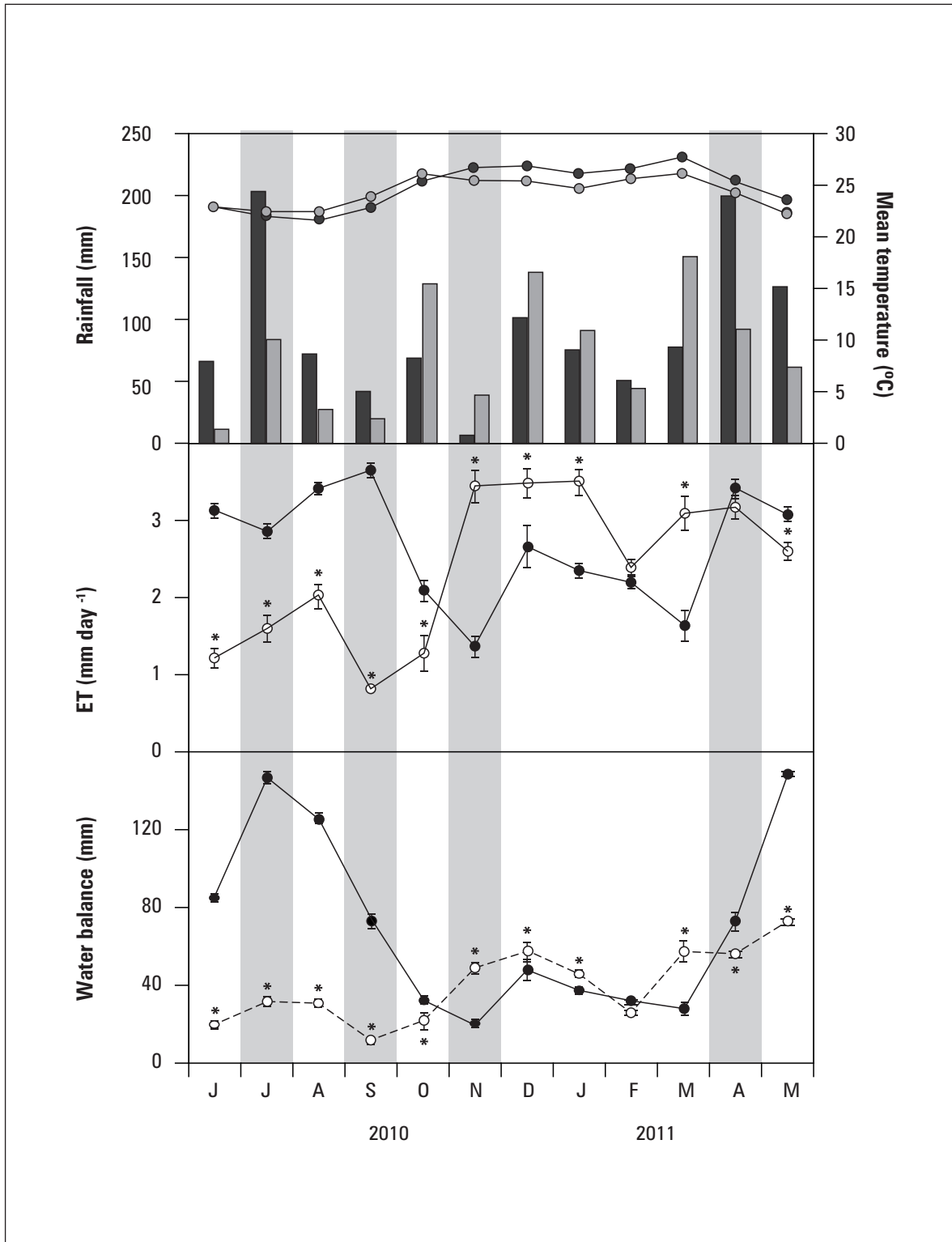


Figure 1. Climatic characteristics: (A) total monthly rainfall (bars) and mean temperatures (lines); (B) evapotranspiration rate [ET] and (C) soil water balance (store) from June 2010 to May 2011 in the tropical wet (filled symbols) and tropical arid (unfilled symbols) ecosystems. Each point followed by asterisks represents statistically significant differences within each month. The values represent the average (\pm SD). Source: Agritempo (2013). Gray vertical bars represent sampling months.

Antioxidant enzymes, including superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX; EC 1.11.1.11), were assayed as described by Pompelli *et al.* (2010a). The H_2O_2 was analyzed according to Chen and Kao (1999). The Malondialdehyde (MDA) was determined following Cakmak and Horst (1991). The electrolyte leakage was assayed according to Campos *et al.* (2012).

The chlorophyll *a+b* and total carotenoids were extracted with 80% (v/v) aqueous acetone and quantified spectrophotometrically according to Pompelli *et al.* (2013). β -carotene, violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z), were assayed by HPLC as reported by Ramalho *et al.* (1999).

Statistical analysis

The data were first analyzed by multivariate analysis of variance (MANOVA) with “month of collection and location” as categorical factors and using as dependent variables “photosynthetic” (P_N , E , g_s , $C_i:C_a$, WUE, T_{leaf} , VPD), “photochemical” (Φ_{PSII} , P , D , P_E) and “enzymatic” (SOD, CAT, APX, H_2O_2 , MDA, electrolyte leakage) group in a GLM Bi-factorial MANOVA, for each variable. Since the interaction effect was highly significant according to the Wilks test in all of the performed analyses ($P \leq 0.0001$), the effective hypothesis decomposition was done with a one-way ANOVA, and the means were compared using Duncan’s or Student’s *t* test. All of the statistical tests were performed using the statistical software package Statistica version 10.0 (StatSoft, Tulsa, OK). The Lilliefors test was conducted to assess whether the experiment errors were normally distributed. As no transformation was needed, all of the statistical analyses were carried out on untransformed data. The results were considered to be significant when $P \leq 0.05$. Only the 8:00h data (when applicable) were used for the photosynthetic variable in MANOVA.

RESULTS

Gas exchange measurements

Annual fluctuations in the net photosynthesis (P_N), stomata conductance (g_s) and transpiration (E) were observed for the two populations. High variations throughout the day were observed, with a 7-fold difference between the extreme values (Fig. 2). The P_N was always greater than $10 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ during the humid season, peaking at $17.4 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for plants evaluated in April under wetter climate conditions. The P_N of the tropical arid plants was consistently lower than the P_N of the tropical wet plants. By means of all of the performed MANOVA, a strong interaction between the categorical variables “month of collect and location” notably pointed to a generalized “dry season effect” (November) and indicated that *S. tuberosa* plants have a differential behavior in wet and arid environments in this season. Consistent with this, the November measurements show that reductions in the P_N were stronger in the plants grown in the tropical humid environment than they for the tropical arid counterparts (Fig. 2). From the humid to the dry season, the P_N decreased by 84% and 34% in the tropical wet and tropical arid plants, respectively (figure 2), and the relationship between P_N and g_s was significantly positive ($r = 0.753$; $P \leq 0.001$). When only the dry season data were analyzed, the correlations were not significant ($P = 0.688$), indicating circumstantially that g_s could be a key factor in determining carbon uptake in a humid environment, but not in a dry one.

A negative relationship between the photosynthetic parameters and PAR ($r = -0.421$, -0.349 , and -0.339 for P_N , E and g_s , respectively; $P \leq 0.05$) was observed. During both seasons, the photosynthetic parameters differed significantly between the ecosystems, but these differences became stronger during the dry season (figure 2).

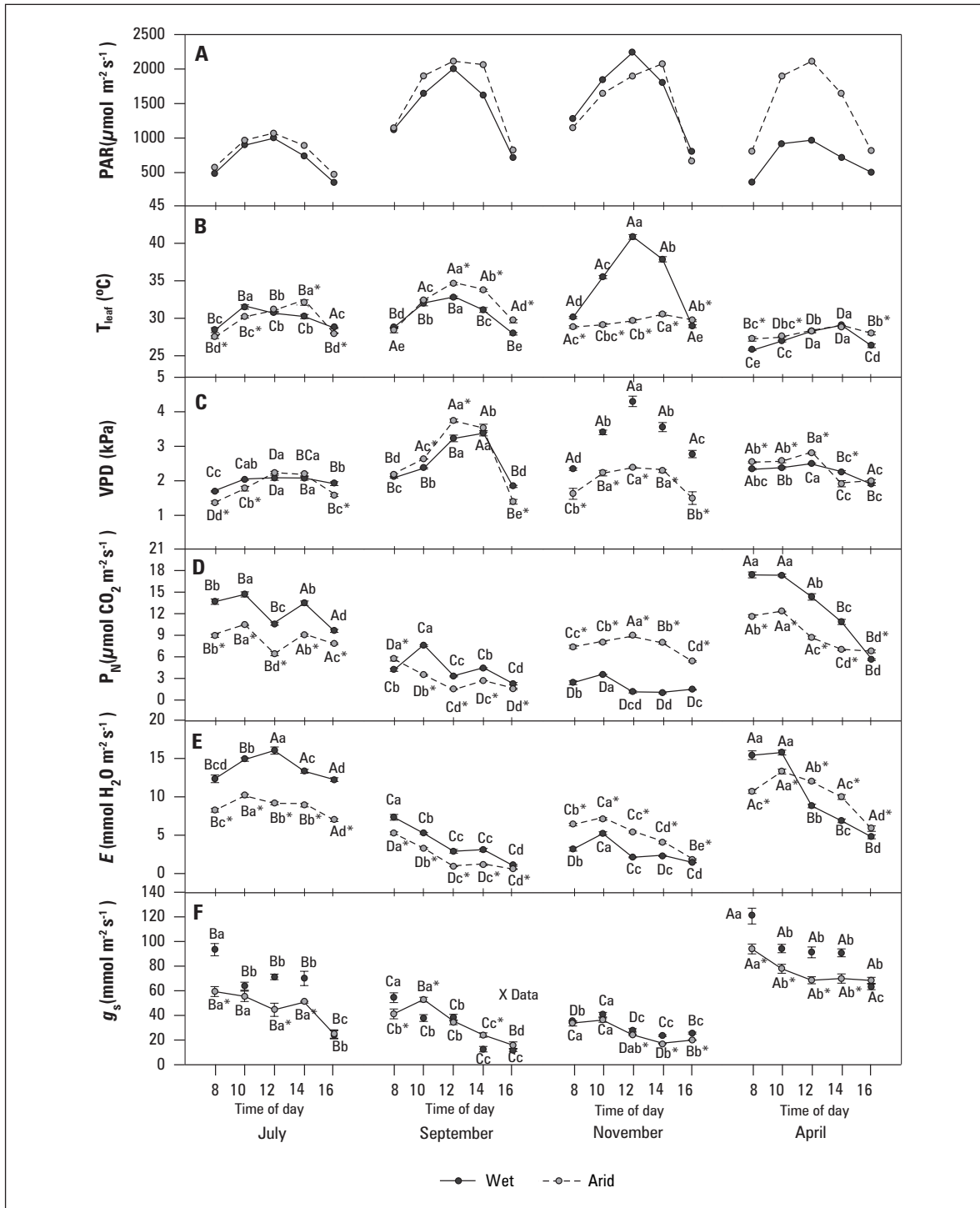


Figure 2. (A) Time course of photosynthetically active radiation [PAR], (B) leaf temperature [T_{leaf}], (C) leaf-to-air vapor pressure difference [VPD], (D) net CO_2 assimilation rate [P_N], (E) transpiration [E] and (F) stomatal conductance [g_s] measured in tropical wet (filled symbols) and tropical arid (unfilled symbols) leaves in four different months in field-grown *Spondias tuberosa* trees from well-exposed leaves between 08:00 and 16:00 h. Within the same schedule, significant seasonal differences among the average values are represented by different capital letters (tropical wet) or lower case letters (tropical arid; Duncan's test, at $P \leq 0.05$). Asterisks denote significant differences between the average values of the tropical wet and tropical arid leaves within the same schedule and month (Student's t test, at $P \leq 0.05$). Each point represents the means (\pm SE). $n=10$.

The P_N , E and g_s may have decreased because of a high vapor pressure deficit (VPD), as confirmed by their strong negative correlations ($r = -0.809$, -0.786 , and -0.849 for P_N , E and g_s , respectively; $P \leq 0.0001$).

In the tropical wet plants, the WUE was strongly reduced during the dry season but sharply recovered at the beginning of the rainy season (figure

3). A similar pattern was not observed for the WUE of the tropical arid plants, which remained constant throughout the year (figure 3). A 50% increase was verified in the $C_i:C_a$ ratio during the dry season, as compared with the humid season in both populations (Fig. 3) circumstantially indicating a nonstomatal limitation of photosynthesis in this season. A high $C_i:C_a$ was correlated with lower P_N and E values ($r = -0.947$ and

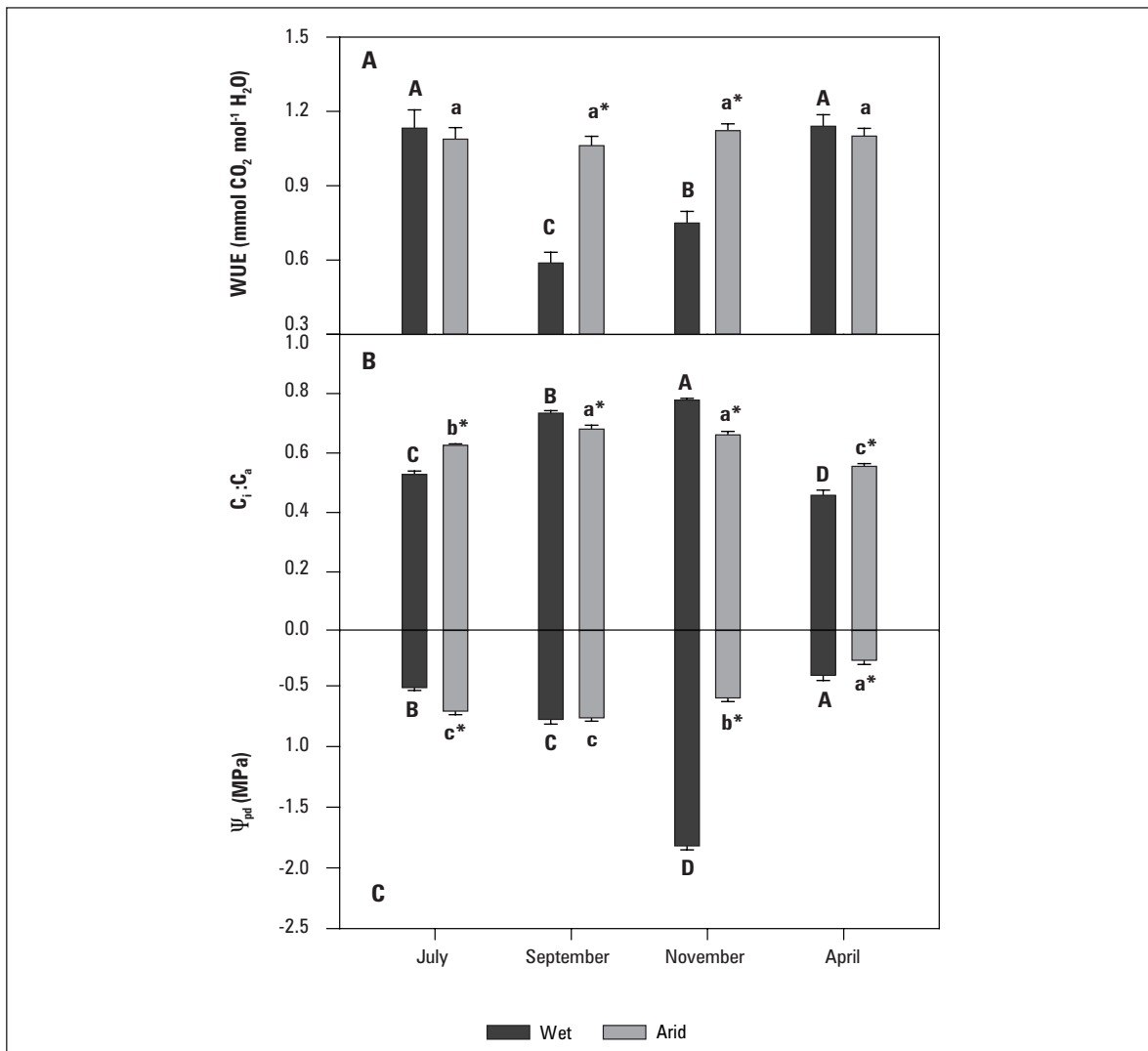


Figure 3. (A) Water use efficiency [WUE], (B) internal-to-ambient CO₂ concentration (C_i:C_a ratio) and (C) leaf water potential [Ψ_{pd}] at predawn as measured in leaves of field-grown *Spondias tuberosa* trees. These measurements were made on plants grown in tropical wet (filled symbols) and tropical arid (unfilled symbols) ecosystems from well-exposed leaves between 08:00 and 09:00 h in four different months, with saving Ψ_{pd} (05:00 h). Different capital letters mean significant ($P \leq 0.05$, Duncan's test) seasonal differences among the means in the tropical wet environment and different lower case letters indicate the same for the tropical arid environment. Asterisk denotes significant differences ($P \leq 0.05$, Student's t test) between the average values of the tropical wet and tropical arid leaves within the same month. The values represent the means (\pm SE). $n=10$.

-0.877, respectively; $P \leq 0.0001$) and a positive relationship ($r = 0.828$; $P \leq 0.0001$) between the $C_i:C_a$ and $\delta^{13}C$ was verified.

The predawn leaf water potential (Ψ_{pd}) was higher than -1.0 MPa during the entire study period, with the exception of November, when the lower values were observed in the plants grown in tropical wet. This result provides evidence that the trees experienced moderate to severe water stress (figure 3).

Chlorophyll fluorescence and quenching

The maximum efficiency of photosystem II ($F_v:F_m$ ratio) showed significant differences ($P \leq 0.05$) between the dry and humid seasons (table 1). During the dry season, there was a small reduction in the $F_v:F_m$ ratio at midday (table 1), with the lowest value found in the tropical wet environment at November. The $F_v:F_m$ ratio (table 1), Φ_{PSII} and P , followed similar trends (figure 4). All of these parameters in both ecosystems reached their minimum values during the dry season and the maximum values during the humid seas ($P = 0.015$), but they were not correlated with the atmospheric temperature ($P = 0.773$). This finding indicates that plants that were exposed to a water shortage were capable of dissipating part of the excess energy as latent heat to minimize the water lost by transpiration ($r = -0.946$; $P \leq 0.0001$, between D and transpiration).

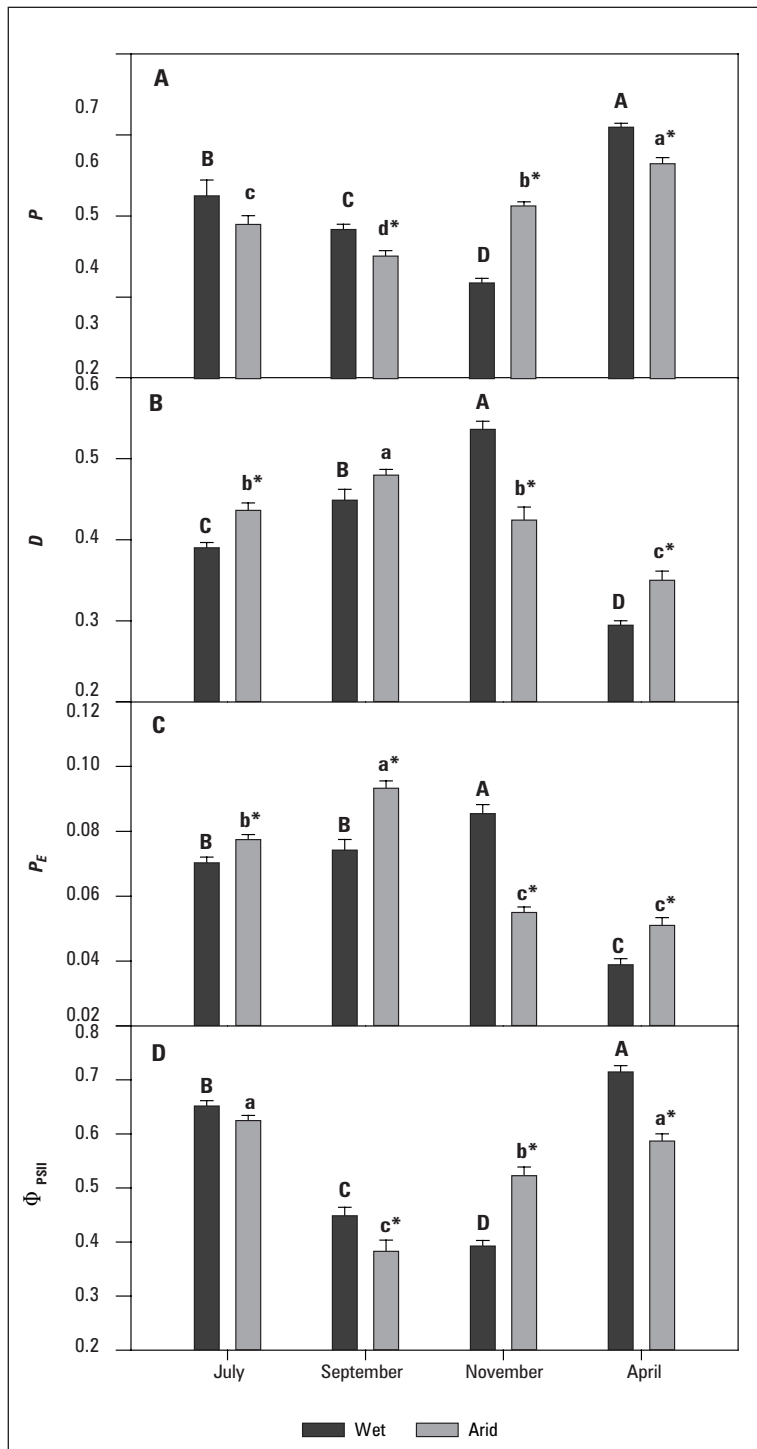


Figure 4. (A) Diurnal changes in the estimated fluxes of photons utilized through photochemistry [P] or (B) dissipated thermally [D], (C) the fraction neither used in photochemistry nor dissipated thermally [P_E] and (D) the actual PSII efficiency during the exposure to sunlight (Φ_{PSII}) as measured in tropical wet (filled symbols) and tropical arid (unfilled symbols) leaves in four different months in field-grown *Spondias tuberosa* trees. The measurements were taken at midday. The values represent the means (\pm SD). $n=10$. For statistical details, see figure 3.

Table 1. Ratios of variable-to-maximum (Fv:Fm) chlorophyll fluorescence measured at predawn (pd) and midday (md) in tropical wet or tropical arid leaves in four different months in field-grown *Spondias tuberosa* trees, using well-exposed leaves. Within the same row, significant seasonal differences among the means are represented by different capital letters (tropical wet) or lower case letters (tropical arid; Newman-Keuls test, at $P \leq 0.01$). Asterisks denote significant differences between the means of tropical wet and tropical arid leaves within the same row and month (Newman-Keuls test, at $P \leq 0.01$). Each point was produced by taking the average of measurements made on ten different plants \pm SD.

Parameters	July		September	
	Tropical wet	Tropical arid	Tropical wet	Tropical arid
Fv / Fm (pd)	0.80 \pm 0.01	0.81 \pm 0.01 Aa	0.82 \pm 0.03	0.77 \pm 0.04 Ba*
Fv / Fm (md)	0.79 \pm 0.01	0.76 \pm 0.01 Ba	0.75 \pm 0.02	0.72 \pm 0.01 Cb*

Parameters	November		April	
	Tropical wet	Tropical arid	Tropical wet	Tropical arid
Fv / Fm (pd)	0.71 \pm 0.01	0.81 \pm 0.03 Ab*	0.83 \pm 0.02	0.80 \pm 0.01 Aa*
Fv / Fm (md)	0.66 \pm 0.03	0.79 \pm 0.03 Ac*	0.81 \pm 0.02	0.75 \pm 0.02 Ba*

Carbon isotope composition

Consistent with the dry season effect predicted by MANOVA's performance, the plants grown in the tropical wet climate showed less enriched $\delta^{13}\text{C}$ values, as compared with the tropical arid plants throughout the study period (figure 5), with exception of the data collected in November. During the rainy season, the $\delta^{13}\text{C}$ ranged from -29.18 to -29.72‰ in the leaves of tropical wet plants

and the value in the tropical arid leaves was approximately -28‰. During the dry season, the $\delta^{13}\text{C}$ composition of the leaves from the tropical wet plants varied substantially (3.1‰); however, the composition of the leaves from the tropical dry plants did not vary substantially (0.6‰). The physiological responses to drought (figure 3) reflected the isotopic signature, or $\delta^{13}\text{C}$, of the leaves. A less negative isotopic signature was observed under the drought conditions (figure 5).

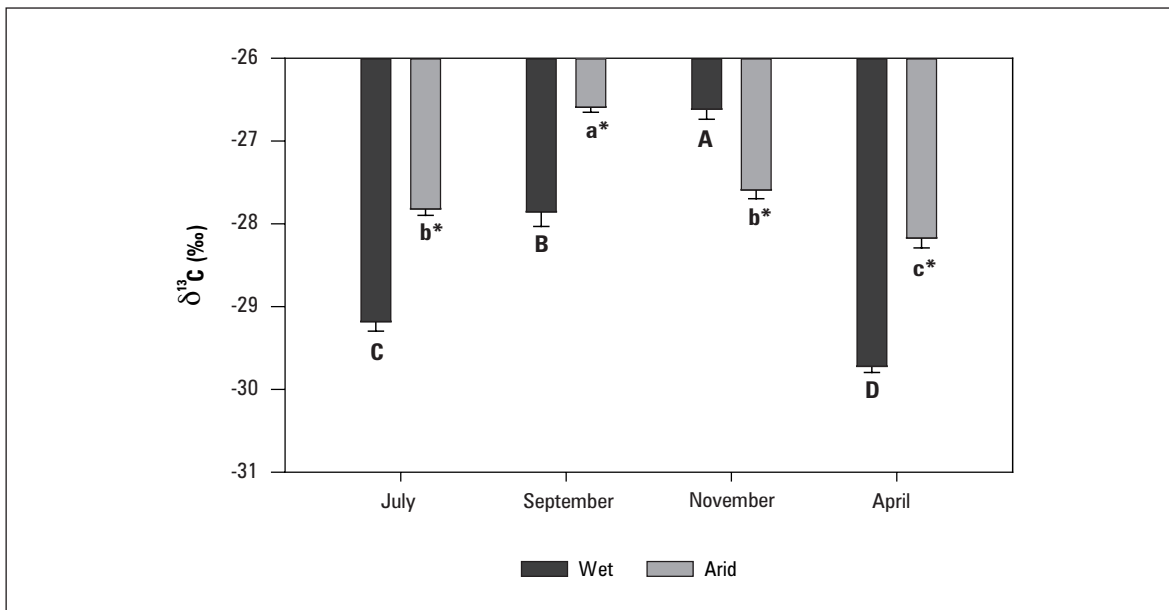


Figure 5. Carbon isotope ratio ($\delta^{13}\text{C}$) measured in the tropical wet (filled bars) and tropical arid (unfilled bars) leaves in four different months in field-grown *Spondias tuberosa* trees. The measurements were taken at midday. The values represent the means (\pm SD). $n=5$. For statistical details, see figure 3.

Biochemical analysis

The chlorophyll content of the tropical wet plants was similar to that of the tropical arid plants; however, the minimum chlorophyll content in both climates was observed at the end of the water deficit period (November), with the tropical arid plants showing a higher concentration than their tropical wet counterparts (table 2). Independent of the locations, the water shortages led to a reduction in the total chlorophyll (~17%).

The β -carotene, antheraxanthin and zeaxanthin concentrations showed similar fluctuations, with gradual increases from July to November and a decrease in April in the next year. Significant correlations between Z and D ($r = 0.558$; $P \leq 0.0001$) and between VAZ and D ($r = 0.557$; $P \leq 0.0001$) ratios were shown during the year. For example, the thermal energy dissi-

pation and D were associated with increased levels of de-epoxidated forms of A and Z within the VAZ cycle (table 2) in November.

The SOD, CAT and APX activities were positively interrelated. Overall, the antioxidant enzymes activities were significantly increased (35%, 51% and 46%, respectively) in the plants grown during the dry season, as compared with the plants grown during the humid season (figure 6). The observed elevated H_2O_2 accumulation may promote membrane damage and the release of MDA ($r = 0.542$; $P \leq 0.0001$). In fact, the MDA level during the dry season was 40% higher than that observed during the humid season. This finding reflects the 80% increase in electrolyte leakage during the dry season in both ecosystems (figure 6). Indeed, the MDA level and the level of electrolyte leakage showed a strong and significant correlation ($r = 0.701$; $P \leq 0.0001$).

Table 2. Chlorophyll and carotenoid composition measured in the tropical wet or tropical arid leaves in four different months in field-grown *Spondias tuberosa* trees. The measurements were taken at midday. Each point was produced by taking the average of measurements made on five different plants \pm SD. For more statistical details, see table 1.

Parameters	July			September		
	Tropical wet	Tropical arid		Tropical wet	Tropical arid	
$g\ kg^{-1}\ DW$						
Chl "a"	3.88 \pm 0.06	3.50 \pm 0.17	Bb*	3.36 \pm 0.27	3.14 \pm 0.12	Cc
Chl "b"	1.68 \pm 0.10	1.76 \pm 0.17	Aa	1.48 \pm 0.05	1.37 \pm 0.05	Bc
Chl "a + b"	5.57 \pm 0.14	5.26 \pm 0.23	Bb*	4.85 \pm 0.27	4.52 \pm 0.12	Cd*
Carotenoids	0.77 \pm 0.07	0.78 \pm 0.03	Ba	0.76 \pm 0.01	0.79 \pm 0.04	Ba
Chl / Carotenoids	7.30 \pm 0.72	6.74 \pm 0.36	Bb	6.34 \pm 0.26	5.69 \pm 0.27	Bc
Carotenoids / Chl	0.14 \pm 0.01	0.15 \pm 0.01	Bb	0.16 \pm 0.01	0.18 \pm 0.01	Ba*
$mg\ kg^{-1}\ DW$						
β -carotene	25.60 \pm 3.01	30.84 \pm 0.70	Ab	35.70 \pm 3.60	38.58 \pm 13.89	Ab
Violaxanthin	56.26 \pm 21.38	42.26 \pm 5.21	Aa*	48.73 \pm 4.42	50.91 \pm 1.75	Aa
Antheraxanthin	51.19 \pm 4.39	61.34 \pm 11.02	Ab*	57.84 \pm 7.63	57.70 \pm 2.37	Aa
Zeaxanthin	119.92 \pm 6.77	126.58 \pm 7.07	Ab	121.75 \pm 17.87	141.33 \pm 18.87	Ab*
V+A+Z	231.38 \pm 9.94	224.18 \pm 4.99	Ab	206.32 \pm 7.17	209.94 \pm 2.71	Bc
V+A+Z / Chl	41.61 \pm 2.57	42.74 \pm 2.60	Ab	42.68 \pm 2.89	46.51 \pm 1.13	Ab*

Table 2. Chlorophyll and carotenoid composition measured in the tropical wet or tropical arid leaves in four different months in field-grown *Spondias tuberosa* trees. The measurements were taken at midday. Each point was produced by taking the average of measurements made on five different plants \pm SD. For more statistical details, see table 1.

Parameters	November			April		
	Tropical wet	Tropical arid		Tropical wet	Tropical arid	
g kg ⁻¹ DW						
Chl "a"	2.89 \pm 0.15	3.46 \pm 0.23	Db*	4.40 \pm 0.17	4.38 \pm 0.34	Aa
Chl "b"	1.18 \pm 0.08	1.46 \pm 0.07	Bd*	1.86 \pm 0.18	1.76 \pm 0.08	Aa
Chl "a + b"	4.08 \pm 0.17	4.92 \pm 0.24	Dc*	6.26 \pm 0.15	6.14 \pm 0.32	Aa
Carotenoids	0.87 \pm 0.09	0.69 \pm 0.03	Ab*	0.50 \pm 0.07	0.58 \pm 0.07	Cc*
Chl / Carotenoids	4.73 \pm 0.57	7.17 \pm 0.47	Cb*	12.80 \pm 2.14	10.78 \pm 1.48	Aa*
Carotenoids / Chl	0.21 \pm 0.02	0.14 \pm 0.01	Ab*	0.08 \pm 0.01	0.09 \pm 0.01	Cc
mg kg ⁻¹ DW						
β -carotene	45.88 \pm 2.75	35.65 \pm 4.86	Aa*	29.68 \pm 11.00	30.34 \pm 8.61	Ab
Violaxanthin	35.78 \pm 2.33	47.19 \pm 3.76	Ab*	55.13 \pm 7.69	46.10 \pm 3.33	Aa
Anteraxanthin	62.17 \pm 1.81	47.78 \pm 4.87	Aa*	41.51 \pm 8.00	48.88 \pm 4.49	Ac
Zeaxanthin	145.41 \pm 6.51	132.28 \pm 5.24	Aa*	87.44 \pm 11.95	104.24 \pm 9.46	Bc
V+A+Z	243.36 \pm 9.05	227.26 \pm 3.37	Aa*	194.08 \pm 9.17	193.22 \pm 10.46	Cd
V+A+Z / Chl	59.77 \pm 3.28	46.26 \pm 2.40	Aa*	31.02 \pm 1.81	31.61 \pm 3.01	Bc

DISCUSSION

The current study provides a seasonal profile of the daily photosynthetic performance of *S. tuberosa* trees grown under wet and arid conditions in natural environments. In general, the umbu plants grown in the tropical wet environments showed a differential behavior mainly at drought when compared to the tropical arid environment with the same stress. The multivariate and physiological analysis points to higher susceptibility to drought for the tropical wet plants. In all of the plants, the diurnal course P_N , g_s , and E values were maximal during the midmorning and decreased in the afternoon and those parameters did not respond to an elevated light stimulus, most likely because T_{leaf} and VPD were increased (figure 2). It is possible that these environmental factors may have somehow affected the stomatal functioning during the day although we cannot rule out the effect of endogenous factors related to the activity of the circadian rhythm in modulat-

ing stomatal functioning and P_N (Mendes and Marenco, 2014). The effect of VPD on P_N , g_s , and E showed that changes in intensity of light can alter the performance of the photosynthetic apparatus over the course of a day irrespective of the seasonal rainfall regime. In this study, we showed that, during the period when the water stress was most severe, *i.e.*, November, the photosynthetic rates of the tropical arid plants were significantly higher than the rates in the tropical wet plants, an effect that was strongly shifted at the beginning of the rainy season, which is the opposite to what should be expected for trees growing in a wet environment. Some investigators previously reported that *S. tuberosa* can maintain its Ψ_{pd} at a relatively constant level during the beginning of the dry season (Lima Filho, 2001; Lima Filho, 2004; Lima Filho, 2007; Lins Neto *et al.*, 2012). A possible explanation for this result is that the umbu tree reduces its stomatal conductance in response to drought early in the morning, resulting in a dramatic decline in plant water loss and assuring

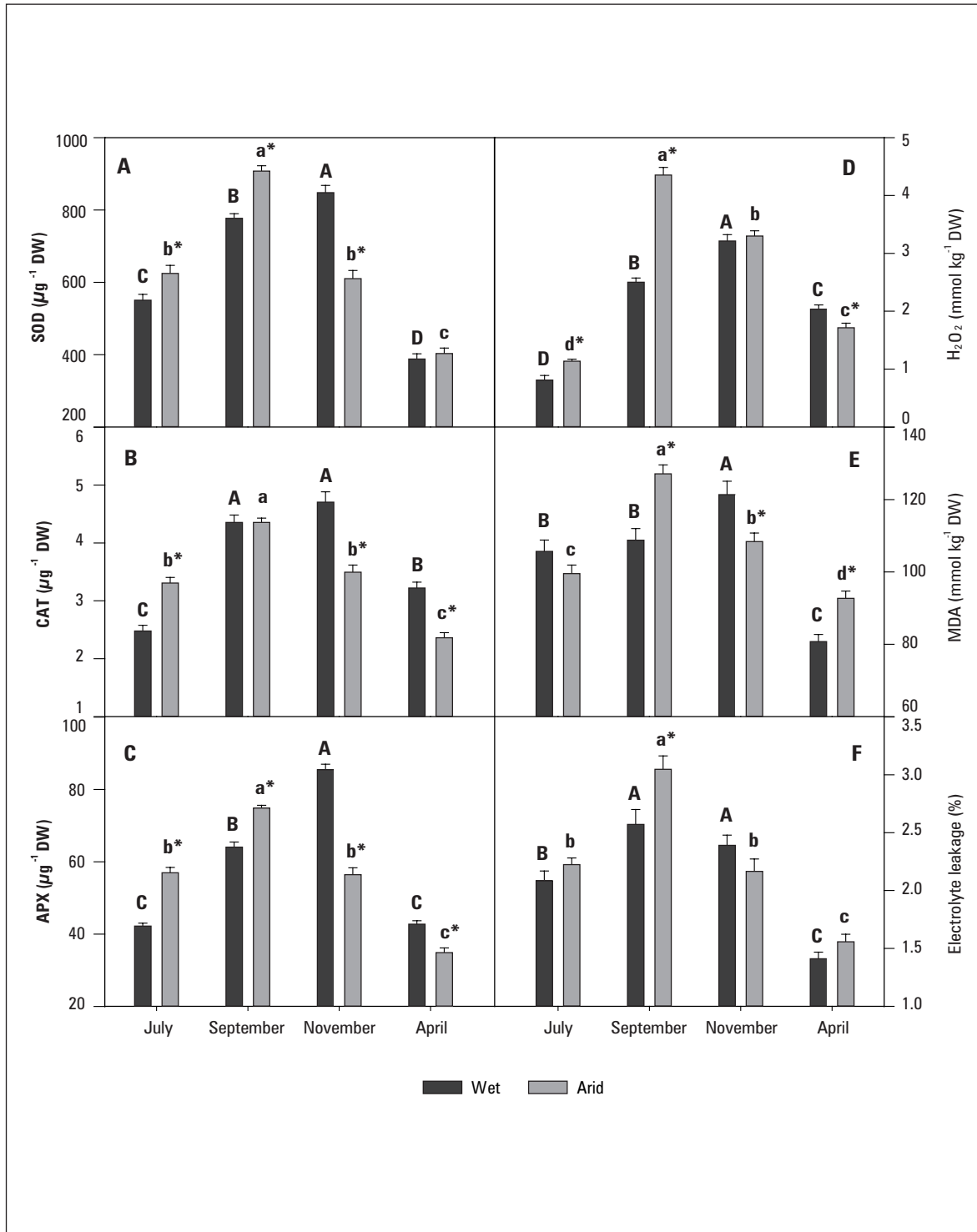


Figure 6. (A) Activities of antioxidant enzymes: superoxide dismutase [SOD], (B) catalase [CAT], (C) ascorbate peroxidase [APX], (D) hydrogen peroxide [H_2O_2], (E) malondialdehyde (MDA) and (F) electrolyte leakage measured in the tropical wet (filled bars) and tropical arid (unfilled bars) leaves in four different months in field-grown *Spondias tuberosa* trees. The measurements were taken at midday. The values represent the means (\pm SD). $n=10$. For statistical details, see figure 3.

significant water storage in the tubers (Lima Filho and Silva, 1988). However, as the water in the soil decreases, which leads to reduced water storage in the tubers, both E and g_s substantially decrease as a function of Ψ_{pd} (Lima Filho, 2004; Silva et al., 2008). *S. tuberosa* root tubers can serve as water storage vessels, and a higher root mass fraction, lower leaf masses and lower total leaf areas are observed in arid environments (Araújo et al., 2009; Lima Filho, 2004; Poorter et al., 2012), which may help the plants to perform better in droughts. These factors may play a substantial role in the amount of water supplied via tubers and the level of water conservation in leaves to maintain a higher P_N under an extreme drought. Despite greater volume of rains in October and November in the arid environment, all of the analyses were done after the rains in both locations throughout the year, excluding small variations of monthly accumulated rainfall as shown in figure 1A and allowing us to explore physiological patterns for seasonal fluctuation. Additionally, it is plausible to speculate that plants growing in areas with a lower water content throughout the year may have xylem vessels with lower diameters, as this represents an important mechanism for avoiding cavitation under extreme droughts (Nardini et al., 2014; Silva et al., 2013), while also restricting water flow when water is plentiful. The annual profile of leaf transpiration (figure 2E), which was focused on the morning period when the stomata are wide open, corroborates this proposition.

In the present study, the E was strongly affected by the VPD associated with a high temperature and high solar radiation, especially during the dry season (figure 2). The increased VPD led to an increase in transpiration, leading to evaporative leaf cooling due to latent heat loss if water was available. This is in agreement with results reported by others (Passos et al., 2009; Santos et al., 2013). If water was not available, a high irradiance (figure 2A), particularly during drought, may have been related to the extremely high leaf temperatures (figure 2B).

An increase in C_i due to stress could occur in response to low photosynthetic activity (Singh and Reddy, 2011); however, this is not a universal response. Stomatal closure typically leads to a decrease in C_i , thereby leading to a decrease in the $C_i:C_a$ ratio. The decreased C_i indicates that stomatal limitations are dominant under moderate but not in severe drought, as observed in tropical wet plants evaluated in November (Fig. 3B). At this time, the umbu leaves showed high $C_i:C_a$ ratios with a low P_N , which indicates that there is a limitation of carbon uptake imposed by nonstomatal factors. The dry season had a detrimental effect on the Rubisco activity or ATP synthesis; moreover, under progressive droughts, the mesophyll conductance (g_m) may decline (Flexas and Medrano, 2002; Lawlor and Tezara, 2009). This allows us to conclude that the non-stomatal limitation likely occurred along with the potential limitation of conductance of the mesophyll, which together must have accounted for the P_N decreases during the driest months (Ni and Pallardy, 1992; Silva et al., 2010).

When the photochemical quenching was reduced, the non-photochemical energy quenching (D and P_E) was increased (figure 4). The deconvolution of fluorescence signals clearly indicates decreases in the photochemical quenching parameters and damage to PSII. The lower extent to which PSII uses the energy absorbed by chlorophyll in photochemical reactions and the increase in non-photochemical signals suggests that trees grown in wet, tropical environments are damaged by excess light under droughts. The lower $F_v:F_m$ ($_{md}$) values (table 1) and the larger D and smaller Φ_{PSII} values (figure 4) observed throughout the year in trees grown in wet environments and subsequently exposed to drought conditions (*i.e.*, in November) supports this conclusion.

The decreased $F_v:F_m$ ratio implies a decrease in the capture and conversion rate of excitation energy by PSII reaction centers and, thus, a reduction in PSII photochemical efficiency (Ramalho et al. 1999). These results strongly suggest that

PSII disorganization appeared to be highly pronounced in sensitive plants, as compared with resistant ones (Huseynova, 2012). A decrease in Φ_{PSII} occurred after the P_{N} decrease, suggesting that nonstomatal limitations, rather than photochemical limitations, primarily limited P_{N} . Moreover, a reduced CO_2 supply is expected to negatively affect the Calvin cycle, which in turn would limit PSII efficiency (Stitt, 1991). These sequential decreases in the P_{N} and Φ_{PSII} under droughts have been described in the literature, as a decrease in photosynthesis intensity is one photoprotection mechanism used by plants to preserve the photochemical apparatus during stress (Genty *et al.* 1989). The decrease in the chlorophyll content in the tropical wet plants in the drought period (table 2), especially from September to November, suggests that photosystem damage occurred during this period and would have limited the speed of post-drought recovery. However, the drought-induced reduction in leaf pigments is considered to be a typical oxidative stress indicator that might be attributed to pigment photooxidation, chlorophyll degradation and/or chlorophyll synthesis deficiency (Campos *et al.*, 2012; Pompelli *et al.*, 2010a; Pompelli *et al.*, 2010b).

The decreased P values were not entirely offset by the increased D during the dry season, leading instead to a higher P_{E} . The increased P_{E} suggests that the down-regulation of PSII to prevent the over-reduction of Q_{A} was not sufficient to compensate for the decreased demand for electrons through NADP^+ consumption (Chaves *et al.*, 2008; Pompelli *et al.*, 2010b; Ramalho *et al.*, 1999). This outcome may in turn result in singlet oxygen formation and damage to membrane components, similar to that which occurs during light stress (Takahashi and Badger, 2011).

As the drought progressed and soil water content declined, the $\delta^{13}\text{C}$ increased and became less negative, with values lower than -27.2‰ , suggesting a carbon limitation induced by water limitation (Schifman *et al.*, 2012), as reported in this study (figure 5). The less negative values of $\delta^{13}\text{C}$ in the

tropical arid plants indicated that the water status was generally lower, with a consequent increase in the stomatal closure and some degree of CO_2 restriction in photosynthesis during the humid months. The opposite situation during the driest month (November) indicates that the tropical wet plants experienced greater restriction of CO_2 influx than the tropical arid plants.

The high carotenoid-to-Chl ratios during the dry season (table 2), particularly in leaves from the wet environments, may help the leaves to avoid photooxidative processes because, in addition to their role as secondary light-absorbing pigments, the carotenoids prevent the photooxidation of the photosynthetic apparatus by reducing the Chl triplet quencher, preventing the formation of singlet oxygen ($^1\text{O}_2$), or by acting directly on $^1\text{O}_2$ scavengers (Krieger-Liszczay 2005). These mechanisms may help to protect the plants, but they are not always sufficient to prevent photo-damage in dry leaves.

Plants subjected to water stress tend to overproduce ROS in different tissues (Carvalho, 2008), and antioxidant enzymes constitute an important line of cellular defense, detoxifying ROS compounds. However, we showed that the increases in the CAT and APX activities during the dry season were not enough to dissipate the excess, reducing the power accumulated in the H_2O_2 that was generated from the water-water cycle or in the SOD activity. In November, the driest month, the H_2O_2 and MDA levels were as high as they were in September, despite the increased activity of the enzymes in the antioxidative system. Although there were increases in the H_2O_2 , MDA and antioxidant enzymes levels, our results showed that *S. tuberosa* plants have the ability to increase their antioxidant activity during the dry season (figure 6) as a defense strategy against the deleterious effects, indicating that APX plays a positive role in the response to water stress. However, this mechanism is not robust enough to prevent damage. We attribute the improved drought tolerance of *S. tuberosa* plants that were grown in the arid environment

to the combination of high leaf Ψ_w , higher g_s and E , and evaporative leaf cooling that allow for the maintenance of a constant leaf temperature. The likely outcome is an increased net CO_2 influx and elevated P and Φ_{PSII} with an efficient sink for electrons from photosynthesis, thus preventing ROS production and reducing cellular oxidative damages. Our data do not indicate that modestly increased carotenoid pools can serve as an effective antioxidant system.

CONCLUSIONS

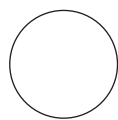
P_N and g_s in *S. tuberosa* trees appear to be highly sensitive to diurnal variations, and even when the photosynthetic parameters are affected by environmental factors (e.g., light and VPD), the stomatal response to a water deficit suggests that stomatal closure is the first line of defense against desiccation in umbu trees. Furthermore, the over-excitation of the reaction centers of PSII during the dry season may increase ROS production in various subcellular organelles, such as chloroplasts and peroxisomes. Although the physiological profile of *S. tuberosa* characterizes it as a drought tolerant species,

the P_N , g_s and E in the plants of the tropical wet population are likely to be limited by the water deficit during the dry season, unlike the umbu plants of the arid population. Such species, when growing under low water conditions, can acclimate to this abiotic stress and are able to survive subsequent drought periods with less damage, as compared with plants from tropical wet regions.

Finally, our results suggest that this species has great potential to acclimate to the altered environment predicted for arid/semiarid regions as a result of climate changes. However, further studies are needed to determine the effects of a prolonged dry season on *S. tuberosa* plants and to develop strategies to mitigate these effects.

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