Braz. J. Bot (2016) 39(1):185–191
DOI 10.1007/s40415-015-0234-y

Effect of shade on emergence, initial growth, and seedling quality in *Physocalymma scaberrimum*

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Received: 11 August 2015 / Accepted: 11 November 2015 / Published online: 14 December 2015

Abstract Research on the ecophysiology of seedling propagation of native species is essential to meet the growing need to remediate environmentally degraded areas. The objective of this study was to evaluate the effect of shade on the emergence, initial growth, and quality of seedlings of *Physocalymma scaberrimum* Pohl. Seeds were sown in cells kept in full sun and under conditions of 30 and 70 % shade. To evaluate the initial growth of seedlings at 80 days after emergence, the seedlings were transplanted to plastic bags containing a mixture of sifted distroferric red latosol, sand, and semidecomposed chicken manure (1:1:0.5) and kept under the same light conditions. Analysis was carried out after 30 days of acclimatization and every 30 days (30, 60, 90, and 120 days after transplantation when seedlings were 110, 140, 170, and 200 days old). The 30 and 70 % shade conditions resulted in greater seed germination and vigor in *P. scaberrimum.* However, seedlings exposed to full sun and 30 % shade showed greater growth, better quality, and more efficient carboxylation.

Keywords Brazilian savanna · Brightness · Seedling production

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Introduction

Human use of natural resources can result in major environmental changes. The consequences of expansion of agriculture and farming and deforestation, among others, are often irreversible, including the extinction of species and loss of biodiversity, as has occurred in the Brazilian Savanna biome. Thus, for the recovery these degraded areas, especially using native species, are fundamental research about ecophysiological behavior and the production of seedlings of these species (Pacheco et al. 2013; Scalon et al. 2014).

Ronquim et al. (2009) in their review noted that the heterogeneity of irradiance in different physiographic areas of the Brazilian Savanna may benefit species with a high capacity for acclimatization. Very little is known about the effects of different light regimens on growth and photosynthetic metabolism although this is one of the most important aspects of Brazilian savanna vegetation.

Responses of different species to shade are variable, and luminosity is a critical factor for the development of a plant and its physiological tolerance to the luminous intensity. Among the various factors that influence the productive performance of plants, light is essential because it directly influences photosynthetic rate, changes the chlorophyll

content, and affects plant growth and adaptation to the environment (Martinazzo et al. 2007; Lima et al. 2008; Mielke and Schaffer 2010; César et al. 2014).

In their review, Soriano et al. (2013) note that after germination seedling roots must be able to absorb water and nutrients from the soil and the shoots must have the photosynthetic efficiency to maintain the growth of seedlings once the nutritional reserve of the seeds is depleted. In seasonal ecosystems, the rapid establishment of seedlings is very important because of variations in rainfall and sunlight. Thus, growth characteristics can indicate seedling adaptability and tolerance to environmental conditions (Gonçalves et al. 2005; Mielke and Schaffer 2010), and these factors vary according to the kind, quality, and quantity of light.

Basic information about germination processes in most Brazilian native species is scarce and even less is known about the needs and quality of seedlings. *Physocalymma scaberrimum* Pohl. (Lythraceae), popularly known as cega-machado or pau-rosa, is a species native to Brazil, widely found in the Brazilian Savanna and semideciduous forests, that may reach heights up to 10 meters. It has lush ornamental flowers between August and September, and it can be used in reforestation (Lorenzi 2002). Basic information on eco-physiological characteristics of its seeds and seedlings is lacking. It is thought to be a species that spreads easily and tolerates high levels of sunlight. This study therefore aimed to evaluate the effect of shade on emergent and initial growth and quality of seedlings of *P. scaberrimum*.

Materials and methods

The research was conducted at the Universidade Federal da Grande Dourados using *Physocalymma scaberrimum* Pohl. (cega-machado) seeds collected from various areas in the Brazilian Savanna. Seeds were disinfected by immersion in 2 % sodium hypochlorite (v/v) for 5 min and then washed under running water.

Seeds were sown in plastic tubes 290 cm^3 in volume containing a mixture of Bioplant® substrate, sifted distroferric red latosol, and sand in the ratio of 1:1:1. The plastic tubes were maintained in full sun and under conditions of 30 and 70 % shade created using black nylon fabric known as Sombrite with four replicates of 25 seeds for each of the three conditions.

Seedling emergence was evaluated weekly by counting seedling hypocotyls beginning at the 8th day after sowing and continuing until the emergent rate was constant in three consecutive observations. We evaluated the final percentage of emergence (%E), the emergence speed index (ESI) according to the method of Maguire (1962), and mean time of emergence (MTE) according to the method of Labouriau (1983).

Assessments of early initial growth were conducted with five seedlings chosen at random from each treatment, up to 75 days after emergence. Length of the shoot (LS), length of the root (LR), and total length (TL) were measured in centimeters (cm). Total fresh mass (TFM) was measured with a precision analytical balance. Fresh leaves were then dried in a forced-air oven at 60 °C for 72 h to a stable weight. The number of leaves and collar diameter (CD) were measured in mm with a digital caliper. The chlorophyll index was measured in the middle leaves of plants with an SPAD 502 chlorophyll meter.

At 80 days after emergence, plants were transplanted to plastic containers 20 × 15 cm containing a mixture of sifted distroferric red latosol, sand, and semidecomposed chicken manure in proportions of 1:1:0.5 (v/v/v). The seedlings were kept in full sun and 30 and 70 % shade using the Sombrite fabric.

After 30 days of acclimatization and every 30 days thereafter (30, 60, 90, and 120 days after transplanting, DAT) when the 110-, 140-, 170-, and 200-day-old seedlings were evaluated for height, CD, number of leaves, and chlorophyll index. At the end of the experiment (120 days after transplanting), we assessed the LR, and fresh and dry mass of leaves, stems, and roots using the Dickson Quality Index (DQI) calculated from the ratio of the height and CD (H/CD) and ratio of the shoot dry mass to the root dry mass (DSM/DRM) according to the formula (Dickson et al. 1960): $DQI = TDM/(H/CD + DSM/DRM)$, in which TDM = total dry mass, H = height, CD = collar diameter, DSM = dry shoot mass, and DRM = dry root mass.

Plants at 120 DAT were also evaluated for the net photosynthetic rate (A), internal carbon concentration (Ci), transpiration rate (E), stomatal conductance (Gs), intrinsic water use efficiency (A/E), and carboxylation efficiency (A/C) with the aid of equipment with open system of photosynthesis with CO_2 analyzer and water vapor by infrared radiation (Infra-red Gas Analyzer—IRGA, model Li-COR 6400). These evaluations were made from each cardinal orientation in the period from 8 to 10 a.m.

All experiments were carried out in completely randomized design for destructive characteristics and photosynthetic metabolism and completely randomized design with a factorial scheme of 3 × 4 (3 levels of shade and 4 times) for the non-destructive, with 4 replicates.

Results were submitted to analysis of variance and having significant difference, the means of treatments were compared by Tukey test and for evaluation of interaction shading and days after transplantation regression equations were adjusted both a 5 % probability using the statistical program SISVAR.

Table 1 Effect of different shading levels on emergence (E), emergence speed index (ESI), and mean time emergence (MTE) of *Physocalymma scaberrimum* seeds in the full sun and under conditions of 30 and 70 % shade in nursery

Treatment	E (%)	ESI	MTE
Full sun	4.0b[1]	0.06b	22a
30 %	54.0a	0.86a	16b
70 %	37.5a	0.63a	16b
C.V. (%)	30.40	34.06	4.55

[1] Means followed by the same letter in the column do not differ at 5 % Tukey test

Results

The seedlings of *P. scaberrimum* emerged both in full-sun conditions and in different levels of shade. However, there were no significant differences in percentage emergence (%E) and ESI in shade conditions as opposed to full sun, and there were no significant differences between the two shade conditions (Table 1). The MTE was higher in full-sun conditions and did not vary between the shade conditions.

The highest values for shoot length (SL), root length (RL), TL, number of leaves, chlorophyll index, and fresh and dry mass (TFM and TDM) were observed under shade conditions and did not differ according to the percentage of shade (Table 2).

Interaction was detected between the levels of shade and days after transplantation for height and number of leaves. Linear growth (Fig. 1a) occurred in all light conditions. The seedlings subjected to greater shade (70 %) maintained greater shoot length throughout the evaluation period. The number of leaves (Fig. 1b) also presented linear growth with greater value to full sun.

The chlorophyll index and the CD did not show significant interaction between treatments throughout the 120 days after transplantation (Fig. 2).

The average CD of the seedlings of *P. scaberrimum* differed statistically according to the different brightness levels (Table 3). Growing in full sun and 30 % shade led to the highest values in average CD and the chlorophyll index than in the 70 % shade condition (Table 3).

At 120 days after transplantation, fresh and dry mass of the root (FRM and DRM) were greater in the full-sun and 30 % shade conditions (Table 4). There were no significant differences in fresh leaf mass (LFM) under any light conditions, but the fresh stem mass was higher in shade. The dry leaf mass and dry stem mass showed no significant differences between light treatments, with averages of 2.75 and 0.80 mg, respectively. The DQI was higher in the full-sun and 30 % shade conditions.

Table 2 Shoot length (SL), root (RL), total (TL) and collar diameter (CD), number of leaves (NF), chlorophyll (CLO), total fresh mass (TFM), and total dry mass (TDM) at 75 days after emergence of *Physocalymma scaberrimum* seedlings in the full sun and under conditions of 30 and 70 % shade in nursery

Treatment	SL (cm)	RL(cm)	TL(cm)	CD (mm)
Full sun	1.4b[1]	8.2b	9.6b	0.54b
30 %	3.4a	17.6a	21.0a	1.08a
70 %	3.4a	18.5a	21.9a	1.08a
C.V. (%)	14.83	4.45	3.93	5.55
Treatment	NL	CLO (SPAD)	TFM (mg)	TDM (mg)
Full sun	5.0b[1]	16.7b	0.0078b	0.021b
30 %	7.0a	19.9a	0.1037a	114.9a
70 %	7.4a	20.4a	0.0829a	104.9a
C.V. (%)	11.43	5.16	32.49	23.99

[1] Means followed by the same letter in the column do not differ at 5 % Tukey test

The highest concentration of carbon (Ci) and stomatal conductance (Gs) occurred with the greatest shade, 70 %, and were lowest in full sun (Table 5). The highest rate of transpiration (E) was detected in the 30 % shade treatment; however, there were no significant differences in the photosynthetic rate and carboxylation efficiency in 30 % shade when compared to full sun.

The seedlings exposed to full sun showed reduced water use efficiency (A/E), feature that ranged from not-shaded seedlings. The results show that the net photosynthetic rate and carboxylation efficiency (A/Ci) of seedlings were higher in full sun and 30 % shade.

Discussion

Seeding *P. scaberrimum* in full sun suppresses emergence and growth and may lead to failure in seedling production and revegetation. Decreased emergence in full sun can be attributed to high light intensity and/or the temperature of the environment, which reduces the water-holding capacity of the soil and may alter the metabolism of seeds and accelerate their degradation (Morris et al. 2000) or the lower water availability as a result of greater evaporation throughout the day (Scalon et al. 2014).

Similar results were observed for other species native to the Brazilian Savanna. Seedlings of baru (*Dipteryx alata* Vog.) showed reduced emergence and ESI in full sun, and the results did not vary significantly between the levels of shade (Queiroz and Firmino 2014). *Stryphnodendron polyphyllum* Mart. and *S. adstringens* (Mart.) Coville showed reduction in percentage and speed of emergence of seedlings in full sun, whereas sowing in 50 and 70 % shade

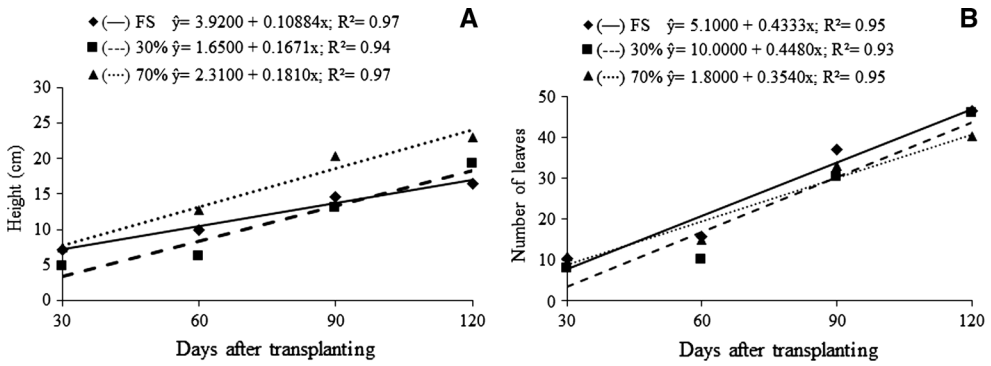

Fig. 1 Height (**a**) and number of leaves (**b**) at the 30, 60, 90, and 120 days after transplantation of *Physocalymma scaberrimum* seedlings in nursery (full sun—FS and under conditions of 30 and 70 % *shade*)

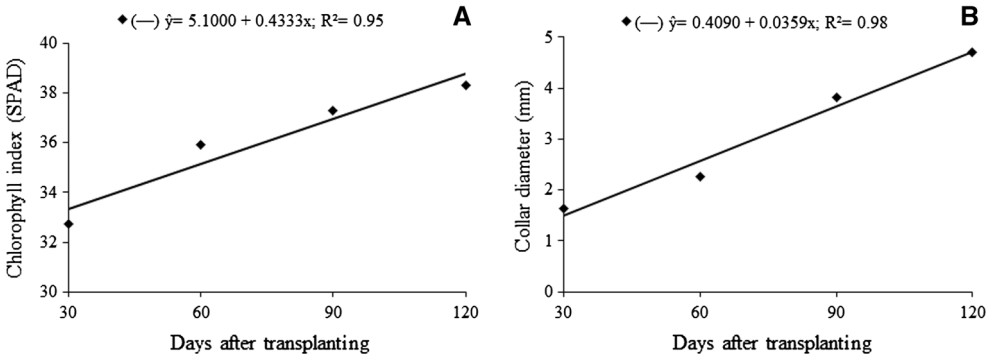

Fig. 2 Chlorophyll index (**a**) and collar diameter (**b**) 30, 60, 90, and 120 days after transplanting the *Physocalymma scaberrimum* seedlings in nursery (full sun—FS and under conditions of 30 and 70 % *shade*)

Table 3 Effect of different shading levels on collar diameter (CD) and chlorophyll (CLO) for *Physocalymma scaberrimum* seedlings in the full sun and under conditions of 30 and 70 % shade in nursery

Treatment	CD (mm)	CLO (SPAD)
Full sun	3.38a[1]	35.1b
30 %	3.22a	35.7b
70 %	2.72b	37.4a
C.V. (%)	12.33	6.54

[1] Means followed by the same letter in the column do not differ at 5 % Tukey test

led to greater and more rapid emergence and greater seedling height and dry mass (Scalon et al. 2014).

The highest number of leaves of seedlings observed under shade may be related to photosynthesis and optimization of the microclimate, resulting in greater accumulation of carbohydrates and acceleration of leaf growth, as also noted for *Pterogyne nitens* Tul. under shade conditions (César et al. 2014). In addition to the increase in the number of leaves, we also observed increased height and dry mass. The greatest height found in 70 % shade also suggests that the seedlings allocate more assimilates in the shoots, and this strategy allows the plant to expose itself to light, escape the shade, and survive (Franco et al. 2007).

We believe that greater growth occurred in the shade as a result of the attenuation of thermal stress because Sombrite reduces the ambient temperature for the plant (Fonseca et al. 2002). In shade, temperatures are milder, which helps leaves keep the stomata opened, reduces water loss, and increases carbon sequestration, thereby optimizing photosynthetic activity and turgidity necessary for growth (Pacheco et al. 2013).

However, if the height increase is not accompanied by an increase in diameter, the plants become etiolated, which is not a desirable feature. Seedlings developed under higher levels of light produce more photoassimilates and growth regulators as a result of greater photosynthesis and is reflected directly in the thickness of the stem. The smaller diameter of the collar under greater shade conditions may be related to the disorder growth of the aerial part of the plant in this condition (Anten et al. 2009). The greater the CD, the greater the possibility of development of the root system, thereby reducing the time before transplanting in the field. Thus, seedlings with well-developed root systems

Table 4 Effect of different shade levels on root length (RL), fresh leaf mass (FLM), fresh stem mass (FSM), fresh root mass (FRM), dry root mass (DRM), and the Dickson quality index DQI) at 120 days after transplantation of *Physocalymma scaberrimum* seedling in the full sun and under conditions of 30 and 70 % shade in nursery

Treatment	RL (cm)	FLM (mg)	FSM (mg)	FRM (mg)	DRM (mg)	DQI
Full sun	30.08a[1]	6.99c	1.61b	5.83a	1.1529a	0.77a
30 %	31.38a	11.42a	2.65a	5.28a	1.2109a	0.71ab
70 %	26.60b	8.78b	2.26ab	3.02b	0.8389b	0.56b
C.V. (%)	3.58	3.58	19.11	10.01	13.05	12.60

[1] Means followed by the same letter in the column do not differ at 5 % Tukey test

Table 5 Average values of CO_2 concentration (Ci), transpiration rate (E), stomatal conductance (Gs), photosynthetic rate (A), water use efficiency (A/E), and carboxylation efficiency (A/Ci) according to the shade levels at 120 days after transplantation of *Physocalymma scaberrimum* seedlings in the full sun and under conditions of 30 and 70 % shade in nursery

Treatment	Ci	E	Gs	A	A/E	A/Ci
Full sun	240.00c[1]	2.82b	0.175c	8.430a	30.30b	0.035a
30 %	264.50b	5.16a	0.260b	8.745a	39.45a	0.033a
70 %	302.50a	2.09c	0.325a	7.175b	39.10a	0.024b
C.V. (%)	4.06	6.72	11.91	5.52	0.98	3.68

[1] Means followed by the same letter in the column do not differ at 5 % Tukey test

have better ability to survive in the field after transplantation (Campos and Uchida 2002). Seedlings of *P. scaberrimum* had a greater CD in full sun and 30 % shade; therefore, extreme shade (70 %) does not favor seedling production in this species.

We believe that the greatest RL found in seedlings in full sun and 30 % shade can be a consequence of greater radiation that produces a higher ambient temperature. The increased temperature triggers greater water loss by evapotranspiration, decreasing water availability to the roots, reducing the growth of the aerial part, and increasing the allocation of assimilates in the root system, which facilitates the absorption of water (Figueroa et al. 2004).

The superior results for fresh root mass (FRM) and dry root mass (DRM) of seedlings similar to LR, in full sun and 30 % shade, indicate that during the initial phase of growth of *P. scaberrimum*, the accumulation of biomass in the root system is promoted by higher irradiation. Resende et al. (2011) observed that the reduction of RDM that occurs in the shade can be related to decreased flow of photoassimilates from photosynthetic organs, because light is one of the determining factors in this transport.

Plants exposed to full sun had a reduced fresh leaves mass (FLM) which can be attributed to lower water availability in this condition that made it hard to maintain the turgidity of the tissues.

Similar results were found by Oliveira and Perez (2012), in a study of initial growth of yellow ipê (*Tabebuia aurea* (Silva Manso) Benth. & Hook. f. Ex S. Moore) according to levels of shade, which showed the highest dry biomass accumulation in brighter conditions. Plants subjected to low solar radiation tend to target the metabolism for the synthesis of non-structural carbohydrates, allowing for greater survivability but smaller accumulation of biomass (Poorter and Kitajima 2007).

Seedlings grown in full sun and 30 % shade had a higher DQI. However, it should be noted that in all three cultivation conditions the DQI was higher than the minimum value of 0.20 recommended by Hunt (1990). The DQI is used by many authors as a parameter to express the quality of seedlings, as it defines the robustness and distribution of biomass, with the simultaneous consideration of many variables. They consider that the greater the DQI, the better the quality of the seedlings (Fonseca et al. 2002; César et al. 2014). Therefore, according to the DQI and DRM, which ensures better performance in the field, better quality seedlings were produced in full sun and 30 % shade in this study.

The intensity and quality of light incident on the leaves can cause changes in the chlorophyll index. Increased chlorophyll content in shade as observed for *P. scaberrimum* has been explained as a compensatory effect for the small amount of light available, which increases light absorption and production of photoassimilates (Martinazzo et al. 2007; Lima et al. 2008; Mielke and Schaffer 2010).

The increase in internal carbon concentration and stomatal conductance Gs in the seedlings in the greatest shade did not increase the photosynthetic rate, showing that the CO_2 available was not sufficient to provide an increase in photosynthesis, resulting in lower carboxylation efficiency (A/Ci) as reported by Rubisco.

Seedlings grown in full sun showed reduced perspiration compared to those grown in 30 % shade, suggesting that these seedlings were able to adapt to ambient radiation conditions, protecting against dehydration by promoting the quicker closure of the stomata, resulting in less water loss by perspiration (Dutra et al. 2012). However, the plants in 70 % shade also showed less perspiration, which

can be attributed to the lower temperature in the shaded environment.

It should be noted that even with lower stomatal conductance in full-sun conditions, seedlings had a high photosynthetic rate and highly efficient carboxylation, probably because this condition did not damage the photosynthetic apparatus and because this species can grow satisfactorily under high light intensity.

The results observed for *P. scaberrimum* corroborate information in the literature that woody species native to the Brazilian Savanna generally show lower growth in the aerial part and greater growth in underground structures as an adaptation to lower water availability (Moreira and Klink 2000; Hoffmann and Franco 2003; Ribeiro and Walter 2008) allowing greater absorption of water and nutrients and providing high rates of photosynthesis and transpiration as a result of the increased availability of light (Claussen 1996).

Full-sun exposure negatively influences growth in many species; however, it can also promote biomass accumulation in root systems, a fundamental response for reforestation in ecologically damaged areas (Hoffmann and Franco 2003). These authors observed that the establishment of the species may be limited by the high luminous intensity and thermal features of the Cerrado in addition to low nutrient and/or water availability. As a result, the establishment and growth of native species in the Cerrado should be facilitated by the ability to allocate more resources to the root system and optimize the capture and storage of water.

Thus, *P. scaberrimum* behaves like a heliophytic species similar to pioneer and early secondary species. We believe that this species can develop well in clearings, similar to the heliophytic behavior observed for *Chorisia speciosa* St. Hil. (Pacheco et al. 2013), characterized as *P. nitens*. This species, initially considered secondary, can behave as a pioneer in sandy soils and degraded sites. It also showed increased height, increased leaf area, and mass reductions in root systems under different levels of light restriction. The highest DQIs have been found in plants maintained in light restricted to 36.5 % (Cézar et al. 2014). The results of this work indicate that the seedlings of *P. scaberrimum* have difficulty developing in extreme 70 % shade conditions in the initial growth phase. The smaller growth of seedlings and consequently lower quality as assessed by the DQI suggest that this level of luminosity represents a light deficit that reduces the metabolic activities of the plant (Silva et al. 2007).

Seedlings of *P. scaberrimum* grown under 30 and 70 % shade have greater germination and vigor; however, seedlings exposed to full sun and 30 % shade exhibit greater growth, quality, and efficiency of carboxylation. Thus, better seedlings in this species can be produced under conditions of 30 % shade or less.

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