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Effects of temperature on the germination of *Diptychandra aurantiaca* (Fabaceae) seeds

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ABSTRACT. *Diptychandra aurantiaca* is a tree belonging to the Fabaceae family. The wood of this tree is moderately heavy and resistant, and it is used to make posts, wooden sleepers and building, among other structures. The aim of this work was to evaluate seed germination and the initial growth of the primary roots in *D. aurantiaca* at different temperatures using seeds collected in the village of Taboco in Mato Grosso do Sul state, Brazil. The seeds were subjected to constant temperatures of 20, 25, 30 and 35°C and alternating temperatures of 20-30 and 25-35°C using four replications of 25 seeds per treatment in a germination chamber. The results showed that most seeds germinated at 25 and 30°C, with 97 and 87%, respectively, thus indicating that these temperatures are efficient to not only promote germination but also to potentiate the speed (1,1 - 25 and 30°C) and decrease the average germination time (9,0 - 25°C and 7,1 - 30°C), highest allocation of dry biomass (0,0067 g at 25°C and 0,0055 g at 30°C) and the greatest primary root size (17,5 mm at 25°C and 16,5 mm at 30°C).

Keywords: broad-leafed, semi-deciduous, savannah (Cerrado), forest seeds, 'true-red-charcoal'.

Efeito da temperatura sobre a germinação de sementes de *Diptychandra aurantiaca* (Fabaceae)

RESUMO. *Diptychandra aurantiaca* é uma árvore da família Fabaceae. Como utilidade, a madeira, moderadamente pesada e resistente, é utilizada na fabricação de postes, dormentes, construção civil, entre outros usos. O objetivo deste trabalho foi avaliar a germinação das sementes e crescimento da raiz primária em diferentes temperaturas, com sementes coletadas na região do Taboco, Mato Grosso do Sul. As sementes foram submetidas a temperaturas constantes de 20, 25, 30 e 35°C e alternadas de 20-30 e 25-35°C, com quatro repetições de 25 sementes por tratamento em câmaras de germinação. Os resultados obtidos evidenciaram maior percentagem de germinação das sementes nas temperaturas de 25 e 30°C, atingindo 97 e 87%, respectivamente, indicando que estas temperaturas são mais eficazes em promover a germinação, propiciando também maior velocidade de germinação (1,1 - 25 e 30°C), menor tempo médio de germinação (9,0 - 25°C e 7,1 - 30°C), maior alocação de massa seca (0,0067 g a 25°C e 0,0055 g a 30°C) e comprimento da raiz primária (17,5 mm em 25°C e 16,5 mm em 30°C).

Palavras-chave: florestas latifoliadas, semidecíduas, cerrado, sementes florestais, carvão-vermelho-verdadeiro.

Introduction

In recent years, an increasing number of studies have been aimed at propagating native forest species due to current environmental problems and the need to restore or recover native vegetation in areas of degradation (OLIVEIRA et al., 2005a). Furthermore, knowledge about Brazilian ecosystems is still scarce, especially with respect to seed germination in native species and in light of the enormous biological potential of these systems (BRANCALION et al., 2010).

Seed germination is considered to be the resumption of the metabolic activities of the embryo-

nic axis that eventually culminates in the critical phase of primary root protrusion, associated with the physiological processes occurring in a seed. It is dependent on environmental factors, such as water, light, temperature and oxygen, thereby confirming our understanding of the ideal conditions required for the germination of seeds in a predetermined species (BRASIL, 2009; CARVALHO; NAKAGAWA, 2000; CASTRO et al., 2004).

Temperature is an environmental factor that significantly affects germination. However, there is no optimum and uniform temperature for all species. Temperature affects the speed and

percentage of germination, primarily influencing water uptake and impacting the biochemical reactions and physiological processes that determine germination (CARVALHO; NAKAGAWA, 2000; TAIZ; ZEIGER, 2009).

Germination occurs within a defined temperature range and will not occur above or below these limits. The ideal temperature will permit maximum germination in the least amount of time (CARVALHO; NAKAGAWA, 2000). Marcos Filho (2005) stated that the optimum temperature allows for the most efficient combination of the percentage and speed of germination.

Temperature is also fundamental for the development of specific parts of seedlings and primary roots, generally the first parts of the plant to protrude during germination. Inadequate temperatures directly affect root growth, a process in which cells are rapidly dividing, and any adverse environmental factor diminishes the capacity of the root for development (LARCHER, 2003).

The tree species *Diptychandra aurantiaca* (Mart.) Tul., commonly known as *carvão-vermelho-verdadeiro* (true-red-charcoal) or *balsaminho* (little Balsam), belongs to the Fabaceae family. This species reaches a height of 8-14 m and has a rectilinear trunk. *D. aurantiaca* is commonly found in the broad-leaved, semi-deciduous forests and dense savannahs of Mato Grosso do Sul, São Paulo, Minas Gerais and Goiás State. Its moderately heavy, hard, resistant wood is extremely durable, even in outdoor environments, and is used in the fabrication of posts, sleepers and charcoal and for civil construction, among other applications. Despite their deciduous nature, these trees are considered to be ornamental species and can be used in landscape gardening. Due to its rapid growth, *D. aurantiaca* can adapt to open areas and is used in heterogeneous re-forestation (LORENZI, 2008; POTT; POTT, 1994).

Because of the lack of studies concerning the germination of Brazilian flora, particularly with respect to understanding of the biology and ecology of native plants in broad-leaved, semi-deciduous savannahs, the objective of this research was to evaluate the effects of temperature on seed germination and the initial growth of the primary root of *Diptychandra aurantiaca*.

Material and methods

Diptychandra aurantiaca fruits were collected from woodlands in the Taboco region of the municipality of Corguinho (19°49'S 54°50'O, 320 m altitude) in the state of Mato Grosso do Sul during the month

of August 2010. Mature, dried fruits were collected from trees using a trimmer, stored in Kraft paper bags and transported to the Laboratory of Research into Bio-diversity and Environmental Systems at Anhanguera-Uniderp University in Campo Grande.

In the laboratory, damaged/collapsed and insect-attacked seeds were eliminated, and a moisture content test was conducted to determine the percentage of water in the seeds following a previously published procedure (BRASIL, 2009).

The seeds selected for germination testing were immersed in a sodium hypochlorite solution (2%) for three minutes and subsequently washed in tap water for one minute. To evaluate the effect of temperature on germination, a total of 600 seeds were exposed to constant temperatures of 20, 25, 30 and 35°C and alternating temperatures of 20-30 and 25-35°C, with four replications of 25 seeds per treatment under a 12-hour photoperiod with white light in B.O.D. germination chambers. The tests were conducted in transparent boxes (11 x 11 x 3.5 cm) over two sheets of germitest paper towels that had previously been humidified with 0.1% (m v⁻¹) Rovral fungicide.

Germination was evaluated daily for a period of 12 days, starting from the first day after sowing. The seeds were considered to have germinated when the primary root protrusion attained a minimum length of 2 mm.

The seedlings from the germination tests (100% of the seedlings for each temperature) were examined to determine the average length and dry biomass of the primary roots. The primary root length was expressed in millimetres (mm) with the aid of a digital paquimeter. Sections of the seedlings were dissected using a scalpel, carefully placed into the designated paper bags, transferred to a ventilated greenhouse and maintained at 80°C for 24 hours, after which the primary roots were individually weighed on analytical scales (the mean weights are given in g).

The germination percentage (G) was evaluated together with the germination speed index (GSI) and the average germination time (AGT). The germination percentage data were converted using an arc-sine ($(x/100)^{0.5}$) function. The data were analysed with Bioestat 4.0 statistical software ($p \leq 0.05$), and the means were compared using Tukey's test ($p < 0.05$).

Results and discussion

The *Diptychandra aurantiaca* seeds contained 7.6% water, within the 5-20% range found in the majority of plant species (BEWLEY; BLACK, 1994), with a few exceptions, including recalcitrant seeds.

Germination occurred under all treatments (Table 1), and the rate of germination was higher at constant temperatures. However, at the highest temperature (35 °C), germination decreased, thus demonstrating that this temperature is not optimal for the germination of *D. aurantiaca* seeds. Using the same species, Lorenzi (2008) demonstrated that the germination percentage is low when seeds are sown using an organic clay substrate, with plants being observed to emerge within two to three weeks under these conditions. Nevertheless, in the laboratory, germination was initiated within four to eight days of the initiation of the experiment, depending on the temperature. The results indicated that the germination percentage was greater than 60% under 20, 25, 30, 20-30 and 25-35°C temperatures.

Table 1. Average germination percentage (G%), germination speed index (GSI) and average germination time (AGT) of *Diptychandra aurantiaca* at six different temperatures.

Temperature (°C)	G (%)	GSI	AGT (days)
20	72 c	0.87 b	8.5 b
25	97 a	1.1 a	9 bc
30	87 ab	1.1 a	7.1 a
35	25 c	0.5 c	10.1 c
20-30	77 bc	0.91 ab	7.3 a
25-35	62 d	0.74 bc	8.9 b

Values followed by the same letter in a column do not differ statistically from each other based on Tukey's test ($p > 0.05$).

Constant temperatures of 25 and 30°C promoted the highest average germination rates and resulted in the highest values for the GSI, indicating that these temperatures promote rapid stand establishment. At a temperature of 20-30°C, germination was also increased, although at a lower rate. The lowest AGT was obtained with temperatures of 30 and 20-30°C. Slow-germinating seeds can result from fungal attack during water imbibition, even resulting in failure to germinate. Therefore, seeds exhibiting the fastest germination speeds are less affected by pathogen attacks (SCREMIN-DIAS et al., 2006); thus, faster germination is better.

Temperatures of 20 and 35°C negatively affected the percentage and speed (AGT and GSI) of germination, showing that these temperatures were inadequate for germination in this species. Alternating temperatures of 20-30 and 25-35°C had similar effects, resulting in lower rates of germination, while at temperatures between 20 and 30°C, the germination speed (GSI and AGT) was high (Table 1).

Figure 1 shows the accumulated germination rates of *D. aurantiaca* seeds during the 12 days of the experiment. At 30°C, germination was positively affected, resulting in the highest values of the studied parameters, with germination occurring prior to the sixth day after sowing and reaching 87% by the end of the experiment under this temperature.

At the 25°C, germination occurred on the eighth day after sowing, reaching 97% after 12 days.

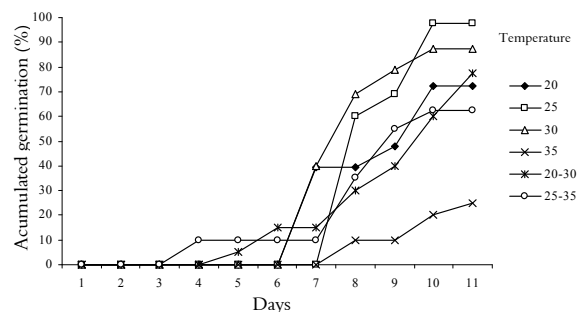


Figure 1. Accumulated germination (%) among *Diptychandra aurantiaca* seeds cultivated at four constant temperatures and two alternating temperatures in a B.O.D chamber.

In accordance with Carvalho and Nakagawa (2000), temperatures above or below the optimum temperature tend to reduce the speed of germination, exposing seedlings to longer periods in less favourable environments. This increased exposure to unfavourable environments could lead to a decrease in the germination percentage, in this study, at 20 and 35°C. These temperatures are therefore below and above optimum, respectively; both of these temperatures resulted in reduced germination speed.

Various authors, including Cassaro-Silva (2001) studying *Senna macranthera* (Collad.) Irwin & Barn., Cetnarski Filho and Nogueira (2005) investigating *Ocotea odorifera* (Vellozo) Rohwer and Oliveira et al. (2005b) using *Tabebuia impetiginosa* (Martius ex A. P. de Candolle), Standley and *T. serratifolia* Vahl Nich., have demonstrated that the optimum temperature falls within a range of 25 to 30°C.

Castro et al. (2004) suggested that the energy of water increases in response to the elevation of temperature, provoking an increase in diffusion pressure, which concomitantly elevates the metabolic activity and diminishes the internal potential of a seed, promoting increased absorption of water. Thus, hydration occurs more rapidly at higher temperatures through physical processes that could accelerate germination.

Germination was observed at constant temperatures of up to 30°C. However, at 35°C, germination was reduced, perhaps as a consequence of damage to the seed structure. Higher temperatures could potentially alter enzymatic activity and reduce the quantity of amino acids available (*via* RNA synthesis), thereby modifying metabolic reactions that reduce embryo

development and restrict seed germination, as reported by Larcher (2003), Marcos Filho (2005) and Taiz and Zeiger (2009).

Cassaro-Silva (2001) studied the enthalpy of germination in *S. macranthera* seeds and reported that at super-optimum or extremely high temperatures, the energy contained in the cells or the cellular membranes of seeds always remains less favourable for embryonic growth until reaching a maximum temperature at which all of the energy is dissipated. At this point, biochemical processes that limit germination occur, such as fusion, evaporation of substances and denaturation of proteins. As a consequence, all enzyme-mediated processes are temporarily interrupted or cease completely until the system is re-established.

A reduction in the rate of metabolic reactions could occur at a temperature of 20°C, affecting the essential processes that initiate germination. Thus, low environmental temperatures could result in the establishment of fewer seedlings and a reduction of biomass, particularly among tropical and sub-tropical species. The extent of the damage incurred depends on the species, the initial water content of the seed, the temperature and the length of exposure time (CARVALHO; NAKAGAWA, 2000; FERREIRA; BORGHETTI, 2004; TAIZ; ZEIGER, 2009), thereby reducing the speed and percentage of germination and increasing the average time period required for germination. This process might be attributed to chemical characteristics, such as the rates of proline and fatty acid synthesis (CASSARO-SILVA, 2001), or the deactivation of enzymes (CASSARO-SILVA, 2001).

Larcher (2003) suggested that the optimum temperature for species in tropical areas typically ranges from 20 to 35°C, which is the most favourable temperature range for the development of many plants in terms of seed germination, growth and flowering, among other processes. According to Cassaro-Silva (2001), the speed of germination is directly dependent on temperature.

Silva and Aguiar (2004), using *Cnidoscylus phyllacanthus* [Pax & K. Hoffm], and Lopes et al. (2005), studying *Basella rubra* L., have suggested that alterations in temperature could facilitate better conditions for germination.

Baskin and Baskin (2001) emphasised that seeds that respond to alternating temperatures exhibit enzymatic mechanisms that function at different temperatures in response to ecological adaptations to the environment. For seeds of the *D. aurantiaca* tree, alternating temperatures were less favourable for germination compared with constant temperatures

between 25 and 30°C; however, the seeds produced at temperatures of 20-30°C were similar in speed.

The results of this study indicate that the minimum temperature for the germination of *D. aurantiaca* seeds lies between 10 and 15°C, while the maximum temperature is between 40 and 45°C. Notably, temperatures of 20 to 35°C were not found to be the most appropriate temperatures for germination.

Figure 2 shows the daily relative frequency of germination, indicating that the peak of germination, for most temperatures, occurred between the seventh and tenth days. However, germination commenced earlier under alternating temperatures and at a constant temperature of 30°C (on the 4th day at 25-35°C and 5th day at 30°C or 20-30°C).

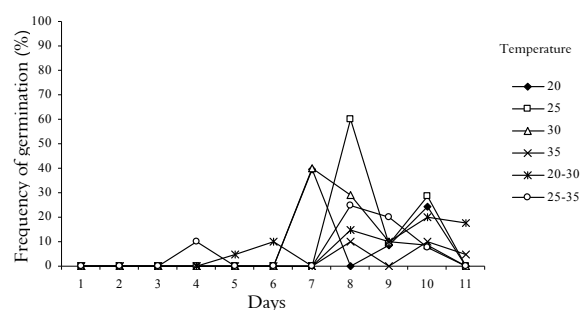


Figure 2. Relative daily frequency of germination in *Diptychandra aurantiaca* seeds cultivated at four constant temperatures and two alternating temperatures.

Diptychandra aurantiaca is typically found in areas of secondary succession and in areas exposed to light. While a standard does not exist, it is generally accepted that plants in these areas display high rates of germination and growth, representing pioneering species, which tend to exhibit a higher tolerance in these areas and, as a consequence, germinate and grow more rapidly. Temperature is therefore an important factor in the germination of *D. aurantiaca* seeds, influencing the total germination, strength and speed of germination. Experiments performed by Carvalho and Nakagawa (2000) showed that temperature is indeed a factor that affects the germination process.

Primary root lengths of 17.5 and 16.5 mm were recorded at temperatures of 25 and 30°C, respectively, with no significant differences. However, the primary root lengths were statistically significantly different under the other treatments, averaging 12.3 mm at 20°C, 11.1 mm at 35°C, and 13.0 mm at temperatures of 20-30 and 25-35°C; these lengths were all statistically equal to each other (Figure 3).

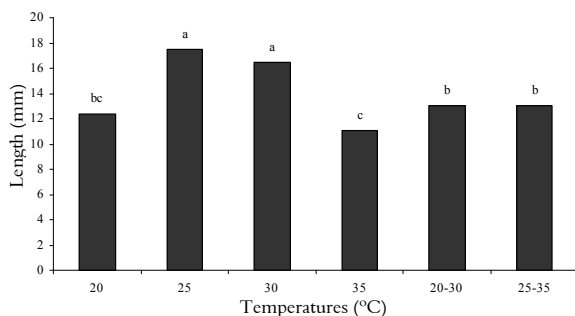


Figure 3. Average length of primary roots (mm) of *Diptychandra aurantiaca* cultivated at different temperatures. Average lengths followed by the same letter were not significantly different when subjected to Tukey's test ($p > 0.05$).

Rosseto et al. (2009) observed an average length of 4 cm for the primary roots of *Parkia pendula* (Willd.), Benth. ex Walp., suggesting that a temperature of 30°C was also more appropriate for the initial growth of the primary root in this species. Oliveira et al. (2011) observed an average length of 20.6 (25°C) and 23.5 (°C) for primary roots of *Aspidosperma tomentosum* Mart., appropriate for the initial growth and similar the this work.

Primary root growth is generally observed to occur within a wide temperature range, varying by at least 2 to 5°C for ligneous species cultivated within temperate zones, and at temperatures above 10°C for plants in tropical regions (LARCHER, 2003). The optimum temperature for cell division is approximately 30°C for the majority of species, which is therefore near the optimum temperature for growth (FERREIRA; BORGHETTI, 2004).

The highest values with respect to the accumulation of dry biomass in the primary roots were observed at temperatures of 20°C (0.0061 g), 25°C (0.0067 g) and 30°C (0.0055 g), with the other examined temperatures resulting in biomass values of 0.0046 g (35°C) and 0.0048 g (20-30 and 25-35°C), less biomass (Figure 4).

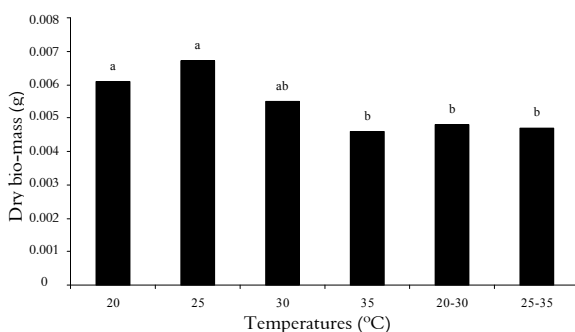


Figure 4. Average dry biomass (g) of the primary roots of *Diptychandra aurantiaca* cultivated at different temperatures (averages followed by the same letter did not differ statistically when subjected to Tukey's test; $p > 0.05$).

Taken together, these data confirm that a temperature of 25°C is the most suitable temperature for seed germination and the initial development (primary roots), producing seedlings that are on average better able to adapt within a smaller time frame. Brancalion et al. (2010) also stated that a temperature of 25°C is appropriate for conducting germination tests in species from a cerrado/savannah biome. The next best temperature is 30°C, which also results in the highest rate and strength of germination and well-formed primary roots.

Conclusion

Temperatures of 25 and 30°C provided the most favourable conditions for germination and initial primary root growth in *D. aurantiaca* seeds.

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