ADEQUACY OF METHODOLOGY FOR GERMINATION OF DIASPORES OF BARAUNA, *Schinopsis brasiliensis* (Anacardiaceae)

ADEQUAÇÃO DA METODOLOGIA PARA GERMINAÇÃO DE DIÁSPOROS DE BARAÚNA, Schinopsis brasiliensis *(Anacardiaceae)*

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ABSTRACT: *Schinopsis brasiliensis* Engl. (Anacardiaceae) is a typical "Caatinga" (savanna) tree, whose seed germination occurs in a slow and irregular. The ideal conditions for seed germination of many tree species are still unknown. The present study objective was to evaluate different temperatures and substrates to establish a protocol for seed germination *Schinopsis brasiliensis*, conducted at the Federal University of Paraiba, Areia, Paraiba State (PB), Brazil. Therefore, different temperatures (25, 30, 35 and 20-30 ºC) and substrates (including sand, vermiculite between, on paper, between paper and paper roll) were used. To assess the effect of treatments for germination (%), first germination count (%), germination velocity index (GSI), lengths of the principal root and of the aerial portion of the seedlings and dry weights of the root system and of the aerial portion of the seedlings. The experimental design was completely randomized with treatments arranged in a 4 x 5 factorial arrangement (temperature and substrate), four replicates of 25 diaspores for each treatment. The means were compared by Tukey test at 1% probability and orthogonal contrasts between temperatures (35 vs 30 ° C, 30 vs 25 ° C and 20-30 vs 30 ° C). The germination tests of *S. brasiliensis* should analyze the numbers of seedlings germinated 10 (first count) and 20 days (final evaluation) after sowing; The combination of a vermiculite substrate and alternating temperature (20-30 ºC) was best for stimulating the germination of *S. brasiliensis* diaspores.

KEYWORDS: *Schinopsis brasiliensis*. Forest seeds. Substrate. Temperature.

INTRODUCTION

Schinopsis brasiliensis Engl. (Anacardiaceae) popularly known as "barauna" or "brauna" is a typical *caatinga* (thorny deciduous vegetation) tree and it has slow and irregular germination. This species has many wood uses and medicinal properties and its exaggerated predatory harvesting has resulted in its being included on the official list of Brazilian species threatened with extinction (BRASIL, 1992).

Even though laboratory germination tests to evaluate seed quality should be undertaken using temperature and substrate conditions that have been established as ideal for each species (as these are usually the two most important factors affecting germination responses), the ideal conditions for seed germination of many tree species are still unknown (ANDRADE et al., 2006).

The seeds of different species require distinct temperature ranges for successful germination, and these ranges reflect their geographical distributions (RAMOS; VARELA, 2003). Optimal temperatures are those that allow germination to occur with the greatest intensity and velocity (HORIBE; CARDOSO, 2001). The maximum and minimum temperatures characterize a critical range above or below which, respectively, germination will not occur (MAYER; POLJAKOFF-MAYBER, 1989).

Another external factor that will influence seed germination as well as seedling development is the substrate (TONIN; PEREZ, 2006). The physical properties of different substrates (such as their structures, aeration, pH, water retention capacities, and degrees of infestation with pathogens) can vary greatly (BARBOSA; BARBOSA, 1985; CARVALHO; NAKAGAWA, 2000; SILVA et al., 2001), and in choosing the material used in an artificial substrate must considerate the size of the seeds, their humidity requirements, their sensibility (or not) to light, and the ease with which it allows the seedlings to develop (BRAZIL, 2009). Certain substrates could cause irregular germination (or inhibit it completely), unformed seedlings or symptoms of nutrient deficiencies or excesses (SETUBAL; AFONSO NETO, 2000).

Examined the interaction of temperature and different substrates on the germination of *Schizolobium amazonicum* - Huber ex Ducke seeds

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and found that the best germination occurred at 25, 30 and 35 ºC using paper substrates (RAMOS et al., 2006) and examinating the germinative behavior of *Apeiba tibourbou* Aubl, known that constants temperatures of 30 and 35 ºC, are the best for analyze the physiological potential (PACHECO et al., 2007).

In spite of the fact that there is a considerable amount of information available concerning the seeds of forest species, much still needs to be known about the ideal conditions for their germination. Therefore the objective this study was evaluated different substrates and temperatures to establish an optimal protocol for their germination in the diaspores of *Schinopsis brasiliensis* Engl.

MATERIAL AND METHODS

The present study was carried out in the Seed Analysis Laboratory at the Center for Agrarian Sciences, Federal University of Paraiba, Areia, Paraiba State (PB), Brazil. Samaras of *Schinopsis brasiliensis* Engl. were collected in 11/2009 from 12 matrixes growing in *Caatinga* (dryland) vegetation in the municipality of Soledade, PB.

The mature (brown) samaras were collected directly from the trees and transported in plastic bags to the Seed Analysis Laboratory where they were manually cleaned by removing the epicarp and mesocarp; poorly formed or damaged diaspores were discarded. The diaspores from each matrix lot were mixed in a Gamet-type seed divider, stored in kraft-type paper bags, and maintained at room temperature.

To obtain a single lot from the unified seeds of the 12 matrixes, equal quantities of diaspores were removed from each of the 12 lots and thoroughly mixed.

Establishing seed germination protocols

Germination tests – The germination tests were conducted in germination chamber at constant temperatures of 25, 30 and 35 ºC, and at alternating temperature of 20-30 ºC, with an eight-hour photoperiod supplied by day light fluorescent lamps (4 x 20 W). The substrates and sowing positions tested included: in sand, in vermiculite, on and between sheets of paper, and in 11 x 11 x 3,5 cm a closed acrylic box. Additionally, rolls of paper towels (Germitest®) were used (BRASIL, 2009). The numbers of germinated diaspores (paper substrates) and emergent seedlings (sand and vermiculite substrates) were evaluated on a daily basis. The final percentages of germinated diaspores were calculated 20 days after sowing considering only normal seedlings (BRASIL, 2009). *First germination count* **-** This measure was made using the same procedures as the germination test, with the germination totals being made on the 10th day after sowing - when most of the seedlings in all of the treatments had already emerged. *Germination velocity index (GVI)* **-** was determined by noting (at the same time every day) the numbers of diaspores germinated (and/or emerged) between the 6th and 20th day after sowing, using the formula proposed by Maguire (1962). *Lengths of the principal root and of the aerial portion of the seedlings* **-** the normal seedlings from each test-group were measured at the end of the germination test to determined the lengths of their principal root (from the root cap to the radical-hypocotyl transition zone); their aerial portions; the hypocotyl (from the radical-hypocotyl transition zone to the insertion of the cotyledons). The results were expressed in centimeters per seedling. *Dry weights of the root system and of the aerial portion of the seedlings* **-** at the end of the germination tests the root systems and hypocotyls of the seedlings measured as above were excised and placed in Kraft paper bags and dried in a forced-air oven at 65 ºC until reaching a constant weight. The material was after weighed using an analytical balance (precision 0.0001 g); the results were expressed in milligrams per seedling. *Experimental design and statistical analyses* **-** four repetitions with 25 diaspores in each treatment were made for each of the four temperature regimes and five substrates. The data were subjected to analysis of variance using the F test to compare the root mean squares; the averages of the substrates were compared using the Tukey test at a 1% level of probability and by orthogonal contrasts between temperatures (35 vs 30 ºC, 30 vs 25 ºC and 20-30 vs 30 ºC). Sisvar 5.1 (FERREIRA, 2008) software was used in the statistical analyses.

RESULTS AND DISCUSSION

The average water content of *S. brasiliensis* diaspores at the start of these experiments was 10.1%. The percentage of germination of *S. brasiliensis* diaspores (Figure 1) as a function of the different substrates and temperatures tested. In the alternating temperature of 20-30 ºC it can be seen that the highest diaspore germination percentages occurred at these temperatures in all of the substrates tested, with the exception of the sand substrate (most likely because the upper layer drained excessively and became dry) (SILVA; AGUIAR, 2004).

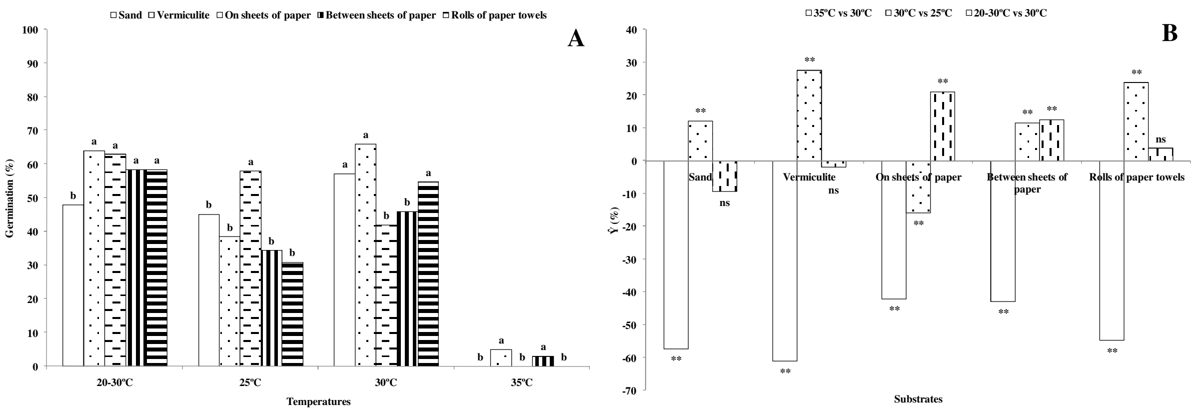

Figure 1. Percentage germinations of *Schinopsis brasiliensis* diaspores exposed to different substrates and temperature regimes. Averages followed by the same letter do not differ at the 1% probability level. ** = significant to the 1% level; ns = not significant

The paper substrates at a constant temperature of 25 ºC, and vermiculite, sand, and the paper rolls at a constant temperature of 30 ºC also favored diaspores germination. The blotting-paper substrates provides the best germinative results with *Schinus terebinthifolius* Raddi ("aroeira vermelha") seeds at 25 ºC (MEDEIROS; ZANON, 1998). There are significant differences between the germination rates of *Myracrodruon urundeuva* Fr. All. seeds when these are submitted to germinate in sandy or blotting-paper substrate, being blotting-paper more efficient at temperatures above 30 ºC or with alternating temperatures (PACHECO et al., 2006). High germination rates of the diaspores in the present study were seen with the vermiculite substrate at 30 ºC or under alternating temperatures of 20-30 ºC, apparently due to this substrate's positive characteristics of aeration, structure and capacity to retain water. Vermiculite was observed to be the best substrate for germinating *Cariniana legalis* (Mart.) Kuntze ("jequitibá-rosa") and *Dalbergia nigra* (Vell.) Allemão ex Benth. ("jacarandá-da-bahia") seeds (RÊGO; POSSAMAI, 2002); and in addition to being an excellent germinative substrate, it is light weight, easy to handle, has excellent water absorption capacities, and does not require daily watering (PACHECO et al., 2006). Contrast estimates (Figure 1.B) showed that alternating (20-30 ºC) and constant (30 ºC) temperatures stand out with the seeds germinated on or between paper, although with decreasing germination of *S. brasiliensis* diaspores when they were subjected to alternating temperature of 20-30 ºC on sand (9%) and vermiculite (2%) substrates, in comparison with a constant temperature of 30 ºC (although they did not statistically differed). The opposite results were seen when using the substrates on-paper, between paper sheets, and in paper rolls, with temperatures of 20-30 ºC resulting in increases of 21.0, 13.0 and 4.0% respectively of the germination percentages when compared with exposure to 30 ºC. When the temperatures were evaluated independently of the substrates, incubation at 35 ºC provided the least favorable condition for the germination of *S. brasiliensis* diaspores (Figure 1.B), often with zero germination in sand, on paper, or within rolls of paper (Figure 1.A). This reduction in germination rates among of *S. brasiliensis* diaspores at 35 ºC indicates that this temperature was higher than the naturally tolerated by the species. The results obtained in the present work concerning the optimal temperature for germinating *S. brasiliensis* diaspores is in agreement with the concept of Ramos and Varela (2003). These authors related ideal germination temperatures to the thermal band normally encountered within a plant's habitat during the ideal season for seedlings emergence and establishment.

The first count data of *S. brasiliensis* diaspores germination did not show any interactions between substrates and temperatures (Figure 2.A). First count had the highest percentages when the diaspores were sown in any of the substrates at alternating temperatures of 20-30 ^{o}C; statistically there were no significant differences between first count germination rates in the sand substrate at a constant temperature of 25 ºC, or in the sand, vermiculite and paper substrates at a constant 30 ºC (Figure 2.A). Likewise, paper-towel substrates at 30 ºC provide the highest first count germination rate with *Peltophorum dubium* (Sprengel) Taubert seeds (OLIVEIRA et al., 2008).

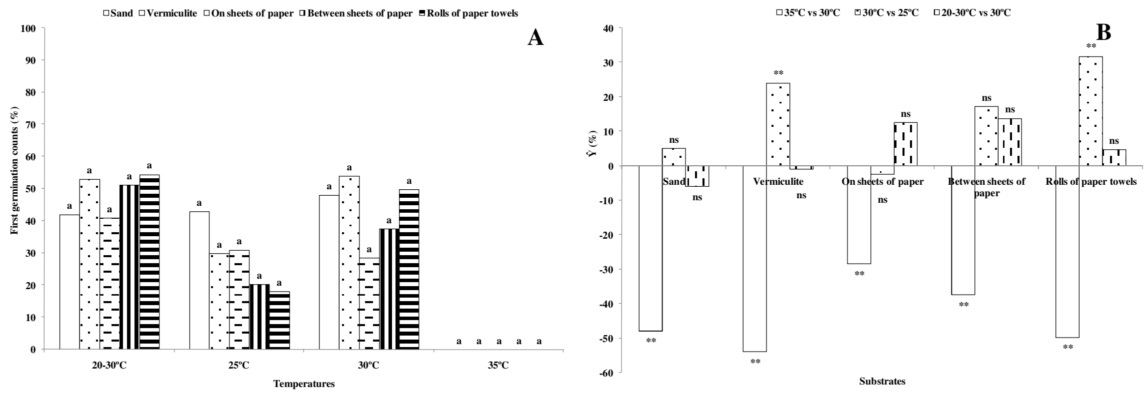

Figure 2. First germination counts (%) of *Schinopsis brasiliensis* diaspores exposed to different substrates and temperature regimes. Averages followed by the same letter do not differ at the 1% probability level. ** = significant to the 1% level; ns = not significant

A 30 ºC environment appears to furnish the most favorable conditions for *caatinga* (dryland) seeds to germinate because this temperature is similar to which they are normally exposed. The 20-30 and 30 ºC temperatures stand out again, with decreasing germination of the *S. brasiliensis* diaspores when subjected to alternating temperature in the sand (6%) and vermiculite (1%) substrates in comparison with a constant temperature of 30 ºC, although these differences were not statistically significant (Figure 2.B). The on paper, between sheets of paper, and paper roll substrates demonstrated the opposite tendency, with 20-30 ºC temperatures resulting in increases of 13.0; 14.0 and 5.0% in germination percentages, respectively, when compared to incubation at 30 ºC (without any statistical differences between them). A constant temperature of 35 ºC affected negatively the first germination count of the diaspores, and no germination was observed (Figure 2.A), the temperature induced large variation in germination response of seeds, some seeds of forest species require specific temperatures in order to germinate, may have been caused by species adaptation to local environmental conditions where this tree species. The temperature at which germination occurs has a very important influence on that process, affecting total germination and germination velocity by influence of water's absorption rates the biochemical reactions involved in the entire germinative process as well (MARCOS FILHO, 2005).

The germination velocity index of *S. brasiliensis* diaspores involve interactions between the different substrates and temperatures tested. The highest germination velocity index values (Figure 3.A) were obtained with: vermiculite substrate combined with alternating temperatures of 20-30 ºC; with the on paper, sand and vermiculite substrates at 25 ºC; and with the paper rolls at 30 ºC.

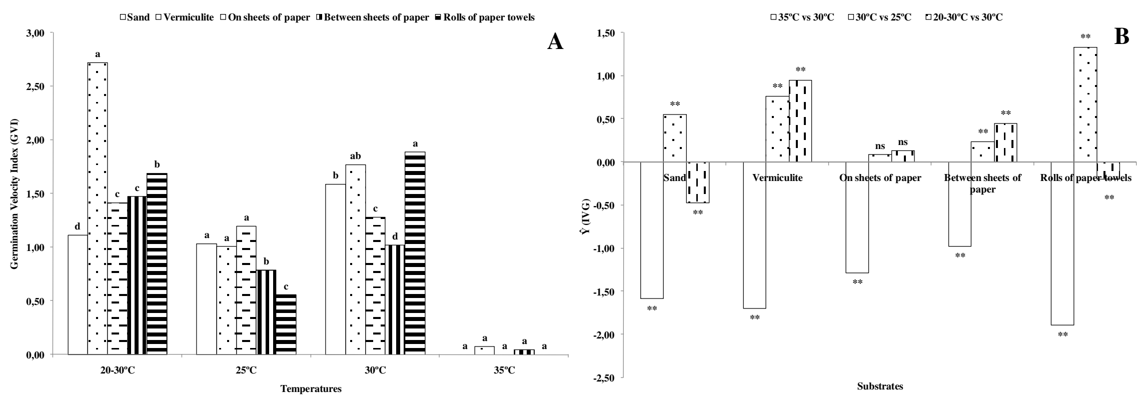

Figure 3. Germination velocity index of *Schinopsis brasiliensis* diaspores exposed to different substrates and temperature regimes. Averages followed by the same letter do not differ at the 1% probability level. ** = significant to the 1% level; ns = not significant

In relation to the different substrates examined, the paper rolls conferred a series of advantages that include the best development of the essential structures of seedlings (thus allowing faster and securer seedling evaluations), greater spacing between the seedlings, and easier forms to evaluate the test results, while occupying less space in germination chambers - thus allowing a greater number of analyses running simultaneously (LIMA; GARCIA, 1996).

Contrast estimates (Figure 3.B) indicated that using the vermiculite and between paper sheet substrates under alternating temperatures, as well as temperatures of 30 ºC with the sand and paper roll substrates, resulted in the greatest emergence velocity index. A optimal temperature which allows any process to occur with the greatest intensity and velocity (HORIBE; CARDOSO, 2001). A constant temperature of 35 ºC affected negatively the germination velocity index of the diaspores (Figure 3.B).

In relation to the data collected from the length of the principal root of *S. brasiliensis* seedlings (Figure 4.A), interactions were observed between the different substrates and temperatures. The greatest root lengths of *S. brasiliensis* were obtained when the diaspores were germinated in vermiculite (at all temperatures tested) and in the sand substrate at a constant temperature of 30 ºC (Figure 4.A).

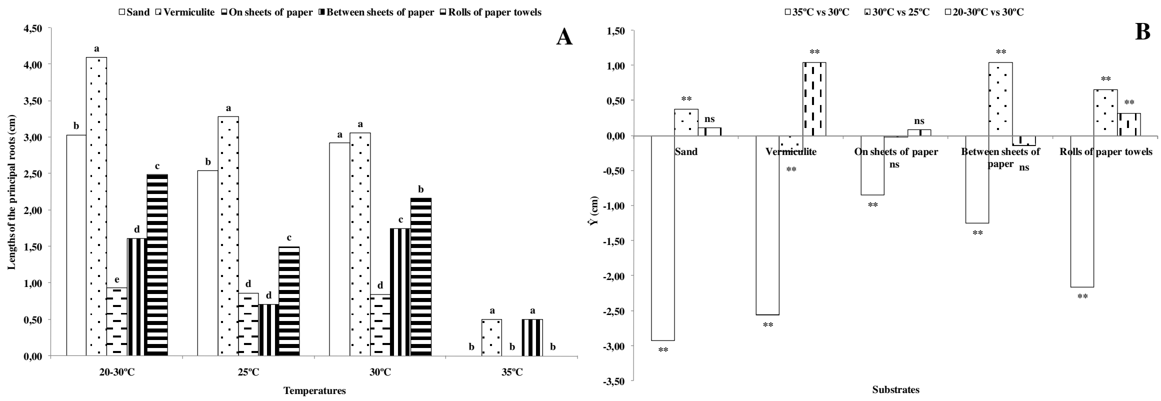

Figure 4. Lengths of the principal roots (cm) of *Schinopsis brasiliensis* seedlings derived from diaspores exposed to different substrates and temperature regimes. Averages followed by the same letter do not differ at the 1% probability level. ** = significant to the 1% level; ns = not significant

Species of *Phoenix roebelenii* O'Brien ("tamareira-anã") and *Dimorphandra mollis* Benth. ("fava d'anta") when submitted to develop in vermiculate and sand substrates at a temperature of 30 ºC provides a long roots lengths when these plants became seedlings (IOSSI et al., 2003; PACHECO et al., 2010). It can be seen in Figure 4.B that alternating temperatures were most favorable to the initial development of the roots of *S. brasiliensis* seedlings in all of the substrates tested, with increases of 0.11% over growth in the sand substrate, 1.04% over the vermiculite substrate, 0.09% over the on paper conditions, and 0.33% more than with paper rolls at a constant temperature of 30 ºC. The average primary roots lengths is reach when the seeds one of *Tabebuia aurea* (Silva Manso) Benth. & Hook f. ex S. Moore seedlings when the seeds were sow on to paper-towels at 30 ºC (PACHECO et al., 2008). It is important to note that shortest root lengths of *S. brasiliensis* were observed among seedlings incubated at 35 ºC, independently of the substrates used (Figures 4.A and B). These results contrast with observations made by Varela et al. (2005) working with *Acosmium nitens* (Vog) Yakovlev. ("itaubarana") seeds, the result found was favored by the 35 ºC temperature, that provides a good root development.

In terms of the length of the aerial portions of *S. brasiliensis* seedling (Figure 5.A) using different substrates and temperature regimes, the greatest stem lengths were observed in the vermiculite substrate at incubation temperatures of 20-30, 25 and 30 ºC. These results reinforce the observation that vermiculite provides the most satisfactory conditions for the initial development of *S. brasiliensis* seedlings.

Contrast estimates (Figure 5.B) indicated that a constant temperature of 30 ºC favored the initial development of the aerial portion of *S. brasiliensis* seedlings in all of the substrates tested, with incubation at alternating temperatures providing results statically similar. Therefore using aerial portions of *Adenanthera pavonina* L. seedlings was possible to not that the greatest lengths were grown at 20-30, 25 and 30 ºC, independent of the substrate used (KISSMANN et al., 2008).

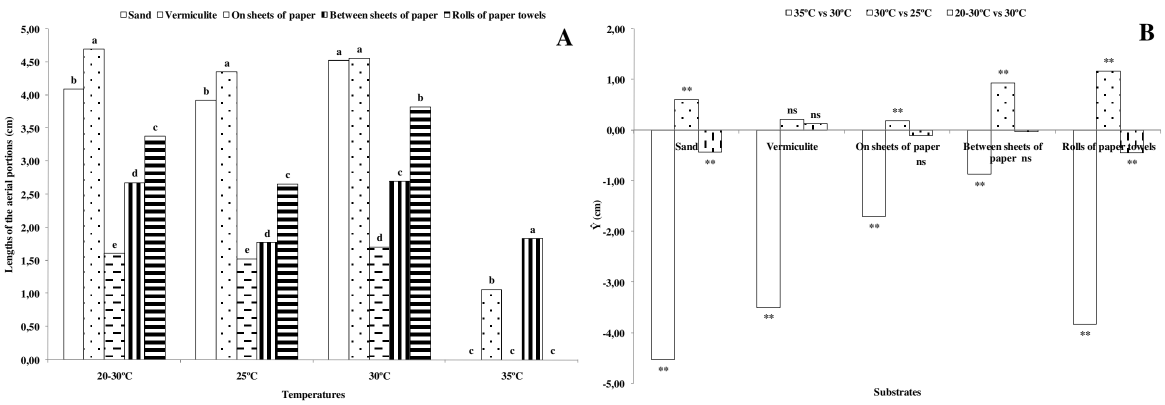

Figure 5. Lengths of the aerial portions (cm) of *Schinopsis brasiliensis* seedlings derived from diaspores exposed to different substrates and temperature regimes. Averages followed by the same letter do not differ at the 1% probability level. ** = significant to the 1% level; ns = not significant

It is important to note that the shortest aerial portions were seen in *S. brasiliensis* at 35 ºC, independent of the substrates used (Figures 6.A and B). On the other hand, high incubation temperatures (30 and 35 ºC) resulted in the longest seedlings lengths of *Adenanthera pavonina* L., indicating that the seedlings of this species can tolerate high temperatures conditions (SOUZA et al., 2007).

In terms of the dry weights of the roots of *S. brasiliensis* seedlings, is possible to say that there were no statistically significant interactions between the different substrates and temperatures tested (Figure 6.A).

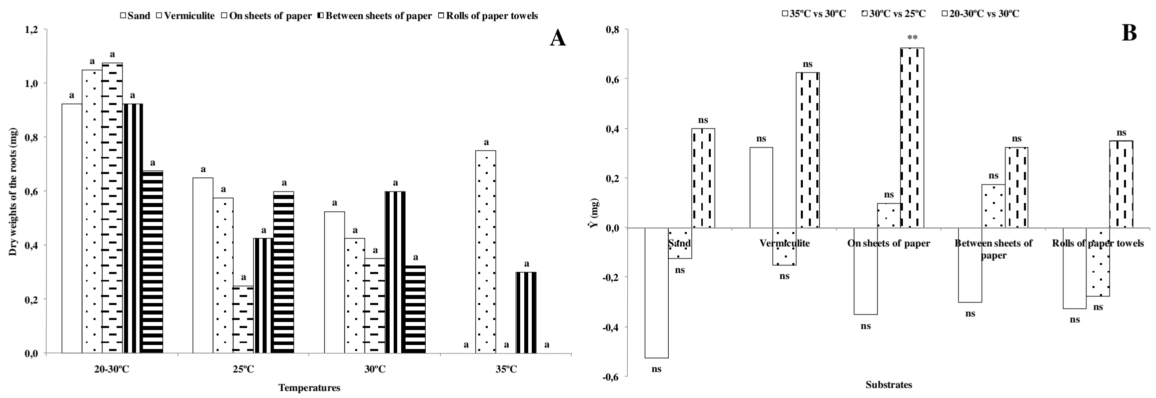

Figure 6. Dry weights of the roots (mg) of *Schinopsis brasiliensis* seedlings derived from diaspores exposed to different substrates and temperature regimes. Averages followed by the same letter do not differ at the 1% probability level. ** = significant to the 1% level; ns = not significant

Alternating temperatures of 20-30 ºC provide the highest root dry weights among *S. brasiliensis* seedlings in all of the substrates tested, but these results were only statistically significant using on paper substrate. Similar to the results for root lengths (Figure 4.B), alternating 20-30 ºC temperatures also resulted in the greatest dry weight accumulation in *S. brasiliensis* seedling roots (Figure 6.B). The greatest dry weight accumulation in the root systems of *Tabebuia aurea* (Silva Manso) Benth. & Hook f. ex S. Moore seedlings occurred when they were subjected to temperatures of 20-30 and 30 ºC in the sand substrate (PACHECO et al., 2008). By contrast, incubation at 35 ºC (Figures 6.A and B) resulted in the lowest dry weight accumulation in the roots of *S. brasiliensis* - suggesting that this species encountered difficulty in translocating nutrients from the cotyledons to the embryonic axis at high temperatures.

The evaluations of the dry weights of the aerial portions of *S. brasiliensis* seedling (Figure 7.A) indicated that plants grown on sand or vermiculite at alternating temperatures of 20-30 ºC or at constant temperatures of 25 and 30 ºC, between sheets of paper or on paper at alternating temperatures of 20-30 ºC or constant 25 ºC, in paper rolls at a constant temperature of 25 ºC, or between sheets of paper at a constant temperature of 35 ºC had the greatest accumulations of dry weight in their aerial portions. A sand substrate likewise gave the greatest dry-weight values of the aerial portions of *Mimosa caesalpiniaefolia* Benth seedlings (ALVES et al., 2002). Santos et al. (1994) evaluated the influence of different substrates and sowing depths on the germinative response and vigor of *Mimosa caesalpiniaefolia* Benth seeds, reported that sowing between sheets of paper resulted in the highest dry weight values at 20 and 25 ºC, and that there were no differences between the other substrates employed; sowing between paper sheets and in sand substrates yielded the greatest dry weight values at 30 and 20-30 ºC.

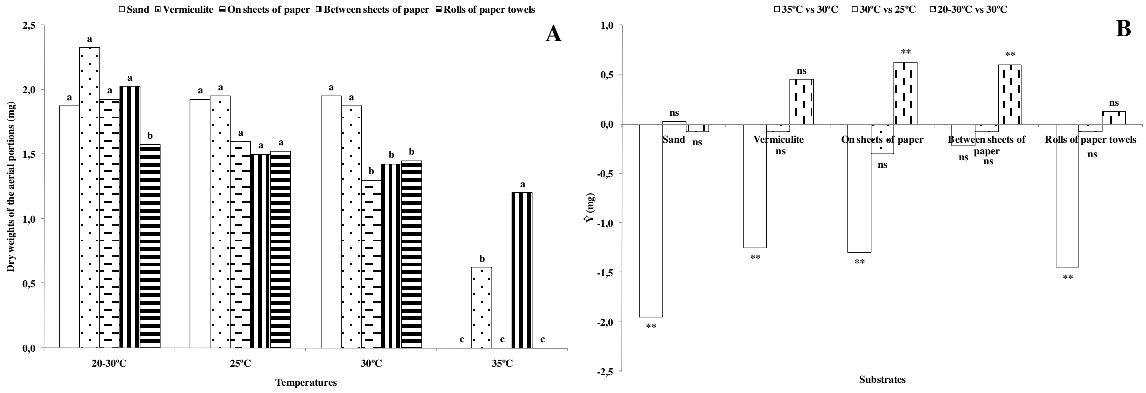

Figure 7. Dry weights of the aerial portions (mg) of *Schinopsis brasiliensis* seedlings derived from diaspores exposed to different substrates and temperature regimes. Averages followed by the same letter do not differ at the 1% probability level. ** = significant to the 1% level; ns = not significant

Contrast estimates (Figure 7.B) indicated that alternating temperature of 20-30 ºC favored the highest dry weight accumulations in the aerial portions of *S. brasiliensis* seedlings sown into all of the substrates. However, this alternating temperature regime statistically differed from the constant temperature regimes of 25 and 30 ºC only when the seeds were sown on the paper substrate or between sheets of paper. Consistent with the results of greater lengths of the aerial portions of the seedlings when incubated under an alternating temperature regime of 20-30 ºC (Figure 5.B), greater aerial portion dry weight accumulations were also observed under these same conditions (Figure 6.B). Additionally, the 35 ºC (Figures 6.A and B) growth regime resulted in the smallest aerial portion dry weight accumulation, contrasting with the observed results for the dry weights of the hypocotyls of *Apeiba tibourbou* Aubl. seedlings, where the best results were obtained at constant temperatures of 30 and 35 ºC (PACHECO et al., 2007), probably because this species is quite tolerant of adverse environmental conditions.

CONCLUSIONS

The germination tests of *S. brasiliensis* should analyze the numbers of seedlings germinated 10 (first count) and 20 days (final evaluation) after sowing;

The vermiculite substrate and alternating temperature of 20-30 ºC are most favorable for germinating *S. brasiliensis* diaspores and for initial development of their seedlings; a constant temperature of 35 ºC is prejudicial to the germination of *S. brasiliensis* diaspores.

RESUMO: *Schinopsis brasiliensis* Engl. (Anacardiaceae) é uma árvore típica da caatinga, cuja germinação das sementes ocorre de forma lenta e irregular. As condições ideais de germinação das sementes de muitas espécies ainda não são bem conhecidas. O presente estudo teve como objetivo avaliar diferentes temperaturas e substratos para o estabelecimento de um protocolo de germinação para sementes de *Schinopsis brasiliensis*, conduzido na Universidade Federal da Paraíba, em Areia-PB. Para tanto, foram utilizados diferentes temperaturas (25, 30, 35 e 20-30 ºC) e substratos (entre areia, entre vermiculita, sobre papel, entre papel e rolo de papel). Para avaliação do efeito dos tratamentos foram realizados testes de germinação (%), primeira contagem de germinação (%), índice de velocidade de germinação (IVG), comprimento da raiz principal e da parte aérea das plântulas e massa seca do sistema radicular e da parte aérea das plântulas. O delineamento experimental utilizado foi o inteiramente ao acaso, com os tratamentos distribuídos em arranjo fatorial 4 x 5 (temperaturas e substratos), em quatro repetições de 25 diásporos para cada tratamento. As médias foram comparadas pelo teste de Tukey, ao nível de 1% de probabilidade e por contrastes ortogonais entre temperaturas (35 vs 30 ºC, 30 vs 25 ºC e 20-30 vs 30 ºC). Para o teste de germinação de *S. brasiliensis*, as plântulas devem ser analisadas aos 10 (primeira contagem) e 20 dias (avaliação final) do semeio; O substrato vermiculita e a temperatura alternada 20-30 ºC são mais indicados para a germinação dos diásporos e o desenvolvimento inicial de plântulas de *S. brasiliensis*. A temperatura constante de 35 ºC é prejudicial à germinação dos diásporos de *S. brasiliensis.*

PALAVRAS-CHAVE: *Schinopsis brasiliensis*. Sementes florestais. Substrato. Temperatura.

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