ORIGINAL ARTICLE - Silviculture



Effect of Different Temperatures on the Germination of Callisthene major (Vochysiaceae)

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Abstract

Knowledge of environmental conditions for seed germination and seedling formation of forest species is essential. The objective of this work is to evaluate the germination of Callisthene major seeds under laboratory conditions using six different temperatures (20, 25, 30, 35, 20-30 and 25-35 °C) and germitest paper (on and between paper). A completely randomized design was used with four replicates of 25 seeds per experimental unit. The variables evaluated were germination (%), germination speed index (GSI), average germination time (AGT) in days and normal seedlings. The results indicated that the species has a high germination index (above 94%) for all tested substrates and temperatures; however, the highest germination vigor (GSI and AGT) was obtained by alternating the temperature between 25-35 °C with the substrate on paper.

Keywords: rupestrian cerrado, forest seed, tapicuru.

1. Introduction and objectives

Callisthene major var. pilosa Warm. (Vochysiaceae) arboreal species, popularly known as "joão farinha", "jacaré" or "tapicuru", among other names, is native and endemic to Brazil, and found in the Northeast (BA), Midwest (MT, GO, DF and MS) and Southeastern (MG) regions in Cerrado areas (Forzza et al., 2010; Lorenzi, 2014).

It is a secondary species which is considered semideciduous, heliophilous and selective xerophilous, reaching up to 12 meters high. It is a moderately heavy wood, resistant, used in carpentry (rafters, slats and beams, among other uses), and for coal production and firewood. It is also recommended for urban afforestation due to its dense and rounded top, and for recovering degraded areas from dry environments. Its bark is also used in folk medicine in rural communities in the form of an infusion bath (Forzza et al., 2010; Lorenzi, 2014; Peixoto, 2002).

Its fruit is semi-woody and capsular $(12-17 \times 11-14 \text{ mm})$, globose or ellipsoid, glabrous, and dehiscent with one to two seeds $(8-10 \times 5-7 \text{ mm})$ per locule with anemochorous dispersion (Shimizu & Yamamoto, 2012), presenting low seed germination rates (Lorenzi, 2014). Despite its dispersion in different regions and potential usage, there is not much information about the basic characteristics of their germination process, such as suitable temperatures or substrates.

The germination process is a sequence of physiological events influenced by external factors (environmental, such as temperature) and internal factors (dormancy and inhibitors or promoters of germination), described as resuming embryo growth and subsequent rupture of the integument by the primary root (Labouriau, 1983).

Each species has basic requirements for germinating its seeds, and one of the main factors conditioning the process when there is no dormancy is temperature. However, there is no standard temperature for all species, and the seeds of plants found in Brazil usually germinate in a wide range, depending on the biome and region, with good germination performance by most species in the range of 20 °C to 35 °C (Brancalion et al., 2010).

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Carvalho & Nakagawa (2012) and Bewley et al. (2013) describe that the germination process does not occur when the seeds are placed at inadequate temperatures, stating that there is an indicated temperature range for the germination of the largest number of seeds in the shortest time.

In addition to temperature, another factor that interferes with germination is the type of substrate, which influences the water retention capacity, aeration and the degree of infestation of pathogens, and it must remain uniformly moist, supplying an adequate amount of water for the metabolic processes (Brazil, 2009; Carvalho & Nakagawa, 2012).

The correct use of these factors accelerates and homogenizes the germination processes, enabling the production of a larger number of seedlings in a shorter period of time. However, due to the great variety of species, the information regarding the germination processes of most of them is still unknown, which makes it difficult to standardize the evaluation procedures and production of seedlings (Brazil, 2009).

For these reasons and considering the potential of the species usage and the existing information gaps regarding its basic germination processes, the objective of this study is to evaluate the germination of *Callisthene major* var. *pilosa* Warm. collected in areas of rupestrian cerrado in the state of Mato Grosso do Sul, and to verify if there is a possible increase in the germination rates when the seeds are kept at different temperatures and substrates.

2. Materials and methods

The fruits were collected in September 2015 in the beginning of the rainy season, being obtained in areas of rupestrian cerrado of the Taboco region, municipality of Corguinho, Mato Grosso do Sul, directly from 12 mother trees. The collection was carried out in a preserved area called Fazenda Colorado Legal Reserve (\pm 1,000 ha), and the fruits were packed in paper bags and transported to the Interdisciplinary Laboratory for Research in Environmental and Biodiversity Systems (LabPSAB), Universidade Anhanguera Uniderp, Campo Grande, Mato Grosso do Sul, Brazil.

After being removed from the fruits, the seeds were surface disinfected by immersion in sodium hypochlorite (2%) for three minutes, and then washed in running water for one minute. A seed lot was used to determine the water content of the seeds (four days after collection for the test), according to the oven-drying method at 105 °C (Brazil, 2009), with the use of four replicates of 20 seeds.

Two substrates (between and on CEL-065 Germitest paper), four constant temperatures (20, 25, 30 and 35 °C) and two alternating temperatures (20-30 and 25-35 °C, 12 h period for each temperature) were used for evaluating the effect of the substrates and temperatures on germination in a germination chamber with 12 h photoperiod of white light (±660 lux). Four replicates of 25 seeds per treatment were used, placed in transparent gerbox plastic boxes (11 cm × 11 cm × 3.5 cm). The paper sheets were previously moistened with 0.1% (w/v) Rovral SC^{*} fungicide, with the solution volume equivalent to 2.5 times the dry mass of the substrate.

The germination was monitored every 24 hours for a period of 30 days. Seeds with primary root of 2 mm were considered to be germinated for calculating the percentage of germination and average germination time (AGT) in days (Labouriau, 1983) and germination speed index (GSI) (Maguire, 1962). The relative germination frequency was calculated according to Labouriau & Valadares (1976). Normal seedling formation was evaluated according to Brazil (2009).

The experimental design was a completely randomized design in a 2 × 6 factorial scheme (substrates × temperatures) with four replications, considering each group of 25 seeds as an experimental unit. The data were submitted to analysis of variance and the Tukey test when significance occurred at a level of 5% (p < 0.05) of probability, with statistical analysis performed through Assistat software. There was no need to transform the germination data (GSI and AGT) according to the normality and homogeneity variance tests.

3. Results and discussion

The water content in the seeds was 11.2%, lower than that obtained for other species of the same genus (*Callisthene fasciculata* Mart.) collected in the Pantanal, which presented values between 24.9% and 26.8% (Oliveira et al., 2015). Other species of the family have values between 9.4% (*Vochysia rufa* Mart.) and 19% (*Qualea grandiflora* Mart.); moreover, *Q. dichotoma* (Mart.) Warm. (11%) and *V. haenkeana* Mart. (12%) (Salomão et al., 2003) species have similar values to *C. major*. The characteristics of *Callisthene major* seeds indicate desiccation tolerance (orthodox), in which the seeds go through a loss of water while still in the mother plant, being dispersed with low water content at between 5% and 10% of their fresh weight (Bewley et al., 2013; Castro et al., 2004).

The results obtained for the accumulated germination (Figure 1) indicated that the seeds were at their highest physiological maturity at which greater germination occurs; a factor related to the moisture degree of the seeds. However, the daily percentages varied according to the studied temperatures. Popinigis (1985) describes that the physiological maturation point is the one at which the seeds present their best physiological quality (maximum germination and vigor).

The formation of normal seedlings occurred at all temperatures with values above 90%, indicating that the

tested conditions did not negatively interfere with the initial formation of the evaluated structures. In evaluating the formation of *Callisthene fasciculata* seedlings, Oliveira et al. (2015) also described that the presence of abnormal seedlings was not noticed under the tested conditions.

The Vochysiaceae family can normally present high germination rates, as reported by Salomão et al. (2003), reaching 100% for Q. *dichotoma*; 95% for *Vochysia tucanorum* Mart.; 90% for *V. rufa*; 80% for *V. haenkeana*; and 75% for *Qualea parviflora* Mart., demonstrating that the *Callisthene* genus could be classified in this group. However, other species of the family have lower rates, such as *Vochysia pyramidalis* Mart., 55%, and *Q. grandiflora*, between 30% and 60% (Figure 1).

It can be observed that the temperature of 20 °C caused a delay in the emission of the primary root in both substrates (Figure 1). In working with *Vochysia bifalcata* Warm., Rickli et al. (2014) also demonstrated that 20 °C led to lower GSI. This situation may be related to slower respiratory rates or increased stiffness of the cell membrane (Bewley et al., 2013; Bradford & Nonogaki, 2007). On the

other hand, in showing germination results for *C. fasciculate*, Oliveira et al. (2015) indicated that the temperature of 20 °C did not negatively interfere with the beginning of the germination process (root emission), demonstrating differences in adaptation to the environment.

The seeds generally germinated in a few days (between two and 15 days), indicating the production capacity of a large number of seedlings in a short period of time. According to Baskin & Baskin (1998) and Fenner & Thompson (2005), the germination behavior of a species is related to habitat, seed size and life cycle, while Bewley et al. (2013) describe that a small average germination time may be related to rapid colonization of the environment. On the other hand, Oliveira et al. (2015) indicated that *C. fasciculata*, another species of the same genus, needs a slightly longer period to finish its germination process (17 days), with it starting on the third day under the best conditions (20 °C, paper). However, *C. fasciculata* seeds were collected in Pantanal, which is a floodplain with a different climatic regime from the environment occupied by *Callisthene major*.

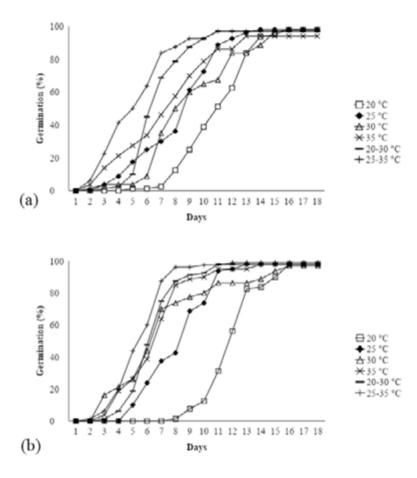


Figure 1. Accumulated germination (%) of *Callisthene major* seeds submitted to different temperatures and substrates (a) on paper and (b) between paper.

The rapid germination process of the species under study may also be related to the collection environment (the rupestrian cerrado), and to its adaptation to this site. The region presents a shallow and rocky soil, and soil moisture does not remain high for too long when rainfall occurs (small water storage capacity). Pinto et al. (2009) describe this environment as of limited environmental conditions, mainly edaphic. In this way, the seeds that germinate quickly would have more chance of establishing themselves and it could be considered that the species adapted its reproductive phenology (seed dispersal from August/September) to the beginning of the rainy season in the region (September/October), germinating rapidly to take advantage of favorable environmental conditions. The rainy season extends until April, when precipitation subsides.

According to Araújo et al. (2005), seedling recruitment of woody species is more intense during the rainy season in

environments subject to water stress. Other authors also report this situation, such as Oliveira & Silva (1993) in evaluating two species of the *Kielmeyera* genus (*K. coriacea* Mart. and *K. speciosa* A. St.-Hil.), and Ranieri et al. (2012) with *K. regalis* Saddi, showing a rapid and numerous germination in the beginning of the rainy season. This strategy of taking advantage of favorable environmental conditions is also the standard used by *Callisthene major*.

The germination frequency is not perfectly synchronized, having a small distribution over the incubation time (Figure 2), and it can be considered heterogeneous for most treatments (except for 20-30 °C on paper); according to Labouriau (1983), this would indicate less dispersion in time. In this way, germination could be considered as having a polymodal character for most of the tested temperatures (except for 20 °C, on paper) (Figure 2).

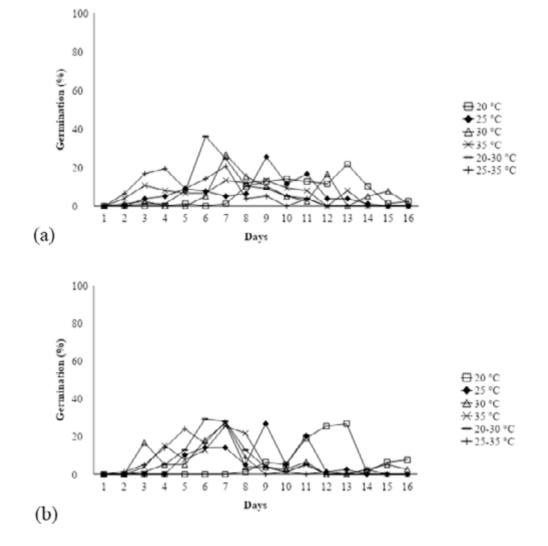


Figure 2. Germination frequency (%) of *Callisthene major* seeds submitted to different temperatures and substrates (a) on paper and (b) between paper.

Labouriau & Agudo (1987) and Brancalion & Marcos Filho (2008) describe that the germination distributed over time may favor the survival of seedlings in environments with variations in environmental conditions. The low water storage capacity in the soil in the collection region can be a decisive factor in the germination distribution in a short period of time. This means the distribution pattern would have an adaptive meaning, compensating for unfavorable environmental conditions.

By evaluating the interaction of factors (Table 1) and considering the two tested substrates, there were significant differences with the use of the substrate on paper with temperatures of 35 °C, 20-30 °C and 25-35 °C, and between paper for 30 °C. However, despite the relevant differences, the germination rates of the species can be considered high, being equal to or greater than 94% for all treatments. The lower efficacy of the substrate on paper may be related to a lower availability of water when seeds are exposed to higher temperatures. This situation, which is of greater water restriction, may have caused damage to the more fragile embryos; a factor probably related to enzymatic alterations, reducing the amount of free amino acids and modifying the rate of metabolic reactions according to Bradford & Nonogaki (2007) and Bewley et al. (2013). However, these

effects were small considering the germination rates obtained, indicating seed vigor.

On the other hand, the germination vigor, measured indirectly by GSI and AGT (Table 2), was strongly affected by the different substrates and temperatures. At the end, the substrate on paper and alternating temperature of 25-35 °C stood out, demonstrating that it was possible to generate a large number of germinated seeds in a short period of time for the evaluated conditions.

The high values of GSI and small values of AGT found for the temperature of 25-35 °C again indicated that the species distributes its germination in a short period of time. According to Borges & Rena (1993), certain species, especially those found in the initial stages of succession, germinate better at alternating temperatures; *Callisthene major* presented good germination and vigor rates at this temperature, although the others also resulted in significant germination rates (Table 1), but with less vigor (Table 2).

The germination results confirmed that the species germinates in a wide temperature range with high germination rates, independently of the substrate and temperature, being able to be considered eurythermic and able to occupy different sites, thus explaining its occurrence in distinct regions such as the Southeast and Midwest.

Table 1. Germination (%) of Callisthene major seeds submitted	to different temperatures and substrates ((on and between paper).
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Interaction			Temperatures (°C)			
Substrates	20	25	30	35	20-30	25-35
On	98 aA	98 aA	98 aA	94 bB	97 bA	97 bA
Between	98 aA	98 aAB	97 bB	98 aAB	98 aAB	99 aA
dms		column 0.97		line 1.41		

Means followed by the same lowercase letters in a column and capital letters on the lines do not differ significantly by the Tukey test (p < 0.05). dms: value of the least significant difference for the chosen test.

Table 2. Germination speed index (GSI) and average germination time (AGT) in days of *Callisthene major* seeds submitted to different temperatures and substrates (on and between paper).

Interaction	GSI		Temperatures (°C)			
Substrates	20	25	30	35	20-30	25-35
On	9.1 aF	13.2 aD	12.0 bE	16.1 aB	14.5 bC	21.5 aA
Between	8.1 bE	12.4 bD	16.7 aB	16.0 aC	15.5 aC	18.4 bA
dms		Column 0.35			line 0.52	
Interaction	AGT		Temperatures (°C)			
Interaction Substratos	AGT 20	25	Temperatures (°C) 30	35	20-30	25-35
		25 8.5 aC	L , ,	35 7.4 bB	20-30 6.9 aB	25-35 5.3 aA
Substratos	20	-	30			

Means followed by the same lowercase letters in a column and capital letters on the lines do not differ significantly by the Tukey test (p < 0.05). dms: value of the least significant difference for the chosen test.

It is probable that the thermal amplitude observed in the germination processes is related to the environment in which the seeds were collected (in areas of rupestrian cerrado), where large variations of temperature can occur due to the presence of rocky outcrops which absorb radiation during the day, and do not maintain their temperature at night. As mentioned by Baskin & Baskin (1998) and Fenner & Thompson (2005), the germination behavior of the species is, among other factors, related to its native habitat.

In working with gaseous exchanges of a *Vochysiaceae* species in an area of rupestrian cerrado in Serra da Canastra, Minas Gerais, Paula et al. (2015) showed that the daily temperature oscillation in October (summer) reached approximately 13 °C (6 a.m to 6 p.m.). Moreover, in researching rocky areas in Chapada Diamantina, Bahia, Neves & Conceição (2010) describe that the temperature in this environment commonly suffers extreme daily fluctuations. The collection of *Callisthene major* seeds occurred in September, indicating that the seeds of the species are adapted to environments with great thermal variation, which occurs in rupestrian cerrado areas, as mentioned by Neves & Conceição (2010) and Paula et al. (2015).

High germination rates of the *Callisthene* genus have already been cited by Oliveira et al. (2015), reaching 100% under the best conditions, with the authors describing that the substrate on paper was the least adequate when compared to vermiculite, sand and between paper. The authors also detected that the temperature of 35 °C in the substrate on paper caused embryo death; a factor probably related to the lower efficiency of this substrate for water retention and greater exposure to heat.

Brancalion et al. (2010) report that about 10% of the 272 Brazilian tree species studied presented optimal germination at alternating temperatures. However, the authors write that the alternating temperatures were exclusively the most favorable for a few species, meaning with no participation of other constant temperatures, thus typically highlighting in this context initial species of forest succession (for example, *Bixa orellana* L., *Trema micrantha* (L.) Blume and *Cnidoscolus phyllacanthus* (Mull. Arg.) Pax & L. Hoffm., among others).

For Bradford & Nonogaki (2007) and Bewley et al. (2013), the alternating temperatures would be related to an alteration in the balance of promoter and inhibitory substances of the germination, either leading to a germination process or to changes in the physical properties of the integument. Baskin & Baskin (1998) and Brancalion et al. (2008) also mention that alternating temperature regimes may be related to overcoming the impermeability of the integument to water, allowing the hydration of the seeds and their germination.

On the other hand, Probert (2000) describes that stimulating the germination by alternating temperatures is common in species of dry zones; in addition, germination at alternate temperatures may be an adaptation of small seeds to germinate when the formation of clearings occurs. According to Liu et al. (2013), who studied the effect of temperature fluctuations on germination of 445 species in Tibet, smaller seeds are benefited by temperature variations, germinating faster and decreasing competition for establishment in clearings; however, this is not a strategy adopted by all species. Masin et al. (2017) confirm that the use of alternate temperatures is adequate to obtain good germination rates, but more research is necessary to understand all the mechanisms involved.

Considering that *Callisthene major* seeds are considered small (Shimizu & Yamamoto, 2012), the good germination performance in alternating temperature would be an adaptation of the species to a restrictive environment, allowing its rapid establishment. However, there is still little information on the influence of this type of temperature on the germination processes of the genus studied, which is confirmed by Salomão et al. (2003), who did not mention any species of *Vochysiaceae* having preference to alternating temperature.

4. Conclusion

The species presents high germination percentages at different temperatures, demonstrating its capacity for adaptation, and that it is recommended to use alternating temperature of 25-35 °C and substrate between paper in order to obtain higher germination percentages and vigor for seeds collected in rupestrian cerrado areas.

Submission status

Received: 10 Aug. 2017 Accepted: 14 June 2019 Associate editor: Juliana Müller Freire

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Financial support

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing the Scientific Start-up Grant (Pibic) and Present Research Grant (PQ2) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) for the scholarship. We would also like to thank the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (Fundect). Finally, we extend our thanks to the Universidade Anhanguera Uniderp for funding the Interdisciplinary Research Group (GIP project).

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