Acta Botanica Brasilica, 2023, 37: e20230119
doi: https://doi.org/10.1590/1677-941X-ABB-2023-0119

Original article

Could the presence of preformed leaves inside the seed be more important than seed size for the establishment and growth of Cerrado seedlings?

Larissa Gabrielle Ferreira da Silva[1], Javier G. Puntieri[2], Nayara Magry Jesus Melo[1] and João Paulo Souza[1*]

Received: May 23, 2023
Accepted: October 3, 2023

ABSTRACT

The internal structure of seeds is relevant to germination, seedling establishment, and early plant growth. The objective of this work was to evaluate whether internal seed structure can influence germination and initial growth in four native woody species of the Cerrado. The species chosen for this study were *Anadenanthera falcata*, *Stryphnodendron adstringens*, *Stryphnodendron polyphyllum*, and *Tabebuia aurea*. Seeds were dissected to evaluate: the lengths of the hypocotyl-radicular axis, the epicotyl and the embryonic axis, mass of the cotyledons and the embryonic axis, and eophyll number (preformed organs). Another group of seeds was germinated in a greenhouse for sixty days to verify the emergence of the epicotyl, eophylls, metaphylls, and to measure seedling biomass. Only *A. falcata* presented a plumule with differentiated eophylls. *Anadenanthera falcata* germinated before the other species in the greenhouse and had faster epicotyl and leaf emergence. Possibly, the presence of eophylls inside the seed enabled *A. falcata* seedlings to carry out photosynthesis, grow faster, and accumulate more aerial biomass than the other species soon after emergence. The presence of eophylls in a differentiated embryo (as in *A. falcata*) may hasten germination and favour seedling establishment.

Keywords: early growth, embryo, organ preformation, seedling establishment, woody species.

---

1 Federal University of Viçosa, Institute of Biology, Highway LMG 818, Km 06, CEP 35690-000, Florestal, Minas Gerais, Brazil.
2 National University of Rio Negro, Research Institute in Natural Resources, Agroecology and Rural Development (IRNAD), Anasagasti 1463, San Carlos de Bariloche, Argentina.

* Corresponding author: joaopaulobio@hotmail.com

Introduction

Seeds have a complex structure and are relevant to the life cycle of woody plants due to their propagation role. In general, a seed consists of an embryo, reserve tissues, and a tegument (Marcos Filho 2015). The formation of a new plant begins with the restart of embryo growth in the seed, during germination (Kramer & Kozlowski 1972; El-Maarouf-Bouteau 2022). Embryo development is influenced by external factors, such as temperature and light, and internal factors, such as genetic background and seed physiology (Sousa *et al*. 2008), and is key for the success of the most vulnerable phases of each species' life cycle: germination (Almansouri *et al*. 2001), and seedling establishment (Fenner & Fenner 1985; Chesson *et al*. 2004; Cipriotti *et al*. 2008; Lamb & Cahill 2006; Yang *et al*. 2008). Mortality may be high at seedling establishment due to conditions at the germination site (Poorter *et al*. 2005). Suboptimal resource availability (water, nutrients, and light), the lack of protective structures, and low levels of resource storage may increase the susceptibility of seedlings to herbivores and irregular water supply (Carreira & Zaidan 2003). Some morphological and functional traits of the seedlings during the establishment period may increase their probability of survival, allowing a sustained resource supply (Ressel *et al*. 2004; Stanik *et al*. 2021).

Several studies have shown that large seeds present advantages for seedling establishment (Westoby *et al*. 2002; Moles & Westoby 2004). Large seeds often have high amounts of nutrient reserves and develop large seedlings (Baskin & Baskin 1998) which in drought-prone environments are more capable of competing with the established vegetation than small seedlings (Tumpa *et al*. 2022). Compared to species with small seeds, those with large seeds can mobilize resources for the photosynthetic seedling structures over a longer period. In this way, more resources could remain uncommitted at any time during the initial period of seedling growth and become available to maintain respiration during a period of carbon deficit (Kidson & Westoby 2000; Gomes *et al*. 2020). In addition to seed size, the development of more preformed organs in the embryo may allow a seedling to establish faster and withstand adverse events. Nonetheless, the significance of embryo size and differentiation within the seed on germination and seedling performance has been little investigated.

Several studies dealt with the germination and establishment of woody Cerrado seedlings (Rizzini 1965; Oliveira & Silva 1993; Ranal *et al*. 2010; Saboya & Borghetti 2012; Sales *et al*. 2013; Silveira *et al*. 2013; Pellizzaro *et al*. 2017; Escobar *et al*. 2018; Daibes *et al*. 2019). However, there is a lack of studies on the relationship between the degree of embryo development inside the seed and the establishment and growth of Cerrado species. Cerrado occupies about 21% of the Brazilian territory, being considered the most diverse savanna in the world (Colli *et al*. 2020). Seasonal precipitation, low soil fertility and drainage, and fire disturbance are important factors determining Cerrado distribution (Oliveira-Filho & Ratter 2002; Lira-Martins *et al*. 2022). The most representative Cerrado physiognomy is the cerrado *stricto sensu* (Paula *et al*. 2021). Cerrado *stricto sensu* has a savannah-like physiognomy in which the two-layered vegetation consists of a continuous herbaceous stratum beneath sparse shrubs and trees (Ribeiro & Walter 2008; Souza *et al*. 2021). In this physiognomy, seedlings should overcome fire events, seasonal droughts, and competition with herbaceous plants in order to establish and grow (Fontenele & Miranda 2022). The ability of seedlings of Cerrado woody species to withstand adverse events such as seasonal drought and fire during their establishment is a characteristic of great importance for species persistence in the Cerrado (Hoffmann & Moreira 2002; Hoffmann *et al*. 2004; Franco *et al*. 2014). Cerrado seedlings invest more biomass in roots than in stems, probably as an adaptation to the high frequency of adverse events, such as seasonal drought and fire, in these communities (Oliveira & Silva 1993; Franco *et al*. 1996; Moraes & Prado 1998; Franco *et al*. 2005; Cirne & Miranda 2008; Rossell *et al*. 2023). In the Cerrado, the relationship between seed mass and seedling survival has been documented (Hoffmann 2000; Lahoreau *et al*. 2006; Ribeiro *et al*. 2021). Cerrado species with large seeds could present greater survival rates after fire and drought events than those with small seeds, as found in other savannas throughout the world (Lahoreau *et al*. 2006).

The seeds of some of the native woody species of the Cerrado may have eophylls (i.e. embryonic leaves other than the cotyledons, Duke 1965), and it has been suggested that this feature would result in faster initial seedling growth compared to that of other coexisting species (Botelho *et al*. 2000; Melo *et al*. 2004). Thus, the woody species that establish themselves faster would be able to withstand adverse events throughout their first growth cycle. The relationship between organ preformation in the buds of adult plants and shoot growth rate (Damascos *et al*. 2005; Damascos 2008; Melo *et al*. 2022) are well documented for the Brazilian Cerrado (Ronquim *et al*. 2003; Rossatto *et al*. 2009). However, the influence of organ preformation (as eophylls) inside seeds, on both germination and seedling establishment for Cerrado woody species is unknown. To test if embryo development influences the germination and establishment of plant species, we choose four Cerrado woody species based on their wide occurrences in cerrado *stricto sensu* areas (Ratter *et al*. 2003). We hypothesized that preformed organs and differentiation of the seed embryo could affect germination rate and seedling establishment. It may be proposed as a prediction that those species whose seeds include more differentiated embryos (with more eophylls) would exhibit the highest rates of germination and probability of seedling establishment.

Materials and methods

Plant species and growth site

The four woody species selected were: *Anadenanthera falcata* (Benth.) Speg., *Stryphnodendron adstringens* (Mart.) Coville, *Stryphnodendron polyphyllum* Mart., and *Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex. S. Moore. We chose these species because they occur widely in cerrado *stricto sensu* areas. *Anadenenthaera falcata* occurs in 10.4%, *S. adstringens* in 25.5%, *Stryphnodendron polyphyllum* in 7.4%, and *T. aurea* in 58.0% of the 376 Cerrado areas sampled by Ratter *et al*. (2003). Cerrado *stricto sensu* is an open phytophysiognomy (savannah-like vegetation), where trees do not form a closed canopy; instead, it is a two-layered system with a continuous herbaceous layer under a discontinuous cover of shrubs and trees (Ribeiro & Walter 1998). Three of the selected species belong to the Fabaceae family: *Anadenanthera falcata* (angico), *Stryphnodendron adstringens* (barbatimão-do-cerrado), and *Stryphnodendron polyphyllum* (barbatimão-da-mata). The fourth species is *Tabebuia aurea* (ipê-amarelo) and belongs to the Bignoniaceae family (Ratter *et al.* 2003).

The study was conducted in a greenhouse (19°52'29'' S, and 44°25'12'' W). The seeds were submitted to two different experiments: (1) dissection and analysis of internal structures, and (2) germination and early growth in a greenhouse. Temperature and radiation factors in the greenhouse were provided naturally, without artificial additions. All seeds used in the experiments were acquired as cleaned seeds (without the fruit covers) from Arbocenter. These seeds were collected in 2017 and showed the same developmental stage.

Seed dissection

Fifty seeds per species were dissected. Initially, the seeds were sanitized with sodium hypochlorite (5% commercial bleach) for five minutes and washed with deionized water. Subsequently, they were placed in a container with deionized water for approximately two hours to facilitate the removal of the tegument; a longitudinal cut was performed on each seed with a carbon-steel scalpel blade n° 20 (ADVANTIVE®). The weights of the embryonic axis and the cotyledons (CEW) were evaluated with an analytical balance to the nearest 0.001 g. Afterwards, we measured the width and length of the cotyledons (CW and CL respectively), the lengths of the embryonic axis (EAL) and of the epicotyl (EL), and the number of eophylls (leaves following the cotyledons and previous to the first metaphylls, Duke 1965) in plumules (ENP), with the aid of a stereomicroscope and calipers.

Germination essay

On August 9 and 10, 2018, we started a germination experiment. Thirty seeds of each species were cleaned with sodium hypochlorite (5%) for five minutes, and then washed with deionized water. Since *S. adstringens* and *S. polyphyllum* present physical dormancy (Martins *et al*. 2008), their seeds were scarified with sandpaper to facilitate water absorption and germination. In the greenhouse, three seeds were placed at 1 cm depth in each of 10 pots per species (n=30 seeds per species). Each pot had a capacity of 0.008 m^3 and was filled with typical Cerrado soil (described below). We determined the germination percentage of all four species after a period of 60 days.

Early growth in the greenhouse and relative growth rate evaluation

The follow-up of the seedlings that emerged in the greenhouse lasted until October 10, 2018, resulting in 60 days of observations after the beginning of the experiment. Measurements of each plant's early growth were performed every day; a 30 cm graduated ruler was used to measure seedling size. Early growth was evaluated from the day when the tegument broke and the embryonic axis emerged from the seed coat; epicotyl length, the number of eophylls, and metaphylls were recorded daily for each seedling.

Relative growth rates in terms of epicotyl length (RGRep, d^{-1}) and number of eophylls (RGReo, d^{-1}) were calculated using the following equations:

RGRep = [(epicotyl length at time 2) - (epicotyl length at time 1)] / [(epicotyl length at time 1) * (time 2 – time 1)]

RGReo = [(number of eophylls at time 2) - (number of eophylls at time 1)] / [(number of eophylls at time 1) * (time 2 – time 1)],

where times 1 and 2 are two successive weeks in which epicotyl length and number of eophylls were recorded. The units of RGRep and RGReo thus obtained were 1/week.

Biomass allocation and morphometric analysis of seedlings

A sample of 50 seeds of each species were dried out (105 °C by 24h) to determine seed mass. Root and stem lengths were measured with a graduated ruler, and eophylls and metaphylls were counted. Eophylls and metaphylls were scanned in black and white and saved in image format; leaf areas were determined using the Image-Pro 5.0 software (Media Cybernetics, Inc., Silver Spring, MD, USA).

At the end of the greenhouse experiment, seedlings were measured by removing the plant from the soil and separating the organs (root, stem, eophylls, and metaphylls). Roots, stems, and leaves were dried separately in an oven at 70 °C for 48 hours; dry matter contents were determined using an analytical balance (to the nearest 0.001 g).

Nutrient analysis of soil used in the experiment

Soil chemical analysis was performed at the Agricultural Chemistry Laboratory of the Instituto Mineiro de Agropecuária (IMA, Brazil). The soil was classified as dystrophic, as the base saturation value was lower than 30% (Embrapa 2009). The soil chemical characteristics were: pH in H_2O=6.1; P=0.8 (mg/dm^3); K=8.0 (mg/dm^3); Ca^{2+}=0.33 (cmolc/dm^3); Mg^{2+}=0.07 (cmolc/dm^3); Al^{3+}=1.83 (cmolc/dm^3); effective cation exchange capacity=2.25 (cmolc/dm^3); saturation aluminum index=81.2%; and base saturation index=10.7%.

Statistical Analysis

The R program, version 3.0.0 (R Core Team 2016) was used to calculate mean and standard deviation (or standard error) for the following parameters: cotyledon length (CL), cotyledon width (CW), cotyledon + embryonic axis weight (CEW), hypocotyl-radicular axis length (HRAL), epicotyl length (EL), eophylls number on plumule (ENP), embryonic axis length (EAL), root dry mass (RDM), stem dry mass (SDM), eophylls dry mass (EDM), metaphylls dry mass (MDM), total dry mass (TDM), root length (RL), stem length (SL), number of metaphylls (NM), number of eophylls (NE), leaf area of metaphylls (LAM), leaf area of eophylls (LAE), and relative growth rate (RGR) of eophylls and stem. The Shapiro-Wilk test was used to verify normality for each data set. Data sets that did not show normal distribution were log-transformed (base 10). Afterward, the Levene test was used to verify variance homogeneity. Analysis of variance (one-way ANOVA) and *a posteriori* Tukey's tests were applied to compare pairs of mean values. For CL, CW, CEW, HRAL, EL, ENP, EAL, RDM, SDM, EDM, MDM, TDM, RL, SL, NM, NE, LAM, LAE, CP, significant differences among species means were sought after. For EL, ENP, and RGR, we used Mixed Design Analysis of Variance (MIXED ANOVA) with a Mauchly's sphericity test with one factor and repeated measures, considering species as a between-subjects factor and time as a within-subjects factor, followed by *a posteriori* Tukey's tests to compare pairs of species means over time. For epicotyl length in seedlings and epicotyl length in seeds we used two-way ANOVA followed by Tukey's tests to compare species means at different times.

Spearman correlation coefficient (rho) was used to verify the degree of correlation between morphological variables. The correlations were performed using the R program, version 3.0.0 (R Core Team 2016). A p<0.05 significance level was adopted in all comparisons.

Results

Germination and seed morphology

Germination rates were 42%, 15%, 78% and 12% for *A. falcata*, *S. adstringens*, *S. polyphyllum* and *T. aurea*, respectively. All four species presented embryos with fleshy cotyledons, *i.e.* cotyledons with storage tissues (Fig. 1). Only *A. falcata* presented seeds in which cotyledons, embryonic axis and plumule with eophylls were differentiated (Fig. 1). The other three species presented seeds with apparent cotyledons and embryonic axis. All four species presented a differentiated but small embryonic axis.

Compared to all other species, *A. falcata* showed higher values of CL (F-test = 1189.0, $p < 0.05$; Table 1), CEW (F-test = 58.4, $p < 0.05$), HRAL (F = 150.6, $p<0.05$), EL (F-test = 109.86, $p < 0.05$), ENP (F-test = 3372.8, $p < 0.05$) and EAL (F-test = 319.0, $p < 0.05$). However, *T. aurea* presented greater CW than the other studied species (F-test = 158.4, $p < 0.05$).

Early growth in the greenhouse

Anadenanthera falcata seedlings had an emerging epicotyl from the second week and, compared to the other species under survey, developed a longer epicotyl from the fourth week onwards (Mauchly's W-test = 1.1, $p < 0.05$, species *x* measurement period interaction; Fig. 2A). Epicotyl emergence was evident from the fifth week in *S. polyphyllum*, and from the sixth week in *S. adstringens* and *T. aurea* (Fig. 2A). After the sixth week and up to the end of the experiment, *S. polyphyllum* plants showed higher numbers of eophylls (Mauchly's W-test = 4.3, $p < 0.05$, species *x* measurement period; Fig. 2B). From the fifth week, only *A. falcata* showed metaphylls production (1.00 ± 0.28). *Anadenathera falcata* showed a higher RGRep (F-test = 6.8, $p < 0.05$, species *x* measurement period; Fig. 2C) in relation to the other species. However, after the sixth week *S. polyphyllum* showed higher RGReo (F-test = 7.8, $p < 0.05$, species *x* measurement period; Fig. 2D).

Morphometry and biomass allocation

Seed dry mass (n=50 seeds of each species) was 5.3 g for *A. falcata*, 4.3 g for *S. adstringens*, 4.5 g for *S. polyphyllum*, and 5.8 g for *T. aurea*. The highest mean values of stem length (F-test = 172.9, $p < 0.05$), number of metaphylls (F-test = 33.3,

Table 1. Mean ± standard deviation of cotyledon length (CL), cotyledon width (CW), cotyledon + embryonic axis weight (CEW), hypocotyl-radicular axis length (HRAL), epicotyl length (EL), eophylls number on plumule (ENP), embryonic axis length (EAL) of seeds of four woody species native to the Brazilian Cerrado. Capital letters in columns compare significant differences between species (p<0.05, Tukey's test). N = 50 seeds per species.

Species	CL (cm)	CW (cm)	CEW (g)	HRAL (cm)	EL (cm)	ENP	EAL (cm)
A. falcata	1.40 ± 0.11 A	1.17 ± 0.16 B	0.19 ± 0.05 A	0.31 ± 0.05 A	0.32 ± 0.07 A	8.88 ± 1.07 A	0.63 ± 0.07 A
S. adstringens	1.07 ± 0.08 B	0.46 ± 0.06 C	0.04 ± 0.01 C	0.15 ± 0.04 B	0.12± 0.04 D	0.00 ± 0.00 B	0.27 ± 0.03 C
S. polyphyllum	0.98 ± 0.12 C	0.41 ± 0.06 D	0.04 ± 0.01 C	0.14 ± 0.04 B	0.20 ± 0.06 B	0.00 ± 0.00 B	0.34 ± 0.08 B
T. aurea	1.06 ± 0.07 B	1.47 ± 0.10 A	0.13 ± 0.12 B	0.14 ± 0.03 B	0.15 ± 0.04 C	0.00 ± 0.00 B	0.30 ± 0.03 C

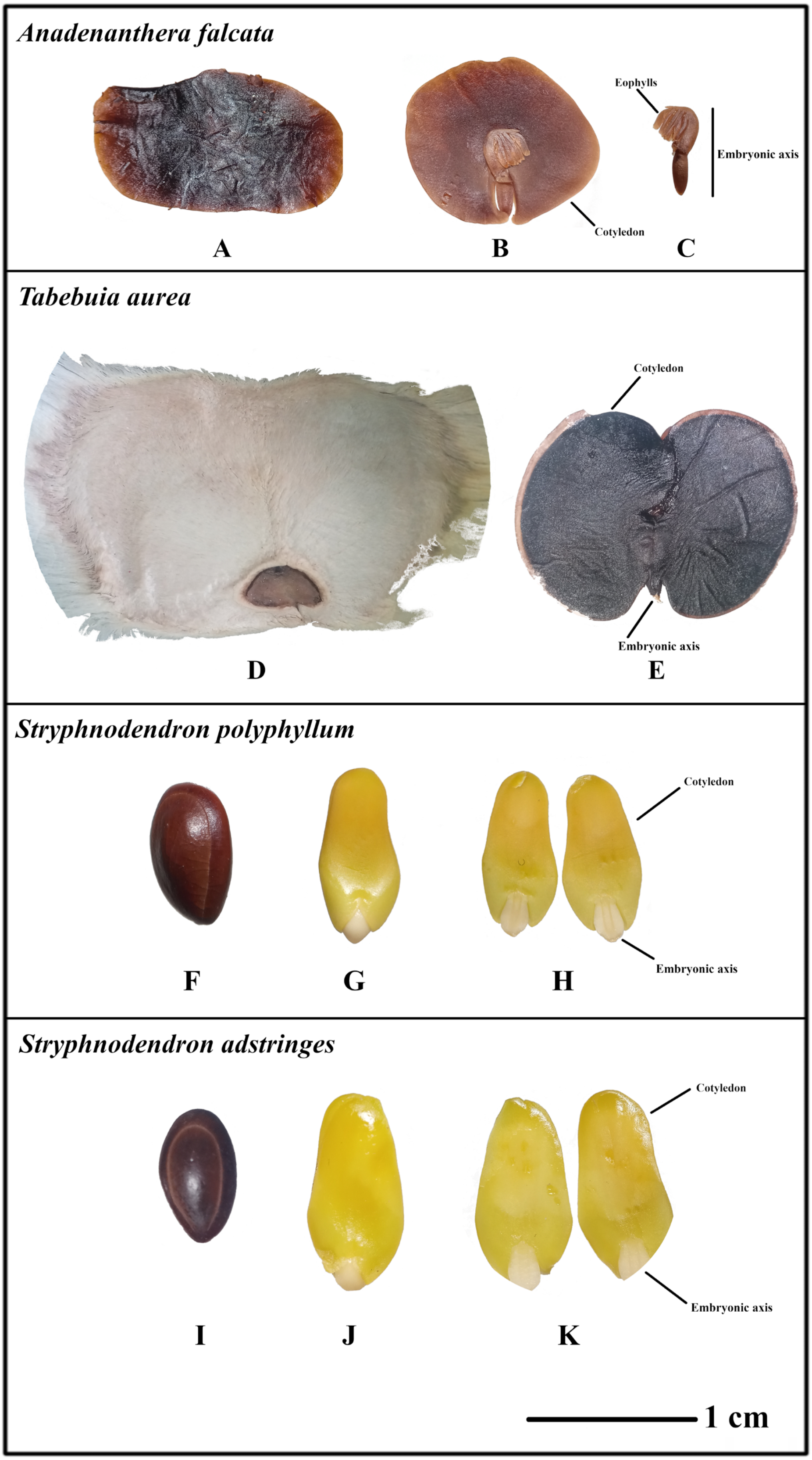

Figure 1. Seeds of four woody species native to the Brazilian Cerrado. A, D, F, and I= seeds with coat; B, E, G, and J= cotyledon and embryonic axis without seed coat; C= embryonic axis without cotyledon; H and K= embryonic axis with cotyledons separated. The seeds were soaked in water to facilitate the coat removal, which is why the cotyledonary leaves, and the embryonic axis are swollen and larger than the unopened seeds.

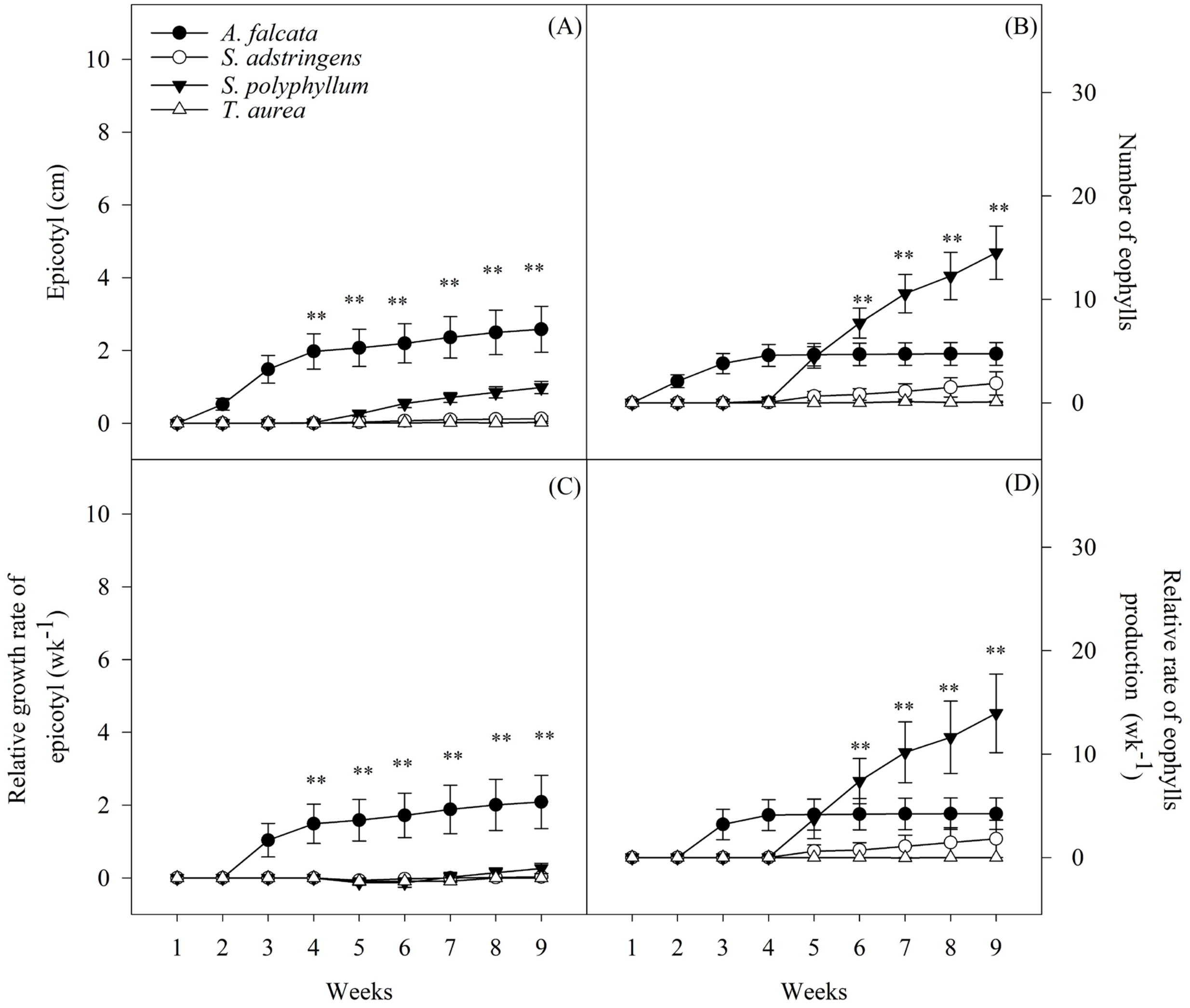

Figure 2. Mean (± standard error) initial growth (A, B) and relative growth rate (C, D) in terms of epicotyl length (A, C) and eophylls production (B, D) for seedlings of four woody species native to the Brazilian Cerrado growing in a greenhouse. Differences between species or among measurement periods are indicated: * main effect, ** double interaction (p <0.05, Tukey´s test). N = 11 (*A. falcata*), N = 3 (*S. adstringens*), N = 2 (*T. aurea*), N = 16 (*S. polyphyllum*).

$p < 0.05$) and leaf area of metaphylls (F-test = 93.0, $p < 0.05$) were those found for *A. falcata* plants (Table 2). *Stryphnodendron polyphyllum*, *S. adstringens* and *A. falcata* plants had more eophylls (F-test = 24.9, $p < 0.05$; Table 2) and area of eophylls (F-test = 24.9, $p < 0.05$; Table 2) than *T. aurea*. There were no differences among species in root length (F-test = 0.9, $p < 0.05$; Table 2).

Anadenanthera falcata, *S. adstringens,* and *T. aurea* had higher values of stem biomass (F = 21.0, $p < 0.05$). In addition, *A. falcata* and *S. polyphyllum* had higher eophylls biomass (F = 7.7, $p < 0.05$; Table 3) and plant biomass (F-test = 6.4, $p < 0.05$). *Anadenanthera falcata* was the only species that presented metaphylls (F-test = 12.2, $p < 0.05$, Table 3). All four species were similar regarding root biomass (F= 5.2, $p < 0.05$; Table 3).

Anadenanthera falcata, *S. adstringens*, and *S. polyphyllum* showed greater epicotyl length in seedlings (T2) than in seeds (T1) (F = 37.4, $p < 0.05$, species x time interaction; Fig. 3). *Anadenanthera falcata* showed greater epicotyl length in both seedlings (T2) and seeds (T1) than all other species (F = 37.4, $p < 0.05$; Fig. 3).

Morphological correlations

Seedlings from seeds with large embryos presented higher RGRep resulting in larger stems (Spearman's rank correlation rho = 0.55, p <0 .05) and total dry matter accumulation after eight weeks (rho = 0.44 $p < 0.05$; Fig. 4). The *A. falcata* seeds presented larger embryos and higher RGRep, and stem and total dry matter production. Accordingly, seedlings with high RGRep showed higher leaf area of eophylls (rho = 0.36, $p < 0.05$; Fig. 4), total dry mass (rho = 0.73, $p < 0.05$; Fig. 4), and embryos dry mass (rho = 0.50, $p < 0.05$; Fig. 4). Also, seedlings with a higher leaf area of eophylls showed a high total dry mass (rho = 0.51, $p < 0.05$; Fig. 4).

Discussion

The seeds of *A. falcata* have larger and more differentiated embryonic axis than those of the other three species. The development of preformed leaves in the embryos of *A. falcata* may explain the higher growth rate reached by this species at early growth stages compared to the other species. Also, during the two months of the survey, *A. falcata* seedlings were the only ones that produced metaphylls, and those that accumulated the highest levels of biomass in roots and stems. Possibly due to the higher embryo mass/total seed mass ratio, *A. falcata* seeds can exhibit faster aerial and underground growth at the critical establishment stage.

Table 2. Mean ± standard deviation of root length (RL), stem length (SL), number of metaphylls (NM), number of eophylls (NE), area of metaphylls (LAM), and area of eophylls (LAE) of four woody species native to the Brazilian Cerrado after 60 days of growth in a greenhouse. Capital letters in columns compare significant differences between species (p<0.05, Tukey's test). N = 11 (*A. falcata*), N = 3 (*S. adstringens*), N = 16 (*S. polyphyllum*), N = 2 (*T. aurea*).

Species	RL (cm)	SL (cm)	NM	NE	LAM	LAE
A. falcata	10.04 ± 3.44 A	7.16 ± 0.44 A	1.09 ± 0.53 A	11.54 ± 1.86 A	17.72 ± 5.24 A	17.12 ± 5.45 A
S. adstringens	10.50 ± 4.09A	2.73 ± 0.51 B	0.00 ± 0.00 B	18.66 ± 12.85 A	0.00 ± 0.00 B	26.70 ± 27.10 A
S. polyphyllum	9.08 ± 4.91 A	1.36 ± 0.82 C	0.00 ± 0.00 B	22.60 ± 13.29 A	0.00 ± 0.00 B	18.10 ± 15.63 A
T. aurea	4.85 ± 1.62 A	1.25 ± 0.07C	0.00 ± 0.00 B	2.00 ± 0.00 B	0.00 ± 0.00 B	2.40 ± 0.56 B

Table 3. Mean ± standard deviation of root dry mass (RDM), stem dry mass (SDM), eophylls dry mass (EDM), metaphylls dry mass (MDM), and total dry mass (TDM) of four woody species native to the Brazilian Cerrado after 60 days of growth in a greenhouse. Capital letters in columns compare significant differences between species (p<0.05, Tukey's test). N = 11 (*A. falcata*), N = 3 (*S. adstringens*), N = 16 (*S. polyphyllum*), N = 2 (*T. aurea*).

Species	RDM(g)	SDM(g)	EDM(g)	MDM(g)	TDM(g)
A. falcata	0.025 ± 0.01 A	0.012 ± 0.006 A	0.023 ± 0.014 A	0.019 ± 0.016 A	0.081 ± 0.036 A
S. adstringens	0.006 ± 0.002 A	0.004 ± 0.001 AB	0.007 ± 0.008 AB	0.00 ± 0.000 B	0.018 ± 0.01 B
S. polyphyllum	0.009 ± 0.008 A	0.001 ± 0.001 B	0.012± 0.010 A	0.000 ± 0.000 B	0.022 ± 0.018 AB
T. aurea	0.006 ± 0.002 A	0.004 ± 0.001 AB	0.0015 ± 0.0007 B	0.000 ± 0.000 B	0.012 ± 0.000 B

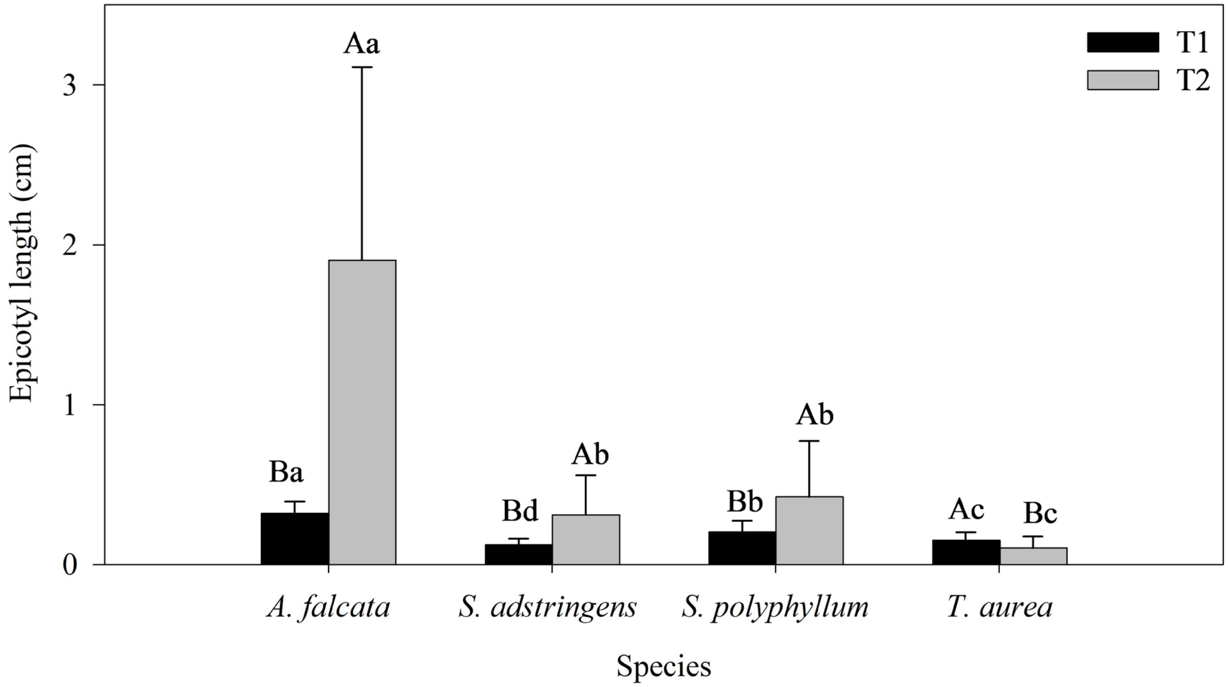

Figure 3. Mean (± standard error) epicotyl length inside the seed (T1) and in the first week after emergence (T2). Capital letters compare values between T1 and T2 within the same species. Lowercase letters compare values between T1 or T2 among different species (p <0.05, Tukey´s test). N = 50 (T1, all species), N = 27 (T2, *A. falcata* and *T. aurea*), N = 6 (T2, *S. adstringens*), N = 30 (T2, *S. polyphyllum*).

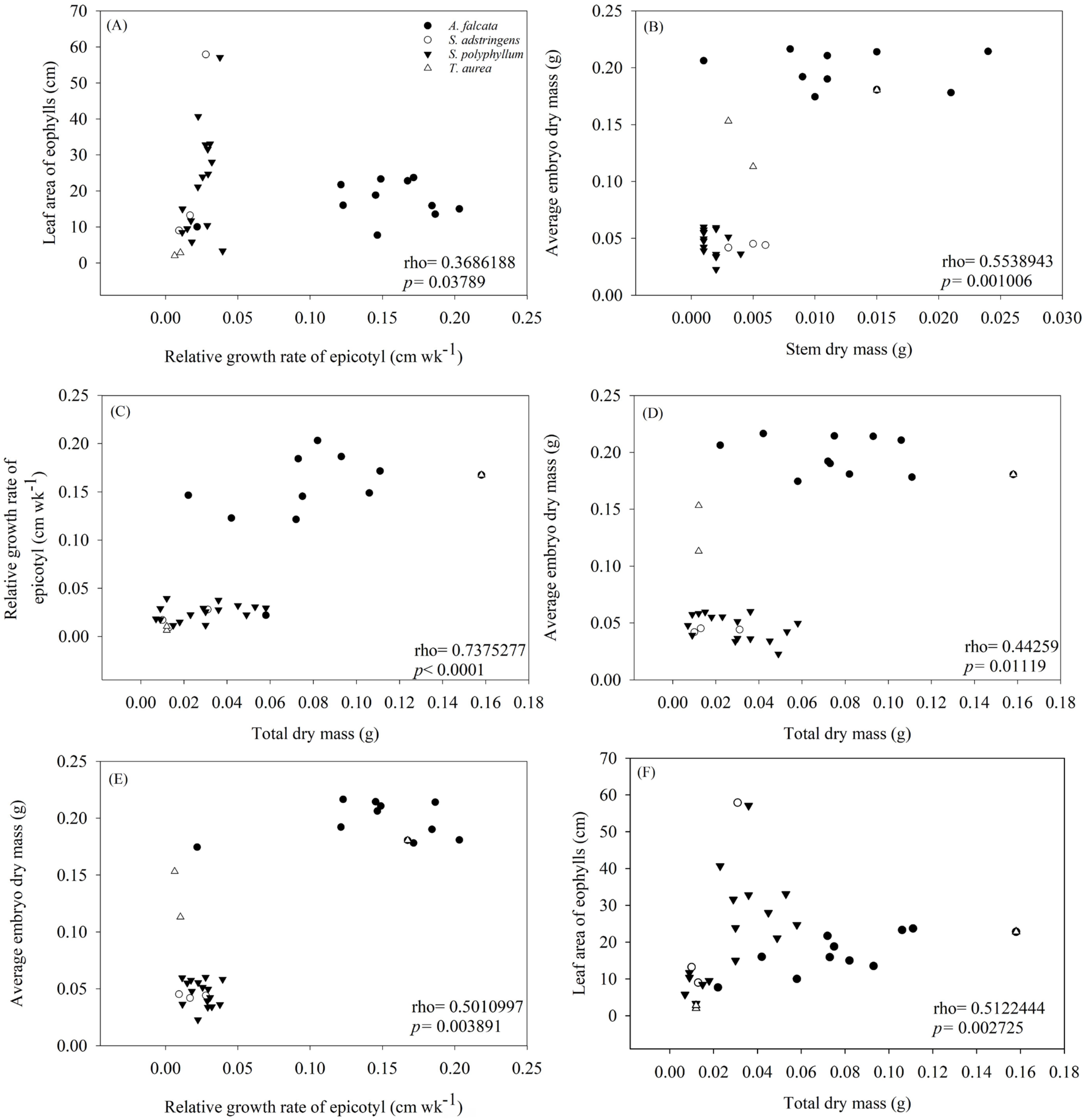

Figure 4. Spearman correlation coefficient (rho) and p-value between morphological variables of four woody species native to the Brazilian Cerrado growing in a greenhouse. N = 11 (*A. falcata*), N = 3 (*S. adstringens*), N = 16 (*S. polyphyllum*), N = 2 (*T. aurea*).

These characteristics may help this species to get established in different savannah phytophysiognomies and explain its high level of dominance in this biome (Ratter *et al.* 2003). Thus, the hypothesis that a larger and more differentiated embryonic axis could exhibit hastened germination and favor seedling establishment is, to some extent, supported by this study. However, even without the presence of leaves inside the seed, *S. adstringens*, *S. polyphyllum*, and *T. aurea* present high distribution ranges in Cerrado areas (Ratter *et al.* 2003). The seedlings of these species present high investment in the dry mass and length of roots (see Table 2 and 3), which may allow their establishment and development in Cerrado areas with seasonal precipitation and oligotrophic soils (Kanegae *et al.* 2000; Ronquim *et al.* 2009).

The establishment of woody plants in the Cerrado of Brazil is a hazardous process (Rossatto *et al.* 2018). The development of large seeds has been envisaged as an attribute that increases the probability of seedling establishment (Cicek 2007; Metz *et al.* 2010; Tilki 2010), although the advantage of large-seeded species may be counterbalanced by the higher numbers of small seeds produced by other species (Moles & Westoby 2004), or by raises in the growth rates of small-seeded plants (Dalling & Hubbell 2002). In general terms, the formation of large seeds is related to high germination rates and fast growth (Deb & Sundriyal 2017; Tumpa *et al*. 2021). However, some species with large seeds present underdeveloped embryos, so that germination may be delayed (Hayat 1963). Our study provides evidence in support of the idea that having seeds with a differentiated embryonic axis, i.e. with preformed organs such as eophylls (as in *A. falcata*) could be more important than seed size for germination and early growth. Also, this differentiated embryonic axis can provide an advantage in growth rate over other species with seeds of similar size, but devoid of preformed leaves. Having a seed with a little-differentiated embryonic axis may imply a longer intra-seed growth period before germination (Simão & Scatena 2003), as found here for *S. adstringens*, *S polyphyllum*, and *T. aurea*.

The seedlings of *S. polyphyllum* – a species with a less differentiated embryonic axis in its seeds compared to *A. falcata* – presented higher eophyll production with a delay of three weeks. This implies that even with small seeds and without an embryonic axis as differentiated as that of *A. falcata*, *S. polyphyllum* could reach high leaf and biomass production. It becomes evident that embryonic axis differentiation may provide an early advantage to a species, but that some species with a less-differentiated embryonic axis in their seeds may be able to make up for their early handicap and increase biomass accumulation. The fleshy cotyledons in *S. adstringens*, *S. polyphyllum*, and *T. aurea* could help these species in early growth and establishment in the natural environment as cited for other species (Polli *et al*. 2020; Gutiérrez-Soto *et al*. 2021; Kowalski *et al*. 2021).

The relative growth rate may be negatively correlated with seed mass (Houghton et al. 2013). Nonetheless, notable differences in relative growth rate may be found even within a species and despite similarities in seed size (Turnbull et al. 2008). Because of this variability, large-seeded species could have low colonization capacity but high competitive ability in the long term (Turnbull et al. 2008). In our study *A. falcata* showed larger embryos and high relative growth rate of the epicotyl, which may provide high competitive ability during early growth. It is important to notice that large seeds with undifferentiated embryos or with a small and little-differentiated embryonic axis could have a delayed germination and thus affect seedling establishment in natural areas. Therefore, it would be important to identify whether the seed has an embryo with preformed structures, which could accelerate germination and favor establishment. Even presenting differentiated embryo *S. adstringens*, *S polyphyllum*, and *T. aurea* presented a delay in germination and production of metaphylls. Lacking leaf preformation in the embryo could be key in delaying seedling growth.

For the Cerrado, it has already been shown that those species that present vegetative buds with preformed organs (leaves) produce leaves throughout a short period before the onset of heavy rains (Damascos *et al*. 2005; Damascos 2008; Souza *et al*. 2009a; b; Melo *et al*. 2022). Thus, even before the onset of the rainy season, these species could present fully developed foliage, reducing the risks of herbivory events (Arasaki 1993) and leaching of foliar nutrients (Araújo & Haridasan 2007). The same pattern could be considered for preformed organs inside the seeds, as found for *A. falcata* species in our study. The high number of leaves inside the embryo of *A. falcata* seeds (average of eight leaves) may help carry out photosynthesis in seedlings resulting in a higher relative growth rate (relative epicotyl growth) and biomass allocation to the roots. In Cerrado, woody plants allocate more biomass to the roots due to intense resource competition (mainly water and nutrients) with grass species (Hoffmann & Franco 2003; Melo *et al*. 2018). So, it is important to consider the embryo structures inside the seed in germination and establishment studies of Cerrado species. Among the four species included in this study, only one species presented a differentiated embryo with eophylls inside the seed, and this trait may be positively influencing its establishment in the Cerrado. In addition to higher RGR, seedlings of *A. falcata* showed higher stem and total biomass production, showing that seedlings directed more resources to growth, which could result in a greater probability of survival.

In species of the Fabaceae family both the seed and the embryo it contains tend to be relatively large (Martin 1946; Baskin & Baskin 2007). Even though the Fabaceae is one of the most studied families regarding seed structure and post-seminal development (Bispo *et al*. 2017; Nonato *et al*. 2022; Cruz *et al*. 2021; Teixeira *et al*. 2023), more studies are needed to better understand the influence of preformed structures within the seed and the degree of differentiation of the embryonic axis on plant establishment. All four species here, including three species of Fabaceae, had well differentiated embryos in their seeds. However, according to our results, the development of preformed structures (as eophylls) within the seeds would not occur in the majority of woody Fabaceae species that are common in the Cerrado. Studies with a larger number of species of this and other plant families would be advisable to test the hypothesis that organ preformation in seeds has been favored throughout the evolution of some plant lineages in the Cerrado. This hypothesis does not rule out the possibility that other ways of achieving rapid seedling establishment may also have been favored.

Our study shows that the degree of embryo development may be more important than seed size for the germination and establishment process of woody species of the Cerrado. The embryo with eophylls preformation may be an advantage for the initiation of photosynthesis at a critical stage of a plant's life cycle. Further studies should be devoted to investigating the relationship between seed content, seed mass, and seedling establishment to improve our knowledge about the mechanisms behind the complex process of establishment and survival of woody species in the Cerrado as well as in other biomes. Seed traits are relevant to surpass the environmental constraints in Cerrado areas (as fire, seasonal precipitation, and poor soils) and allow the coexistence of woody species with large seeds with small-seeded grasses to generate and maintain the structure (two-layered system) and the high biodiversity of Cerrado. Additional experimental studies with species presenting large and small seeds and with variable environmental traits, such as light and soil, are needed to elucidate the early growth constraints of Cerrado species.

Acknowledgments

The authors would like to thank the members of the Plant Functional Ecology Laboratory and the Seed Laboratory at UFV for their support in carrying out the experiment.

References

Almansouri M, Kinet JM, Lutts S. 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant and Soil 231: 243-254.

Arasaki FR. 1993. Crescimento foliar em árvores e crescimento inicial em plântulas de *Kielmeyera coriacea* Mart. PhD Thesis, Universidade Estadual de Campinas, Campinas.

Araújo JFD, Haridasan M. 2007. Relação entre deciduidade e concentrações foliares de nutrientes em espécies lenhosas do cerrado. Brazilian Journal of Botany 30: 533-542.

Baskin CC, Baskin JM. 1998. Seeds: Ecology, biogeography, and evolution of dormancy and germination. 2nd. edn. San Diego, Academic Press.

Baskin CC, Baskin JM. 2007. A revision of Martin's seed classification system, with particular reference to his dwarf-seed type. Seed Science Research 17: 11-20.

Bispo JDS, Costa DCCD, Gomes SEV *et al*. 2017. Size and vigor of *Anadenanthera colubrina* (Vell.) Brenan seeds harvested in Caatinga areas. Journal of Seed Science 39: 363-373.

Botelho SA, Ferreira RA, Malavasi MDM, David AC. 2000. Aspectos morfológicos de frutos, sementes, plântulas e mudas de jatobá-do-cerrado (*Hymenaea stigonocarpa* Mart. ex Hayne) -Fabaceae. Revista Brasileira de Sementes 22: 144-152.

Carreira RC, Zaidan LBP. 2003. Estabelecimento e crescimento inicial de *Miconia albicans* (Sw.) Triana e *Schizocentron elegans* Meissn., sob fotoperíodos controlados. Hoehnea 30: 155-161.

Chesson P, Gebauer RL, Schwinning S *et al*. 2004. Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. Oecologia 141: 236-253.

Cicek E. 2007. Seed size effects on germination, survival and seedling growth of *Castanea sativa* Mill. Journal of Biological Sciences 7: 438-441.

Cipriotti PA, Flombaum P, Sala OE, Aguiar MR. 2008. Does drought control emergence and survival of grass seedlings in semi-arid rangelands?: An example with a Patagonian species. Journal of Arid Environments 72: 162-174.

Cirne P, Miranda HS. 2008. Effects of prescribed fires on the survival and release of seeds of *Kielmeyera coriacea* (Spr.) Mart. (Clusiaceae) in savannas of Central Brazil. Brazilian Journal of Plant Physiology 20: 197-204.

Colli GR, Vieira CR, Dianese JC. 2020. Biodiversity and conservation of the Cerrado: Recent advances and old challenges. Biodiversity and Conservation 29: 1465-1475.

Cruz MSFV, Malavasi MDM, Ristau ACP, Malavasi UC, Dranski JAL. 2021. Maturidade de sementes de *Anadenanthera colubrina* (Vell.) Brenan. Ciência Florestal 31: 515-532.

Daibes LF, Pausas JG, Bonani N, Nunes J, Silveira FA, Fidelis A. 2019. Fire and legume germination in a tropical savanna: Ecological and historical factors. Annals of Botany 123: 1219-1229.

Dalling JW, Hubbell SP. 2002. Seed size, growth rate and gap microsite conditions as determinants of recruitment success for pioneer species. Journal of Ecology 90: 557-568.

Damascos MA. 2008. Conteúdo das gemas, momento da brotação e padrão de produção de folhas em espécies lenhosas do cerrado. In: Prado CHBA, Casali CA (eds.). Fisiologia Vegetal: Práticas em relações hídricas, fotossíntese e nutrição mineral. Barueri, Editora Manole. p. 1-9.

Damascos MA, Prado CHBA, Ronquim CC. 2005. Bud composition, branching patterns and leaf phenology in cerrado woody species. Annals of Botany 96: 1075-1084.

Deb P, Sundriyal RC. 2017. Effect of seed size on germination and seedling fitness in four tropical rainforest tree species. Indian Journal of Forestry 40: 313-322.

Duke JA. 1965. Keys for the identification of seedlings of some prominent woody species in eight forest types in Puerto Rico. Annals of the Missouri Botanical Garden 52: 314-350.

El-Maarouf-Bouteau H. 2022. The seed and the metabolism regulation. Biology 11: 168.

Embrapa. Centro Nacional de Pesquisa de Solos (Rio de Janeiro, RJ). 2009. Sistema brasileiro de classificação de solos. 2nd. edn. Rio de Janeiro, EMBRAPA-SPI.

Escobar DF, Silveira FA, Morellato LPC. 2018. Timing of seed dispersal and seed dormancy in Brazilian savanna: Two solutions to face seasonality. Annals of Botany 121: 1197-1209.

Franco AC, Rossatto DR, de Carvalho Ramos Silva L, da Silva Ferreira C. 2014. Cerrado vegetation and global change: The role of functional types, resource availability and disturbance in regulating plant community responses to rising CO_2 levels and climate warming. Theoretical and Experimental Plant Physiology 26: 19-38.

Franco AC, Nardoto GB, Souza MP. 1996. Patterns of soil water potential and seedling survival in the cerrados of central Brazil. In: Pereira RC, Nasser LCB (eds.). Proceedings do 1st International Symposium on Tropical Savannas: Biodiversity and sustainable production of food and fibers in the Tropical savannas. Planaltina, EMBRAPA-CPAC. p. 277-280.

Franco AC, Bustamante M, Caldas LS *et al*. 2005. Leaf functional traits of Neotropical savanna trees in relation to seasonal water deficit. Trees 19: 326-335.

Fenner M, Fenner M. 1985. Soil seed banks. In: Fenner M, Thompson K (eds.). Seed Ecology. Cambridge, Cambridge University Press. p. 57-71.

Fontenele HG, Miranda HS. 2022. Seed ecology of post-fire flowering species from the Cerrado. Seed Science Research 32: 222-229.

Gomes L, Miranda HS, Soares-Filho B, Rodrigues L, Oliveira U, Bustamante MM. 2020. Responses of plant biomass in the Brazilian savanna to frequent fires. Frontiers in Forests and Global Change 3: 507710.

Gutiérrez-Soto MV, Meoño-Piedra S, Guerrero-Barrantes M, Rocha OJ. 2021. Acorn characteristics, seed germination, seedling development, and leaf traits of three oak species from Talamanca, Costa Rica1. The Journal of the Torrey Botanical Society 148: 85-96.

Hayat MA. 1963. Morphology of seed germination and seedling in Annona squamosa. Botanical Gazette 124: 360-362.

Hoffmann WA, Moreira AG. 2002. The role of fire in population dynamics of woody plants. In: Oliveira PS, Marquis RJ (eds.). The cerrados of Brazil: Ecology and natural history of a neotropical savanna. New York, Columbia University Press. p. 159–177.

Hoffmann WA. 2000. Post-Establishment seedling success in the Brazilian Cerrado: A comparison of Savanna and forest species 1. Biotropica 32: 62-69.

Hoffmann WA, Franco AC. 2003. Comparative growth analysis of tropical forest and savanna woody plants using phylogenetically independent contrasts. Journal of Ecology 91: 475-484.

Hoffmann WA, Orthen B, Franco AC. 2004. Constraints to seedling success of savanna and forest trees across the savanna-forest boundary. Oecologia 140: 252-260.

Houghton J, Thompson K, Rees M. 2013. Does seed mass drive the differences in relative growth rate between growth forms? Proceedings of the Royal Society B: Biological Sciences 280: 20130921.

Kanegae MF, Braz VDS, Franco AC. 2000. Efeitos da seca sazonal e disponibilidade de luz na sobrevivência e crescimento de *Bowdichia virgilioides* em duas fitofisionomias típicas dos cerrados do Brasil Central. Brazilian Journal of Botany 23: 459-468.

Kramer J, Kozlowski T. 1972. Fisiologia das árvores. Lisboa, Fundação Calouste Gulbenkian.

Kidson R, Westoby M. 2000. Seed mass and seedling dimensions in relation to seedling establishment. Oecologia 125: 11-17.

Kowalski VK, Tardivo RC, Oliveira FMC, Mourão KSM. 2021. Morphology and anatomy of seedlings of Bromeliaceae from the perspective of ecophysiological types. Flora 285: 151959.

Lahoreau G, Barot S, Gignoux J, Hoffmann WA, Setterfield SA, Williams PR. 2006. Positive effect of seed size on seedling survival in fire-prone savannas of Australia, Brazil and West Africa. Journal of Tropical Ecology 22: 719-722.

Lamb EG, Cahill JF. 2006. Consequences of differing competitive abilities between juvenile and adult plants. Oikos 112: 502-512.

Lira-Martins D, Nascimento DL, Abrahão A *et al*. 2022. Soil properties and geomorphic processes influence vegetation composition, structure, and function in the Cerrado Domain. Plant and Soil 476: 549-588.

Marcos Filho J. 2015. Fisiologia de sementes de plantas cultivadas. Londrina, Abrates.

Martin AC. 1946. The comparative internal morphology of seeds. The American Midland Naturalist 36: 513-660.

Martins CC, Camara ATRD, Machado CG, Nakagawa J. 2008. Métodos de superação de dormência de sementes de barbatimão. Acta Scientiarum. Agronomy 30: 381-385.

Melo FD, Aguiar Neto AD, Simabukuro EA, Tabarelli M. 2004. Recrutamento e estabelecimento de plântulas. In: Ferreira AG, Borghetti F (eds.). Germinação: Do básico ao aplicado. Porto Alegre, ARTMED. p. 237-250.

Melo NMJ, Prado CHBDA, Branco DRV, Souza JP. 2022. Bud structure and development in trees of a neotropical savanna differing by leaf persistence. Austral Ecology 47: 316-325.

Melo NMJ, Rosa RSEG, Pereira EG, Souza JP. 2018. Rising $[CO_2]$ changes competition relationships between native woody and alien herbaceous Cerrado species. Functional Plant Biology 45: 854-864.

Metz J, Liancourt P, Kigel J, Harel D, Sternberg M, Tielbörger K. 2010. Plant survival in relation to seed size along environmental gradients: a long-term study from semi-arid and Mediterranean annual plant communities. Journal of Ecology 98: 697-704.

Moles AT, Westoby M. 2004. Seedling survival and seed size: a synthesis of the literature. Journal of Ecology 92: 372-383.

Moraes JAPV, Prado CHBA. 1998. Photosynthesis and water relations in cerrado vegetation. Oecologia Brasiliensis 4: 45-63.

Nonato ERL, de Oliveira CHS, da Silva Ferreira T *et al*. 2022. Morfometria de estruturas reprodutivas, germinação e crescimento inicial de *Hymenaea courbaril* L. para fins de reconhecimento em campo. Concilium 22: 325-342.

Oliveira PE, Silva JC. 1993. Reproductive biology of two species of *Kielmeyera* (Guttiferae) in the cerrados of Central Brazil. Journal of Tropical Ecology 9: 67-79.

Oliveira-Filho AT, Ratter JA. 2002. Vegetation physiognomies and woody flora of the Cerrado biome. In: Oliveira PS, Marquis RJ (eds.). The Cerrados of Brazil. New York, Columbia University Press. p. 91-120.

Paula GA, Fischer E, Silveira M, Almeida H, van den Berg E. 2021. Woody species distribution across a savanna-dry forest soil gradient in the Brazilian Cerrado. Brazilian Journal of Biology 83: e243245.

Pellizzaro KF, Cordeiro A, Alves M *et al*. 2017. "Cerrado" restoration by direct seeding: field establishment and initial growth of 75 trees, shrubs and grass species. Brazilian Journal of Botany 40: 681-693.

Polli A, Romagnolo MB, de Souza LA, Pastorini LH. 2020. Influence of the functional traits of seeds on germination dynamics and morphofunctional pattern of the seedlings. Acta Scientiarum. Biological Sciences 42: e52154.

Poorter L, Bongers F, Sterck FJ, Wöll H. 2005. Beyond the regeneration phase: Differentiation of height-light trajectories among tropical tree species. Journal of Ecology 93: 256-267.

Ranal MA, DG Santana, Schiavini I. 2010. Are there germination patterns for cerrado species? In: Del-Claro K, Oliveira PS, Rico-Gray V, Barbosa AAA (eds.). Encyclopedia of life support systems. Tropical biology and conservation management, savannah ecosystems. Oxford, Encyclopedia of Life Support Systems (Eolss) Publishers. p. 1575-1671.

Ratter JA, Bridgewater S, Ribeiro JF. 2003. Analysis of the floristic composition of the Brazilian cerrado vegetation III: Comparison of the woody vegetation of 376 areas. Edinburgh Journal of Botany 60: 57-109.

R Core Team. 2016. A language and environment for statistical computing. http://www.R-project.org/. 1 Jan. 2016.

Ressel K, Guilherme FA, Schiavini I, Oliveira PE. 2004. Ecologia morfofuncional de plântulas de espécies arbóreas da Estação Ecológica do Panga, Uberlândia, Minas Gerais. Brazilian Journal of Botany 27: 311-323.

Ribeiro JF, Walter BMT. 1998. Fitofisionomias do bioma Cerrado. In: Sano SM, Almeida SP (eds.). Cerrado: Ecologia e Flora. Planaltina, EMBRAPA Cerrados. p. 89-166.

Ribeiro JF, Walter BMT. 2008. Fitofisionomias do bioma Cerrado. In: Sano SM, Almeida SP, Ribeiro JF (eds.). Cerrado: Ecologia e Flora, Planaltina, EMBRAPA Cerrados. p.151-212.

Ribeiro JW, Pilon NA, Rossatto DR, Durigan G, Kolb RM. 2021. The distinct roles of water table depth and soil properties in controlling alternative woodland-grassland states in the Cerrado. Oecologia 195: 641-653.

Rizzini CT. 1965. Experimental studies on seedling development of cerrado woody plants. Annals of the Missouri Botanical Garden 52: 410-426.

Ronquim CC, Prado CHBDA, Paula NFD. 2003. Growth and photosynthetic capacity in two woody species of cerrado vegetation under different radiation availability. Brazilian Archives of Biology and Technology 46: 243-252.

Ronquim CC, Prado CHBDA, Souza JP. 2009. Growth, photosynthesis and leaf water potential in young plants of *Copaifera langsdorffii* Desf. (Caesalpiniaceae) under contrasting irradiances. Brazilian Journal of Plant Physiology 21: 197-208.

Rossatto DR, Hoffmann WA, Franco AC. 2009. Differences in growth patterns between co-occurring forest and savanna trees affect the forest–savanna boundary. Functional Ecology 23: 689-698.

Rossatto DR, de Araújo PE, da Silva BHP, Franco AC. 2018. Photosynthetic responses of understory savanna plants: Implications for plant persistence in savannas under tree encroachment. Flora 240: 34-39.

Rosell JA, Marcati CR, Olson ME, Lagunes X, Vergilio PC, Jiménez-Vera C, Campo J. 2023. Inner bark vs sapwood is the main driver of nitrogen and phosphorus allocation in stems and roots across three tropical woody plant communities. New Phytologist 239: 1665-1678.

Saboya P, Borghetti F. 2012. Germination, initial growth, and biomass allocation in three native Cerrado species. Brazilian Journal of Botany 35: 129-135.

Sales NM, Pérez-García F, Silveira FAO. 2013. Consistent variation in seed germination across an environmental gradient in a Neotropical savanna. South African Journal of Botany 87: 129-133.

Silveira FA, Fernandes GW, Lemos-Filho JP. 2013. Seed and seedling ecophysiology of neotropical melastomataceae: Implications for conservation and restoration of savannas and rainforests1. Annals of the Missouri Botanical Garden 99: 82-99.

Simão DG, Scatena VL. 2003. Morphological aspects of the propagation in *Heliconia velloziana* L. Emygd. (Zingiberales: Heliconiaceae). Brazilian Archives of Biology and Technology 46: 65-72.

Sousa MP, Braga LF, Braga JF, Delachiave MEA. 2008. Germinação de sementes de *Plantago ovata* Forsk. (Plantaginaceae): Temperatura e fotoblastismo. Revista Árvore 32: 51-57.

Souza JP, Prado CH, Damascos MA, Albino ALS. 2009a. Influence of shoot inclination on irradiance and morphophysiological leaf traits along shoots in cerrado trees with distinct leaf deciduousness. Brazilian Journal of Plant Physiology 21: 281-289.

Souza JP, Prado CH, Albino ALS, Damascos MA. 2009b. Shoot-foliage relationships in deciduous, semideciduous, and evergreen cerrado tree species. Brazilian Journal of Plant Physiology 21: 76-86.

Souza GF, Almeida RF, Bijos NR, Fagg CW, Munhoz CBR. 2021. Herbaceous-shrub species composition, diversity and soil attributes in moist grassland, shrub grassland and savanna in Central Brazil. Brazilian Journal of Botany 44: 227-238.

Stanik N, Peppler-Lisbach C, Rosenthal G. 2021. Extreme droughts in oligotrophic mountain grasslands cause substantial species abundance changes and amplify community filtering. Applied Vegetation Science 24: e12617.

Teixeira AGR, Pacheco KR, Peixoto JC. 2023. Caracterização morfológica das sementes de *Vigna unguiculata* (L.) Walp (FABACEAE). Ipê Agronomic Journal 7: 1-8.

Tilki F. 2010. Influence of acorn size and storage duration on moisture content, germination and survival of *Quercus petraea* (Mattuschka). Journal of Experimental Biology 31: 325-328.

Tumpa K, Šatović Z, Vidaković A, Idžojtić M, Stipetić R, Poljak I. 2022. Population variability of almond-leaved willow (*Salix triandra* L.) Based on the Leaf Morphometry: Isolation by distance and environment explain phenotypic diversity. Forests 13: 420.

Tumpa K, Vidaković A, Drvodelić D *et al*. 2021. The effect of seed size on germination and seedling growth in sweet chestnut (*Castanea sativa* Mill.). Forests 12: 858.

Turnbull LA, Paul-Victor C, Schmid B, Purves DW. 2008. Growth rates, seed size, and physiology: do small-seeded species really grow faster. Ecology 89: 1352-1363.

Yang QH, Wei X, Zeng XL *et al*. 2008. Seed biology and germination ecophysiology of *Camellia nitidissima*. Forest Ecology and Management 255: 113-118.

Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ. 2002. Plant ecological strategies: Some leading dimensions of variation between species. Annual Review of Ecology and Systematics 33: 125-159.