



Research paper

Hydraulic architecture and photoinhibition influence spatial distribution of the arborescent palm *Euterpe edulis* in subtropical forests

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Physiological characteristics of saplings can be considered one of the most basic constraints on species distribution. The shade-tolerant arborescent palm *Euterpe edulis* Mart. is endemic to the Atlantic Forest of Argentina, Brazil and Paraguay. At a local scale, saplings of this species growing in native forests are absent in gaps. We tested the hypothesis whether sensitivity to photoinhibition or hydraulic architecture constrains the distribution of *E. edulis* saplings in sun-exposed forest environments. Using shade houses and field studies, we evaluated growth, survival, hydraulic traits and the susceptibility of Photosystem II to photoinhibition in *E. edulis* saplings under different growth irradiances. Survival rates in exposed sites in the field were very low (a median of 7%). All saplings exhibited photoinhibition when exposed to high radiation levels, but acclimation to a high radiation environment increased the rate of recovery. Petiole hydraulic conductivity was similar across treatments regardless of whether it was expressed per petiole cross-sectional area or per leaf area. At the plant level, investment in conductive tissues relative to leaf area (Huber values) increased with increasing irradiance. Under high irradiance conditions, plants experienced leaf water potentials close to the turgor-loss point, and leaf hydraulic conductance decreased by 79% relative to its maximum value. *Euterpe edulis* saplings were able to adjust their photosynthetic traits to different irradiance conditions, whereas hydraulic characteristics at the leaf level did not change across irradiance treatments. Our results indicate that uncoupling between water demand and supply to leaves apparently associated with high resistances to water flow at leaf insertion points, in addition to small stems with low water storage capacity, weak stomatal control and high vulnerability of leaves to hydraulic dysfunction, are the main ecophysiological constraints that prevent the growth and survival of *E. edulis* saplings in gaps in the native forest where native lianas and bamboos show aggressive growth.

Keywords: gap, hydraulic conductivity, leaf hydraulic conductance, leaf water potential, semideciduous Atlantic Forest, understory.

Introduction

Understanding the mechanisms that determine plant species abundance and distribution has been an important goal in plant ecology over the last few decades (Brown 1984, Schnitzer 2005), gaining new relevance under different global climate

change scenarios (Parmesan and Yohe 2003). Although species occurrence in any given site is the result of a series of filters, i.e., historical, physiological and biotic factors such as dispersal ability and competition (Lambers et al. 1998, Kempel et al. 2013), physiological limitations can be considered one

of the most basic and fundamental constraints on the distribution and abundance of organisms (Kearney et al. 2008, Kearney and Porter 2009). For example, drought vulnerability has an important, if not central, role in determining the distributional limits of plant species at both regional and local scales (Brodribb and Hill 1999, Engelbrecht et al. 2007, Baltzer et al. 2008, Kursar et al. 2009, Blackman et al. 2012). In the case of arborescent palms, hydraulic architecture (Renninger et al. 2013), water use and water balance have received less attention as determinants of spatial-pattern distribution than in woody trees.

Euterpe edulis Mart., a monopodial arborescent palm endemic to the Atlantic Forest of Argentina, Brazil and Paraguay, is conspicuous in the understory and canopy of many stands (Henderson et al. 1995). In the southern part of the Atlantic Forest (Misiones Province, Argentina), with a slightly undulating topography, temperatures under 0 °C are a major determinant of its distribution at a landscape scale, restricting this species to raised areas of this land, where freezing temperatures are nearly absent (Gatti et al. 2008). In these higher parts of the landscape, where minimum temperatures never drop below 4 °C (Gatti et al. 2008), this species is highly abundant, reaching >500 stems ha⁻¹ larger than 10 cm dbh (diameter at breast height) (Chediack 2008). However, even in forests without freezing temperatures, the distribution of *E. edulis* saplings is heterogeneous, being mostly absent in gaps and open sites (Gatti et al. 2011). The overall aim of this study was to understand ecophysiological mechanisms underlying local spatial patterns of distribution of this species in native forest ecosystems.

In the studied forest stands, the uprooting and snapping of canopy trees are a frequent cause of gap formation. The size of the gap depends on the size of fallen trees and on the effect of abundant lianas attached to the host and neighbouring trees, as well as selective harvesting of commercial trees in non-protected forests (Campanello et al. 2007a, Montti et al. 2014). The sudden increase in irradiance as a consequence of gap formation may result in physiological stress in plants exposed to the newly formed environment. Among the physiological stresses that these plants might experience, photoinhibition (Langenheim et al. 1984) and low rates of water supply to leaves, resulting in water deficits when evaporative demands are high (Maherali et al. 1997, Renninger et al. 2007), have a substantial impact on plant growth (Caquet et al. 2009, Krause et al. 2012), survival and distribution (Long et al. 1994). The extent of photoinhibition depends, in part, on the degree of acclimation of leaves to different light levels (Mulkey and Pearcy 1992, Durand and Goldstein 2001). Given that *E. edulis* is mainly absent in gaps in the Argentinean Atlantic Forest, we hypothesized that its susceptibility to photoinhibition plays an important role in determining its distribution at a local scale.

Palms exhibit low drought tolerance (Eiserhardt et al. 2011 and references therein). In adult individuals, the lack of secondary growth may impose hydraulic limitations through a decreased functioning in conductive tissues with age, while in saplings a sustained growth of conductive tissues occurs until the final stem diameter is attained (Zimmermann 1973, Tomlinson 1990). Consequently, phenotypic plasticity in hydraulic traits could be expected during this establishment phase in palm saplings. On the other hand, *E. edulis* is a shade-tolerant species (Sedrez dos Reis et al. 2000). Compared with fast-growing species, slow-growing, shade-tolerant woody species appear to have low plasticity with regard to adjusting their hydraulic traits to accommodate higher transpirational demands associated with increased irradiance (Campanello et al. 2008, 2011). We hypothesized that the low plasticity of hydraulic traits may inhibit the development of *E. edulis* saplings under high irradiance conditions. We expected that palm saplings would show leaf water deficits under high radiation levels and a low ability to adjust hydraulic traits to different levels of irradiance.

Materials and methods

Study site and species characteristics

The climate in the subtropical Atlantic Forest of Argentina is characterized by average monthly temperatures of 25 °C in January and 15 °C in July, the hottest and coldest months, respectively. Annual precipitation ranges from 1900 to 2100 mm (Ligier et al. 1990). Rainfall is evenly distributed throughout the year, but short dry spells can occur. Freezing temperatures during winter are frequent in low topographic areas near streams and rivers (Gatti et al. 2008). The forest physiognomy is characterized by mature trees ranging from 20 to 45 m in height and abundant lianas, epiphytes and bamboo species in the understory. The canopy is mostly dominated by Lauraceae, Fabaceae, Meliaceae and Myrtaceae trees (Campanello et al. 2007b). Gaps ranging between 4 and 30 m² are common in this forest (Montti et al. 2014).

Euterpe edulis is a single-stemmed arborescent palm species. Reproductive individuals are at least 15 m tall, dominating the middle canopy of some Atlantic Forest stands (Henderson et al. 1995). Because it is a monopodial palm species, individuals do not survive after cutting the apical meristem, which is required to harvest palm hearts (Galetti and Fernandez 1998).

Experimental design and ecophysiological variables

An outline of the experiments performed, treatments and variables measured in each case is shown in Figure 1. We carried out a shade-house experiment with a completely randomized factorial design, with four fixed levels of radiation and a minimum of 60 plants per treatment. We grew *E. edulis* plants from seeds collected in July 2000 from 10 different individuals growing in a

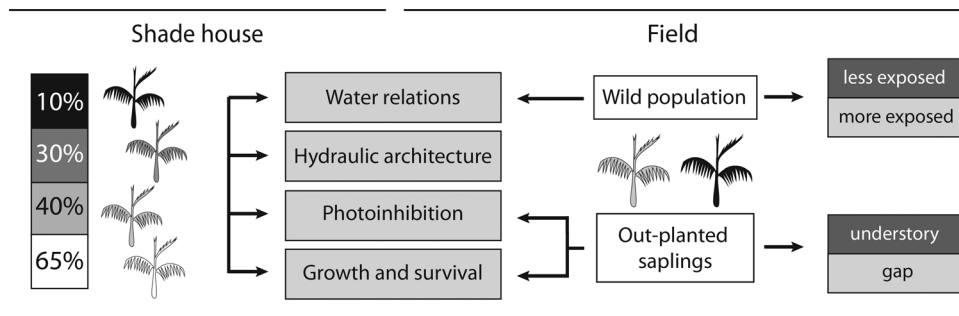


Figure 1. Outline of experiments, radiation treatments and traits under study in the present work. Shade-house experiments (left): percentages indicate the four different growth irradiance treatments (% of incident solar radiation) evaluated in *E. edulis* saplings. Field experiments (right) were developed both in a wild population under two different conditions of plant radiation exposure (more and less exposed) and with out-planted saplings growing in two different radiation environments: gap and understory. Arrows link the different experiments developed with variables under study.

wild population at the Iguazú National Park, 25°39'S—54°12'W (see details in Gatti et al. 2011).

We created four different growth irradiance conditions using neutral density filters, reproducing a range of microclimatic conditions normally found in this forest (Campanello et al. 2007b). The irradiance treatments were 10, 30, 40 and 65% of the maximum incoming solar radiation, resulting in average photosynthetic photon flux densities (PPFDs) of 2.6 ± 0.4 , 8.1 ± 1.5 , 10.9 ± 1.7 and $16.4 \pm 2.6 \text{ mol m}^{-2} \text{ day}^{-1}$ (means \pm SE, $n = 4$ clear summer days), respectively. Saplings were 1 year old when we randomly assigned 240 plants to the four irradiance treatments. The youngest leaf in each individual was marked in order to identify new leaves developed under the experimental growing conditions. Within each irradiance treatment, we monitored PPFD, relative humidity and air temperature. Air saturation deficits were similar across all treatments (Campanello et al. 2008).

In addition, we conducted two different field experiments with out-planted individuals and with wild populations of saplings growing in the understory of native forests exposed to relatively high and low light levels (Figure 1). We out-planted to the field *E. edulis* saplings, obtained from seeds, on 10 permanent $15 \times 15\text{-m}$ plots: five natural gaps and five understory sites. The mean incident solar radiation was 26 and 11% of the maximum incoming solar radiation for gaps and understories, with a mean maximum temperature of 28.6 and 30.8 °C, respectively (Villagra 2012). In each plot, 15 6-month-old *E. edulis* saplings were out-planted in June 2004. Each plant was tagged with a numerical label. We monitored growth and survival (see below) for 2 years.

We selected 12 plants from a wild population of *E. edulis* inside the Iguazú National Park, similar in size to those studied in the shade house. In each plant, we measured incident photosynthetic active radiation in tagged leaves (one leaf per plant) during the daytime. Plants were classified into two groups depending on the different levels of irradiance: more exposed and less exposed plants (16 and 2% of the full radiation

reaching the canopy, respectively). Average PPFDs at midday were 166 ± 59 and $18 \pm 7 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ (means \pm standard error, SE), respectively. All the saplings found were growing in the forest understory (they are absent in gaps) and consequently the PPFD experienced by the saplings during the study period was low.

Growth and survival

We used changes in stem diameter as an indicator of plant growth (Gatti et al. 2011) because during this initial stage of palm development stems increase in diameter until most of the xylem tissue is formed, and then start growing in height (Tomlinson 1990). Stem diameter was measured at the beginning and at the end of the experiments in saplings growing in the shade house and out-planted in the field, and we calculated growth as

$$\text{Growth} = \frac{\varphi_f - \varphi_i}{\varphi_i}, \quad (1)$$

where Growth is a dimensionless variable (mm mm^{-1}), φ_i is initial stem diameter and φ_f is final stem diameter.

We estimated survival rate as the percentage of green plants remaining in each treatment in the shade house and light environment in the field. *Euterpe edulis* saplings in the shade house had an initial mean stem diameter of 13.8 mm (± 0.4 SE, $n = 30$) and a plant height of 42.3 cm (± 1.2 SE, $n = 29$). Plants in the field had a mean initial stem diameter of 3.7 mm (± 0.1 SE, $n = 40$) and a height of 6.2 cm (± 0.3 SE, $n = 40$) at the height of the uppermost leaf insertion.

Photoinhibition

We assessed susceptibility to photoinhibition in *E. edulis* grown under different light environments with a PAM fluorometer (Mini-PAM, Walz, Germany) following the procedures described by Durand and Goldstein (2001).

Shade-house experiment Four plants per light treatment were randomly selected. In the morning, we placed plants

under sub-saturating light levels for 20 min followed by 10 more minutes in the dark to ensure that all Photosystem II (PSII) reaction centres were oxidized (open). We measured the maximum quantum efficiency of PSII photochemistry (F_v/F_m) three times in different sites in one leaflet, per leaf per plant, waiting 5 min between measurements. Plants were then subjected to full solar radiation ($>2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 90 min. At the end of the full sun exposure period, we placed plants under sub-saturating light levels ($<20 \mu\text{mol m}^{-2} \text{s}^{-1}$) and we monitored the recovery of F_v/F_m for 80 min. We used a decrease in F_v/F_m values from the maximum as an indicator of the degree of PSII inactivation (Barth et al. 2001, Durand and Goldstein 2001).

Field measurements We evaluated PSII photoinhibition to high radiation levels on saplings which grew in gap and understory environments following the same procedure mentioned above, on three plants per treatment (Figure 1). Plants were exposed to $2500 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with a halogen lamp for 20 min, and we monitored the recovery of F_v/F_m for 40 min.

Hydraulic architecture

We measured leaf hydraulic conductivity and biomass allocation in conductive tissues relative to leaf area under different irradiance conditions in the shade house (Figure 1).

Leaf hydraulic conductivity Hydraulic conductivity was measured in fully developed leaf petioles under the four irradiance treatments in the shade house. Before dawn, we moved potted plants to a laboratory, where they were kept in darkness and well irrigated. At least 12 leaves from 12 different plants from each light treatment were cut and immediately re-cut under water—their cut ends were smoothed under water with a razor blade. The basal portion of the leaf blade, where the petiole ends and the rachis begins, was also cut and separated. We removed leaflets and bagged them for leaf area determination. As petioles have a longitudinal split, they were filled with inert putty and petiole ends were sealed with parafilm around the cuts in order to prevent water leakage. Petiole segments at least 7 cm long were perfused with degassed and filtered ($0.1 \mu\text{m}$) water under a hydrostatic pressure head of $3.4\text{--}7.4 \times 10^{-3}$ MPa, and allowed to reach equilibrium for 5 min before the flow rate was volumetrically measured. We maintained the room temperature at 25°C during measurements. We measured hydraulic head pressure and the length of the segment for hydraulic conductivity calculations (k_h ; $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$). We used petiole cross-sectional area as an analogue of sapwood area to obtain a proxy of specific hydraulic conductivity (k_s ; $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) (Renninger and Phillips 2011). The leaf area for each sample was scanned and measured using image analysis software (Scion Image, National Institute of Health, Bethesda, MD,

USA), thus enabling hydraulic conductivity to be expressed as leaf-specific conductivity (k_l ; $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$).

Huber value We calculated the Huber value, which relates the stem cross-sectional area with the leaf area distal to the stem segment (Tyree and Ewers 1991), at two different levels—one for leaves and one for whole plants—for palm saplings under the four irradiance treatments in the shade house. For the leaf Huber value, we divided the petiole cross-sectional area (m^2) by the leaf surface area (m^2 , $N = 12$). Leaf area was measured in leaves used for conductivity studies (see above). For the plant Huber value, we divided the stem cross-sectional area (m^2) (estimated from the stem diameter) by the total leaf area of the plant (m^2 , $N = 54\text{--}58$). Stem diameter and total leaf area were measured at the same time. Total leaf area was calculated using the number of leaves per plant and the average leaf size of harvested plants growing under similar light conditions (Gatti et al. 2011).

Water relations

Shade-house experiment We determined midday leaf water potential on four plants randomly chosen from the lowest and highest irradiance treatments (10 and 65%). The stem water potential (Ψ_s) was estimated from covered, non-transpiring leaves (Begg and Turner 1970). We enclosed one leaf portion containing three to four leaflets per plant in aluminium foil and plastic bags at predawn. Leaflet water potential (Ψ_l) was determined in exposed leaflets. At midday, we cut one covered and one uncovered leaflet of the same leaf per plant and we immediately measured their water potential using a pressure chamber (PMS, Albany, OR, USA).

Field study in a wild population During summer (December), we took a daily course of leaf water potential and stomatal conductance in the two groups of plants selected in the field. A fully expanded leaf in each of the 12 plants selected (six per group) was tagged the day before the experiment. Leaf water potential was measured on exposed leaflets. For this, we cut one exposed leaflet every 2 h, and immediately bagged it and kept it in a cooler to prevent desiccation. We measured water potentials with a pressure chamber in the laboratory within 2 h of leaf excision. In addition, the course of stomatal conductance was measured with a steady-state porometer (LI-1600, Li-Cor, Lincoln, NE, USA) in three plants randomly selected from each group. Stomatal conductance measurements began at 9 am because of dew formation in the early morning.

Pressure–volume curves and vulnerability of leaf hydraulic conductance to drought We constructed pressure–volume curves and calculated leaf hydraulic conductance (K_{leaf}) in leaflets of saplings growing at 30% irradiance in the shade house (Figure 1). Saplings grown under this treatment exhibited the

highest rates of photosynthesis, growth and survival (this work and Gatti et al. 2011). Pressure–volume relationships (Tyree and Hammel 1972) were obtained from six leaflets of *E. edulis* saplings under 30% growing irradiance in the shade house using the dehydration method (see details in Villagra et al. 2013). We estimated the turgor-loss point from plots of $-1/\Psi_L$ against the relative water deficit. These data allowed the estimation of mass of water per unit leaf area, leaf dry mass per unit area and absolute leaf capacitance before and after the turgor-loss point, which was necessary for the calculation of K_{leaf} (see below).

We constructed a vulnerability curve of hydraulic conductance of the leaf lamina (K_{leaf}) following the method by Brodrigg and Holbrook (2003). Plants were well irrigated the night before the experiment. Early in the morning we cut a leaf section containing five to seven leaflets and left it to dehydrate to different levels of Ψ_L on the laboratory bench. The samples were then bagged for 30 min in order to reach Ψ_L equilibrium between leaflets. We cut two leaflets and measured the initial Ψ_L . If differences >0.1 MPa were observed, we discarded the leaf section, and a new one was used. We determined initial Ψ_L in leaflets adjacent to those used for partial rehydration (Brodrigg and Holbrook 2003). The rachis supporting the basal part of a leaflet was then re-cut under water and allowed to rehydrate for 15 s (the time required for rehydration was previously determined). We immediately blotted the submerged portion of the leaflet with a paper towel and new Ψ_L determination was made in order to estimate Ψ_L after partial rehydration. The K_{leaf} of the sample was calculated according to Brodrigg and Holbrook (2003). We repeated this procedure with several leaflets from six individuals, with different initial Ψ_L . We built vulnerability curves by plotting K_{leaf} against initial Ψ_L and fitting a cumulative normal probability curve to the data.

Data analysis

We statistically assessed irradiance effects on photosynthetic traits and hydraulic conductivity by running analysis of variance (ANOVA) tests; we normalized data or used non-parametric tests when necessary. We assessed treatment effects on survival rate in the shade house by calculating the frequencies of live and dead plants per treatment with a Chi-squared 4×2 table using a G-test. We analysed irradiance effects on hydraulic variables, leaf water potentials in the shade house, as well as the survival rate in the field using Kruskal–Wallis tests.

Results

Growth and survival under different light environments

Sapling growth rate was affected by irradiance in the shade house and also by the radiation environment in out-planted saplings in gaps and in the understory (Table 1). In the shade-house experiment, intermediate irradiance (i.e., 30%) increased the growth of *E. edulis* saplings, whereas the out-planted saplings that survived in gaps showed higher growth rates than plants in the understory.

The survival rate was high across all treatments in the shade house, reaching an average of 87%. Nevertheless, plant survival decreased by 15% with increasing growth irradiance in the shade house. In the field, the average survival rate in out-planted saplings was lower than in the shade house, and the radiation environment had a strong effect on this variable. Survival was six times lower for out-planted saplings growing in the gaps compared with plants in the understory. In all the five understory plots, some plants survived. In contrast, in two of the five gaps no plants survived, and in one of them only one plant was still alive 2 years after out-planting (Table 1).

Table 1. Growth (stem relative increment, dimensionless variable mm mm^{-1}) and survival rate (%) of *E. edulis* saplings grown in shade houses under different growth irradiance conditions (10, 30, 40, 65% incident solar radiation), and in the field under two different radiation environments (understory and gap). Values are expressed as mean \pm SE and median (25 and 75 quartile). Number of replicates (N), statistical test used for comparing results across columns (Stat; F : ANOVA; G : Chi-squared 4×2 table using the G-test; H : Kruskal–Wallis) and P -values are also shown. Different letters indicate significant differences between irradiance treatments. For the shade-house experiment, N represents the number of individuals in each treatment; for the field experiments, N indicates the number of plots with 15 out-planted individuals at the beginning of the experiment. For relative growth rate in the field, mean \pm SE values were estimated only for those plots with at least one green plant surviving.

Shade house	Irradiance treatment (%)				N	Stat	P
	10	30	40	65			
Growth	1.30 \pm 0.07 ^a	1.97 \pm 0.11 ^b	1.82 \pm 0.10 ^b	1.73 \pm 0.11 ^b	55–58	$F = 8.41$	<0.001
Survival	94	91	83	80	58–62	$G = 7.73$	0.05
Field	Radiation environment		N	Stat	P		
	Understory	Gap					
Growth	0.54 \pm 0.12	2.95 \pm 0.92	3–5	$F = 12.08$	0.01		
Survival	47 (20, 60)	7 (0, 33)	5	$H = 4.06$	0.04		

Photoinhibition

After 90 min of exposure to high radiation levels ($>2000 \mu\text{mol m}^{-2} \text{s}^{-1}$), the F_v/F_m values of all *E. edulis* plants in the shade house were very low. After this period of irradiance saturation, all plants were exposed to non-saturation light conditions ($20 \mu\text{mol m}^{-2} \text{s}^{-1}$) and showed a gradual recovery of the maximum quantum efficiency of PSII photochemistry (F_v/F_m) with time (Figure 2a). However, the recovery of F_v/F_m was influenced by growth irradiance (Figure 2a, one-way ANOVA $F_{3,11} = 3.85$, $P = 0.04$, $n = 4$). Plants growing under low irradiance (10%) showed a lower recovery rate, attaining on average 64.4% of the maximum F_v/F_m after 80 min, while plants growing under high irradiance (65%) exhibited the highest rate of recovery reaching almost 90% of maximum F_v/F_m in the same time interval (Figure 2a).

Out-planted saplings in the field also exhibited photoinhibition, as evidenced by a decrease of F_v/F_m after exposure to high radiation. Similar to the patterns observed in the shade-house plants, saplings in the understory showed a lower rate of recovery of maximum F_v/F_m compared with saplings growing in gaps, reaching 62 and 90% recovery, respectively (Figure 2b).

Hydraulic architecture

Hydraulic conductivity, expressed either per petiole cross-sectional area (specific hydraulic conductivity, k_s) or per leaf area (leaf-specific conductivity, k_l), was not significantly affected by irradiance in the shade house (Table 2). The Huber value at the leaf level did not show differences between growth irradiance conditions (Table 2). In contrast, Huber values at the plant level significantly increased with increasing irradiance, showing a higher conductive cross-sectional area per unit of leaf surface area (Table 2).

Water relations

Plants growing in the shade house under contrasting irradiances (10 and 65%) had similar stem water potential (Ψ_s , estimated from covered leaflets). In contrast, exposed leaflet water potential (Ψ_L) was influenced by irradiance, being 40% higher (less negative) in plants growing under 10% irradiance than in plants growing at 65% of the maximum solar radiation (Table 2). In saplings growing in the wild population, Ψ_L decreased towards midday followed by a recovery in the afternoon (Figure 3a). Leaf water potentials were lower in the more exposed group of plants (repeated-measures ANOVA $F_{6,5} = 5.00$, $P = 0.049$), particularly around 15:00 h. At midday (12:30 h), similar radiation levels in leaves of the two groups of plants resulted in similar values of Ψ_L (Figure 3a). Stomatal conductance in sapling leaves did not change with exposure to solar radiation and was highly variable between plants (repeated-measures ANOVA $F_{1,3} = 0.13$, $P = 0.74$; Figure 3b).

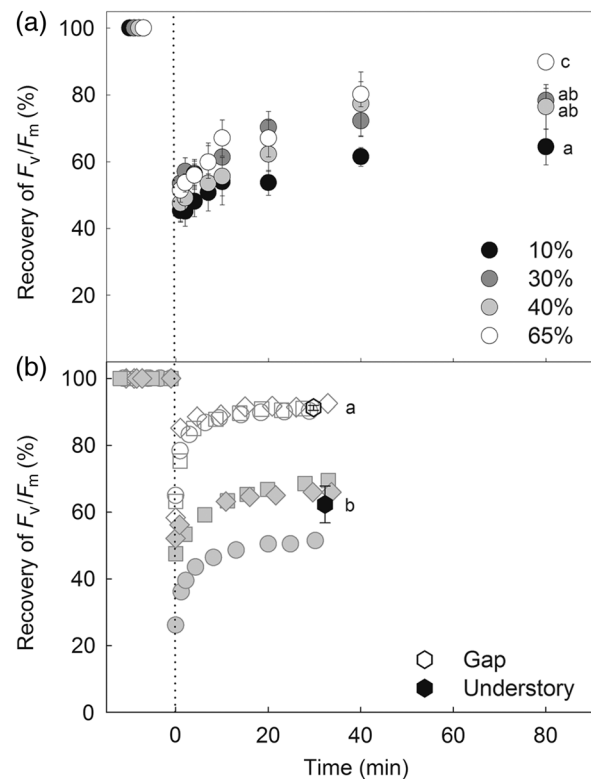


Figure 2. Percentage of recovery of PSII quantum yield (F_v/F_m) over time in non-saturation radiation levels for (a) plants growing in the shade house after 90 min of exposure to high radiation levels ($>2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) indicated by a dashed line, and (b) out-planted saplings in the field after 20 min of exposure to high radiation with a halogen lamp ($2500 \mu\text{mol m}^{-2} \text{s}^{-1}$) (dashed line) for leaflets of *E. edulis* plants developed under four different light treatments (% of maximum incoming solar radiation, shade house) and two radiation environments (gap and understory, field). (a) Data points are mean \pm SE; $n = 4$ leaves; each leaf from a different individual. Letters on the right side indicate statistical differences between treatments for the F_v/F_m recovery at the end of the experiment (80 min, $P < 0.05$, see the Results section). (b) Each shape in grey and white represents a different individual (grey and white symbols represent out-planted saplings growing in the understory and in gaps, respectively); 'gap' and 'understory' symbols represent the mean \pm SE of the percentage of recovery for each light environment at the end of the experiment (30–35 min). Letters on the right side indicate statistical differences between treatments ($P < 0.05$).

The leaflet turgor-loss point was -1.65 MPa as estimated from pressure–volume relationships. Absolute leaf capacitance values before and after the turgor-loss point were 254 and $1030 \text{ mmol m}^{-2} \text{ MPa}^{-1}$ respectively, calculated from the slopes of the two-phase curve fitted to our data (Figure 4a). These leaflet capacitance values were used for leaf hydraulic conductance calculations (K_{leaf}) to generate the hydraulic vulnerability curve (Figure 4b), which showed that K_{leaf} decreased substantially with leaf water potentials near the turgor-loss point, losing 79% of leaf hydraulic conductance at that point.

Table 2. Specific hydraulic conductivity (K_s ; $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$), leaf-specific conductivity ($K_l \times 10^{-4}$; $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$), Huber value for the leaf (leaf Hv, $\times 10^{-3}$) and for the plant (plant Hv), total leaf area (TLA, m^2) and water potential (Ψ , MPa) on the stem (Ψ_s) and leaflets (Ψ_L) of *E. edulis* saplings growing at different irradiance conditions (10, 30, 40, 65% incident solar radiation) in shade houses. Values are expressed as median (25 and 75 quartile) and mean \pm SE. Number of replicates (N), statistical test used for comparing between columns (Stat; H : Kruskal–Wallis; F : ANOVA) and P -values are also shown. Different letters indicate significant differences between irradiance treatments.

	Irradiance treatment (%)				N	Stat	P
	10	30	40	65			
K_s	0.15 (0.01–0.20)	0.14 (0.08–0.15)	0.14 (0.10–0.26)	0.11 (0.08–0.15)	12–13	$H = 3.27$	0.35
K_l	0.57 (0.42–0.77)	0.48 (0.31–0.61)	0.56 (0.46–1.09)	0.56 (0.37–0.59)	12–13	$H = 2.84$	0.42
Leaf Hv	0.41 ± 0.020	0.38 ± 0.019	0.42 ± 0.022	0.45 ± 0.020	12–14	$F = 1.76$	0.17
Plant Hv	0.135 ± 0.008^a	0.140 ± 0.008^{ab}	0.157 ± 0.011^{ab}	0.173 ± 0.013^c	54–58	$F = 2.97$	0.03
TLA	0.608 ± 0.008^a	0.902 ± 0.026^b	0.873 ± 0.023^b	0.757 ± 0.120^c	54–58	$F = 47.9$	<0.001
Ψ_s	–0.400 (–0.512, –0.325)	–	–	–0.425 (–0.550, –0.300)	4	$H = 0.02$	0.88
Ψ_L	–0.987 (–1.100, –0.962)	–	–	–1.425 (–1.537, –1.350)	4	$H = 5.33$	0.02

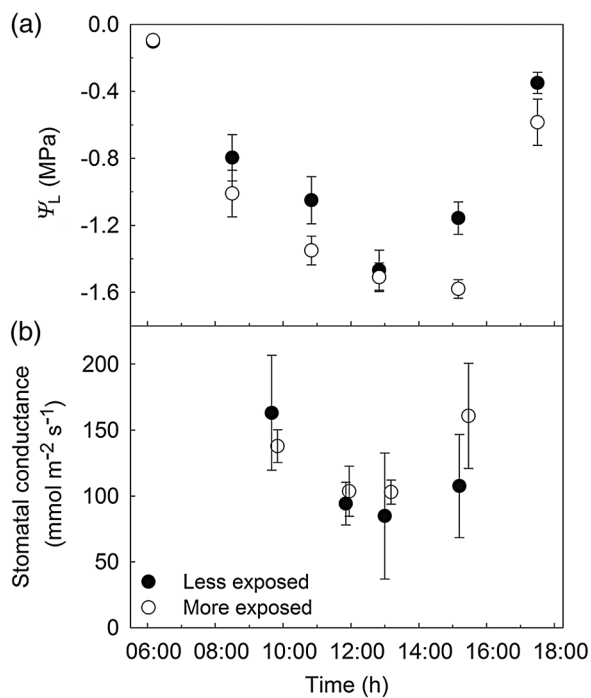


Figure 3. Daily leaf water potential (a) and stomatal conductance (b) over a day in *E. edulis* saplings. Plants are classified into two groups as they have more (open symbols) or less exposed leaves (black symbols); 16 and 2% of the full sun radiation reaching the canopy, respectively). Each symbol represents mean \pm SE.

Discussion

The degree of photoinhibition and hydraulic phenotypic plasticity in *E. edulis*, addressed in the present study, help explain the local spatial pattern of its distribution in native forests. Photosynthetic adjustments to different light environments may prevent long-term photoinhibition in *E. edulis* when gaps are opened in the forests. The lack of hydraulic adjustment to new light and evaporative-demand conditions, on the other hand, constrains the survival and growth of *E. edulis* saplings in gaps and open areas. In particular, the results of this study

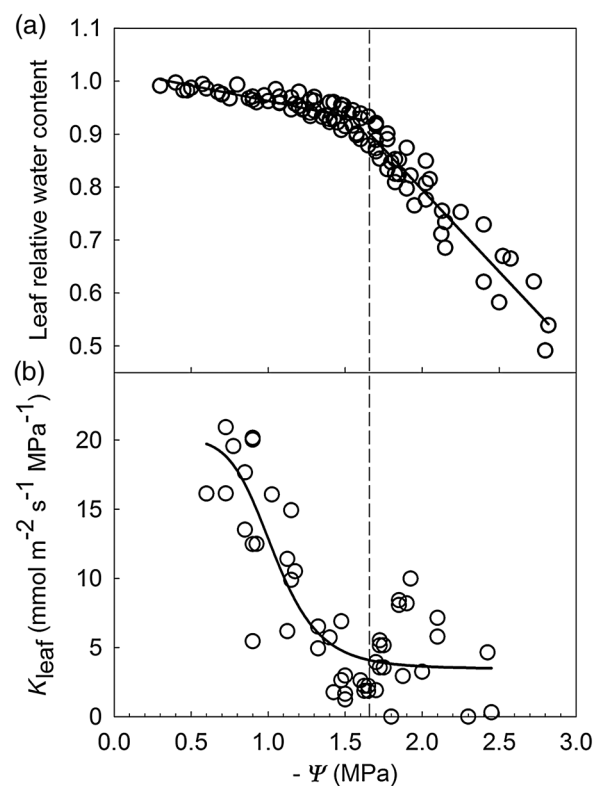


Figure 4. (a) Two-phase function fitted to pressure–volume data allowing leaf capacitance estimation, before and after the turgor-loss point (dotted line), from the slope of the relationship between leaf relative water content and leaf water potential (Ψ_L); and (b) vulnerability curve of leaf hydraulic conductance (K_{leaf}) for leaflets of six different plants from the 30% growth irradiance condition in the shade house. A sigmoid function was fitted to the data ($r^2 = 0.72$; $P < 0.001$).

suggest that the local distribution of *E. edulis* is constrained by the inability of the leaf's hydraulic system to maintain high Ψ_L values when irradiance levels increase due to gap formation in forests.

No plant can perform optimally over the entire light gradient that occurs in highly diverse forests, from open gaps to

deep-shade understories, as morphological and physiological adaptations to both environments are usually incompatible (Valladares and Niinemets 2008). In this study, *E. edulis* saplings showed positive growth responses and the highest survival rates under low irradiance (10–11%), both in the shade house and under field conditions, suggesting that this species is shade tolerant, consistent with previous studies (Sedrez dos Reis et al. 2000, Gatti et al. 2011). We also observed that all plants, regardless of growth irradiance conditions, showed transient PSII photoinhibition when exposed to full sun in both shade-house and field experiments. However, when *E. edulis* plants grow under high light conditions for long periods of time, the photosynthetic system of the leaves can acclimate to avoid strong photoinhibition effects. Plants grown with at least 30% of solar radiation exhibited faster recovery rates (faster F_v/F_m increase) compared with plants grown under lower irradiances. Similar patterns of photosynthetic acclimation have been observed in other plant species (Durand and Goldstein 2001, Karim et al. 2003, Krause et al. 2012). In a previous study, Gatti et al. (2011) observed that *E. edulis* saplings showed increased photosynthetic capacity at high irradiances. Taken together, these responses indicate that *E. edulis* saplings are able to adjust their photosynthetic activity to different levels of solar radiation. However, growth rates and survival tend to decrease under high solar radiation, suggesting that other factors constrain *E. edulis* development under high irradiance conditions.

Hydraulic traits at the leaf level, such as specific hydraulic conductivity (k_s), leaf-specific conductivity (k_l) and the Huber value (petiole cross-sectional area/leaf area), show no acclimation behaviour with changing irradiance. Low phenotypic plasticity in hydraulic traits has also been observed in co-occurring shade-tolerant tree species (Campanello et al. 2008, 2011). Renninger and Phillips (2011) found similar k_l values across individuals who differed widely in height and growing environments in three different palm species, exhibiting different compensatory responses for maintaining k_l , such as a decrease in the number or size of leaves (Renninger et al. 2009). We found a similar compensatory response to different light conditions in *E. edulis* saplings. At the whole-plant level of analysis, the higher Huber values obtained at high radiation levels are indicative of an increase in stem conductive cross-section relative to leaf area, suggesting a potential increase in resource allocation to stem tissue per unit of leaf area. These changes in plant Huber values across growth irradiance conditions are associated with changes in leaf size. Leaf size plasticity can provide an efficient way for adjusting hydraulic conductance under different evaporative-demand conditions (Murphy et al. 2012): large leaves are common in moister and/or shaded habitats, and smaller leaves are more common in drier and exposed habitats (Givnish 1987, Fonseca et al. 2000). *Euterpe edulis* saplings grown under high irradiance exhibit smaller leaves than saplings grown under

low radiation when leaves of a similar ontogenetic stage are compared between treatments (Gatti et al. 2011). Therefore, *E. edulis* plants maintain a similar water supply to leaves (k_l) when grown under high irradiance by developing the same area of conductive tissue, while reducing the transpiration surface area (Gatti et al. 2011). Despite changes in Huber values, Ψ_L decreased under high irradiances even when Ψ_S values were similar. Under high radiation levels, leaf water potentials were near the turgor-loss point, which may have triggered partial stomatal closure. This result needs additional studies in order to be confirmed given that pressure–volume curves were only done at the 30% irradiance treatment and osmotic adjustment was not assessed.

An uncoupling between stem and leaf water potential may be related to leaf resistance to water flow, given that leaves are an important bottleneck in tree hydraulics (Sack et al. 2003, but see also Villagra et al. 2013). In particular, for palms, high resistance points at the level of the leaf junction have been observed (Sperry 1986). These observations are consistent with the ‘segmentation hypothesis’ proposed by Zimmermann (1983) in which the points of hydraulic failure are localized in redundant and replaceable points of the plant water transport system, thereby increasing the ability of plants to survive and recover from drought, by decreasing the transpiration surface area (Sperry et al. 2002). In the case of palms, hydraulic segmentation is considered an important safety mechanism for stems, which do not have secondary xylem and represent many years of carbohydrate investment; consequently, xylem functionality must be maintained at all costs (Zimmermann 1983). Leaves are expendable, but if their stem is massively embolized, the palms may never recover (Tyree and Ewers 1991). High resistance points at the leaf insertion could be protecting the stem from cavitation, but also uncoupling water supply from the stem with demands from the leaves under high irradiance. In field-grown *E. edulis* plants, even a small increment in sun exposure resulted in leaf water potential decreasing to levels near the turgor-loss point, which would represent a predicted loss of K_{leaf} equal to 75% from its maximum value. The observed decrease in K_{leaf} was likely the result of embolism formation during dehydration (Johnson et al. 2012). Saplings recovered and had higher values of Ψ_L at the end of the day, likely associated with a K_{leaf} recovery (Brodribb and Holbrook 2004), but also with a decline in transpirational demand while water flows into the leaves at a low rate (i.e., low K_{leaf} values). Vessel refilling at the end of the day and overnight has been observed in arborescent palms, and positive root pressure may be a mechanism operating to restore the integrity of the water flow pathway in small individuals (Renninger and Phillips 2011). The strong decline in Ψ_L under moderate light conditions, as well as similar stomatal conductance irrespective of sun exposure in the field, suggests a relatively weak stomatal control.

Survival followed the same trend for plants growing in the shade house and under field conditions, with high radiation increasing plant mortality. In the field, the saplings that survived in gaps were growing in the less exposed sites, i.e., on gap borders or near dead, fallen trees, where incident solar radiation was on average 18% (Villagra 2012). However, adult *E. edulis* plants naturally occur in the mid-canopy (Henderson et al. 1995) with their whole crown frequently exposed to direct solar radiation. In several arborescent palm species, the stem acts as a water-storage reservoir, subsidizing water-resource needs (Holbrook and Sinclair 1992a, 1992b, Gomes and Prado 2007). In another study, young individuals of the oil palm, *Elaeis guineensis* Jaq., were not able to maintain adequate leaf water status under water deficits, since they did not have a voluminous stem and an extensive root system (Gomes and Prado 2007). The same may be occurring in *E. edulis* saplings, with an incipient stem that does not allow water storage.

In order to take advantage of the increase in light following gap formation, understory plants not only need to increase their photosynthetic capacity, but also must be able to deliver more water to the leaves to help sustain photosynthetic gas exchange throughout the day (Campanello et al. 2008). *Euterpe edulis* increased its photosynthetic capacity (Gatti et al. 2011) and decreased its sensitivity to photoinhibition (this study) when grown under high radiation environments. But, under these conditions, *E. edulis* saplings show an uncoupling between water demand and supply to leaves, apparently due to strong hydraulic segmentation that restricts water delivery to leaves, weak stomatal control and the absence of a fully developed stem that could function as a water reservoir. These hydraulic constraints in the growth and survival of *E. edulis* saplings may result in competitive exclusion in the more exposed sites of the forest, where lianas and native bamboos (e.g., *Chusquea ramossissima*) have aggressive growth behaviour arresting gap phase regeneration (Campanello et al. 2007b, 2012, Montti et al. 2011, 2014).

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Conflict of interest

None declared.

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