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Shading as a conditioning factor to forest species planting: a study with *Apuleia leiocarpa*

Intensidad de sombra como condición para la plantación de especies forestales: estudio con *Apuleia leiocarpa*

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SUMMARY

This study aimed at identifying the most appropriate level of shading for initial growth of *Apuleia leiocarpa* seedlings, through their survival, morphological and physiological/biochemical parameters. The following treatments were evaluated: 0 (full sun), 18, 50 and 70 % of shading. Survival was assessed at 30 and 360 days after planting and morphological parameters such as height (H), stem diameter (SD) and H/SD ratio were measured bimonthly for a year. Physiological parameters as contents of chlorophyll *a*, chlorophyll *b*, chlorophyll *a*/*b* ratio, carotenoids, lipid peroxidation, acid phosphatase enzyme activity, initial fluorescence, maximum fluorescence, maximum quantum yield of photosystem II and electron transport rate were estimated at 180 and 360 days after planting. At 30 days after planting (d.a.p) there was no mortality of *Apuleia leiocarpa* seedlings influence of different levels of shading. In the assessments performed at 180 and 360 d.a.p., there was no significant difference among levels of shading for H and SD. However, high mortality rates were observed at 360 d.a.p., especially in full sun. Morphological and physiological parameters of *Apuleia leiocarpa* plants showed this species requires shading (18 and 50 %) in their early stages of growth.

Key words: Fabaceae, morphological and physiological parameters, native species.

RESUMEN

El objetivo de este estudio fue identificar la intensidad de sombra más adecuada para el crecimiento inicial de plantas de *Apuleia leiocarpa*, por medio de la supervivencia y parámetros morfológicos y fisiológicos/bioquímicos. Los tratamientos fueron: 0 (pleno sol); 18, 50 y 70 % de sombra. La supervivencia se evaluó a los 30 y 360 días después del trasplante y los parámetros morfológicos altura (H), diámetro del cuello (DC) y la relación H/DC, bimensualmente durante un año. Los parámetros fisiológicos contenido de clorofila *a*, clorofila *b*, relación clorofila *a/b*, carotenoides, peroxidación lipídica, actividad de la enzima fosfatasa ácida, fluorescencia inicial, fluorescencia máxima, rendimiento cuántico máximo del fotosistema II y la tasa de transporte de electrones fueron estimados a los 180 y 360 días después del trasplante. A los 30 días después de la siembra (d.a.p) no hubo mortalidad de plántulas de *Apuleia leiocarpa* ni influencia de diferentes niveles de sombreado En las evaluaciones realizadas a 180 y 360 d.a.p. No hubo diferencia significativa entre los niveles de sombreado para H y SD. Los parámetros morfológicos y fisiológicos de las plantas de *Apuleia leiocarpa* evidencian que la especie necesita sombra (18 y 50 %) en su etapa inicial de crecimiento.

Palabras clave: Fabaceae, parámetros morfológicas y fisiológicas, especie nativa.

INTRODUCTION

Anthropic activities have been causing significant changes in the structure and functioning of ecosystems, resulting in new conditions that lead to alterations of the original environments, consequently, contributing to decrease and loss of species diversity and habitat extinction (Bogaert *et al*. 2011).

According to a study of endangered native flora of Rio Grande do Sul State (Brazil) (SEMA 2014), several tree species with economic value were identified, including *Apuleia leiocarpa* J.F. Macbr (grápia), which is among the critically endangered species. *Apuleia leiocarpa* was described as having commercial value due to high wood qualities, such as easy workability, high natural resistance and multiple uses. Seedlings of *Apuleia leiocarpa* do not present significant regeneration in forests understory being replaced by others with superior tolerance to shade. Furthermore, this species has commercial biotechnology potential due to its antimicrobial effect for combating phytopathogenic bacterium of *Xanthomonas campestris* (Carvalho *et al*. 2015). Carvalho (2003) describes that species as having semi-heliophile behavior while Backes and Irgang (2009) classify it as an aggressive pioneer in fallow vegetation, with wide distribution in Brazil and other Latin American countries.

Apuleia leiocarpa has a wide geographic distribution that includes from the state of Pará to Rio Grande do Sul, Brazil, as well as Argentina, Bolivia, Paraguay, Peru and Uruguay (Carvalho 2003). Furthermore, this species is associated with environmental variation of the regions might be a determining factor in the classification of behavior when it comes to light demand. In this context, species with such importance in ecological and timber production should be studied regarding the effects of growth environment, thus enabling its establishment in pure and/or enrichment plantations, with low risk of losses in the field.

The selection of tree species must be established based on the characteristics of the site, planting purpose and, crucially, their physiological, ecological and silviculture requirements (Lamprecht 1990). Lamprecht (1990) also mentions that the main obstacle in native forest refers to insufficient knowledge about tropical tree species. Regarding that, Kageyama and Castro (1989) analyzed forest planting of native species, aiming at systematizing some criteria that would serve as a basis to such research. Currently, despite the available and highly relevant work, however, there is a lack of detailing that encourages the use of native tree species of high timber value for outplanting, which could provide species conservation associated with timber use. Although Brancalion *et al*. (2012) describe the existence of information of Brazilian native species with exception of publications such as Carvalho (2003) and Kageyama and Castro (1989), other experiences are only restricted to queries.

The species requirements regarding intensity, duration and periodicity of light are among the factors that interfere in the production of forest plants (Azevedo *et al*. 2015). Response of plants to light availability has a central role in determining their distribution and success. Demand for light by native forest species is commonly described based on phytosociological studies (Lima *et al*. 2010). However, no studies were found evaluating shading in post-planting, whose limitations become more significant for the plants and require adaptation to the new environment. This information is important because, with plasticity, species are more likely to survive in harsh conditions for their ability to adapt physiologically, biochemically and morphologically.

Evaluating plants in shading experiments addresses physiological parameters such as chlorophylls and carotenoids contents, which provides important information on the establishment, growth and plasticity of the photosynthetic apparatus (Afonso *et al*. 2012). In addition to morphological parameters as height and stem diameter. These photosynthetic pigments are used in works as quality indicators for the seedlings. It is expected that shading may affect the growth of *Apuleia leiocarpa* seedlings in the field, presenting higher growth as the intensity of shading increases. Thus, to establish the suitable planting condition for *Apuleia leiocarpa*, the study aimed to identify the most appropriate level of shading for the initial growth of seedlings of species, through plant survival and morphological and physiological/biochemical parameters.

METHODS

Characterization of the study area. The experiment was conducted for a year (2012-2013) in an area near to the Forestry and Nursery Laboratory of the Department of Forest Sciences of the Federal University of Santa Maria (UFSM) (29º 43' 12.65" S; 53º 43' 08.74" O), Rio Grande do Sul State (RS), Brazil. According to Köppen's classification, the local climate is 'Cfa' (humid subtropical), with mean temperature in the coldest month between 3 and 18 °C, and in the warmest month above 22 °C; and mean annual precipitation of 1,600-1,900 mm (Alvares *et al*. 2013). Temperature (minimum, average and maximum) and monthly precipitation data are according to Alvares *et al*. (2013) during the study period and can be observed in figure 1.

Seedlings of *Apuleia leiocarpa* were produced from October to July in the Forestry Laboratory in 110 cm³ containers. The commercial substrate peat based on *Sphagnum*, plus 20 % of carbonized rice husk, was used. In the base fertilization Osmocote® (15-9-12), 6 g L^{-1} substrate was used. They were randomly selected for planting in the field and presented the following averages: 31.60 (± 3.94) cm of height (H); 3.94 (± 0.40) mm of stem diameter (SD) and 8.05 (± 0.89) cm mm^{-1} of H/SD ratio.

Prior to planting, soil characterization of the area was performed in the Soil Analysis Laboratory (UFSM), which showed the following characteristics: low organic matter content (1.3 to 2.0 %), phosphorus level from very low to low (3.4 to 5.3 mg dm^{-3}), high potassium level (60 to 82 mg dm^{-3}), high magnesium level (1.3 to 2.5 $cmol_c$ dm^{-3}), calcium level from medium to high (3.5 a 7.4 $cmol_c$ dm^{-3}), average cation exchange capacity ($CEC_{pH\ 7.0}$) (12.4 to 13.2 $cmol_c$ dm^{-3}), SMP index ranging from 5.4 to 6.4 and texture 3 (SBCS/CQFS 2004). Based on chemical properties, soil pH was adjusted to 5.5 with lime (PRNT 75.1 %) in the total area and it was incorporated into the soil one month before planting. Different intensities of shading were used as treatments: T1 – 0 % (full sun - control), T2 – 18 %, T3 – 50 % and T4 – 70 %. The different shading intensities were obtained using nylon mesh on 2-meter-high arches in the center of the tunnel.

Planting spots were opened (30 cm of diameter x 35 cm depth) and aligned in the east/west direction, spaced 1 x 1 m. Spots were filled with the same soil as described

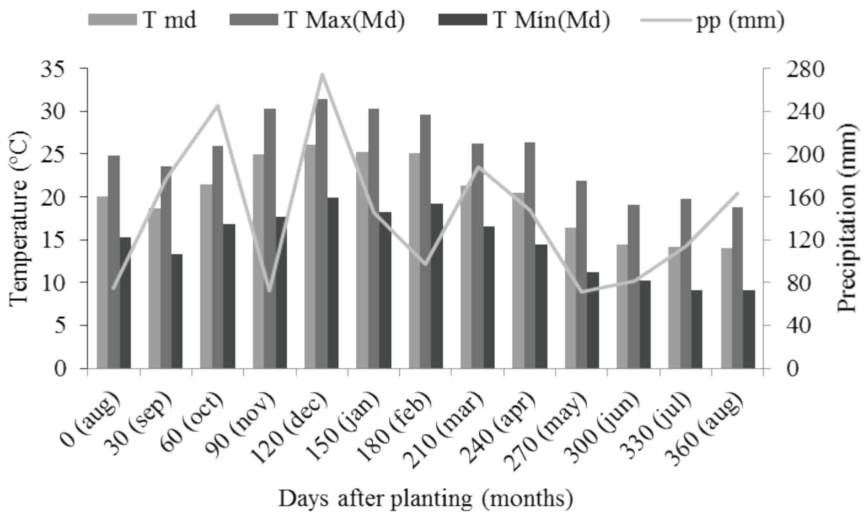

Figure 1. Monthly climatic data of Santa Maria (RS) in "year" during the experiment with *Apuleia leiocarpa* seedlings. T = temperature (md = average; Max = maximum; Min = minimum); pp = precipitation. Source: Climatological Station of Santa Maria, Federal University of Santa Maria.

Datos climatológicos de Santa Maria (RS) durante el experimento a campo con plantas *Apuleia leiocarpa*. T = temperatura (md = media; Max = máxima; Min = mínima); pp = precipitaciones. Fuente: Estación Climatológica Principal de Santa María, Universidad Federal de Santa María.

previously and added with 500 mL of commercial substrate containing barley residue, to improve both physical and chemical qualities of the soil. Substrate used showed these characteristics: pH 4.66, 560.12 kg m^{-3} dry density, 81.05 % total porosity, 5.83 % aeration space, 23.39 % easily available water and 18.72 % buffering water

In absence of precipitation, seedlings were irrigated every two days in the first month, with two liters of water per plant and subsequently once a week. Dry vegetation cover (mulching) was placed around the seedlings with approximately 35 cm in diameter and 5 cm in height. Cover fertilizing was performed at 30 and 180 days after planting (d.a.p), with NPK (5-20-20). Two lateral holes were opened for each seedling for application of 100 g of fertilizer (50 g in each side) at a distance of approximately 10 cm from the plant. Silvicultural treatments, such as insecticide application, ant control, crowning with manual weeding surrounding the seedlings and in line control of weed competition by herbicide application on two occasions (120 and 180 days after planting), were performed during the experiment.

Survival evaluation was performed at 30 and 360 d.a.p and morphological parameters such as height (H) and stem diameter (SD) were measured at the time of planting and, afterward, bimonthly (60, 120, 180, 240, 300 and 360 d.a.p). Height was measured with a ruler (cm) and stem diameter with a digital caliper (mm) to provide the H/SD ratio. In addition, an analysis of the absolute growth rate (AGR) for H and SD was performed by using the following formula (Benincasa 2003):

$$AGR = (H_1 - H_0)/(T_1 - T_2) \quad [1]$$

Where: AGR = Absolute growth (AGR); H_1 = Height at time n; H_0 = Height at time n-1; T_1 = Number of days of time n; T_0 = Number of days of time n-1.

Physiological characteristics such as chlorophyll content, carotenoids, lipid peroxidation and activity of acid phosphatase enzyme were analyzed in the Plant Biochemistry Laboratory of the Biology Department (UFSM) at 180 and 360 d.a.p. For these analyses, the fourth expanded leaf from the apical meristem of three plants was collected per treatment, which were immediately frozen in liquid nitrogen and stored at -80 °C in an ultrafreezer until quantification. The contents of chlorophyll *a*, chlorophyll *b* and carotenoids were determined by following the methodology described by Hiscox and Israeslstam (1979) and estimated using the formula of Lichtenthaler (1987). The fresh samples of leaves (0.1 g) were incubated at 65 °C with dimethylsulfoxide (DMSO) for two hours. Subsequently, the absorbance of the solution was measured in a spectrophotometer at 663, 645 and 470 nm. The peroxidation of membrane lipids was estimated according to the methodology of El-Moshaty *et al*. (1993) and expressed as nmol MDA mg^{-1} of protein. The activity of the acid phosphatase enzyme was determined according to the methodology of Tabaldi *et al*. (2007).

Determination of chlorophyll *a* fluorescence was performed using a portable fluorometer (Junior-Pam®) in fully expanded leaves from plants at 180 and 360 d.a.p. The selected leaves were submitted to 30 minutes in the dark. After that, they were exposed to saturated light pulses at an induced wavelength by red light (peak at 650 nm) for approximately 3.000 µmol m^{-2} s^{-1}, obtaining the initial fluorescence signs (Fo), maximum fluorescence (Fm), maximum quantum yield of the photosystem II (Fv/Fm) and electron transport rate (ETR).

Design and statistical analysis. Experimental design was set up as randomized blocks, with four treatments (shading levels) in five blocks totaling 80 seedlings. The statistical analysis was carried out using SISVAR software 5.3 (Ferreira 2011), submitting the data to normality and homogeneity and subsequently to the ANOVA analysis. Means were compared by Tukey test at 5 % probability.

RESULTS

At 30 days after planting (d.a.p), there was no mortality of *Apuleia leiocarpa* seedlings. Survival rate was high and without influence of different levels of shading. However, high mortality rates were observed at 360 d.a.p., especially in full sun (table 1).

Table 1. Survival rate of *Apuleia leiocarpa* seedlings at 30 and 360 days after planting in the field, at different levels of shading.

Tasa de supervivencia de plantas de *Apuleia leiocarpa* a los 30 y 360 días después del trasplante a campo, en diferentes niveles de sombra.

Level of shading (%)	Survival (%)	
	30 d.a.p	360 d.a.p
0	100 a*	50 b
18	100 a	90 a
50	100 a	80 ab
70	100 a	95 a

* Averages followed by different letters in the column differ from each other by the Tukey test ($P < 0.05$).

Absolute growth rate (AGR) in height and stem diameter shows the same behavior, which is observed zero or reduced growth at 60 days. However, after the establishment during the spring, there was significant growth in subsequent seasons in up to 240 days, which coincides with the fall (figure 1), followed by the decrease in the dormant period (figures 2 and 3).

In the assessments performed at 180 and 360 d.a.p., there was no significant difference among levels of shading for H and SD (table 2). The H/SD showed lower seedling stability under 50 and 70 % of shading, especially at 180 days, when there was a reduction of photoperiod and temperature (figure 1).

Shading levels and age of the plants (180 and 360 days) significantly influenced ($P < 0.05$) the content of photosynthetic pigments (table 3). Chlorophyll contents were higher at the highest shading condition, especially at 360 d.a.p (winter) under 70 %. Moreover, it is important to emphasize that *Chl a* was always higher in the winter, while *Chl b* showed the highest oscillation (table 3). *Chl a/b* ratio shows more variation in data when comparing both shading and different season growths (winter and summer). At 360 d.a.p, a higher content of 18 and 50 % levels was observed.

Carotenoid content was higher in plants with 50 % of shading at 180 days and with 70 % at 360 days (table 3). Similarly, *Chl a* content values were the highest at 360 days under any level of shading.

There was no significant difference among treatments for lipid peroxidation content at 180 days, although, at 360 days that value was higher in absence of protective mesh (full sun) (table 4). For acid phosphatase enzyme, there was no difference among treatments at 180 days, while at 360 days that activity was higher with 0 and 50 % of shading. The acid phosphatase enzyme was enhanced at 180 days, although there was no difference among treatments. On the other hand, at 360 days, full sun and 50 % of shading were higher.

Table 2. Height (cm), stem diameter (mm) and ratio H/SD averages of *Apuleia leiocarpa* seedlings in full sun (0 %) and different intensities of shading (18, 50 and 70 %), at 180 and 360 days.

Medias de altura (cm), diámetro del cuello (mm) y relación H/DC de plantas de *Apuleia leiocarpa*, a pleno sol (0 %) y diferentes niveles de sombra (18, 50 y 70 %) a 180 y 360 días.

Level of shading (%)	Height (H)	Stem diameter (SD)	H/SD ratio	Height (H)	Stem diameter (SD)	H/SD Ratio
	180 days			360 days		
0	46.04 a*	7.41 a	6.25 ab	79.65 a	11.50 a	6.53 a
18	47.60 a	7.92 a	6.01 a	82.18 a	12.36 a	6.68 ab
50	51.02 a	7.25 a	6.97 ab	92.33 a	10.19 a	9.04 c
70	59.19 a	7.23 a	8.33 b	92. 63 a	10.41 a	8.79 bc
CV (%)	33.46	29.02	18.23	41.95	33.07	14.75

* Averages followed by different letters in the column differ from each other by the Tukey test ($P < 0.05$). CV: Coefficient of variation.

There was no significant interaction between shading x time for chlorophyll *a* fluorescence variables. Initial fluorescence (Fo), maximum fluorescence (Fm), Fv/Fm ratio and ETR showed significance for isolated factors ($P < 0.05$). Fo values increased at higher levels of shading (50 and 70 %), similarly to Fm. During winter (360 days) Fo was significantly higher in the summer (360 days), which was similar to Fm, however, in this case there was no statistical difference ($P = 0.2980$) (table 5).

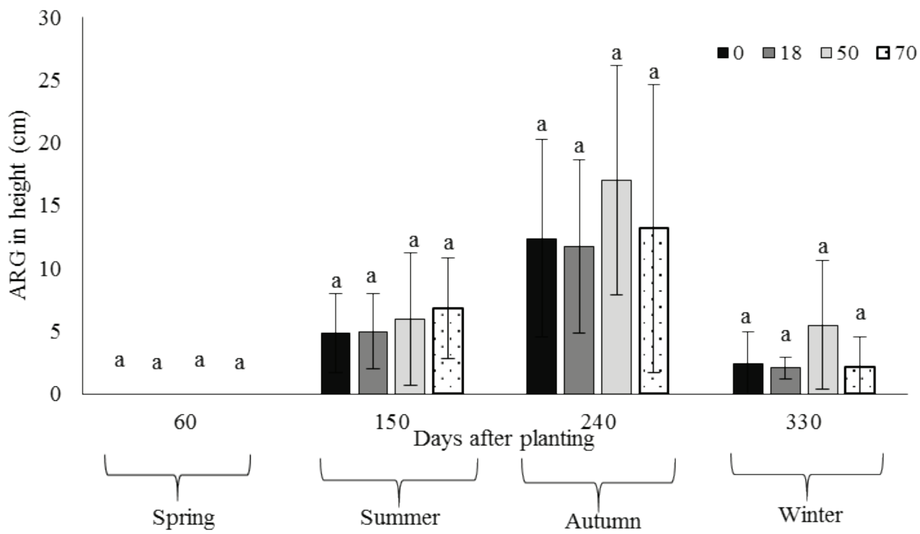

Figure 2. Absolute growth rate (AGR) in height of *Apuleia leiocarpa* seedlings in different levels of shading (0, 18, 50 and 70 %), as a function of days after planting. Averages followed by different letters in each season differ from each other by the Tukey test ($P < 0.05$).

Tasa de crecimiento absoluto (TCA) en altura de plantas *Apuleia leiocarpa* en diferentes intensidades de sombra (0, 18, 50 y 70 %), en función de los días después del trasplante. Medias seguidas por letras diferentes en cada estación, se diferencian por la prueba de Tukey ($P < 0,05$).

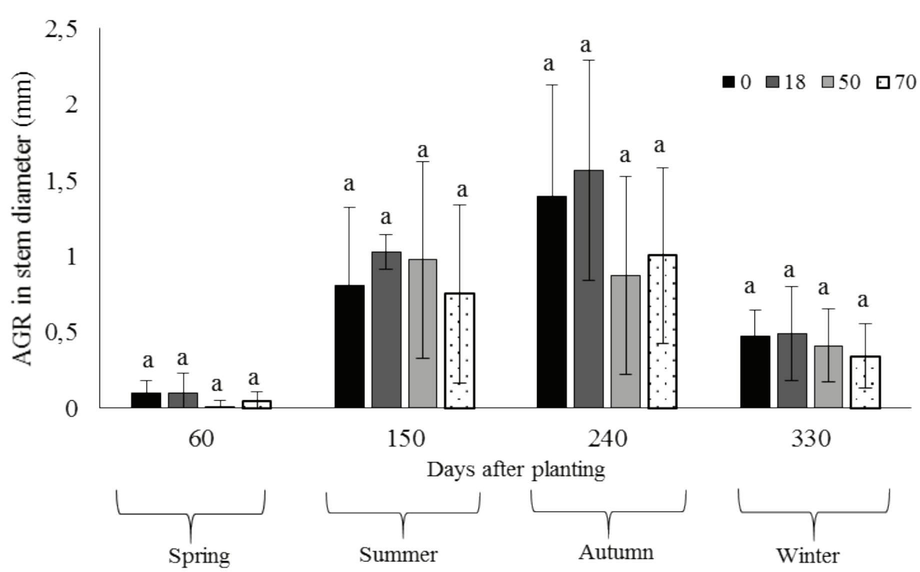

Figure 3. Absolute growth rate (AGR) in stem diameter of *Apuleia leiocarpa* seedlings in different levels of shading (0, 18, 50 and 70 %), as a function of days after planting. Averages followed by different letters in each season differ from each other by the Tukey test ($P < 0.05$).

Tasa de crecimiento absoluto (TCA) del diámetro del cuello de plantas de *Apuleia leiocarpa* en diferentes intensidades de sombra (0, 18, 50 y 70 %), en función de los días después del trasplante. Medias seguidas por letras diferentes en cada estación, se diferencian entre sí por la prueba de Tukey ($P < 0,05$).

Table 3. Averages of chlorophyll *a*, *b* and Chl *a*/*b* ratio of *Apuleia leiocarpa* seedlings in full sun (0 %) and in different levels of shading (18, 50 and 70 %), at 180 and 360 days after planting.

Medias de clorofila *a*, clorofila *b*, y relación *Chl a/b* de plantas de *Apuleia leiocarpa* en pleno sol (0 %) y diferentes intensidades de sombra (18, 50 y 70 %) después de 180 y 360 días en campo.

Level of shading (%)	Chlorophyll *a* (mg g^{-1} FM)		Chlorophyll *b* (mg g^{-1} FM)		*Chl a*/*b* ratio		Carotenoids (mg g^{-1} FM)	
	Days after planting							
	180	360	180	360	180	360	180	360
0	0.82 Bc*	1.26 Ab	0.15 Bc	0.27 Ab	5.57 Aa	4.65 Ba	0.28 Bb	0.45 Ab
18	1.00 Bb	1.15 Ab	0.24 Ab	0.24 Ab	4.24 Acb	4.72 Aa	0.17 Bd	0.40 Ac
50	1.10 Bb	1.28 Ab	0.33 Aa	0.26 Bb	3.33 Bc	4.86 Aa	0.32 Ba	0.42 Ac
70	1.32 Ba	1.84 Aa	0.29 Bab	0.69 Aa	4.63 Aab	2.68 Bb	0.25 Bc	0.55 Aa
CV (%)	5.45		7.78		12.19		3.74	

*Averages followed by different capital letters on the line and lower in the column differ from each other by the t (line) and Tukey (column) tests ($P < 0.05$); CV: Coefficient of variation and FM = fresh mass.

Table 4. Averages of lipid peroxidation and acid phosphatase enzyme of *Apuleia leiocarpa* seedlings in full sun (0 %) and in different levels of shading (18, 50 and 70 %), at 180 and 360 days after planting.

Medias de peroxidación lipídica y actividad de la enzima fosfatasa ácida de plantas de *Apuleia leiocarpa* en pleno sol (0 %) y diferentes intensidades de sombra (18, 50 y 70 %), a los 180 y 360 días en campo.

Level of shading (%)	Lipid peroxidation (mmol de MDA g^{-1} FM)		Acid phosphatase enzyme (nmol Pi g FW^{-1} min^{-1})	
	Days after planting			
	180	360	180	360
0	0.77 Aa	1.23 Bc	5.97 Aa	8.90 Ba
18	0.59 Aa	0.77 Aab	5.93 Aa	9.47 Bb
50	0.48 Aa	0.44 Aa	5.96 Aa	8.59 Ba
70	0.49 Aa	0.89 Bb	5.88 Aa	9.52 Bb
CV (%)	20.27		1.87	

*Averages followed by different capital letters on the line and lower in the column differ from each other by the t (line) and Tukey (column) tests ($P < 0.05$); CV: Coefficient of variation and FM = fresh mass.

Table 5. Chlorophyll *a* initial fluorescence (Fo), maximum fluorescence (Fm), maximum quantum yield of photosystem II (Fv/Fm) and electron transport rate (ETR) in *Apuleia leiocarpa* seedlings in different levels of shading (0, 18, 50 and 70 %), at 180 and 360 days after planting.

Fluorescencia inicial de la clorofila *a* (Fo), fluorescencia máxima (Fm), rendimiento cuántico máximo del fotosistema II (Fv/Fm) y tasa de transporte de electrones (ETR) en plantas de *Apuleia leiocarpa* en diferentes intensidades de sombra (0, 18, 50 y 70 %), a los 180 y 360 días en campo.

Level of shading (%)	Fo	Fm	Fv/Fm	ETR
0	206.83 b*	393.92 b	0.53 a	22.37 a
18	252.50 b	527.00 b	0.52 a	19.67 a
50	306.33 ab	794.17 a	0.62 a	17.29 a
70	457.33 a	879.33 a	0.52 a	15.71 a
Time	Fo	Fm	Fv/Fm	ETR
180	227.92 b	617.04 a	0.6267 a	21.82 a
360	383.58 a	680.17 a	0.4675 b	15.70 b
CV (%)	31.35	22.16	21.74	31.94

*Averages followed by different letters in the column differ from each other by the Tukey test (levels of shading) and the t test (time) ($P < 0.05$) e CV: Coefficient of variation.

The Fv/Fm ratio was not significant among shading levels, corresponding to 55 % of photons arriving in the plant and being transferred to the photochemical route. The largest luminous energy transfer occurred in the summer (table 5). These results correspond to the electron transport rate as observed, regarding both, level of shading and evaluation season.

DISCUSSION

The present study shows the effect of shading during the initial establishment of *Apuleia leiocarpa* seedlings in the field, required to achieve higher survival rates and to increase the physiological performance of the plants (tables 3, 4 and 5). In this study, the effect of shading levels on survival, growth and physiological characteristics of *Apuleia leiocarpa* seedlings in the initial field establishment is reported for the first time.

Among the environmental factors, solar radiation has an important role in photosynthesis, regulating the survival, growth and adaptation of plants (Zervoudakis *et al.* 2012). The survival of *Apuleia leiocarpa* seedlings in 360 days was positively influenced by increased levels of shading. High mortality of *Apuleia leiocarpa* in full sun could be associated with the fact that the species requires more protection in the early stage of development, which possibly is related to the reduced shading and the influence of lower temperatures. This result could be associated with the level of lipid peroxidation, which was higher in full sun at 360 days, and with the content of carotenoids, which were lower in less shaded treatments at 360 days. Probably, carotenoids did not protect *Apuleia leiocarpa* plants from the excess of light and, consequently, there was higher lipid peroxidation and plant mortality. Some of these results corroborates Carvalho (2003), who classifies *Apuleia leiocarpa* as a species with semi-heliophile behavior. Under natural conditions, in the regeneration phase, this species has a tolerance to low light intensities, due to closed canopy.

Apuleia leiocarpa plants showed similar behavior for TCR in height and stem diameter. Variation of AGR is an expected response, since any increase over a certain period is directly related to the dimensions previously reached (Lima *et al*. 2007).

The species behavior in relation to the irradiance is distinguished, mainly when seedlings come to growth in the field, indicating different tolerance levels demanded, which is important for plant development and use in forestry. Aimi (2014) and Tonetto (2014), studying respectively *Cabralea canjerana* Vell. Mart. (canjerana) and *Handroanthus heptaphyllus* (Mart.) Mattos (ipê-roxo), observed antagonistic behavior of species in relation to light requirement, with the highest growth occurring under 50 % of shading and full sun, respectively.

In addition to the high survival of *Apuleia leiocarpa* seedlings under 18 % of shading, we also found a higher carbon allocation in stem diameter in relation to height, a fact opposed to the condition of 50 % of shading. The acid phosphatase enzyme was more active at 360 days under 70 % shading, indicating that the phosphorus mobilization promoted by this enzyme may have contributed to increasing stem diameter. Several studies have shown that height/stem diameter ratio of trees decreases with light availability, suggesting that shading affects the growth in diameter more negatively than does height growth (Petritan *et al*. 2009).

Almeida *et al*. (2004) reported that plant growth might reflect the ability of species adaptation to environmental radiation conditions in which they are developing. Furthermore, Scalon *et al*. (2002) reported that growth characteristics are used to infer the degree of species tolerance to low light availability.

The increase of photosynthetic pigment content in leaves of plants due to the increase in shading is a response widely described in literature (Lichtenthaler *et al*. 2007). In this study, increasing the level of shading for *Apuleia leiocarpa* plants caused an increase in photosynthetic pigment content. At 180 days (summer), an increase of chlorophyll *a* and *b* was observed in higher shading levels in order to meet the minor incident radiation, as well as at 360 days.

It is noteworthy that, possibly, the species needs to invest in the production of pigments to better capture incident light energy. This response allows the plant to maximize the gain in carbon and changes in light interception. Chlorophyll is constantly being synthesized and destroyed (photooxidation) in the presence of light, nevertheless, under higher light intensities further degradation occurs and balance is established at a lower concentration (Almeida *et al*. 2004). This is confirmed by the increase of carotenoids, which absorb different wavelengths, from those absorbed by *Chl a* and *b*. There was an increase in chlorophyll production and 70 % shading at 360 days. Typically, carotenoids acquire the ability to increase with decreasing light intensity and may perform different functions during photosynthesis, such as absorption in the light-harvesting complex and photoprotection of the photochemical apparatus (Kerbauy 2004).

The behavior of *Apuleia leiocarpa* in the south region of Brazil demonstrated to be similar to which was found in other studies, such as Rêgo and Possamai (2006); when evaluating light (full sun, 34, 44, 64 and 70 %) in *Cariniana legalis Cariniana legalis* Kuntze (jequitibá-rosa), they observed that the contents of chlorophyll *a, b* and total were higher in plants under lower light condition. Lima *et al*. (2010) confirmed that the highest carotenoid content in *Hymenaea courbaril* L. (jatoba) and *Enterolobium contortisiliquum* (Vell.) Morong. (timbaúva) occurred under shading, thus differing from those in full sun.

In the present study, it was found that the chlorophyll *a/b* ratio showed high values under full sun (5.5 and 4.6, respectively at 180 and 360 d.a.p) with reduction due to shading increase (2.6; 70 % shading to 360 d.a.p). In general, chlorophyll *a/b* ratio increases with higher irradiance in plants, however this feature could respond differently given the availability of nitrogen (Maina and Wang 2015). Under shading conditions, plants show less chlorophyll *a*/*b* compared to plants in full sun due to the high portion of chlorophyll *b* assigned to the antenna complex. Streit *et al*. (2005) describe this relationship in plants occurs at 3:1 ratio.

According to Taiz and Zeiger (2013), some plants have plasticity to adapt to a range of light regimes and can grow in full sun or in shady environments. The Fv/Fm ratio has been the most commonly used parameter for characterizing conditions affecting the photochemical efficiency of plants under stress conditions (Lichtenthaler *et al*. 2007, Bussotti and Pollastrini 2015). In general, low values of Fv/Fm ratio indicate foliar photoinhibition (Cascio *et al*. 2010). According to our results, there was a significant difference between the values of Fv/Fm estimated in leaves of *Apuleia leiocarpa* in different seasons. Plants subjected to stress caused by low winter temperatures had lower values of Fv/Fm (0.47) compared to the value observed at higher temperatures (0.63).

Even there was higher initial and maximum fluorescence in the treatments with 50 and 70 % of shading, no significant difference was found among treatments for maximum quantum yield of photosystem II (Fv/Fm) and electron transport rate (ETR). That indicates the biomass production was not affected in high shading treatments, since there was no significant difference between treatments for height and stem diameter of *Apuleia leiocarpa* under 18 % and 50 % of shading, they showed values of Fv/Fm closed to 0.55 and electron transport rate around 18 µmol electrons m^{-2} s^{-1}. Ritchie *et al*. (2010) described that Fv/Fm below 0.6 and electron transport rate higher than 300 µmol electrons m^{-2} s^{-1} represent stress to plants. However, there is wide variation between species, and we believe that in these environments (18 % and 50 % of shading) in which there was high survival rate, such values were not limited to the establishment of the species and could be considered appropriate to be a reference on the photochemical efficiency of *Apuleia leiocarpa*.

The significant reduction of photochemical efficiency (Fv/Fm) of the plants of *Apuleia leiocarpa* between 180 and 360 days after planting was probably due to the inhibitory effect of photosynthesis because of the average temperature difference among times. Probably, at 360 days, higher temperatures were observed, which might have reduced Fv/Fm values. This pattern of reducing the photochemical efficiency due to injuries caused by low temperature has already been observed in *Pinus sylvestris* L. (Linkosalo *et al*. 2014).

Adaptation to light intensities is a genetic characteristic, since leaves have anatomical structure and physiological properties able to, effectively, use the available solar radiation. In this sense, considering that *Apuleia leiocarpa* has wide distribution, it could be planted in different latitudes, light intensities and temperatures, except in the condition of full sun and high shading.

CONCLUSIONS

The initial hypothesis of this study is confirmed: shading affects the growth of seedlings *Apuleia leiocarpa* in the field.

Morphological and physiological parameters of *Apuleia leiocarpa* plants evidence that this species requires shading (18 to 50 %) in its early stage of development in the field.

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