Water deficit responses of *Euterpe edulis* Martius seedlings at different growth stages

Respostas ao déficit hídrico em mudas de *Euterpe edulis* Martius em diferentes estádios de desenvolvimento

Rodrigo Minici de Oliveira¹, Angelo Albano da Silva Bertholdi², Vera Lex Engel³, José Raimundo de Souza Passos⁴ e Luiz Fernando Rolim de Almeida⁴

**Resumo**

Mudas de *Euterpe edulis* de dois estádios de desenvolvimento (Classe A, 2 anos de idade, e Classe B, 4 anos de idade, com altura média de 16±1.3 e 49±3.3 cm, respectivamente), foram submetidas a três regimes de disponibilidade hídrica (déficit hídrico, reidratação e nova submissão ao déficit hídrico) em casa de vegetação. Avaliaramos parâmetros fisiológicos e morfológicos para detectar diferenças nas respostas ao déficit hídrico. Plantas da classe B mostraram fechamento estomático mais rapidamente, 14 dias após início do regime (DAIR) em comparação às plantas da Classe A (que só responderam após 17 DAIR). No regime de reidratação, a Classe B alcançou valores de condutância estomática semelhantes ao controle mais rapidamente (14 DAIR) em comparação às plantas da classe A (25 DAIR). Quando submetidas novamente ao déficit hídrico, plantas da Classe B apresentaram condutância estomática duas vezes maior do que o controle, enquanto a Classe A apresentou valores cinco vezes inferiores ao controle. Este padrão de resposta deve-se às diferenças morfológicas entre os estádios de desenvolvimento, que refletem diferente comportamento hidrodinâmico. Além disso, a Classe B apresentou relação raiz/parte aérea sistema radicular mais balanceada (0.91±0.32) em comparação à Classe A, que apresentou apenas 0.51±0.17. Concluímos que plantas em estádio de desenvolvimento superior são mais tolerantes a ciclos de déficit hídrico recorrentes. Os resultados apontam para o potencial de promover a rustificação de plantas de *Euterpe edulis* em viveiro.

**Palavras-chave:** palmeira-juçara, estádios de desenvolvimento, déficit hídrico, reidratação, respostas ecofisiológicas e morfológicas.

**Abstract**

*Euterpe edulis* plants at two developmental stages (Class A, two-year-old seedlings, and Class B, four-year-old-saplings, with a mean height of 16.0±1.3 and 49.0±3.3 cm, respectively), were subjected to three successive water availability regimes (water deficit, re-watering and subsequent water deficit after re-watering) in greenhouse conditions. We assessed physiological and morphological parameters to detect differences in the water deficit response. Saplings showed a more rapid stomata closure 14 days after the beginning of the regime (DABR) than seedlings (which only showed a response after 17 DABR). Furthermore, the stomata conductance of saplings subjected to the re-watering regime reached the same value as the controls faster (14 DABR) than that of seedlings (25 DABR). When again subjected to water deficit, saplings showed a two-fold higher stomata conductance than the control plants, whereas seedlings showed a five-fold lower value. This response pattern can be attributed to morphological differences between the developmental stages, which is reflected in different hydrodynamic behaviors. Saplings showed a more balanced root/aerial shoot ratio (0.91±0.32), whereas the ratio for seedlings was 0.51±0.17. We conclude that more developed plants are more tolerant to recurrent cycles of water deficit. The results suggest the potential to harden “jussara” palm plants in nurseries.

**Keywords:** “jussara” palm, growth stages, drought, rehydration, eco-physiological and morphological responses.
INTRODUCTION

The global distribution of the *Arecaceae* family is restricted to tropical and subtropical climates, as its members prefer regions with a constant high humidity and high temperature throughout the year (EISERHARDT et al., 2011; SALM et al., 2007). This limited geographical distribution has been attributed to physiological and morphological traits, mainly due to the relative absence of drought-tolerance and avoidance mechanisms (HENDERSON et al., 1995; TOMLINSON, 2006). However, even typical plants from humid habitats might experience severe water deficit, especially in atypical drier years (COMITA et al., 2009). Thus, the capacity to tolerate water stress is an important attribute for the persistence of the *Arecaceae* family in ecosystems.

*Euterpe edulis* Martius ("jussara" palm) is the main representative of the *Euterpe* genus in the Brazilian Atlantic Forest. It has gained considerable commercial interest for palm hearts since the mid-twentieth century, and due to overexploitation, the species came close to extinction in its natural habitats (REIS et al., 2000). Originally, *E. edulis* populations occurred at a high density and frequency in rainforests, and were more restricted to riparian habitats in seasonally dry environments (REIS et al., 2000), demonstrating the preference of the species for humid environments (MATOS et al., 1999).

Soil moisture has been considered a key factor for the establishment, development and initial growth of *Euterpe edulis* (BOVI et al., 1987). Previous studies have indicated that the soil-water deficit is the main limiting factor for the initial establishment of *E. edulis* in areas undergoing restoration with the occurrence of a dry season (RM OLIVEIRA et al., unpubl. res.). Together with severe water deficit, unfavorable light incidence conditions can result in high mortality rates (NAKAZONO FILHO et al., 2001), as this species is shade-tolerant. Nevertheless, the species responds differentially to soil water availability, according to its developmental stage. Although plants in early stages of development require wet habitats for their initial establishment (BOVI et al., 1987), older saplings and juvenile plants show higher growth rates on well-drained sites (NOGUEIRA Jr. et al., 2008).

The mortality rate of *E. edulis* in natural forests is considerably higher for seedlings, and decreases for plants over 50 cm in height (CONTE et al., 2003; FANTINI; GURIES, 2007). This is compatible with the species’ reverse "J" population structure (CONTE et al., 2003); these authors found expressive mortality rates for plants less than 10 cm tall in natural regeneration dynamic studies. They also found reduced mortality rates for plants between 11–50 cm tall, and a mortality rate of almost zero for individuals taller than 50 cm. These initial slow growth and high mortality rates were attributed to several factors, such as herbivory, leaf and branch fall from the surrounding trees, disease, as well as to intra- and inter-species competition. Abiotic factors, such as inadequate light levels, soil fertility, precipitation, temperature, and plant fragility due to the early developmental stage might also be constraints. The survival of plants over 50 cm tall was attributed, however, to a greater phenotypic plasticity, resulting in greater competition ability within the forest (CONTE et al., 2000). Nevertheless, to date, no eco-physiological studies have been undertaken to test these hypotheses.

Knowledge concerning the eco-physiological responses to environment conditions is especially relevant for *E. edulis*, due to the urgent need to restore populations of this endangered species (ME-LITO et al., 2014), as well as due to the need for its successful *ex situ* cultivation.

To understand better the eco-physiological responses of *E. edulis* to soil-water deficit, we tested the effect of different water regimes on plants at two developmental stages. We hypothesized that seedlings of *E. edulis* Martius respond differentially to soil water deficit at different developmental stages. Our prediction was that older plants (saplings) can cope better with soil-water deficit and climate change than small seedlings, through the development of physiological and morphological adaptations.

MATERIAL AND METHODS

Site description

The study was conducted in the nursery of the Forest Science Department, School of Agronomic Sciences, UNESP, Botucatu, São Paulo, Brazil (22°51’S, 48°25’W), from August to November 2013. The site is located at 786 m above sea level, and the local climate is Cfa, according to the Köppen system, i.e., warm-temperate humid (mesothermal), with a mean temperature of the hottest month abo-
April, July and August are the months subjected to water deficits. The annual potential evapotranspiration reaches 945.15 mm, 33% of which occurs during the summer (CUNHA; MARTINS, 2009).

The experiment was performed in a semi-controlled environment (a transparent plastic-covered greenhouse). The environmental conditions (vapor pressure deficit – VPD and photosynthetic photon flux density – PPFD) of the greenhouse were monitored whenever the plants were evaluated, between 9:00 and 11:00 a.m. The mean values of VPD and PPFD during the study period were 33 ± 0.02 % and 1228 ± 238 μmol m⁻² s⁻¹, respectively.

Plant material

We used plants of *Euterpe edulis* belonging to two developmental stages: Class A (two-year-old seedlings, 16 ± 1.3 cm tall, 1.4 ± 0.2 cm base diameter, 4.0 ± 0.5 fully-expanded leaves, 228 ± 90.5 cm² leaf area), and Class B (four-year-old saplings, 49 ± 3.3 cm tall, 4.6 ± 0.5 cm neck diameter, 5.0 ± 0.7 expanded leaves and 2,643 ± 708 cm² leaf area).

Class A plants were grown in root-trainers from seeds collected in August 2011. Class B plants were cultivated initially on a sphagnum and vermiculite substrate (Carolina Soil ®) in 90-mL root trainers and were subsequently transplanted into 7-dm³ plastic containers, with a substrate composed of mineral soil and cattle manure (2:1); seeds were collected in August of 2009. The seeds of both classes were collected at Carlos Botelho Forest Reserve, São Paulo State.

At the beginning of the experiment, all seedlings were transplanted into 50-dm³ plastic containers, containing a clayey soil substrate of medium texture collected from Dark-Red Latosol topsoil (EMBRAPA, 2013).

Experimental design

The experiment consisted of a complete random factorial 2 × 3 design with two developmental stages, three water regimes, and 12 plants per treatment.

We subjected 48 plants (24 from each developmental stage) to acclimatization in the greenhouse for forty days, and kept that soil at about field capacity (FC). A soil water tension of 0.03 MPa was used as the measure of FC, which corresponds to 25.03% of retained water (dm³/dm³).

The water content necessary to maintain FC was determined daily using a digital tensiometer, in addition to the correspondence of the observed tension values with the retained water values, which were estimated by adjusted soil-water retention curves using Equation 1:

\[ y = -0.0334x^3 + 0.0567x^2 - 0.2553x + 0.5019; \]  

(Equation 1, \( R^2 = 0.9805 \)),

where: \( x = \) observed soil moisture (KPa) and \( y = \) corresponding value of water potential (MPa).

From the beginning of the experiment, 24 plants were maintained at FC by irrigation: 12 plants from Class A and 12 from Class B. The same number of plants from each class was subjected to different water-availability regimes:

1. Water Deficit 1 (WD1) – 31 days of water deficit;
2. Re-watering (R) – maintenance at FC for 25 days,
3. Water Deficit 2 (WD2) – plants were subjected to water deficit after re-watering.

Plants of classes A and B were acclimatized under irrigation at field capacity (FC), in the beginning of the WD1 regime. The soil amount used for the two classes was the same. In the beginning of experiment, water availability was the same in both classes, independent of the morphological differences relating to classes.

Evaluations were always performed on clear, cloudless days, with a minimum interval between evaluations of one day. The end of the regime was considered to be the death of at least four plants from the same treatment, either for WD1 or WD2. The duration of the re-watering regime was determined by the full recovery of stomata conductance values to the control level.

Physiological Evaluations

Stomata conductance (\( g_s \)) was evaluated using a leaflet from the intermediate region of the youngest and fully expanded leaf. Data were collected using a Steady State Porometer (Leaf Porometer SC-1; Decagon Devices, USA), from 9:00 to 11:00. The VPD was calculated using air temperature
and humidity data, collected by the porometer, using Equation 2 after Jones (1992):

\[
VPD = 0.61137e^t \times (1 - \frac{RH}{100}) \quad \text{(Equation 2)}
\]

Where: VPD is the percentage vapor-pressure deficit, RH is the relative air humidity and t is obtained by the following equation:

\[
t = \frac{(17.502 \times T_{air})}{(240.97 + T_{air})} \quad \text{(Equation 3)}
\]

Where: T_{air} represents air temperature.

We assessed the stomata conductance (gs) throughout the different water-availability regimes evaluating four plants per treatment at 3, 8, 10, 14, 17, 22, 28, and 31 DABR for WD1; three plants per treatment at 6, 14, 18 DABR for R1; and three plants per treatment at 25 DABR for WD2.

**Morphological evaluations**

We assessed the root/aerial shoot ratio via the destructive analysis of four plants per treatment, at the end of the WD1 and WD2 regimes. The plants were oven-dried to constant weight and the shoot and root dry mass were quantified. All plants in each treatment which had fully expanded leaves were counted at the beginning and end of WD1.

**Data analysis**

A completely randomized design with a factorial treatment structure was used to measure the gs response, with time (days) and water availability regime as factors. The same design was used to analyze the root/aerial shoot ratio response variable, considering only the water availability regimes as factors. In each case, we adjusted generalized linear models (GLM) with a gamma distribution response and logarithmic linking function (DIGGLE et al., 2002; NELDER; WEDDERBURN, 1972). We used a completely randomized design to assess the response variable, using the water deficit regime according to treatments as factors. Generalized linear models, with a Poisson response and logarithmic linking function were adjusted, considering repeated measures (paired, at the beginning and end of the experiment) (DIGGLE et al., 2002; NELDER; WEDDERBURN, 1972).

For all models, the goodness-of-fit was assessed by deviance analysis. To perform comparisons among treatments within each factor, we used the lsmeans test from the genmod procedure in SAS Software (release 9.2, 2012), with 95% confidence intervals.

**RESULTS AND DISCUSSION**

The first significant reduction in gs values compared to the controls occurred at 14 DABR for Class B plants and at 17 DABR for Class A plants, considering individuals subjected to the WD1 regime. From 17 DABR until the end of WD1, both classes showed gs values of about zero (Figure 1).

Stomata closure prevents excessive water loss by transpiration. Thus, partial stomata closure can indicate adaptation mechanisms to limit water loss (CHAKHCHAR, 2015; FIGUEIREDO et al., 2015). This rapid stomata closure observed initially for Class B plants in the WD1 regime might reflect existing morphological differences between classes, which triggered hormonal changes at different time-points and intensities. For example, the greater leaf area of Class B plants than Class A plants might have led to a higher water loss and consequently to a more rapid consumption of available substrate water. Therefore, stomata response occurred more rapidly for plants in Class B, as a result of the detection of water deficit in the substrate by the roots (MA et al., 2015; SCHACHT-MAN; GOODGER, 2008).

Compared to other palm species cultivated in similar conditions, the triggering of stomata closure occurred earlier in *E. edulis*, indicating that this species is less tolerant than others. In five-month-old “buriti” palm (*Mauritia vinifera*) seedlings, stomata closure occurred following about day 40 of water deficit with near zero values (CALBO; MORAES, 1997). In contrast, in four-month-old plants of *Euterpe oleacea*, the “assai” palm, stomata closure occurred after 53 days of water deficit (CALBO; MORAES, 2000).

At the end of the WD1 regime, an adjustment of the root/aerial shoot ratio to values near 1.0 was detected only for Class B plants (Table 1).
Values represent means ± confidence intervals (WD1, n = 4; R1 and WD2, n = 3). Comparisons were assessed by the lsmeans test (p < 0.05).

**Figura 1.** Stomata conductance ($g_s$) of *Euterpe edulis* plants (Classes A and B), according to the water-availability regime.

As a response to water deficit, plants prioritize root growth rather than that of shoot parts, to favor water absorption and to minimize transpiration (HOJATI et al., 2011; KOZLOWSKI; PALLARDY, 2002). A reduction in the leaf number contributes to an increase in the root/aerial shoot ratio through a decrease in the dry weight of aerial parts in Class A and B plants. The leaf number of Class A plants decreased from 4.0 ± 0.9 to 1.0 ± 0.9, whereas that of Class B plants decreased from 5 ± 0.5 to 2.0 ± 0.6, and the control plants did not lose any leaves (Class A - 4.0 ± 0.4; Class B - 5.0 ± 0.6).

The recovery of $g_s$ values to the control level occurred more rapidly in Class B plants than class A plants, at 14 DABR of re-watering for Class B (day 45 of the experiment) and only at 25 DABR after re-watering (day 56 day of the experiment) in Class A (Figure 1). This response pattern might be due to morphological differences between the plants in each class. The larger root and shoot system of class B plants suggests a greater ability to exploit the available water in the container and more water being lost through transpiration, leading to the rapid rehydration of tissues and consequent stomata opening. Calbo and Moraes (1997, 2000) showed that “buriti” palm seedlings recovered 70% of their $g_s$ 5 days after re-watering, whereas “assai” palm plants recovered 90% $g_s$ 12 days after re-watering.

Both classes of plants were unable to restore $g_s$ values to the control levels at 6 DABR of re-watering (37 days after the experimental start). We believe that the slow stomata response observed in this study is related to the species’ characteristic of shade tolerance. Plants that are tolerant to high shade levels usually have large, low-density stomata, which results in a slow stomata response that might be harmful in the dry season (AASAMAA et al., 2001; VALLADARES; NIINEMETS, 2008). Other studies have also demonstrated a weak stomata regulation in *Euterpe edulis* (GATTI et al., 2014; SANTOS et al., 2013).
After the return to water deficit in the WD2 regime, Class A plants showed a five-fold lower g\(_{sva}\) value than the control, whereas Class B plants showed a two-fold higher g\(_{sva}\) value than the control. This emphasizes further the different responses to water deficit between plants of each class. The shoot-root ratio of saplings of Class B was significantly higher than that of Class A saplings, although plants of neither class differed significantly from their respective controls. The difference might be due to a more equivalent root/aerial shoot ratio (closer to 1.0) for plants in Class B (Table 1). These results indicate the potential to increase the tolerance of \(E. edulis\) plants to water stress by hardening them in the nursery, especially at more advanced developmental stages. This would result in more vigorous plants, with a greater survival rate and greater initial establishment growth after planting (KOZLOWSKI; PALLARDY, 2002; YANG et al., 2015).

**CONCLUSIONS**

\(Euterpe edulis\) plants respond differently to water deficit at different developmental stages: older plants control water loss more efficiently, due to morphological traits that affect the efficiency of stomata closure.

After a drought and rehydration treatment, \(Euterpe edulis\) saplings show a great ability to tolerate a recurring cycle of drought than younger seedlings.

**ACKNOWLEDGMENTS**

We thank the "Brazilian Federal Agency for Support and Evaluation of Graduate Education" for the MSc scholarships to Rodrigo Minici de Oliveira and Angelo Albano da Silva Bertholdi. “National Council for Scientific and Technological Development” (CNPq), Proc. no. 485253/2013-5 and the “São Paulo Research Foundation” (FAPESP) for financial support. VL Engel has a Research Productivity Fellowship level 2 from CNPq.

**REFERENCES**


