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Emergence and vigor of *Euterpe edulis* seedlings under shading levels and the presence and absence of the pericarp

Emergencia y vigor de plántulas de *Euterpe edulis* bajo niveles de sombreado y presencia y ausencia del pericarpio

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ABSTRACT

Euterpe edulis Mart., known as palm juçara, is a palm tree with high economic and ecologic value, but since it is threatened with extinction, the best propagation methods for the species must be researched. This study evaluated the effects of shading levels (0, 16, 54, 72 and 92%) and of the presence and absence of the pericarp on the emergence and vigor of *E. edulis* seedlings. Ninety-nine days after sowing, the total number of emerged seedlings was counted to represent the emergence percentage (%), root length (RL) and stem length (SL) were measured and the total dry matter mass of the seedlings (DM) was determined. The emergence velocity index (EVI) was also calculated. The increase in shading levels resulted in linear increments of the variables related to germination and to vigor. Seedlings originated from seeds with pericarp displayed higher vigor only at 54% shading compared to seedlings from seeds without pericarp. The EVI was higher with pericarp removal. We conclude that the germination and establishment of *E. edulis* seedlings are favored by elevated shading levels, independent of the presence or absence of the pericarp. Pericarp removal can accelerate and increase the germination percentage, but sowing whole fruits might select the most vigorous individuals for cultivation.

Key words: juçara palm, native flora, seeds, species at risk of extinction.

RESUMEN

Euterpe edulis*, conocida como la palmera juçara, es una palmera con un alto valor económico y ecológico, que está en peligro de extinción. Este estudio evaluó los efectos de los niveles de sombreado (0, 16, 54, 72 y 92%) y la presencia y ausencia del pericarpio en la emergencia y el vigor de las plántulas de* E. edulis*. Noventa y nueve días después de la siembra, se contó el número total de plántulas emergidas para representar el porcentaje de emergencia (%), longitud de la raíz (RL) y las partes aéreas (SL), el peso de la materia seca total de las plântulas (DM y el índice de velocidad de emergência (EVI)). El aumento en los niveles de sombreado resultó en incremento lineal de variables relacionadas con la germinación y el vigor. Las plántulas de semillas con el pericarpio tienen mayor fuerza que el de 54% de sombra. En comparación con las plantas de semillero se originaron a partir de semillas sin pericarpio. El EVI fue mayor con la eliminación del pericarpio. Se deduce que la germinación y el establecimiento de las plántulas de* E. edulis *son favorecidos por los altos niveles de sombreado, independientemente de la presencia o ausencia del pericarpio. La remoción del pericarpio puede acelerar y aumentar el porcentaje de germinación, sino sembrar la fruta entera puede seleccionar los individuos más vigorosos para el cultivo.*

Palabras clave*: palmera juçara, flora nativa, semillas, especies en peligro de extinción.*

Introduction

Euterpe edulis Mart. (Arecaceae) is a palm tree that is widely distributed in well-preserved rainforests, occurring throughout the whole Atlantic Forest domain in Argentina, Brazil and Paraguay (Gatti *et al.* 2014). Popularly known as "juçara palm" or "palmiteiro", *E. edulis* is considered a key species in the Atlantic Forest, both for its ecological relevance and for its value to human societies (Reis *et al.* 2000). However, the historical exploitation of palm heart has led the plant to the Brazilian list of threatened species under the vulnerable category (Leitman *et al.* 2013).

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The juçara palm is a non-cespitous plant which takes from eight to ten years to reproduce. Harvesting the palm heart causes the immediate death of the plant and the cessation of its descendance (Matos *et al.* 1999). Palm heart extracting in natural forests is unsustainable since it impacts not only the palm itself, but also the animals that depend on palm fruits for food (Galetti and Aleixo 1999). The effects on the plant demography can be even more pronounced considering density-dependent mortality due to seed predation by different animals (Pizo and Vieira 2004; Rother *et al.* 2013). Mortality rates during the initial development stages vary according to landscape heterogeneity, whose direct influence happens to be in the form of herbivory and pathogens (Rother *et al.* 2013). *E. edulis* occurs in high densities in humid areas where interspecific competition is high, mainly among seedlings (Matos and Alves 2008). Seeds that are isolated by animal dispersal have a higher probability of germination and survival than the clustered ones, since clustering increases intraspecific competition (Pizo and Simão 2001).

One of the main issues related both to the cultivation and the ecology of *E. edulis* is its apparent preference for partially shaded locations; it is usually characterized as a sciophilous species (Reis *et al.* 2000). The availability of irradiation seems to be the limiting factor for the growth of *E. edulis* seedlings under the forest canopy (Illenseer and Paulilo 2002). In closed forest tracts only about 0.5 to 2% of solar light reaches the floor (Clark *et al.* 1996), which makes light availability a limiting factor for seedling growth for most species. Like *E. edulis*, most neotropical understory palm species are shade tolerant and have a number of adaptations to these shaded conditions such as photosynthetic tissues, epidermal chloroplasts, low compensation points for photosynthesis (Chazdon 1986a), horizontally oriented leaves (De Granville 1992) and long life spans (Chazdon 1986b). Regardless of their capacity for positive growth rates even under low irradiation (understood as the amount of light per area unit), the development and reproduction of neotropical palms are also limited by low light incidence (Chazdon 1986a; Gatti *et al.* 2011; Lavinsky *et al.* 2014).

When conservation and sustainability are sought in the economic exploitation of a given species, the most adequate form of cultivation is by using seeds, hence preserving genetic intrapopulation diversity. This is also the usual propagation method for the juçara palm. However, the germination of *E. edulis* seeds is heterogeneous, hampering seedling production and the upscaling of cultivation (Matos and Watkinson 1998). Many factors influence germination behavior, such as the degree of ripeness of the fruit, presence or absence of the pericarp, the time interval between harvest and sowing, environmental conditions, substrate humidity and seed dormancy (Matos and Watkinson, 1998). Studies have reported that pericarp removal, scarification and soaking in water might all improve the germination of the recalcitrant *E. edulis* seeds (Bovi 1990; Reis *et al.* 2000; Panza *et al.* 2007). In this context, the present study aims to evaluate the effect of shade levels and of the presence and absence of the pericarp on the emergence and vigor of *E. edulis* seedlings.The hypothesis is that seeds without pericarp placed under intermediate levels of shading will result in the highest germination rates and the most vigorous saplings.

Materials and Methods

The experiment was performed in the Capixaba Institute of Research and Rural Assistance (INCAPER), located in Domingos Martins, Espírito Santo, Brazil. The average annual rainfall is around 1260mm, while the average temperature is 14.6 °C in the coldest month and 21.2 °C in the warmest month, with an annual average of 18.2 °C (Alvares *et al.* 2013). The region is characterized by a subtropical climate, Cfb in the classification of Köppen (Alvares *et al.* 2013). It is located in the Atlantic Forest domain of mountainous and sub-mountainous dense ombrophilous subtypes (Veloso 1991), where *E. edulis* occurs naturally in abundance. The fruits were collected in a neighboring location, Venda Nova do Imigrante, less than 30km away, in December, 2014. Fruits were harvested from different plants in a small agroforestry yard circumscribed within a circular area of about 50 meters radius. Fruits from all plants were mixed together and only fully ripened fruits were selected for the experiment.

Seeds with and without pericarp (pulp) were subjected to five levels of shading: 0%, 16%, 54%, 72% and 92%. The pericarp was removed using a common pulping method among farmers from the highlands of the Espírito Santo: the fruits were immersed in hot water (65 °C) for approximately 20 minutes and afterwards were processed in a pulping machine with four straight rods for approximately 25 minutes. The pericarps had been completely removed by the end of the process.

Shading levels were obtained by combining different nylon shade cloths and measuring the resulting percentages with a portable digital Lux Meter. The shade cloths were fixed onto pieces of wire bent into small arches in the shape of small greenhouse tunnels of 25x10x09cm (length x width x height). The experiment was structured inside 20L plastic containers each filled with 1kg of medium grade vermiculite, watered initially with 2 L of regular water. Each container represented an experimental block.

The experiment was designed in randomized complete blocks with treatments arranged in a split plot design, in which shading levels (tunnels) were established in plots and the removal or not of the pericarp was established in subplots, by splitting each plot in two (Figure 1). In one subplot, sixteen fruits without pericarp were lined up while in the subplot next to it, sixteen fruits with pericarp were lined in parallel. The fruits were buried completely in the vermiculite. Afterwards, each plot with two lines of seeds (two subplots, one with seeds with pericarps and the other without) was covered with a tunnel of a different shading level. There were four blocks in total, composing five plots and ten subplots each.

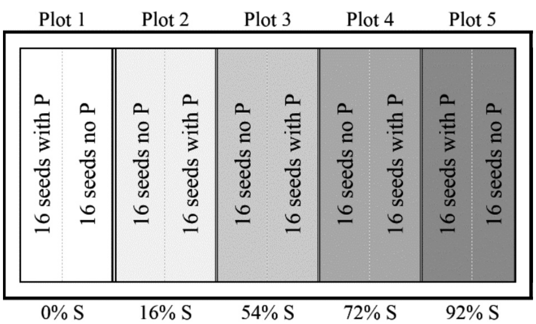

Figure 1. Exemplification of the arrangement of treatments in an experimental block (plastic container). P: pericarp. S: shading level.

The containers were kept in a greenhouse under daily irrigation, with the number of emerged plants being recorded on a weekly basis. After 99 days, the total number of emerged seedlings was counted to represent the emergence percentage (%). Additionally, root length (RL) and stem length (SL) (up to the top of the youngest leaves) of all seedlings were measured. All plant material was then placed into a forced air lab oven at 60 °C until it reached constant mass. The material was then weighed for determination of the total dry matter mass of the seedlings (DM). The emergence velocity index (EVI) was calculated according to equation 1 (Maguire 1962) based on the number of emerged seeds counted once a week from the thirtieth day on, when the first seedling emerged from the substrate, amounting to ten counts.

$$EVI = \sum_{i=1}^{n} \frac{N_i}{t_i} \qquad (1)$$

In which N_i represents the number of emerged seedlings in the first, second, etc. up to the last count (n); and t_i is the number of days from the sowing at the first, second, etc. up to the last count (n).

The Lilliefors normality test was applied for all dependent variables. Variables that did not conform to normality (emergence percentage, EVI and DM) had their values transformed in arcsine $(x/100)^{0.5}$ and were subjected again to the Lilliefors test.

Data were statistically analyzed by means of analysis of variance with slicing of significant interactions (RL, SL and DM). The mean values of treatments with and without pericarp were compared using the Tukey test ($p<0.05$) and the responses of variables to shading levels were evaluated with regression analyses. Linear, quadratic and cubic models were tested.

Results and Discussion

The interaction between factors (shading levels x fruits with pericarp and with no pericarp) was non-significant for the variables emergence percentage and EVI. For all other variables, the interaction between factors was significant.

Seedling emergence from fruits without and with pericarp began 30 and 44 days after sowing, respectively. The presence or absence of the pericarp did not significantly affect the emergence percentage (average values of 18% with pericarp and 20% without pericarp). However, pericarp removal significantly increased the EVI (average values of 0.281 without pericarp and 0.146 with pericarp).

An increase in shading levels resulted in linear increments of the variables related to germination and to vigor (Figures 2 and 3). The presence of the pericarp statistically favored variables related to vigor (SL, RL and DM) only at 54% shading (Table 1). Above and under 54% shading, the absence or presence of the pericarp did not affect these variables.

Similar to the results in this study, Bovi *et al.* (1990) observed that depulping accelerates seedling emergence, decreasing the average time of the germination process. However, the present

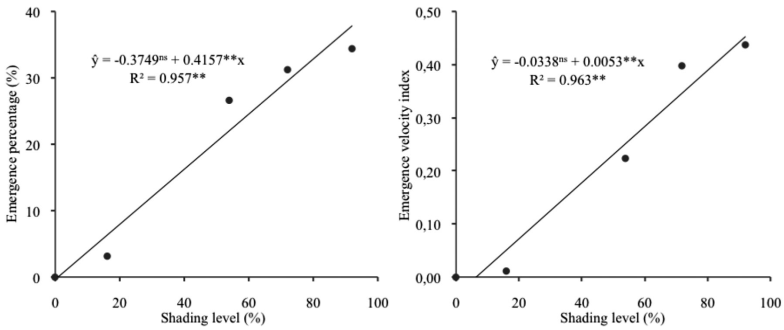

Figure 2. Emergence percentage and emergence velocity index of *Euterpe edulis* seedlings 99 days after sowing fruits with pericarp and with no pericarp under five shading levels. ns: non-significant (probability >10%); **: significant at 1% probability

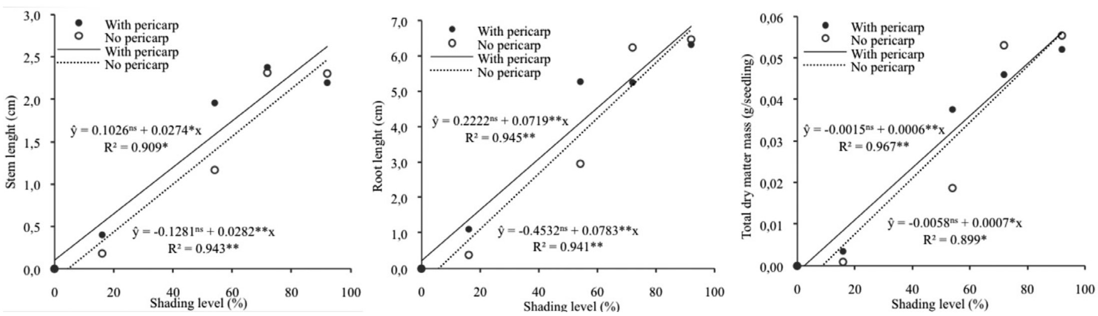

Figure 3. Stem length, root length and total dry matter mass of *Euterpe edulis* seedlings 99 days after sowing fruits with pericarp and with no pericarp under five shading levels. ns: non-significant (probability >10%); *, **: significant at 5% and 1% probability, respectively.

Table 1. Average values of variables related to the emergence and vigor of *Euterpe edulis* seedlings 99 days after sowing fruits with pericarp (WP) and with no pericarp (NP) under five shading levels.

	Shading level (%)					Shading level (%)				
	0	16	54	72	92	0	16	54	72	92
	Stem length (cm)					Root length (cm)				
WP	0.0a	0.4a	2.0a	2.4a	2.2a	0.0a	1.1a	5.3a	5.3a	6.3a
NP	0.0a	0.2a	1.2b	2.3a	2.3a	0.0a	0.4a	3.0b	6.2a	6.5a
	Total dry matter mass (g/seedling)									
WP	0.000a	0.004a	0.038a	0.046a	0.052a					
NP	0.000a	0.001a	0.019b	0.053a	0.055a					

Average values followed by the same letter within a column are not statistically different from each other according to the Tukey test, at 5% probability.

results show that pericarp removal, although it speeds up germination, does not significantly impact emergence percentage. Pericarp removal apparently increases the water and oxygen influx that triggers the germination process. According to Bovi *et al.* (1990), *E. edulis* shows two major limits to germination: the fruit pulp and a waxy operculum that prevents water from reaching the seed. It is relevant to comment on the water temperature in which the seeds were submersed before pericarp removal, since it may have altered the waxy operculum structure and thus favored seed soaking and the germination process.

The positive effect of pericarp removal on the physiological performance of *E. edulis* seeds was also verified by Cursi and Cícero (2014) on fruits immersed in water at 40 °C for 20 minutes before depulping. Nonetheless, the authors do not advise immersion in temperatures over 55 °C, stating that it can compromise seed quality. This recommendation is not confirmed by the present results, since fruits immersed at 65 °C kept high germination rates compared to fruits with pericarp.

Early emergence is a desirable characteristic for sapling production and pericarp removal would bring satisfactory results. Although having emerged more slowly, plants with preserved pericarp are more vigorous, which could be related to nutritional reserves present in this structure. However, Neuburger *et al.* (2010) found that until 135 days after germination, the expenditure of nutritional reserves was similar in seeds with and without pericarp and independent of the light or nutritional variables. Significant nutrient intake through the roots was detected only after this period. Given that in the present study plants were evaluated up to 99 days after sowing, nutritional reserves should not be considered as a relevant factor to explain the superior vigor of seeds with pericarp.

Pulping reduces the average length of the germination process, consequently increasing the density of seedlings. In natural conditions, seeds that have been just dispersed by animals suffered an intragastric process of pericarp removal. However, in many cases, *E. edulis* seeds fall on the soil and germinate with the pericarp on, just below the mother plant. In the former case, the absence of the pericarp contributes to plant establishment in new locations by increasing the velocity of seed germination and favoring the final percentage of seedling emergence (Bovi *et al.* 1990). In the latter case seeds tend to be found in higher densities, favoring intraspecific competition. In such a situation, natural selection of the most vigorous individuals and competition itself act as means of regulating demographic density (Pizo and Simão 2001). This might help understand why the physical barrier represented by the pericarp ends up by selecting, in each generation, the most vigorous individuals. However, as Pizo and Simão (2001) point out, the reproductive success of *E. edulis* individuals is influenced by a series of multiple interactions.

The good fit of linear models for all variables confirms an increase in both the emergence and the vigor of seedlings under higher shading levels. At the same time, the absence of a plateau indicates that the shading levels tested were below that which would possibly result in the maximum expression of the emergence and vigor potential of *E. edulis* seedlings. This means that shading levels above 92% might further improve results regarding these features. Such a tendency is conceivable only for the phases of seedling germination and establishment, since high shading levels may not have a positive effect on other phases of the life cycle of this species. Gatti *et al.* (2011) found that sapling stem growth in diameter and maximum photosynthetic activity were both lower with 10% light incidence than with unshaded conditions.

Also working with saplings, Illenseer and Paulilo (2002) observed increments in measurements related to growth under higher luminosity, whereas Neuburger *et al.* (2010) recorded the positive influence of light on seedling dry matter. Survival rates were higher in shaded conditions both in the shade house and in field conditions as opposed to more lighted environments (Gatti *et al.* 2014).

In spite of the relevance of light, Gatti *et al.* (2011, 2014) suggest that photoinhibition and water limitation can have a negative effect on *E. edulis* growth rates in forest gaps. Together with the vigorous growth of pioneer species under high light incidence, this could explain the absence of *E. edulis* in large forest clearings. Under this perspective, the results of the present study indicate that exclusion from forest clearings could have resulted, in the long term, in an adaptation to shaded conditions that would be more evident during the phases of seed germination and seedling establishment. Accordingly, planting recommendations involve initial location in the shade since its seedlings do not develop under full sun. Shade might then be gradually eliminated, exposing plants to full sun around the third year after planting (Martins and Souza 2009).

Braz *et al.* (2014) report that juçara seeds are insensitive to light in conditions of high water saturation (overflow). Thus water availability might even be more important than luminosity during the germination phase. In field conditions, it is possible that the species' preponderance in shaded environments is not due to shade itself, but due to moist soil. However, the results of the present study show that seeds under high luminosity did not germinate, even though the substrate was irrigated daily. It is possible that the vermiculite in the plots under full light dried up faster than in the shaded plots. However, since all levels of shade were represented in each container, the difference in humidity between plots was probably not enough to affect the factors under evaluation. Vermiculite is an extensively used substrate that has shown satisfactory results on germination. Among other factors, this is due to a good capacity for water retention, which is very important for recalcitrant seeds.

Conclusion

As preliminary recommendations derived from our results, although depulping as a practice speeds up germination and sapling production, it compromises vigor and might be detrimental to survivorship in challenging conditions. Shading favors both emergence and vigor of *E. edulis* plants and environments subjected to high solar incidence must be avoided in the plant initial phase. Nonetheless, the species' need for light during later life cycles must be considered as well as the possibility for canopy thinning in agroforestry systems. In evolutionary terms, two possible implications of these results could be further tested in the future: i) *E. edulis* adaptation to shaded environments, shown during the phases of seed germination and seedling establishment, is selected by interspecific competitive exclusion from environments with higher light incidence; ii) given that the pericarp represents an initial limiting factor to germination, the greater vigor of seedlings from seeds with pericarp results from selection in a context of intraspecific competition in the natural environment, where most fruits fall directly on the ground. These hypotheses, derived from the present study, open a vast field for further research on the reproductive mechanisms of *E. edulis*.

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