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Frost resistance in the tropical palm *Euterpe edulis* and its pattern of distribution in the Atlantic Forest of Argentina

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ABSTRACT

Frost resistance and subzero temperature effects on photosynthesis, survival and distribution were studied in *Euterpe edulis*, a tree palm species of the Atlantic Forest, near the southern limit of the species distribution. *E. edulis* grows under the forest canopy and is absent from forest stands located near bottom valleys. This palm species has been commercially exploited for palm hearts, making it the most important non-timber forest species in North-Eastern Argentina. Its distribution was studied in relation to the frequency and intensity of subzero temperatures along a topographic gradient. *E. edulis* abundance was higher at the highest site and decreased or became absent towards the lowest site. Subzero temperatures during each of three winter seasons were observed in the lowest site. The medium and high elevation sites never experienced absolute minimum temperatures below 0 °C. Forest structure, fraction of solar radiation transmitted through the canopy and soil water potentials did not change substantially along the gradient. After a low temperature period in winter, the maximum quantum yield in *E. edulis* leaflets was relatively low in individuals at the low elevation site while having normal values at the medium and high elevation sites. Thermal analysis indicated that *E. edulis* saplings can supercool down to about −10 °C and do not tolerate extracellular ice formation. Ice formation was observed at about −4 °C, relatively close to the equilibrium freezing temperature, only after an increase in ambient humidity resulted in dew formation on the plant surface. Dew formation is commonly observed in valleys during the winter season. This observation gives further support to the hypothesis that strong infrequent frost events could be an important environmental factor determining the spatial distribution pattern of *E. edulis* in the Atlantic Forest of Argentina.

1. Introduction

Understanding the determinants of species distributions and community composition is an important goal in plant ecology. A central issue is whether spatial patterns of plant species distribution mainly reflect environmental conditions and the ecological requirements of the species, or dispersion and extinction processes (Svenning and Skov, 2002 and references therein). In particular, for sustainable forest management and restoration, a poor knowledge in species physiology and ecology is an important limitation for successful management techniques design and implementation (Guariguata and Pinard, 1998).

Temperature has long been recognized as one of the primary factors influencing vegetation distribution. Both, low and high temperatures represent a major environmental constraint of plant growth, and development (Melcher et al., 1994; Lambers et al., 1998). Low temperature, in particular, is an important limiting factor in subtropical regions and high elevation tropical sites, where frosts are of relatively short duration. Most lowland tropical plant species lack the ability to adapt to subzero temperatures and are typically injured by temperatures below 10 °C (Xin and Browse, 2000). Tolerance to extracellular freezing and avoidance to intracellular ice formation is a species-specific phenomenon in the high tropics and subtropical regions (e.g. Goldstein et al., 1985; Rada et al., 2001). In the tropical mountains of South America, the upper limit of distribution of giant rosettes, for example, is determined by their supercooling capacity, i.e. their ability to avoid ice formation below the species-specific equilibrium freezing temperature (Goldstein et al., 1985; Rada et al., 1987). Miniature

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giant rosette plants that tolerate tissue freezing, on the other hand, can grow at even higher elevations in tropical mountains (Azócar et al., 1988). Plants that tolerate tissue freezing (extracellular ice formation) are able to cope with intracellular dehydration for long periods of time without damage (Pearce, 2001).

The tree palm species *Euterpe edulis* Mart., endemic to the Atlantic Forest, is conspicuous in the understory of many forest stands (Henderson et al., 1995). In the southern part of the Atlantic Forest where the topography is predominantly irregular and temperatures may drop below 0 °C during the short winter season, natural populations of *E. edulis* are usually absent in the lower portions of the landscape (Mortara, 2000; Gatti, 2005). It is expected that in the lowest part of this topography, downward turbulent heat flux cools the air immediately adjacent to the slopes, resulting in relatively low air temperatures (Chung et al., 2006) and increasing the probability of night-time frosts (Blennow and Persson, 1998). Seedlings of *E. edulis* tend to be absent from large gaps (i.e. gaps receiving more than 40% full solar radiation) near the southern limit of the species distribution because of high mortality rates and relatively low growth rates of this species under high irradiance (Bovi et al., 1987; Nakazono et al., 2001; Gatti, 2005), and perhaps because radiative cooling may enhance low freezing temperatures during winter nights in these open areas. In Northern sites of the Atlantic Forests, where freezing temperatures do not occur, *E. edulis* recruitment in forest openings has been observed (Santos Marcos and Silva Matos, 2003).

E. edulis is one of the most important Atlantic Forest non-timber forest products (NTFPs) and has been highly exploited for extraction of palm hearts, which has caused a large reduction of the entire population as well as local extinctions in several areas (Henderson et al., 1995; Galetti and Fernandez, 1998). It has been considered an important economic alternative for landowners trying to accomplish the goals of producing a sustainable income while retaining a forest canopy suitable for biodiversity conservation (Fantini and Guries, 2007). *E. edulis* seeds are recalcitrant (Silva de Andrade and Sampaio Pereira, 1997; Panza et al., 2004) and loose their viability 3 or 4 months after falling (Silva Matos and Watkinson, 1998) depending on microclimatic conditions (Reis et al., 1999). Because of its short viability and high proportion of germination, this species produces a substantial seedling bank (Sedrez dos Reis et al., 2000). In consequence, individual survival and development depends on seedlings and saplings ability to withstand unfavourable environmental conditions.

The objective of this research was to assess the effects of low temperatures on *E. edulis* physiology, survival and distribution in subtropical forests of North Eastern Argentina. Distribution of the species was studied in relation to the frequency and intensity of subzero temperatures along a topographic gradient in the field. Additional environmental factors that could influence *E. edulis* distribution, such as soil water potential and light availability, were studied in the field. Also, the effect of low temperatures on photosynthetic performance was assessed by measuring the maximum quantum yield of the photosystem II. Potential mechanisms of avoidance or tolerance to freezing of *E. edulis* were studied under experimental conditions in the laboratory.

2. Material and methods

2.1. Study area

The climate in the Atlantic Forest of Argentina is subtropical with average monthly temperature variations of about 10 °C between the warmest and coldest month. Annual precipitation ranges from 1900 to 2100 mm (Ligier, 1999). Rainfall is evenly distributed along the year, but short dry spells can occur. The period with certain probability of freezing temperatures is from middle June to middle September (Fig. 1). The number of days with temperatures below 0 °C ranges from 0 to 9, depending on proximity to rivers, altitude of the site (Ligier, 1999) and year (Servicio Meteorológico Nacional, 2005).

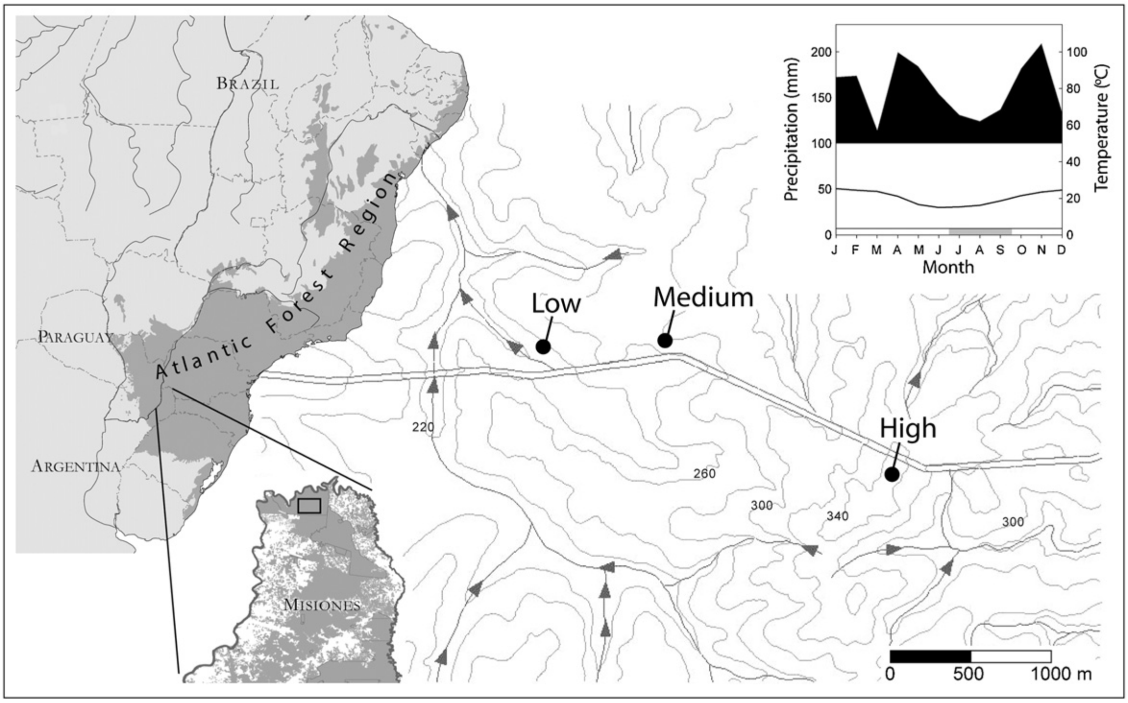

Fig. 1. Geographic distribution of Atlantic Forest ecosystems in South America on the left of the figure (adapted from Conservation International, 2002), the study area location in Misiones Province, Argentina, indicated with a rectangle, and the location of the three study sites along the topographic gradient: low (230 m a.s.l.), medium (270 m a.s.l.) and high (340 m a.s.l.). Contour lines are every 20 m elevation (double line is a small dirt row). In the right upper corner a climate diagram shows seasonal changes in precipitation and temperature. In grey colour are indicated the months when frosts may occur (Servicio Meteorológico Nacional, Argentina, 2005).

The undulating topography of this region in North Eastern Argentina is the result of a dense network of rivers eroding a basalt formation (Tujchneider et al., 2006). The lowest parts of the landscape close to rivers are at an elevation of 150–250 m a.s.l., while the upper parts of the plateau are at 550–800 m a.s.l. The resulting topographic gradient between rivers and plateaus is not very steep. The soils are derived from basaltic rocks containing a high concentration of Fe, Al and Si, and are well drained (Ligier, 1999).

2.2. Field studies

A representative topographic gradient was chosen in the central part of the Iguazú National Park, Misiones Province, Argentina (25°39′S–54°14′W; Fig. 1). The gradient was selected inside this natural protected area due to minimal human influence since 1934 on the forest structure and dynamics, and in particular on the growth and distribution of *E. edulis*. Three different study sites (hereafter termed as "sites") along the gradient were selected (Fig. 1): high, at the higher portion of the gradient (340 m a.s.l.); low, almost at the lower part (230 m a.s.l.); and medium (270 m a.s.l.). The medium site was established at the altitudinal limit of distribution of *E. edulis* populations along the gradient. The topographic gradient was 4.5 km long with an average slope of 2.4%, typical of the region. Flooding never occur at any of the sites.

Four circular plots of 5 m radius (78.5 m^2) were established randomly at each site, following contour lines, at least 25–30 m apart. The height and diameter at breast height (dbh) of palms (*E. edulis* and *Syagrus romanzoffiana* (Cham.) Glassman) and trees ≥5 cm dbh were measured inside the plots. At the centre of each plot, a subplot of 1.5 m radius (7.1 m^2) was used to estimate *E. edulis* seedlings and saplings abundance (seedlings: individuals with up to three primary fan-shaped leaves and saplings: individuals that have already produced the fourth leaf; according to Carvalho et al. (1999) and Freckleton et al. (2003)). In all individuals, stem diameter and height (from ground level up to the youngest leaf) were measured.

Ambient temperature, soil water potential and the fraction of solar radiation transmitted (FRT) through the canopy were estimated at the centre of each plot. Temperature was recorded every 10 min with cooper–constantan temperature sensors (HOBO Type T, Onset Corporation, MA, USA) located 10 cm above the ground, during the winter (June–September) of 2002, 2003 and 2004. Soil water potential was estimated from soil samples (0–5 cm depth) inside and between plots (4 inside and 3 between, 7 per area) using the filter paper technique (Deka et al., 1995). Soil samples were obtained in 2004 after a moderate dry spell in the spring (27 September 2004, 11 days without rain) and during a more intense drought during the summer (18 February 2005, 16 days without rain). The FRT was estimated from hemispherical photographs taken at 0.7 m above the soil surface, with a digital camera Nikon Coolpix 950, fitted with a Nikkor 8 mm lens, on a self-level platform.

During the autumn (April) of 2004 a total of 96 potted *E. edulis* seedlings were out-planted in the three sites along the topographic gradient (8 seedlings per 78.5 m^2 plot). During winter, after a short period of temperatures between 0 and 5 °C at night, the maximum quantum yield of PS II ($\Delta F/Fm$) of seedling leaves was measured with a portable chlorophyll fluorometer (MiniPam, Walz, Effeltrich, Germany). Chlorophyll fluorescence can provide insights of the plant's ability to tolerate environmental stresses and the extent to which those stresses have damaged the photosynthetic apparatus. A low $\Delta F/Fm$ indicates a decreased photosynthetic efficiency (Maxwell and Johnson, 2000). Chlorophyll fluorescence has been previously used to assess effects of low temperature on plants that are not otherwise visible, manifesting itself as a loss of photosynthetic efficiency (e.g. Lamontagne et al., 2000; Major et al., 2003).

2.3. Thermal analysis in the laboratory

Two heat exchangers located inside a Styrofoam box were connected to a pump that circulated a 50% ethylene glycol solution between the heat exchangers and a refrigerated water bath (Ultralow Refrigerated Circulator F81, Julabo, Germany). This experimental chamber was used for thermal analysis of *E. edulis* plant tissues. The box had an opening at the base where whole plants were inserted, leaving pots with the root system outside the chamber. Whole saplings were used for the laboratory experiments because small tissue volume or detached leaves used in thermal analysis are known to give erroneous determinations of ice nucleation temperatures (Ashworth et al., 1985). Roots with soils were left outside the chamber to mimic conditions in the field where roots are usually protected from freezing temperatures by the high thermal capacity of the soil. Leaf and petiole temperatures were monitored with copper–constantan thermocouples connected to a data logger (CR10X, Campbell Scientific Inc., Logan, UT, USA) and recorded every second while the ambient temperature in the chamber was decreased from ambient to the desired air temperature inside the chamber. The thermocouples were placed in contact with the leaf tissue and held in place using small pieces of surgical tape. Three types of experiments were performed. The temperature inside the chamber was continuously lowered from room temperature at a rate of 30 °C/h until: (a) −20 °C was reached ($n = 6$); (b) −2 or −5 °C were reached and then kept constant for at least 100 min ($n = 2$); and (c) −5 °C was obtained and then kept constant for 60 min; at the end of the 60 min period the experimental chamber was briefly opened to increase the ambient humidity with a fine spray of water resulting in a film of water on the leaf surfaces, and the tissue temperature was recorded for an additional 40 min period ($n = 3$). The absence of surface moisture can result in artifactual supercooling (Wisniewski et al., 1997), for this reason, supercooling capacity of *E. edulis* saplings was also tested while simulating dew formation at the leaf surfaces. After the completion of each experiment, the plants were kept in low

Table 1
Forest basal area (m^2), abundance (number of individuals on 10 m^2 basis), and average height for saplings and trees (≥5 cm dbh), and only trees (≥10 cm dbh), along the topographic gradient

Site	Basal area		Abundance		Height
	Saplings + trees	Trees	Saplings + trees	Trees	Trees
High	0.0296 (0.0279–0.0534)	0.0267 (0.0235–0.0495)	1.15 (0.76–1.53)	0.45 (0.19–0.64)	10.46 ± 0.66^a
Medium	0.0381 (0.0175–0.0610)	0.0371 (0.0170–0.0599)	0.76 (0.57–0.96)	0.64 (0.45–0.83)	11.86 ± 1.04^{ab}
Low	0.0461 (0.0330–0.0872)	0.0450 (0.0320–0.0863)	0.70 (0.53–0.83)	0.45 (0.32–0.51)	16.04 ± 1.18^b

Values are for the three different sites along the topographic gradient (high, medium, low: 340 m, 270 m, and 230 m a.s.l., respectively). *Note*: Basal area and height includes all trees and palms; abundance includes all individuals except *E. edulis* palms. Basal area and abundance data are median (1st and 3rd quartiles), and height is mean ± standard error, $n = 4$, different superscripts indicate statistical differences inside each column, $p < 0.05$.

light, well watered and at room temperatures to monitor the recovery of the tissues after cooling or freezing. Injury was visually determined after a week as wilting and necrosis of tissues in plants. Individual survival was determined 1 month later.

2.4. Data analysis

The FRT was calculated as the proportion of the solar radiation reaching the understory relative to the solar radiation on top of the canopy. The non-parametric test Kruskal–Wallis was performed in order to compare high, medium and low sites along the topographic gradient for FRT, forest structure (basal area and abundance), *E. edulis* abundance, and soil water potential. For variables with sample size $n \geq 6$ differences across sites were tested with ANOVA. In the case of quantum yield, a one-way ANOVA was performed, and for ice nucleation temperatures a nested ANOVA was used (leaf parts, i.e. petioles and leaflets, nested in leaves and leaves nested in individuals).

3. Results

3.1. Field studies

The stand basal area was similar across all sites along the topographic gradient for all individuals (trees + palms) ≥5 cm, and for all individuals ≥10 cm dbh (Table 1; $H_{2,12} = 0.35$, $p = 0.84$; $H_{2,12} = 0.46$, $p = 0.79$, respectively). When considering only trees without *E. edulis* palms, the mean basal area tended to decrease with elevation but differences were not significant (≥5: $H_{2,12} = 1.19$, $p = 0.55$; ≥10: $H_{2,12} = 2.42$, $p = 0.29$) (data not shown).

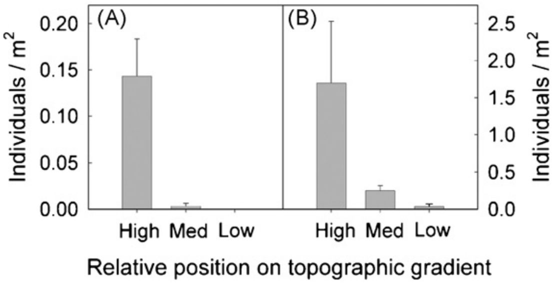

Fig. 2. *Euterpe edulis* density (individuals/m^2) at three different sites along the topographic gradient (high, medium, low: 340 m, 270 m, and 230 m a.s.l., respectively) for two palms size classes: A, with conspicuous stem at 1.3 m above ground (height: 5–15 m) and B, established individuals without stem (height: 0.1–0.5 m). Bars are means + S.E. ($n = 4$). The value corresponding to the low site in panel A is zero. Scales are different for panels A and B.

Tree abundance (without palms) was similar along the gradient for individuals ≥5 and ≥10 cm dbh (Table 1; $H_{2,12} = 2.57$, $p = 0.28$; $H_{2,12} = 2.16$, $p = 0.34$, respectively). Average height of individuals ≥10 cm dbh was different across sites along the topographic gradient (Table 1; $F_{2,59} = 8.47$, $p < 0.001$). The high site exhibited individuals of smaller size compared to individuals in the low site, reflecting the high abundance of *E. edulis* palms (65% of all the individuals) in the higher site, which have a lower stature compared to trees (8.98 ± 0.58 and 13.46 ± 0.69 mean and standard error for *E. edulis* and tree height, respectively; $F = 1.94$, $p = 0.032$).

E. edulis density changed along the topographic gradient following a similar trend for both adults and, seedlings and saplings (Fig. 2; $H_{2,12} = 9.37$, $p = 0.009$; $H_{2,12} = 5.16$, $p = 0.07$,

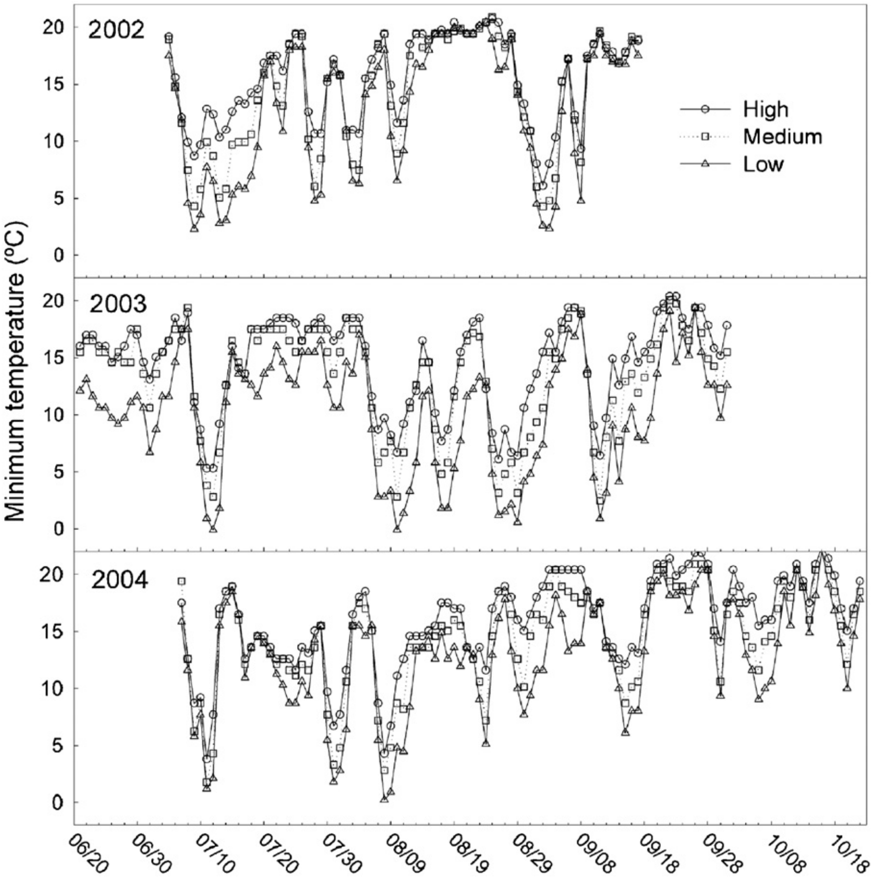

Fig. 3. Daily minimum air temperatures recorded at 10 cm above ground during three winter seasons (from 2002 to 2004), and at 3 different sites along the topographic gradient: high, medium and low (340 m, 270 m, and 230 m a.s.l., respectively). Each point represents the daily minimum temperature and is the mean of 3–4 measurements.

Table 2

Absolute minimum temperatures (Min Temp) recorded during three consecutive winter seasons (from 2002 to 2004), soil water potential estimates for two dry periods (spring (27 September 2004) and summer (18 February 2005)) and fraction of solar radiation transmitted (FTR) through the canopy measured at 0.7 m aboveground for 3 sites (high, medium and low: 340 m, 270 m, and 230 m a.s.l., respectively), along the topographic gradient

Site	Min Temp (°C)			Soil water potential (MPa)		FRT
	2002	2003	2004	Spring	Summer	
High	4.8	4.8	3.8	−0.55 (−0.74 to −0.24)	-3.81^{ab} (−4.65 to −2.60)	0.44 (0.28–0.69)
Medium	1.8	1.8	1.8	−0.47 (−0.81 to −0.39)	-2.77^{a} (−3.28 to −2.28)	0.50 (0.28–1.04)
Low	−1.1	−0.1	−0.1	−0.65 (−0.85 to −0.42)	-4.85^{b} (−5.54 to −3.33)	0.29 (0.20–0.62)

Note: Water potentials are median (lower–upper quartiles), $n = 7$, different superscripts indicate statistical differences inside each column, $p < 0.05$ and FRT are median (lower–upper quartiles), $n = 4$.

respectively), being abundant at the high site, scarce at the medium site, and absent in the low site; also seedlings and saplings were highly abundant at the high site, medium at the medium site and scarce at the low site (Fig. 2).

Patterns of minimum daily air temperature variations were similar in the three sites, but there were consistent minimum temperatures differences among sites during three consecutive winter seasons (Fig. 3). The medium and high elevation sites did not experience minimum temperatures below 0 °C throughout the study period, while the lowest site exhibited subzero temperatures every year (Table 2). The soil water potentials along the topographic gradient were similar among sites, after a moderate dry spell in the spring (September 2004) ($H_{2,20} = 1.15$, $p = 0.56$). However, after a longer dry spell during the summer (February 2005), statistical differences were observed between the medium and low site but not between the high and the two lower sites (Table 2) ($H_{2,21} = 6.46$, $p = 0.04$), with the higher site having the highest abundance of *E. edulis* and the other two sites having substantially lower density of palms. The FRT through the canopy was similar across all sites (Table 2; $H_{2,12} = 0.65$, $p = 0.72$). Even though stand leaf area is slightly lower during the winter compared to the summer in the study area, substantial seasonal variations in FRT at 0.7 m above ground were not observed in a study done concurrently with this one (Campanello et al., 2007).

At the end of July and beginning of August 2004, minimum air temperatures dropped considerably (Fig. 3). On 1 August, the average minimum temperature was 7.7 °C at the high site, 4.8 °C at the medium site and 2.8 °C at the low site. One day after these relatively low air temperatures occurred, maximum quantum yield was lower for out-planted *E. edulis* seedlings in the lowest part of the topographic gradient, while it remained near non-stressed predawn values of about 0.78 at the medium and high sites (Fig. 4; $F_{2,66} = 5.03$, $p = 0.009$). On December 2004 (8 months after out-planting), no differences were observed in out-planted seedling growth ($F_{2,82} = 1.28$, $p = 0.28$) or survival between sites. This field experiments was only done with the purpose to assess chilling temperature effects on photosynthetic performance. No subzero temperatures were recorded during the 8-month period.

3.2. Thermal analysis in the laboratory

Ice formation was observed in laboratory studies of *E. edulis*, but the ice nucleation temperature depended on tissue characteristics, rate of air temperature decrease in the experimental chamber, and the presence or absence of dew on the tissue surfaces. Ice nucleation was observed as a transient increase in temperature (exotherm) due to the release of heat of fusion during freezing (Fig. 5). When air temperature was decreased continuously at a rate of 30 °C/h down to −20 °C, average leaflet ice nucleation temperature was observed at -10.62 ± 1.32 and -9.01 ± 0.76 °C for younger and older leaves, respectively (Fig. 5, upper panel), but no statistical differences were found ($t = 1.57$, $p = 0.18$; $n = 6$). There were significant differences in ice nucleation temperatures between leaflets and petioles, being approximately 2 °C higher in leaflets than petioles ($F_{2,10} = 10.75$, $p = 0.003$). When the air temperature in the experimental chamber was decreased continuously down to −2 °C and then kept at this temperature for 120 min, ice formation was not observed (Fig. 5, middle panel). However, when air temperature was lowered to −5 °C, ice nucleation occurred only after the increase in ambient humidity resulted in dew formation (Fig. 5, lower panel). Plants that did not exhibit tissue freezing remained healthy for 1 month after the experiment. When ice nucleation occurred, permanent injury was detected as wilting and necrosis of tissues, resulting in plant death.

4. Discussion

Field and laboratory results indicate that freezing temperature is an important environmental constraint on *E. edulis* establishment and growth in the lower portions of the landscape. Other factors that may influence *E. edulis* distribution, such as forest structure, and solar radiation did not change in the study area along the topographic gradient. Soil water potentials also tended to be similar along the gradient during short drought periods, but statistical differences were observed after a long dry spell between the medium and the low site. Those differences were not consistent with variations in *E. edulis* abundance along the topographic gradient. It is well known that topography affects plant distribution, and consequently plant community composition in tropical and subtropical forests (e.g. Tuomisto et al., 1995; Chen et al., 1997; Oliveira Filho et al., 1998; Vormisto et al., 2000; Enoki and Abe, 2004). Likewise, it has been suggested that topography influences *E. edulis* (Mortara, 2000) and other palm species distribution (Svenning, 1999; Vormisto et al., 2004), but factors such as air temperature were not measured in those studies. Topography could not be considered as a limiting factor per se (Mortara, 2000; Vormisto et al., 2004). Topographic variations are associated to changes in environmental conditions such as soil type, water and nutrient availability, and air temperatures, which can directly affect plant establishment and growth, through its effects on metabolism and survival (Clark et al., 1998, 1999; Davies et al., 1998; Pacheco, 2001; Bjorholm et al., 2005; Wang et al., 2006).

Leaves of subtropical palms in general can resist temperatures between −5 and −14 °C without permanent injury (Larcher, 2003). In the present study *E. edulis* leaves were able to supercool to temperatures as low as −14 °C in the laboratory when dew formation was not experimentally induced. This low temperature never occurred at the study site. Supercooling is an unstable state (Mason, 1958) and several factors may induce ice formation closer to the equilibrium freezing temperature in plants that permanently supercool, especially under field conditions. The results of laboratory experiments support this idea because the extent of supercooling in *E. edulis* was limited by dew formation on the plant surface. Experimentally inducing the formation of a water-film on the leaf and petiole surfaces resulted in ice formation at −4.6 °C, a temperature markedly above the ice nucleation temperatures observed in thermal analysis studies when dew formation was prevented. The rate of cooling used was higher than rates of temperature decrease observed in nature. However, the most important thermal analysis experiments in the present study were done with temperature ramps (lowering down to −2 or −5 °C and then maintaining the temperature constant for a certain period of time). Consequently, the initial rate of temperature decrease was not crucial for the process under study. Plants that supercool avoid the potentially damaging effects of cells dehydration during extracellular freezing (typical of plants that tolerate ice formation) but exhibit tissue damage when freezing occurs (Goldstein et al., 1985; Rada et al., 1985; Goldstein and Nobel, 1991; García-Varela and Rada, 2003). Consistent with this, the general pattern of damage and ice formation in plants that permanently supercool (sensu Larcher, 2003) ice formation in *E. edulis* was always followed by non-reversible tissue injury during the laboratory experiments. In most cases the individuals died after a few weeks.

At a weather station (Puerto Iguazú) near the study sites the minimum temperature recorded over a 30-year period was −5 °C, and temperatures lower than −1.5 °C were observed 11 times (Servicio Meteorológico Nacional, Argentina, 2005). However, the minimum temperatures observed at this weather station were higher and the frequencies of frost events were lower than in the field study sites, during the same time period. During the winter of 2002 we recorded two consecutive days with subzero temperatures at the low site in the topographic gradient with a minimum temperature of −1.1 °C, while at the Puerto Iguazú weather station no days with freezing temperatures were recorded, being 2 °C the minimum temperature observed. Consequently, it should be expected that freezing temperatures at the low site in the topographic gradient might occur with a relatively high frequency. These subzero temperatures can be low enough that freezing of *E. edulis* tissues will result in plant damage. When the study sites were selected during the autumn of 2002, before the field measurements began, approximately 75% of young and reproductive palms were dead with the foliage completely dry at the medium site as well. It is possible that these individuals were damaged during the intense frost which occurred in the winter of 2000. During this year, temperatures at the Puerto Iguazú weather station dropped to −1.3 °C and 5 days with temperatures below zero were recorded.

After low air temperatures occurred during the winter, the maximum quantum yield, in out-planted *E. edulis* seedlings, was substantially lower at the low site, compared to out-planted seedlings at the medium and high sites, suggesting that relatively low temperatures may also affect the photosynthetic performance of *E. edulis* without necessarily causing permanent injury. During cold periods in winter, the recorded minimum temperature differences in the field were 3.9–8.5 °C between the highest and the lowest sites. Even though not damaging to the plant, low temperatures, even above 0 °C, may be detrimental for carbon assimilation and growth of *E. edulis* plants. Hendrickson et al. (2004) noted that even in plants non-sensitive to chilling such as the grape, the cumulative effect of small differences in mean minimum air temperature (1–3 °C) decreased growth rates by 34–63%.

E. edulis depends on animal dispersion (especially birds) for long-distance seed dispersal (Pizo and Simáo, 2001). For some palm species, such as *Maximiliana maripa*, it was observed that long-distance seed dispersal by tapirs is responsible for the formation of palm patches (Fragoso, 1997). Seed dispersal is not a limiting factor in *E. edulis* distribution in North-Eastern Argentina. There is evidence that seeds of this species reach the lower elevation site because seedlings were found at this site (see results), but no *E. edulis* adults were observed. This suggests that post dispersal processes result in tissue injury (Souza and Martins, 2002) and consequently individuals do not survive to reach reproductive age.

On a scale of a few kilometres, along the topographic gradient in this study, there were no substantial changes in forest structure,

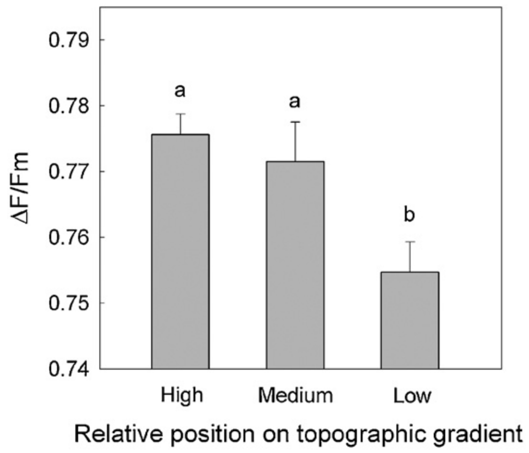

Fig. 4. Maximum quantum yield for leaves of *Euterpe edulis* palms early in the morning after a night with low air temperatures (see Fig. 1, 1 August 2004 and text), for out-planted seedlings in 3 different sites along the topographic gradient (high, medium and low: 340 m, 270 m, and 230 m a.s.l., respectively). Bars are means + standard error, *n* = 21–25. Different letters indicate statistical differences, $p < 0.05$.

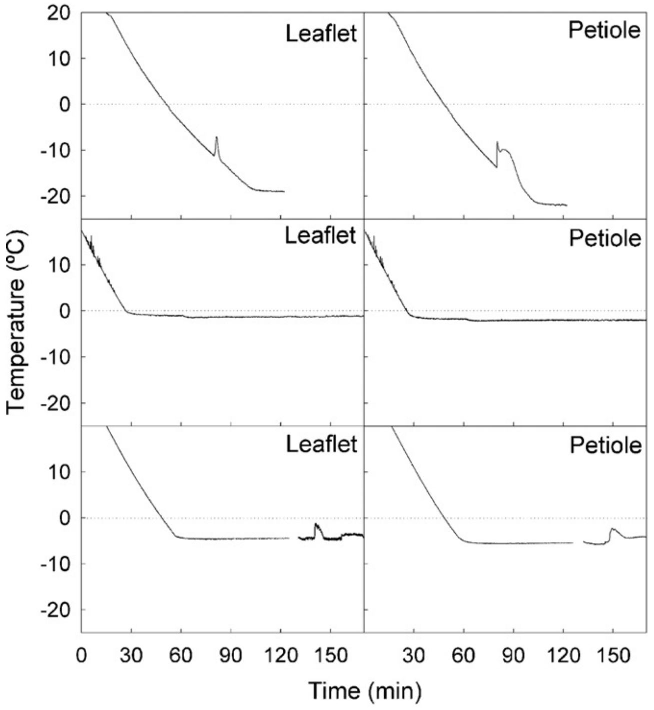

Fig. 5. Thermal analysis of leaflets and petioles in *Euterpe edulis* saplings. A short increase in leaf temperature in the upper and lower panels indicates an exothermic event due to ice nucleation inside foliar tissues. Upper panel: air temperature inside the experimental chamber was constantly decreased until the lowest temperature during cooling was obtained. Middle panel: air temperature constantly decreased down to −2 °C was obtained, and then kept constant for at least 100 min. Lower panel: air temperature was constantly decreased down to −5 °C, and then kept constant for 60 min; after this period, the experimental chamber was briefly opened to increase the ambient humidity (line disruption) with a fine spray of water to induce dew formation.

soil water potential and levels of solar radiation reaching the forest floor. In consequence, these factors cannot explain the pattern of *E. edulis* distribution in the southern limit of its range in Argentina. On the other hand, the frequency and intensity of subzero temperatures exhibited consistent trends along the topographic gradient. Ice nucleation was observed in laboratory experiments only after an increase in ambient humidity resulted in dew formation on the plant surface. Dew formation is commonly observed in valleys and low sites in the study area. This gives further support to the idea that infrequent frost events could be an important determinant of *E. edulis* distribution in the Atlantic Forests of North-Eastern Argentina, by explaining its absence from lower elevation sites, where subzero temperatures and high air humidity occur during the winter.

The findings of this study are useful for conservation, management and restoration plans of *E. edulis* populations and Atlantic Forest remnants. Predictive mapping vegetation (i.e. predicting the geographic distribution of the vegetation composition across a landscape from mapped environmental variables), an important need for resource use and conservation planning, is based on relationships between climatic variables and plant physiological processes (Franklin, 1995). On the Atlantic Forest as a whole, there exist controversies about altitude effects on *E. edulis* distribution (Mortara, 2000), perhaps because the direct factors influencing the distribution of this palm species were not studied. The identification of freezing temperatures as a limiting factor for the growth and survival of *E. edulis* palms provided in the present study represent an important predictive tool, which could be used for modelling potential distribution of this species at very different scales, decreasing the uncertainty of restoration efforts and helping in forest management. Conservation efforts and protected areas design should take into account that during relatively warm periods, *E. edulis* populations could extend its distributions into frost-risky sites, which will be damaged in colder years. One of the most common techniques for increasing the production of palm hearts is to spread *E. edulis* seeds in the forest understory (Bovi et al., 1987), however the use of seeds is not always appropriated because they tend to desiccate rapidly (Silva de Andrade and Sampaio Pereira, 1997) or are predated if germination is delayed. Another approach is out-planting forest patches with *E. edulis* seedlings. Enrichment with *E. edulis* is generally done without taking into account the site-specific differences in the frequency and intensity of frosts. Forests that regularly experience frosts have to be avoided which could help to minimize economic losses and improve restoration plans of this important non-timber forest species.

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