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Substrate and temperature on seed germination of *Aspidosperma vargasii* A. DC. (Apocynaceae)

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Abstract
The Amazon has a wide diversity, including forest species with timber and non-timber potential, which often have their use restricted due to a lack of silvicultural information. Thus, the present study aimed to evaluate the effect of substrates and temperatures on seed germination of *Aspidosperma vagarsii*. Therefore, the premise of a completely randomized design was followed, in a 3 x 3 factorial scheme, with three substrates (paper, sand and vermiculite) and three temperatures (20, 25 and 30 °C). The germination percentage, germination speed index and mean germination time were determined, as well as germination relative frequency graphs. In addition, the weight of a thousand seeds, the number of seeds per kilo, and the moisture content was determined. The weight of thousand seeds was equal to 276.4 g, with 3,618 seeds per kilo, and a moisture content of 9.5%. There was no interaction between the studied factors. The sand substrate and temperatures of 20 and 25 °C presented the best results for seed germination of *A. vargasii*.
Keywords: Amazon; native forest species; SGI; germination test.

Substrato e temperatura na germinação de sementes de *Aspidosperma vargasii* A. DC. (Apocynaceae)

Resumo
A Amazônia é detentora de uma ampla diversidade, incluindo espécies florestais com potencial madeireiro e não madeireiro, as quais muitas vezes tem seu uso restringido por carência de informações silviculturais. Assim, o presente estudo objetivou avaliar o efeito de substratos e temperaturas na germinação de sementes de *Aspidosperma vagarsii*. Para tanto, seguiu-se a premissa de um delineamento inteiramente casualizado, em esquema fatorial 3 x 3, sendo três substratos (papel, areia e vermiculita) e três temperaturas (20, 25 e 30 °C). Foram determinados a porcentagem de germinação, índice de velocidade de germinação e tempo médio de germinação, além de gráficos de frequência relativa de germinação. Ainda, determinou-se o peso de mil sementes, o número de sementes por quilo e o grau de umidade. O peso de mil sementes foi igual a 276,4 g, com 3.618 sementes por quilo, e grau de umidade de 9,5%. Não houve interação entre os fatores estudados. O substrato areia e as temperaturas de 20 e 25 °C apresentaram os melhores resultados para germinação das sementes de *A. vargasii*.
Palavras-chave: Amazônia; espécies florestais nativas; IVG; teste de germinação.

Introduction

Aspidosperma vargasii A. DC., belonging to the Apocynaceae family, is known as “amarelão” and occurs naturally in the Brazilian Amazon, being widely used in logging as it provides hardwood with great durability (LORENZI, 2002). This species produces a large amount of seeds which are dispersed by wind, flowering between August and November, and fruiting in July and August (LORENZI, 2002). In addition to their recognized timber potential (MARANHO *et al.*, 2013), bioactive compounds, especially indole alkaloids, provide to species of *Aspidosperma* genus a wide range of therapeutic

uses, due to the biological activities attributed to these compounds, such as antitumor, antiplasmodic, consistent antimicrobial, and antibacterial (HENRIQUE *et al.*, 2010). Despite its economic potential and pharmacological application, there are few studies reporting the silvicultural and ecological characteristics of *A. vargasii*. Problems related to seeds and general conditions for germination are among the primary information to be investigated.

Seed germination involves a sequence of biochemical and physiological events. It is essential to know the ideal conditions for this process to occur normally, mainly since species have varied responses regarding several factors, such as dormancy, viability, environmental conditions, involving water, light, temperature, oxygen, and the type of substrate used (CARVALHO; NAKAGAWA, 2012). The germination potential of seeds can be evaluated from germination tests, conducted in laboratories, under controlled conditions (DUARTE *et al.*, 2015). Through these tests, it is possible to compare the quality of different seed lots (BRASIL, 2009), evaluating viability and vigor, with temperature and substrate considered basic components for conducting germination tests.

As already mentioned, seeds have variable germination performance when subjected to different temperatures and substrates, and it is of fundamental importance to know the responses inherent to each species related to these factors (MONDO *et al.*, 2008). Each species has an optimal temperature for germination, determined as a function of the maximum germination percentage in the shortest time (OLIVEIRA; BARBOSA, 2014). Seeds of native forest species can germinate in a wide thermal range, depending on the Biome and the region, with a supposed positive relationship between the optimum temperature and the thermal regime of the natural place of occurrence (BORGHETTI, 2005). In addition, there is no determination of an optimal and uniform value for all species; however, the range of 20 to 30 °C is adequate for germination of a large number of forest species (MARCOS FILHO, 2015).

In addition to temperature, the substrate has a direct influence on the germination tests results. This is due to its characteristics, such as structure, aeration, water retention capacity, and disposition to infestation by pathogens, favoring or not germination (MARTINS *et al.*, 2011). The Rules for Seed Analysis (BRASIL, 2009) reveal that paper and sand are the most used substrates in laboratory for germination analyzes. Furthermore, vermiculite is also used in germination tests, especially for forest species, due to its low contamination by microorganisms, good absorption, and water retention, promoting good aeration and enabling the proper development of seedlings (BRASIL, 2013; PADILHA *et al.*, 2020).

Although *A. vargasii* is an important native forest species, there is still a gap of basic information, with no reports in the literature on procedures and recommendations for germination of this species. Therefore, this study aimed to evaluate the effect of substrates and temperatures on seed germination of *Aspidosperma vargasii*.

Material and methods

The fruits were donated by the Technological Foundation of the State of Acre – FUNTAC, and collected directly from the canopy of mother trees, in the State Forest of Antimary (located between the coordinates 9°13' to 9°31'S; 68°01' to 68° 23'W), Western Amazon, Acre. After processing, seeds were placed in semipermeable plastic bags and sent to the Forest Seeds Laboratory of Federal University of Paraná (25°26'51.68"S; 49°14'15.89"W), in Curitiba, Paraná, where they were stored in a cold chamber for 90 days, until the installation of the experiment.

For seeds physical characterization, the weight of a thousand seeds, the number of seeds per kilogram, and the moisture content of seeds were determined, adopting the methodology described in the Rules for Seed Analysis (BRASIL, 2009), after storage period. The weight of a thousand seeds was obtained from eight subsamples of 100 seeds, weighed on a precision scale (0.001 g), and the number of seeds per kilo was calculated from the weight of a thousand seeds. The moisture content was obtained from three samples of 20 seeds with wings, dried in a drying oven at 105 ± 3 °C, for 24 hours.

The effects of temperature and substrate on *A. vargasii* seed germination were evaluated under three different constant thermal regimes: 20, 25 and 30 °C, associated with three substrates: sand, germitest paper, and vermiculite (medium particle size). The sand was sterilized in a drying oven at 200 °C for two hours and uniformed in a 0.8 mm mesh sieve (BRASIL, 2009). The vermiculite was sterilized in a drying

oven at 200 °C and the germitest paper at 105 °C, both for two hours. Seeds were sown in plastic trays (29 x 20 cm) on the substrates. For sand and vermiculite, the trays were filled with 2/3 of the substrate, moistened with distilled water at 60% retention capacity. For the paper, two sheets were used, saturated in distilled water. The trays were sealed with plastic film and placed in *Mangelsdorf* germination chambers, with water replenishment whenever necessary.

The experiment was carried out in a completely randomized design, in a 3 x 3 factorial scheme (substrate x temperature), with six replications of 20 seeds, in a photoperiod of 10h of light and 14h of dark. Were considered germinated seeds that emitted radicles with a length greater than 2 mm and were evaluated daily until the absence of germination for three consecutive days. After, germination percentage, germination speed index (GSI), and mean germination time were calculated. In addition, graphic analysis of the relative frequency of germination was performed.

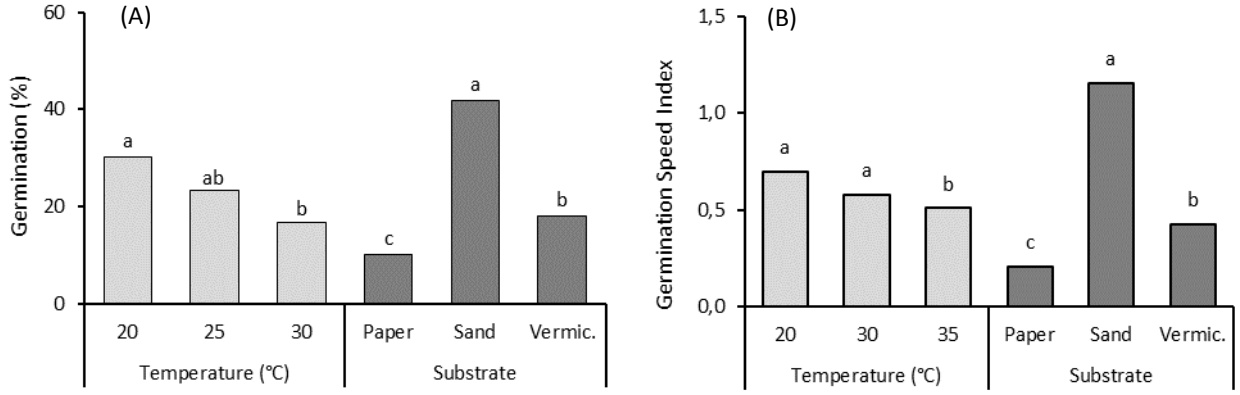
Data were submitted to the Bartlett test. After verifying the inhomogeneity of the variances, the original data were submitted to transformation. Then, the analysis of variance was carried out, with 5% significance. The values of GSI and mean germination time were transformed by square root ($\sqrt{x}$), and germination percentage by the arcsine of the square root divided by 100 ($\text{arcsen}\left[\sqrt{x/100}\right]$). To compare treatment means, the Tukey test was used, at 5% significance, using the RBio software (BHERING, 2017).

For the seed lot studied, the weight of a thousand seeds of *A. vargasii* was equal to 276.4 g (CV = 2.19%), totaling 3,618 seeds per kilo, with a 9.5% of moisture content. The water content observed in this study is above that literature mentions for species of this genus. Oliveira *et al.* (2015) report 4% moisture content for *Aspidosperma subincanum* Mart. ex A. DC and Oliveira *et al.* (2011) observed a content of 7.7% in newly harvested seeds of *Aspidosperma tomentosum* Mart., and also point out that seeds of this genus usually have little stored water.

Germination and GSI revealed, by the F test, non-significant effects for interaction between substrate and temperature (Figure 1), indicating that the effects of the factors were independent. However, results showed significant differences within the temperatures and within the substrates. Germination occurred at the three temperatures evaluated, and the seed's behavior showed a reduction in germination potential as temperature increased (Figure 1A). The temperature of 20 °C provided the highest percentage of germination (30.3%) differing significantly only from the temperature of 30 °C (16.7%). In Brancalion *et al.* (2010) it is possible to observe that the optimal temperature for germination of Amazonian Forest species was 30 °C, with significant participation of the 35 °C class, but this behavior was not observed, as shown in Figure 1A. In contrast, Oliveira *et al.* (2011) demonstrated that *A. tomentosum* has its highest germination potential expressed at temperatures of 20 and 25 °C, corroborating the results observed in this study.

Results and discussion

Figure 1. Germination percentage (A) and germination speed index (B) of *Aspidosperma vargasii* seeds in different substrates and temperatures.



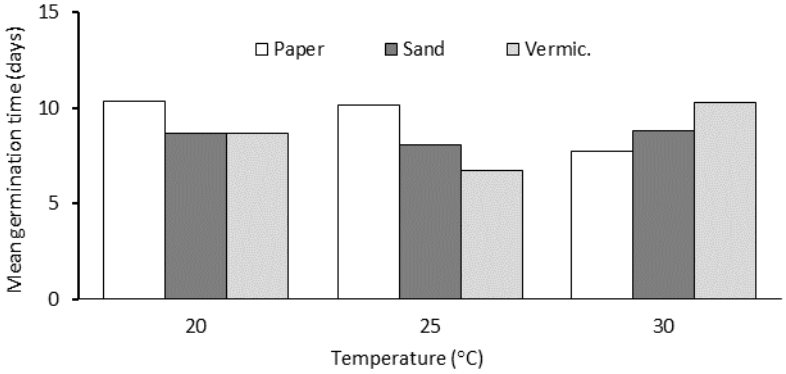
Means followed by the same letter do not differ through the Tukey test at 5% significance.

Regarding the substrates, sand proved to be the most effective for the germination of *A. vargasii*, differing statistically from the others, providing 42% of germination (Figure 1A). Among the main characteristics for this substrate, we highlight the high porosity, good water drainage, and aeration. Ramos *et al.* (1995) observed germination of *Aspidosperma polyneuron* Müll. Arg. above 80% with the use of sand substrate at a temperature of 20 °C, and when the seeds were subjected to 30 °C, in the same substrate, germination reduced to 23%. In contrast, Silva *et al.* (2007) observed germination percentages between 68 and 82% in *Aspidosperma ramiflorum* Müll seeds. Arg. using germitest paper at a temperature of 25 °C.

Although germination studies for *A. vargasii* are not observed, when compared to other species of the *Aspidosperma* genus, the results found for germination percentage can be considered low. This behavior may be due to numerous factors, including specific characteristics of the seed lot used, such as water content, storage time, and the occurrence of fungi during the germination test, probably intrinsic to the seeds, given the sterilization of the substrates used. Moreover, Guollo *et al.* (2016), when studying the germination of *Aspidosperma parvifolium* as a function of different collection forms, obtained germination percentages ranging from 48 to 76%, and associate this variation with the physiological maturation point of the seeds.

Germination speed, measured by the germination speed index (GSI), reached a maximum value (1.2) in the sand substrate and at temperatures of 20 and 25 °C (0.70 and 0.60), which did not differ between each other (Figure 1B). Low GSI values being evident at the temperature of 30 °C and, above all, for the paper substrate. For the mean germination time, there were a non-significant difference between temperatures and substrates. The shortest mean germination time was verified at 25 °C in vermiculite substrate (6.7 days), indicating that germination started from the sixth day after the imbibition start (Figure 2). According to Fisch *et al.* (1998), the mean temperature values in the Amazon region are between 24 and 26 °C, which corroborates the performance of *A. vargasii* seeds in this temperature range observed in the present study.

Figure 2. Mean germination time of *Aspidosperma vargasii* in different substrates and temperatures.

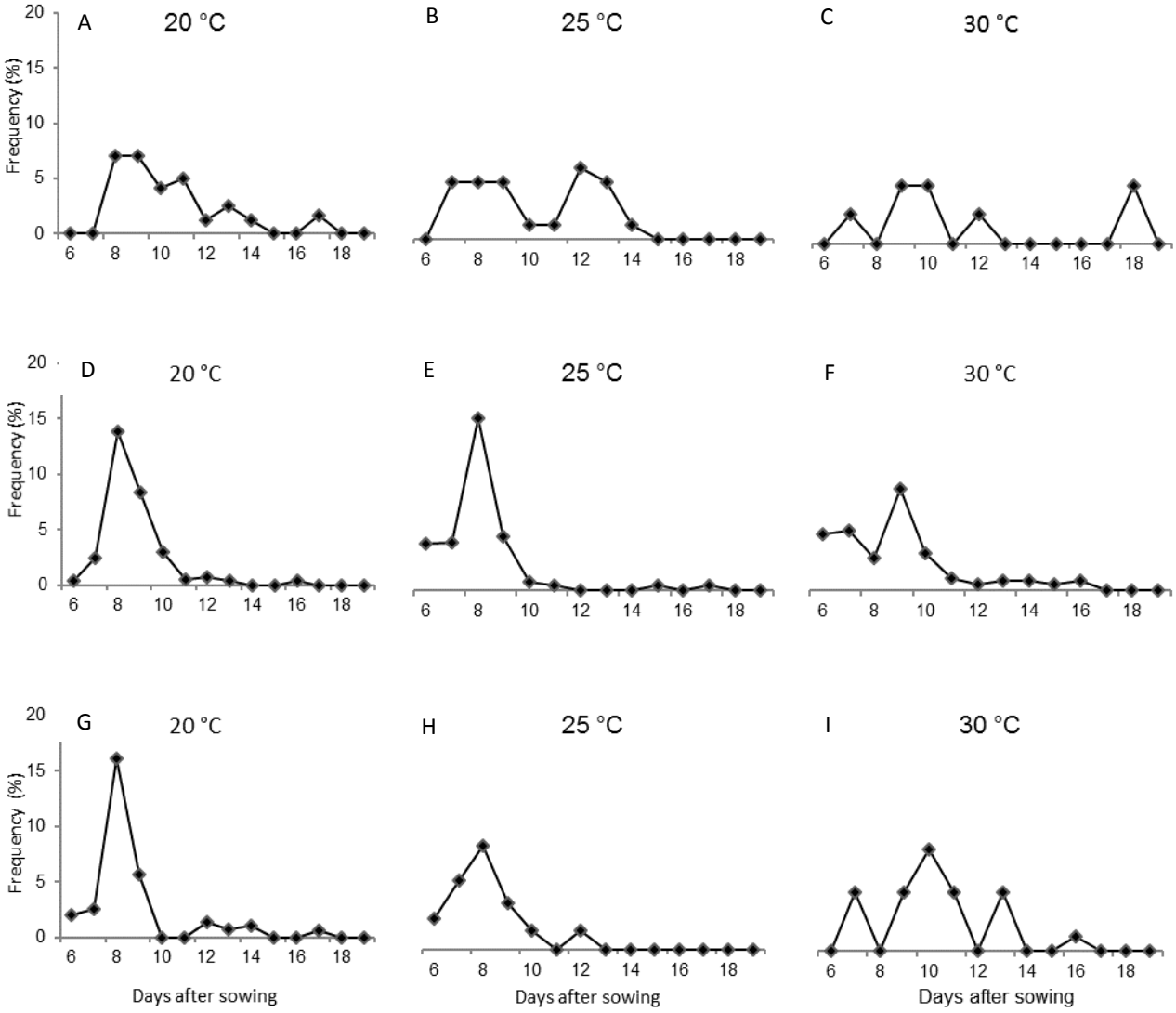


Oliveira *et al.* (2015) observed that temperature of 25 °C, both for GSI and for mean germination time, presented the best result for the germination of *A. subincanum*, demonstrating that, in addition to the best germination percentage obtained, the seeds germinated in the shortest time. As an ecological criterion, the mean germination time is a good index to assess the speed of occupation of a species in a given niche or territory (FERREIRA *et al.*, 2001). Oliveira *et al.* (2011) concluded that at temperatures of 20, 25, and 30 °C, *A. tomentosum* seeds had a mean germination time of 5 to 6 days. The mean time for *A. vargasii* ranged from six to ten days and, for this parameter, there was no interaction or significant effect for the sources of substrate variation and temperature.

The relative frequency distributions in *A. vargasii* seeds germination clearly demonstrate the differences in the number, position, and mode frequencies, in which greater heterogeneity is observed at the highest

temperature (30 °C) in all substrates tested (Figure 3). For the substrates, especially in the paper, the distributions showed a multimodal character, suggesting a loss of synchrony in the germination process (LABOURIAU, 1983) at all temperatures.

Figure 3. Relative frequency of *Aspidosperma vargasii* germination on paper (A, B and C), sand (D, E and F), and vermiculite (G, H and I) substrates at different temperatures.



The paper substrate was unfavorable to *A. vargasii* germination, as it provided the lowest percentages and germination speed, with longer mean time and greater heterogeneity in the distribution of relative frequencies. The use of paper as a substrate generated satisfactory results in other studies, such as Oliveira *et al.* (2013) for seeds of *Diptychandra aurantiaca* (Mart.) Tul., Silva *et al.* (2017) for seed germination of *Parkia platycephala* Benth. and Soares *et al.* (2015) when evaluated the germination of *Psidium rufum* seeds. At temperatures of 20 and 25 °C, in sand and vermiculite substrates (Figures 3D and 3G), the frequency of germination was almost unimodal. Rossatto and Kolb (2010) found greater heterogeneity in the relative frequency at lower temperatures for the germination of *Pyrostegia venusta* seeds and a unimodal character for the optimal temperature for germination of this species (35 °C).

Based on the results, we observed that temperature and substrate were important factors and both influence *A. vargasii* seeds germination. Thus, the importance of searching for methodologies is highlighted, especially for germination tests, in order to determine the most suitable conditions for each species. In Brazil,

studies like this are becoming increasingly important, given the great diversity of native species, in which it is impossible and inconsistent to generalize and extend results from one species to another, due to the peculiarity of each one.

Conclusion

Among the conditions studied, sand substrate and temperatures of 20 and 25 °C showed the best results for *A. vargasii* seeds germination in laboratory.

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