UNIVERSIDADE FEDERAL DE SÃO CARLOS

CENTRO DE CIÊNCIAS BIOLOGICAS E DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E RECURSOS NATURAIS

ECOFISIOLOGIA DA GERMINAÇÃO E ESTABELECIMENTO DE PLÂNTULAS DE *Dalbergia miscolobium* Benth.

Celso Alfredo Barbieri Junior

SÃO CARLOS – SP

BRASIL

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CELSO ALFREDO BARBIERI JUNIOR

Dissertação apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais, do Centro de Ciências Biológicas e da Saúde da Universidade Federal de São Carlos, como parte dos requisitos para obtenção do título de Mestre em Ecologia e Recursos Naturais.

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A natureza mortal procura, segundo os seus meios, perpetuar-se e imortalizar-se; o único meio que dispõe para o seu fim é a geração que, perpetuamente, substitui o ser antigo por um novo... Tal é o estratagema (mecano) através do qual o mortal participa da imortalidade.
Platão (*O Banquete, 207 d, 208 b*)

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RESUMO

Práticas de recuperação de áreas degradadas no cerrado são praticamente desconhecidas e, o estudo da ecologia da reprodução, dispersão, estabelecimento das espécies vegetais deste bioma é a etapa principal e fundamental para uma possibilidade de restauração ambiental. As sementes podem ser usadas durante um longo período de tempo como um estoque de propágulos para programas de reflorestamento em áreas degradadas e recuperação de áreas nativas. Foram avaliados alguns parâmetros biométricos das sementes e a influência da planta matriz de *D. miscolobium*, sobre a germinação, a influencia da luminosidade e do tipo de substrato na emergência das plântulas. Estudou – se também a influencia do estresse termo hídrico e de sua interação com estresse térmico o efeito do armazenamento e do envelhecimento acelerado na viabilidade e vigor das sementes. *D. miscolobium* é uma espécie arbórea, característica dos cerrados e das florestas estacionais, com ocorrência em todo o território nacional, do Piauí ao Paraná. Pertencente à família Fabaceae, é uma árvore com grande potencial paisagístico Espécie semidecídua, heliófita, pioneira e seletiva xerófita, característica de terrenos arenosos e bem drenados, floresce na maior parte da sua área de ocorrência entre janeiro e fevereiro, frutificando entre maio e junho, porém os frutos podem ficar na árvore por mais um ano antes de serem dispersos de forma anemocórica. A partir dos resultados obtidos nos testes de germinação pode-se concluir *que D. miscolobium* é uma espécie quiescente e ortodoxa para germinação de sementes.Não houve relação do tamanho ou da massa da semente na velocidade de germinação e na germinabilidade. Não houve diferença estatística entre a planta matriz e classes de tamanho das sementes na germinabilidade e na velocidade de germinação. A emergência de plântulas sob 50% de sombreamento foi estatisticamente menor que a ocorrida a pleno sol. As sementes de *D. miscolobium* se mostraram para a germinação, resistentes ao estresse hídrico, e à

combinação de estresse hídrico e térmico, tendo este ultimo favorecido a germinabilidade. O melhor método observado para armazenamento foi vidro hermeticamente fechado dentro de refrigerador comum, onde as sementes permaneceram viáveis por no mínimo quatro anos, sem perder o vigor.A condição luminosa exerceu influencia sobre a sobrevivência das plântulas. A maior taxa de mortalidade foi observada nos tratamentos sob 50% de sombreamento. Com estes resultados foi possível inferir que a espécie estudada tem como estratégia ecológica a germinação de sementes e o estabelecimento de plântulas na primeira estação chuvosa após a dispersão dos diásporos.

INTRODUÇÃO GERAL

Como as sementes interagem com sinais do ambiente para determinar quando iniciar o crescimento radicular culminando no desenvolvimento da muda? Esta é uma questão chave no estudo da fisiologia da germinação de sementes. Esta decisão crítica para a semente, bem como para as plântulas, é dependente, sobretudo, da disponibilidade da água, temperatura e luz. Devido ao grande número de sementes produzidas na maioria das espécies de plantas, somado à ação de pressões seletivas do ambiente como a sazonalidade do clima, por exemplo, e fatores intrínsecos como a variabilidade genética e fisiológica das sementes, não é de surpreender que existam diversas formas de comportamento germinativo o que está intimamente relacionado com o ambiente natural de cada espécie (Bradford 2002). A disponibilidade de água está direta e indiretamente envolvida com as fases da germinação de sementes, influenciando as reações enzimáticas, solubilização e transporte de compostos metabólicos (Bradford, 1995).

A água é tão importante para o sucesso do estabelecimento da plântula que virtualmente todas as espécies mesofiticas e xerofiticas têm mecanismos para armazenar água ou aumentar o potencial osmótico celular com consideráveis alterações na sua fisiologia para que as sementes germinem e as plântulas continuem seu desenvolvimento apenas quando houver elevada probabilidade de que exista disponibilidade hídrica para seu estabelecimento (Bradford 1995).

O clima do Cerrado é sazonal, com verões úmidos e invernos secos, classificados também como Aw ou Cwa segundo o sistema de Köppen (1931). A estação seca é muito agressiva e em alguns casos durando até seis meses (Eiten 1972). Com isso, a seca representa um momento de estresse marcante para as plantas do cerrado, afetando drasticamente a vegetação herbácea com raízes superficiais (Sarmiento 1996).

A vegetação do cerrado é considerada um dos 25 mais importantes "hotspots" terrestres e é o único dominado por uma savana tropical (Myers *et al*., 2000). Nos últimos 35 anos, mais de 50% dos seus aproximadamente dois milhões de km^2 foram transformados em pastagens e terras para agricultura (Klink & Machado, 2005).

Práticas de recuperação de áreas degradadas no cerrado são praticamente desconhecidas e, o estudo da ecologia da reprodução, dispersão e estabelecimento das espécies vegetais deste bioma é a etapa fundamental para uma tentativa de restauração ambiental. As sementes podem ser usadas durante um longo período de tempo como um estoque de propágulos para programas de reflorestamento em áreas degradadas e recuperação de áreas nativas.

Devido à escassez de estudos sobre germinação e o estabelecimento das espécies vegetais arbóreas na enorme biodiversidade do cerrado, no primeiro capítulo desta dissertação foram avaliados alguns parâmetros biométricos das sementes e a influência da planta matriz de *D. miscolobium*, sobre a germinabilidade e velocidade de germinação. Ainda neste capítulo foram analisados a influência da luminosidade e do tipo de substrato na emergência das plântulas. No segundo capítulo foi avaliada a influência do estresse hídrico e de sua interação com estresse térmico na germinabilidade e velocidade de germinação, o efeito do armazenamento e do envelhecimento acelerado na viabilidade e vigor das sementes e, por último, a influência do sombreamento artificial e do tipo de substrato na sobrevivência e crescimento de plântulas de *D. miscolobium*.

O gênero *Dalbergia* compreende cerca de 100 espécies de árvores e lianas com distribuição pantropical, mas principalmente neotropical. Muitas espécies deste gênero são valorizadas por causa da resistência, maleabilidade e grande durabilidade da madeira, com características físicas ideais para a construção de móveis e instrumentos musicais.

D. miscolobium é uma espécie arbórea, da família Fabaceae, característica dos cerrados e das florestas estacionais, com ocorrência em todo o território nacional, do Piauí ao Paraná (Batalha & Mantovani, 2001; Durigan *et al.*, 2002; Ratter *et al.*, 2003, Gibbs & Sassaki, 1998). A altura varia de 8 a 20 m, com 0,3 a 0,5 m de diâmetro de tronco, sendo uma árvore com grande potencial paisagístico devido aos tons azulados das folhas e flores, ainda prescrita por arquitetos e paisagistas (Lorenzi 1992). Espécie semidecídua, heliófita, pioneira e seletiva xerófita, característica de terrenos arenosos e bem drenados, floresce na maior parte da sua área de ocorrência entre janeiro e fevereiro, frutificando entre maio e junho, porém os frutos podem ficar na árvore por mais um ano antes de serem dispersos de forma anemocórica.

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CAPITULO 1

SEED ECOLOGY OF *Dalbergia miscolobium* Benth., A SAVANNA TREE SPECIES FROM BRAZIL.

Seed ecology of *Dalbergia miscolobium* Benth., a savanna tree species from Brazil.

Key words: Cerrado, biometry, *Dalbergia*, emergence, imbibition curve, water content, maternal effect, germination, seed ecology.

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Resumo

O objetivo deste trabalho foi estudar alguns aspectos básicos da biometria da semente, teor de umidade, curva de embebição, efeito do tamanho da semente e da árvore matriz sobre a germinação, influência da luz e do substrato sobre a emergência em viveiro de plântulas de *Dalbergia miscolobium* Benth. O teor de umidade das sementes foi aproximadamente 7,3% e o melhor preditor para o peso foi o comprimento da maior dimensão da semente. A fase I da germinação terminou próximo a 21 horas e a fase 2 próximo a 72 horas após o inicio da embebição. A emergência de plântulas sob condições de céu aberto apresentou os maiores valores de velocidade e de porcentagem de germinação, quando comparadas com plântulas emergidas sob 50% de sombreamento independente do tipo de substrato utilizado. Nenhuma diferença estatística foi encontrada entre velocidade e porcentagem de germinação quando comparadas entre classes de tamanho de semente e da árvore matriz (P >0,05). *D. miscolobium* é uma espécie quiescente e tem como estratégia ecológica a germinação de semente e o estabelecimento de plântulas na primeira estação chuvosa após a dispersão dos diásporos, tendo como sítios preferenciais para seu estabelecimento locais co solo bem drenado e pouco sombreados.

Abstract

The aim of this work was study some basic aspects of seed biometry, water content, imbibition curve, seed size and maternal effects on germination, light and substrate influence on field emergence of *Dalbergia miscolobium* Benth. The water content was around 7.3% and the best predictor for the seed weight was the larger axis length. The phase I of germination ends around 21 h and the phase 2, around 72 h after imbibition starts. Seedling emergence under open sky conditions presented the highest values of rate and germination percentage, when contrasted with seedlings under 50% of shading, independently of the germination substratum. No statistical difference was found when rate and germination percentage were contrasted inside a length classes or seed provenance ($P > 0.05$). So, *D. miscolobium* is a quiescent species, and it has like ecological strategy the seed germination and seedling establishment at the first rainy season after seed dispersal on sunny well drained places.

Introduction

Tropical savanna covers about 15,0 to 24,6 x 10^6 km^2 of the surface in South America, Africa and Asia. The largest, richest and possibly most threatened tropical savanna in the world is the Cerrado in Brazil (Myers *et al*., 2000; Silva & Bates, 2002). This vegetation was ranked among the 25 most important terrestrial hotspots and it is the only dominated by a tropical savannah (Myers *et al*., 2000). In the last 35 years, more than 50% of its approximately 2 million km^2 has been transformed into pasture and agricultural lands planted in cash crops (Klink & Machado, 2005).

In Brazil, which possesses the vast majority of Cerrado areas, the situation of the conservation strategies is worse, in contrast to others countries in South America (Durigan *et al*., 2002). There are few large Cerrado reserves in Brazil, and they are not distributed evenly across the biome, and consequently, an important part of the Cerrado's environmental diversity has not been incorporated in a network of protected areas (Durigan *et al*., 2004; Silva & Bates, 2002). Nowadays 20% of the Cerrado area remains undisturbed, only 2.2% of its area is under legal protection and an estimated 20% of threatened and endemic species do not occur in protected areas (Klink & Machado, 2005).

In 1962 the Cerrado covered 33,929 km^2 of São Paulo state area (Borgonovi *et al*., 1965). Eleven years later, the biome was reduced to 10,388 km^2 and in 1992 the reminiscent area was about 2,379 km^2 (Durigan *et al.* 2002). The restoration practices on Cerrado areas are almost unknown and the study of the ecology plant species reproduction of this biome is the main foot step for a future possibility of restoration. In this work, some basic aspects were evaluated as seed biometry size, water uptake, parental and seed size effects on germination, light and substratum influence on field emergence of a commonly and widely spread tree species of Cerrado, *Dalbergia* *miscolobium* Benth. (Batalha & Mantovani, 2001; Durigan *et al.*, 2002; Ratter *et al.*, 2003).

Methods

Fruits of *D. miscolobium* were harvested from 13 different trees in the cerrado reserve of São Carlos Federal University, São Carlos City, São Paulo State, Brazil (21°58′, 22°00′ south latitude and 47°51′, 47°52′ east longitude) on July of 2004. The seeds were handly separated from the pericarpe, and after, they were stored under laboratorial conditions (20^{o}C ± 5^{o}C), in opened containers until the beginning of the experiments.

I - Water content and Biometry:

The water content of the *D. miscolobium* seed was determinate using sampling of 50 replicates of 10 seeds, reaching 500 seeds and 53.69g of fresh seeds (Brasil, 1992; Piña-Rodrigues *et al.*, 2004). Each replicate was weighted (fresh weight) and dried at 105^{o}C in dissecator oven during 24 h, when they were weighted again and contrasted with the initial fresh weight (Brasil, 1992, Figliolia & Piña-Rodrigues, 1995, Piña-Rodrigues *et al.*, 2004).

The aim of the biometry experiments was search about the measurement that better described seed mass, in order to be used like a classification parameter for *D. miscolobium* seed, as related in the literature, where the mass and volume play an important role on seed dispersion, germination and plant establishment (Baskin & Baskin, 2001, Fenner & Thompson, 2005). Three different lengths were measured with a digital paquimeter (Figure 1) and the weight for each one of 197 *D. miscolobium* seeds was obtained in a digital balance. Therefore, the metric results were submitted to analysis of covariance (Zar, 1999) using the fresh weight. The initial hypothesis evaluated was:

Ho: Are all tree correlation indices equals?

Ha: Are at least one correlation index different?

II - Imbibition curve

The experimental design for the imbibition curve was as the following: for each imbibition time evaluation, four replicates of ten seeds were used, which were imbibed inside a Petri dish lined by filter paper moistened with distilled water and maintained inside standard incubators (B.O.D.) at 30°C ± 0,5°C, the optimal germination temperature (Barbieri Junior *et al*. 2005). During the imbibition period, seeds were sampled and weighed. After this, the seeds were dried in a dessicator oven under 105°C through 24 h and weighed again to water content determination. Performing a regression curve (Zar, 1999), the water uptake behavior and the imbibition phases of *D. miscolobium* were described.

III - Seed size and maternal effect on germination

The questions considered with this experiment were: Are the rate and germination percentage affected by seed size? Are the rate and germination percentage affected by the maternal influences? Are these two phenomena occurring at the same time?

To verify these questions *D. miscolobium* seeds were harvested from 10 different adult plants and utilizing the previous biometry test results, separated in two groups: a) bigger and /or equal to the best predictor length mean and;

b) smaller then the best predictor length

Seeds were submitted to a germination test according to a factorial design: four replicates for each treatment with 25 seeds each. The seeds germinated inside a 15cm

Petri dish lined with filter paper, moistened with distill water and enclosed with PVC film to prevent drying out.

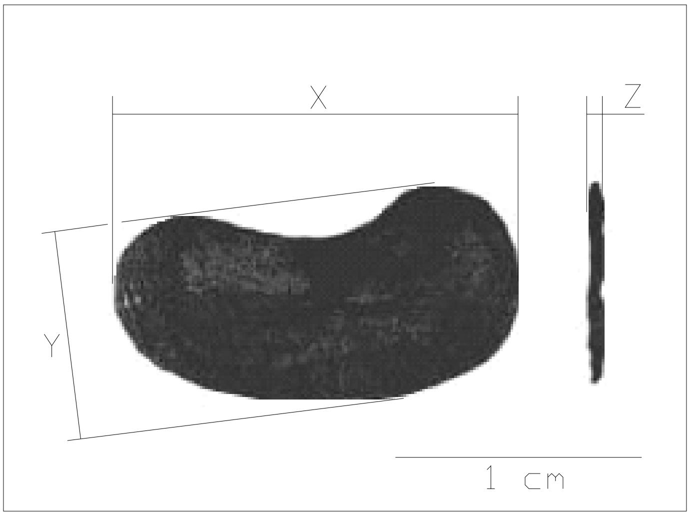

Figure 1: Representation of the *D. miscolobium* seed and the axe lengths X, Y and Z.

The germination tests were conducted inside standard incubators (B.O.D.) at optimal temperature ($30^oC \pm 0.5^oC$) (Barbieri Junior *et al.*, 2005). The germination tests were daily monitored; at the same time and the germinated seeds were handly removed from the Petri dishes, as soon as germination was identified. The experiments were enclosed when all seeds germinated or the last ones were deteriorated (Brasil, 1992). It was assumed as a germinated seed, when root protrusion was larger than 2mm, with a positive geotropic curvature (Duran & Tortosa, 1985; Perez *et al*. 2001).

The rate and germination percentage was obtained according to Labouriau & Valadares (1976), Labouriau (1983), and Labouriau & Agudo (1987). The data was submitted to the Shapiro-Wilk normality test, followed by ANOVA and the Tukey test (Zar, 1999). For all the tests was assumed $\alpha = 0.05\%$. The germinability data was transformed into arcsin $\sqrt{(X/100)}$ before the statistical tests (Sokal & Rohlf, 1997; Santana & Ranal, 2004).

IV - Field emergence

The substratum and shade influence on seedlings emergence of *D. miscolobium* were analyzed with a factorial designed experiment comparing three different substratum: sand, cerrado soil, and a mixture (five parts of cerrado soil, tree parts of organic compound and two of sand). The substratum was distributed in the 1.5 L polyethylene bags and so, exposed to two light conditions: 100 and 50% of open-sky light. In each polyethylene bag a single seed was sowed. The experimental design consisted of 10 replicates with 10 polyethylene bags each, reaching 100 seeds per treatment.

The polyethylene bags were disposed upon a triturate rock floor, without cover (100% open-sky light) or in a shade house (30m x 15m x 3m) with one layer of monophilament cloth (50% of transmittance). The light density was measured with a luximeter (Extech Instruments, USA), confirmed that 50% of open-sky light was achieved. All polyethylene bags received 5mm of water supply through an irrigation system (Naan-dan ® 5024 agricultural sprinkler) once a day, during all the experimental period. The observations were performed daily at the same time and the seedlings emergence registered. The experiments were finished when all seeds emerged and/or the lasting ones were deteriorated (Brasil, 1992). It was considerated emerged seedlings with cotyledonal overture larger than 45^{o} and primary leaves visible.

The data offsprouting was transformed to angular values (arcsin√%) before the statistics tests (Sokal & Rohlf, 1997; Santana & Ranal, 2004). A two way ANOVA and a Tukey test ($\alpha = 0.05$) was performed to contrast the observed values (Zar, 1999).

All the experiments were conducted in the Botany Department of Federal University of São Carlos, São Paulo State, except that where seedling emergence and survival emergency was evaluated. So, in this case, Ymyrá Nursery Tree Farm at Jacareí. Town, in the same State, 100 km away from São Paulo City was used.

Results

I - Water content and Biometry:

The *D. miscolobium* seeds water content was 7.3 % with 1.8 % of standard deviation. The **Table 1** presents the biometry results and the **Table 2** shows the correlation coefficients for the biometry results. The results showed that at least one of three correlation coefficients is different ($X^2 = 27.5664 > X^2crit = 5.991$, $\alpha = 0.05$). Performing a Tukey Test was found that fresh weight versus "X" coefficient is different from other two coefficients. With these results was concluded that longer axis length (Figure 1, X) is the best predictor for the fresh weight.

Table 1: Biometry results of 197 *D.miscolobium* seeds; Fresh Weight (g), SD=Standard deviation, X, Y and Z (mm) position are show in Figure 1.

Variable	Fresh weight	X	Y	Z
Average	0.0947	17.4228	10.0873	1.0091
SD	0.0249	1.5355	1.0042	0.0975

Table 2: Correlation coefficient (r) performed according to Zar, 1999 (α= 0,05).

	weight	X	Y	Z
weight	1.0000			
X	0.8496	1.0000		
Y	0.6810	0.6427	1.0000	
Z	0.5535	0.4351	0.3281	1.0000

II - Imbibition curve

The phase I for *D. miscolobium* seed imbibition curve ends around 21 h and the phase II around 72 h of imbibition with the beginning of the germination process (i.e. root protusion). A massive and fast water uptake was observed before 24 h (phase I of imbibition) uprising 200% of the initial fresh weight (Figure 2).

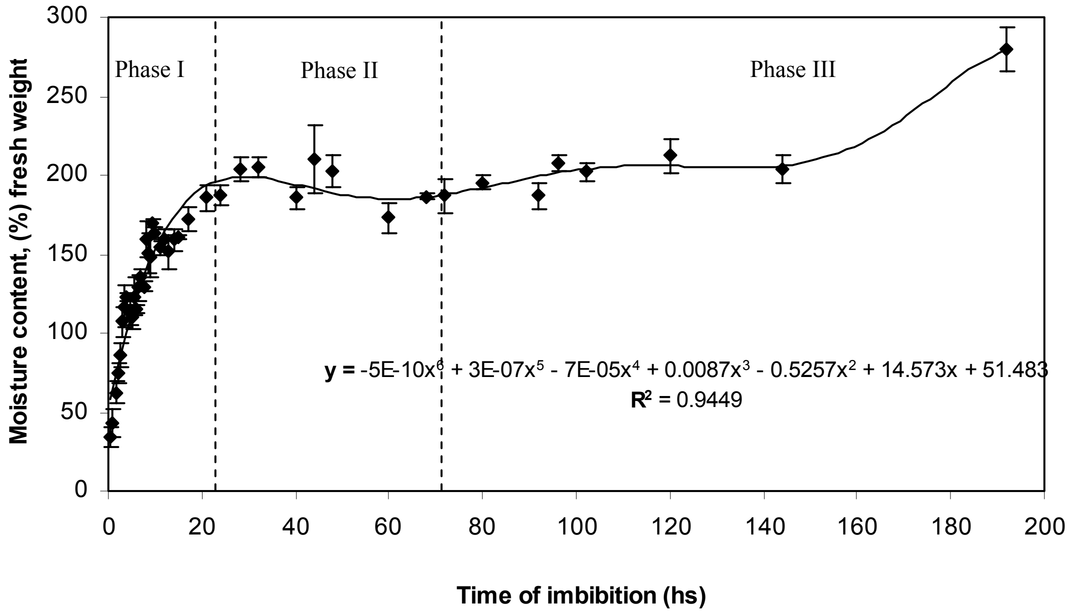

Figure 2:*D.miscolobium* water uptake during 8 days. Mean ± Standard deviation. R^2 = 0,9449 (P<0,05).

III - Seed size and maternal effect on germination

Any statistical difference was not found (P>0.05) between seed provenance (maternal effect) and length classes, using like variables the germinability and rate (Table 3).

Table 3: Effect of seed size and mother plant on germinability of *D. miscolobium*. Small seeds < median value of X (figure 1) Big seeds ≥ median value of X. The values are not significantly different (P>0,05).

Seed length	Mother plant	Germinability	Vel. (Days^{-1})
big	***1***	98.8 ± 2.5	0.19 ± 0.010
big	***2***	100.0 ± 0.0	0.19 ± 0.008
big	***3***	98.8 ± 2.5	0.20 ± 0.001
big	***4***	97.5 ± 2.9	0.20 ± 0.011
big	***5***	97.5±2.9	0.19 ± 0.010
big	***6***	98.8 ± 2.5	0.20 ± 0.011
big	***7***	96.3 ± 4.8	0.19 ± 0.008
big	***8***	100.0 ± 0.0	0.19 ± 0.010
big	***9***	100.0 ± 0.0	0.19 ± 0.014
big	***10***	97.5 ± 2.9	0.20 ± 0.001
small	***1***	100.0 ± 0.0	0.19 ± 0.008
small	***2***	97.5 ± 2.9	0.19 ± 0.010
small	***3***	96.3 ± 2.5	0.19 ± 0.003
small	***4***	96.3 ± 4.8	0.19 ± 0.015
small	***5***	96.3 ± 4.8	0.21 ± 0.035
small	***6***	100.0 ± 0.0	0.19 ± 0.008
small	***7***	98.8 ± 2.5	0.19 ± 0.010
small	***8***	97.5 ± 2.9	0.19 ± 0.005
small	***9***	100.0 ± 0.0	0.19 ± 0.010
small	***10***	98.8 ± 2.5	0.19 ± 0.010

IV - Field emergence

Seedlings emerged under open sky conditions had a better rate and emergence percentage when compared with seedlings obtained under 50% of shade, independently of the substratum (Figure 3). Cerrado soil was the worst media for seedlings emergence, under open sky conditions, than sand and the mixture of sand, soil and organic compound. Seedlings grown under 50% of shading conditions do not differ in relation to the rate and emergence percentage.

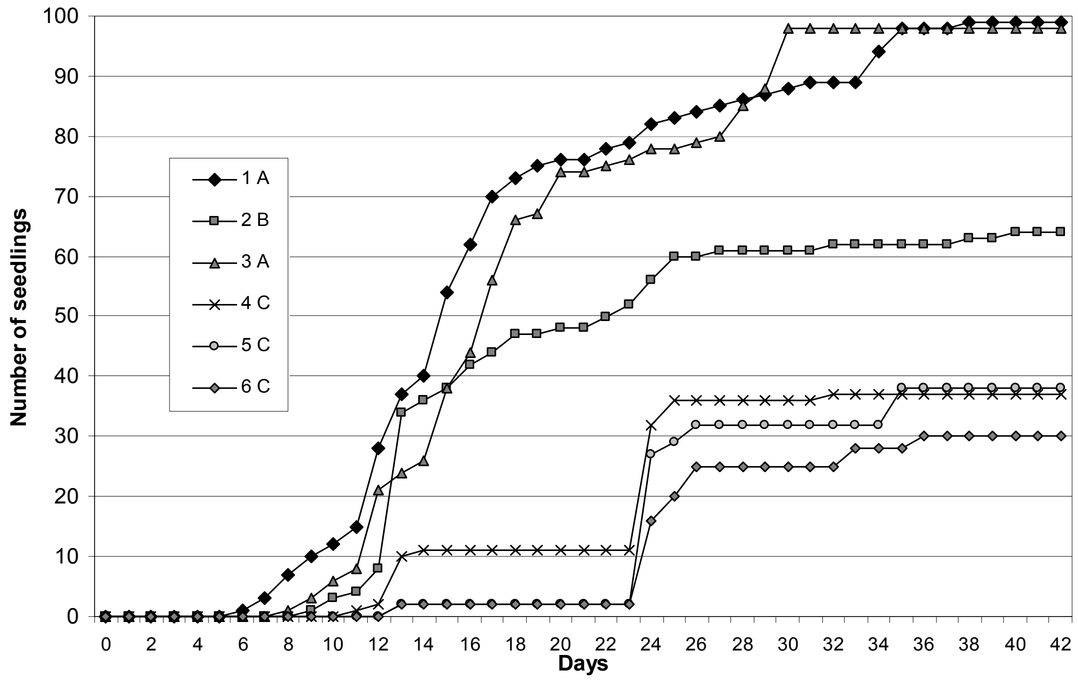

Figure 3 : Number of emergent seedlings thru the days of experiment: 1 e 4= Sand, 2 e 5= Soil plus organic substratum and sand, 3 e 6 = Soil; 1, 2 e 3 open sky conditions; 4, 5 e 6 50% of shade. Numbers followed by same letter are not statistical different (P<0,05).

Discussion

I - Water content and Biometry:

Three physiological stages of development are recognized in most seeds: histodifferentiation (stage I), cell expansion with food reserve storage (stage II), and maturation drying (stage III) (Bewley and Black, 1994;. Hartman *et al*., 2002) Orthodox seeds in stage III reached the physiological maturity (i.e. maximum dry weight). This stage is characterized by a fast water loss, preparing the seeds to the following stage, the dispersion (Hartman *et al.*, 2002). Despite of low water content verified (7.3 ±1.8%) *D. miscolobium* seeds could be considered a quiescent seed, only moisture and a favorable temperature on the species habitat is necessary to begin the germination process (Taylor *et al*., 2004).

II - Imbibition curve

Germination of orthodox seeds start up with the imbibition and could be described using a three phase process: phase I, imbibition; phase II, Lag phase with the metabolic activity and phase III with root protrusion (Bewley and Black, 1994, Hartman et all, 2002;). The results allow us to conclude that *D. miscolobium* has as ecological strategy for seed germination and seedling establishment, the first seasonal rain, after the seed dispersion. Non dormant seeds germinate after being exposed to favorable temperatures and water potentials during an adequate period of time (Bradford, 1995, Taylor *et al*. 2004).

III - Seed size and maternal effect on germination

It is well known that germination characteristics are not only affected by the current environmental conditions but also by the conditions experienced by mother plants in the previous generation (Bischoff *et all.*, 2005). Bischoff *et al*. (2005) described a positively correlation between seed mass and germination percentage in *Cichorium intybus* L. (Asteraceae) and *Origanum vulgare* L. (Lamiaceae) but in the same experiment the authors does not found any correlation of seed mass and germination in *Legousia speculumveneris* (L.) CHAIX (Campanulaceae) and a negative one, in *Echium vulgare* L. (Boraginaceae).

The results recorded at the present study could not corroborate with Sassaki & Felippe (1999) observations, because these authors found a statistical differences in germinability of *D. miscolobium* seeds with different mass. Bangarwa *et al.* (1995) registered variations on the rate and germination percentage characteristics when *Dalbergia sissoo* seeds were collected from different sites and so, these results are correlated with longitudinal habitat variation. Variation between seed length and weight is a commonly relational with dispersion distance from the mother plant and with the colonization of different safe sites for germination and seedling establishment (Ferner & Thompson, 2005; Urbanska *et al*., 2000). These variations are described as a result of predator's pressure on seeds and seedlings (Fenner & Thompson, 2005). The relationship between resource availability, seed size and germination is constrained by many trade-offs (e.g. seed size and seed number, predation**,** dispersion) and there is also evidence for an increased seed mass in stressful, competitive environments (Bischoff *et al.*, 2005; Galloway, 2001).

IV - Field emergence

It is widely recognized that seed dispersal have a profound effect on vegetation structure (Wang & Smith, 2002). Species with a fast growing answer, positively to the light, increase the germination and seedling recruitment index (Chazdon *et al*. 1996) and so, the higher temperatures on soil surface increased of seed respiration rate (Baskin & Baskin, 2001). Corroborating to this hypothesis, the emergence under open sky conditions, occurred under more elevated temperatures, when contrasted with the environment under 50% of shading (Figure 4). An exception was found when pure soil was used as substratum, where low emergency percentage was recorded. This situation presents a correlation with soil moisture level because the clay percentage on pure soil is higher than the others two substrata (Figure 3).

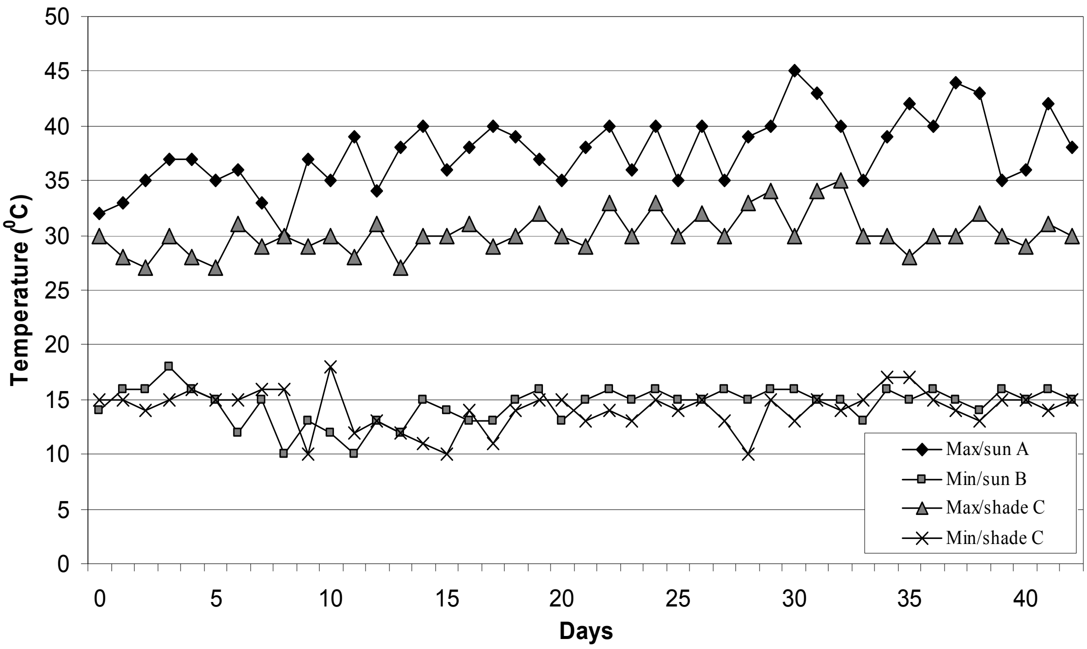

Figure 4: Variation of temperature on open sky condition and 50% of shade. Max/sun and Min/sun= Maximum and minimum temperatures under open sky conditions; Max/shade and min/shade= Maximum and minimum temperatures under 50% of open sky light conditions respectively. Values followed by the same letter are not significantly different (P>0,05).

Extreme seedbed conditions, created by soil mechanical impedance and reduced oxygen supply could produce overriding effects on seedling emergence (Kolb *et al.*, 2002; Whalley *et al.*, 1999). The clay soil texture may partially be responsible for the high mortality on field emergence, since mechanical impedance reduces the elongation of the seedlings (Wang *et all.*, 2005, Whalley et al., 1999). In addition, biotic factors in the soil, such as pathogenic fungi could significantly reduce seedling survival. Dissimilarity between seed germination and field emergence are common even under similar temperature regimes mainly because substratum physical characteristics (Romo and Young, 2002). On the other hand, deficiencies in water availability significantly reduced size and germination rate of the seeds (Akhalkatsi & Losch 2005). So*, D. miscolobium* presents an ecological strategy of establishment upon lighting, warm and well drained gaps on Cerrado soil surface.

Overall Conclusions

1: *D. miscolobium* seeds are a quiescent and orthodox seed.

2: There is no relation between seed size, or between seed mass with the rate and germinability. There is no statistical difference between seed provenance and length classes when the rate and germinability were evaluated.

3: The emergence under open sky conditions was higher than at 50% of artificial shading.

4: The ecological strategy for the final stage of *D. miscolobium* dispersion is seed germination and seedling establishment at the first rainy season after the dispersion.

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CAPITULO 2

WATER STRESS, STORAGE AND ACCELERATED AGING ON SEED GERMINATION AND SHADDING, SOIL INFLUENCE ON NURSERY SEEDLING OF *Dalbergia miscolobium* Benth., A SAVANNA TREE SPECIES FROM BRAZIL.

Water stress, storage and accelerated aging on seed germination and shading, soil influence on seedling establishment of *Dalbergia miscolobium* Benth., a savanna tree species from Brazil.

***Key words*:** Cerrado, water deficit, viability, vigor, temperature, germination, seed ecology.

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Resumo

Com o intuito de acrescentar informações aos estudos realizados no capítulo anterior, o objetivo deste trabalho foi avaliar o limite de tolerância ao estresse hídrico, interações entre estresse hídrico e temperatura na germinação, viabilidade da semente após armazenamento e a influência do tipo de substrato e luz na sobrevivência de plântulas de *Dalbergia miscolobium* Benth. O limite de tolerância para a germinação de sementes sob estresse hídrico foi entre -1,0 e 1,2 MPa. A temperatura sub-ótima para germinação interagiu com o estresse hídrico, aumentando a porcentagem final de germinação. Após quatro anos de armazenamento usando recipientes de vidro herméticos em ambiente de refrigerador ($4^{o}C \pm 2^{o}C$), as sementes apresentaram 82,16 ± 9,33 de germinabilidade e uma taxa de germinação de 0,217 ± 0,050. Como *D miscolobium* não apresenta tegumento duro foi checado o vigor utilizando envelhecimento acelerado e as sementes resistiram até 24 horas à $50^{o}C$ e 100% de umidade relativa Luz e substrato influenciaram a sobrevivência de plântulas. *D. miscolobium* é uma espécie tolerante ao estresse hídrico para a germinação de sementes e tem como estratégia ecológica, estabelecimento de plântulas em locais ensolarados e preferencialmente em terrenos arenosos bem drenados.

Abstract

In order to add information to the studies already initiated, the aim of this work was evaluated the tolerance limit to water stress, the interaction water deficit and temperature on germination, seed viability after storage and influence of artificial substratum and light on seedling survival of a commonly, wide spread tree *Dalbergia miscolobium* Benth. The tolerance limit for seed germination, under water deficit was between -1.0 and -1.2MPa. Suboptimal temperature of germination has interacted with water stress and increase the germination percentage. After stored during four years using hermetic glass recipients inside refrigerator (4^{o}C ± 2^{o}C), the seeds presented 82.16 ± 9.33 of germinability and 0.217 ± 0.050 of germination rate. As *D miscolobium* does not present a hard coat and it was check the vigor using the accelerated aging and the seeds resisted until 24h at 50^{o}C and 100% of RH. Light and substratum play an important rule on seedling survival. *D. miscolobium* is a water tolerant species for seed germination, and it has as ecological strategy, seedling establishment on sunny well drained places preferable on sandy soil.

Introduction

The major savanna region of Neotropics in Brazil is the Cerrado, covering 90% of total neotropical savanna area (Sarmiento, 1983). According to Ratter *et al.* (1997) cerrado domain is the second largest phytogeographic province in Brazil, originally covering about 23% of the Brazilian territory (around two million km^2). Nowadays, 20% of the Cerrado area remains undisturbed, only 2.2% of its area is under legal protection and an estimated 20% of threatened but endemic species do not occur in protected areas (Klink & Machado, 2005).

With an elevated richness, high degree of endemism, and current threats to its biodiversity, the cerrado was included among the biodiversity hotspots for highest priority conservation in the world (Mittermeyer et al. 1999) and it is the only hotspot dominated by a tropical savanna (Myers *et al*., 2000).

The Cerrado climate is seasonal, with wet summers and dry winters, classified either as Aw or Cwa, following Köppen's (1931) system. The dry season is quite pronounced and, in some cases, may last six months (Eiten 1972). According to Sarmiento (1996) one of the most important ecological limitations for the growth of savanna plants is water availability. During the dry season, soil water potentials decreased as low as the permanent wilting point (Sarmiento and Acevedo 1991; Sarmiento 1996). However, drought does not affect strongly the woody plants (with deep roots) because deeper water is constantly available (Sarmiento 1996).

Water availability is directly and indirectly involved with seed germination and seedling establishment phases playing an important rule on enzymatic reactions, solubilization and transportation of metabolic compounds (Bradford, 1995), besides plant water balance. Thus, drought represents one marked stressing moment to cerrado species (Sarmiento 1996).

Restoration practices on Cerrado areas are almost unknown and the study of the ecology plant production of this biome is the main foot step for a future possibility for recovery. The purpose seed storage is to secure the supply of good quality seed for planting programs (Schmidt, L. 2000). In seasonal climates, like cerrado weather, with a relatively short planting season, sowing time is normally determined by the wish to have plantable size seedlings at the beginning of the growing season. Hence, seeds must often be stored during the period from harvest to sowing. Seed storage serves as a buffer between demand and production and has a regulatory turnover: seeds are stored during periods of seed availability and shipped to nurseries or other recipients when required to raise plants (Schmidt, L. 2000).

Species with orthodox seeds could be stored and it is a positive factor to be used in restoration of degraded areas and for recovery of native forests programs. Seed moisture content and storage temperature are considered the main factors in maintaining vigor viability. Seeds can then be used for a longer period of time a stock of viable seeds maintained to for reforestation programs in degraded areas or for the recovery of native forests. The measurement of seed qualities with the germination tests allow the researchers observe the maximum germination under favorable conditions. On the other hand , under natural conditions, seeds are submited to pressures, like soil moisture variation, radiation and competition, unfavorable conditions to the germination.

Seed vigor is one of seed proprieties that determine the potential for a fast and uniform emergence with a normal seedlings development above a large range of ambient conditions. (AOSA, 1983)

The methods of vigor evaluation (vigor tests) are classified direct or indirect. The direct methods are test realized on the field or in laboratory, simulating field conditions. On the other hand, indirect methods may be tests, conduced under laboratorial conditions, evaluating physical, biochemical and physiological characteristics of seeds determining seed qualities. The artificial aging is an indirect method to evaluating seed vigor, simulating stress conditions and consequently, with high respiration rate and reserves consume, accelerating the process that drive the seeds to deterioration.

Dalbergia miscolobium is a widely spread tree species of Cerrado, (Batalha & Mantovani, 2001; Durigan *et al.*, 2002; Ratter *et al.*, 2003). It is a Fabaceae species, with 8 to 20 m of height, 0.3 to 0.5m of trunk diameter. Composts leaves present between 15 to 20 cm length. The wood is heavy, malleable and very decorative with natural durability under open sky conditions, appropriate to posh furniture .It is a semidecidual, heliophyte, pioneer and selective xerophytes species, characteristics of well drained sandy grounds. The flowering process is registered between January and February and the fruit maturation between May to June.

In order to add information to the studies already begun, the aim of this work was evaluated the tolerance limit to water stress, the interaction water deficit and temperature on germination, seed viability after storage and influence of artificial substratum and light on seedling survival of a commonly, wide spread tree *Dalbergia miscolobium* Benth..

Methods

Biological material

Fruits of *D. miscolobium* were harvested from 13 different trees in the cerrado reserve of São Carlos Federal University, São Carlos City, São Paulo State, Brazil (21°58´, 22°00´ south latitude and 47°51´, 47°52´ east longitude). The seeds were handly isolated from the pericarpe, and after, they were stored under laboratorial conditions ($20^oC \pm 5^oC$), in opened containers until the beginning of the experiments.

Water stress

The response of *D. miscolobium* seed germination to different levels of simulate water stress were verified using PEG 6000 solutions prepared at seven different concentrations: -0.2, -0.3, -0.4, -0.6,-0.8, -1.0, -1.2 MPa according to Vilella *et al.,*(1991) plus a control group (distilled water), at the optimal temperature for germination (Barbieri Jr *et. al.* 2005, Perez *et al*. 2001). To verify the synergistic effect of temperature variation and water stress (Bradford, 2002), seeds were submitted to germinated in PEG 6000 solutions at -0,2 and -0,3 MPa, that are the higher water potential limit and three temperatures: 20, 30 and 44^{o}C, respectively sub optimal, optimal and supra optimal temperature for germination (Barbieri Jr *et. al.*, 2005, Bradford, 2002, Perez *et al.*, 1998).

Storage and accelerated aging

First was determined the water content of the *D. miscolobium* seed using sampling of 50 replicates of 10 seeds, reaching 500 seeds and 53.69g of fresh seeds (Brasil, 1992; Piña-Rodrigues *et al.*, 2004). Each replicate was weighted (fresh weight) and dried at 105^{o}C in a dessicator oven during 24 h, when they were weighted again and contrasted with the initial fresh weight. (Brasil, 1992, Figliolia & Piña-Rodrigues, 1995, Piña-Rodrigues *et al.*, 2004).

Seeds were stored inside glass bottles hermetically closed at two temperature conditions, inside a refrigerator (4 ^{o}C ± 2^{o}C) or under laboratory temperature conditions (20 ^{o}C ± 5^{o}C). For each time of storage seeds were stored in different bottles. For the accelerated aging the *D. miscolobium* seed were disposed inside a modified gerbox upon a perforate metal plate that separate seeds from the 20mL of distilled water on the box bottom. For each temperature and each aging time were four replicates. Each replicate was made with one modified gerbox with 25 seeds inside. The gerbox were put inside a water jacked incubator with 100% of relative humidity for tree different temperatures, 45, 50 and 60^{o}C and during 24, 48 and 72h. Past the artificial aging treatment the seeds were transfer to Petri dishes and a standard germination test (describe below) was performed.

Standard germination test

The seeds germinated inside a 15cm Petri dish lined with filter paper, moistened with distilled water or PEG 6000 solutions and enclosed with PVC film to prevent drying out**.** The germination tests were conducted inside standard incubators (B.O.D.) at optimal temperature (30^{o}C ± 0.5^{o}C) (Barbieri Junior *et al.*, 2005). The germination tests were daily monitored; at the same time and the germinated seeds were handly removed from the Petri dishes, as soon as germination was identified. The experiments were enclosed when all seeds germinated or the last ones were deteriorated. It was assumed as a germinated seed, when radicular protrusion was longer than 2mm, with a positive geotropic curvature (Duran & Tortosa, 1985; Perez *et al*. 2001).

The rate and germination percentage was obtained according to Labouriau & Valadares (1976), Labouriau (1983), and Labouriau & Agudo (1987).

The data were submitted to the Shapiro-Wilk normality test, followed by ANOVA and the Tukey test (Zar, 1999). For all the tests was assumed $\alpha = 0.05\%$. The germinability data were transformed into $\arcsin\sqrt{(X/100)}$ before the statistical tests when necessary (Sokal & Rohlf, 1997; Santana & Ranal, 2004).

Seedlings survival and growth

The substratum and shade influence on *D. miscolobium* seedlings survival and growth were analyzed with a factorial designed experiment, comparing three different substratum: sand, cerrado soil, and a mix (5:3:2) being five parts of cerrado soil, tree parts of organic compound and two parts of sand. The substratum was distributed in the 1.5 L polyethylene bags and so, exposed to two light conditions: 100 and 50% of open-sky light. In each polyethylene bag a single seed was sowed. The experimental design consisted of 10 replicates with 10 polyethylene bags each, reaching 100 seeds per treatment.

The polyethylene bags were disposed upon a triturate rock floor, without cover (100% open-sky light) or in a green house (30m x 15m x 3m) with one layer of monophilament cloth (50% of transmittance). The light density was measured with a luximeter (Extech Instruments, USA) checking that 50% of open-sky light was achieved. All polyethylene bags received 5mm of water supply through an irrigation system (Naan-dan ® 5024 agricultural sprinkler) once a day, during all the experimental period. The observations were performed on 180 and 360 days old seedlings. They were washed with a fine sieve one by one, to prevent lost of root material. A digital paquimeter was used to measure the diameter of shoot and primary root, high above ground, and a rule was used to evaluate the root length.

After seedlings were cut with a scissor and separate in above and underground parts, whose were conditioned inside different paper pack and dried inside a oven at 105^{o}C during 48hs after what the dried seedlings parts were weighted with a digital balance. A two way ANOVA and a Tukey test ($\alpha = 0.05$) was performed to contrast the observed values (Zar, 1999).

All the experiments were conducted in the Botany Department of Federal University of São Carlos, São Paulo State except that one, evaluating seedling growth and survival, was conducted at Ymyrá Nursery Tree Farm at Jacareí city in the same State, 100 km away from São Paulo City.

Results

Water stress

The rate and germination percentage were affected by temperature and osmotic potential variations. The **Figure 5** shows that at -0.2 MPa was no statistically different of the control group for germinability parameter, but for germination rate a statistical difference was observed **(Figure 5)**. For all others osmotic potentials statistical differences were observed on the rate and germination percentage **(Figure 5 and 6)**. The maximal tolerance limit for seed germination was between -1.0 and -1.2MPa **(Figure 5)**. The theoretical point P50 **(Figure 6)** was situated at -0.37MPa.

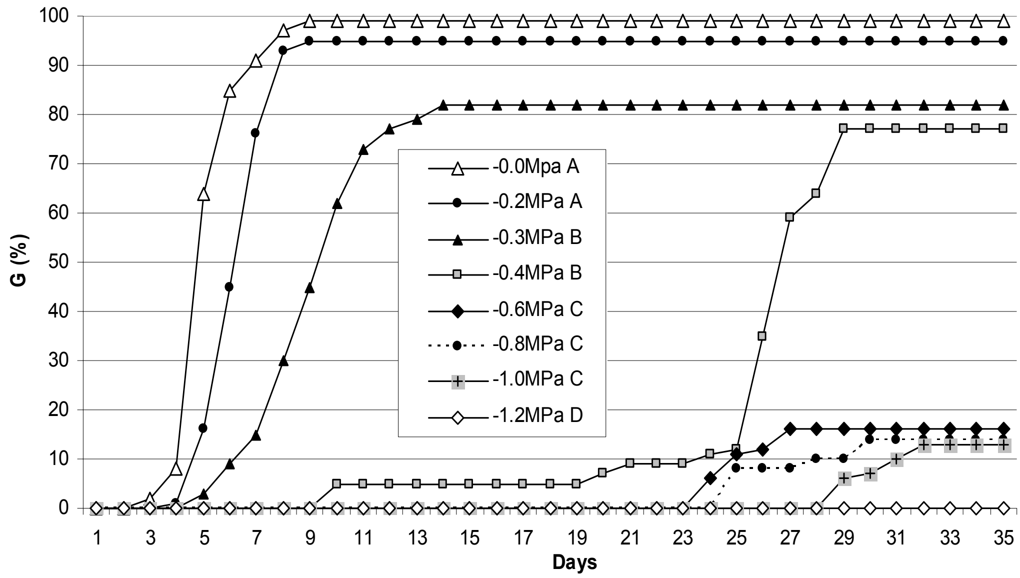

Figure 5: Seed germination under water stress Values on the legend followed by the same letter are no statistical different (P>0,05).

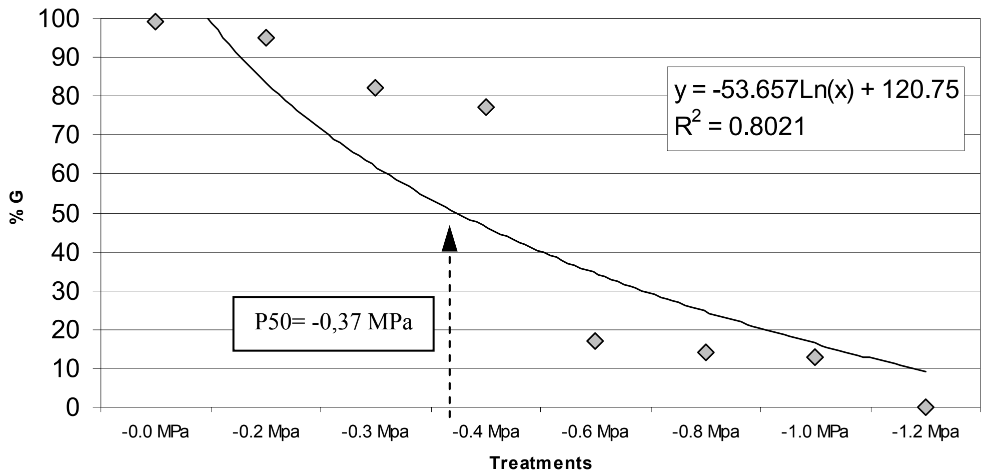

Figure 6: Seed germination (%) in function of water stress treatments. Values on the legend followed by the same letter are not statistical different (P>0,05). Black arrow indicate P50.

When the osmotic potential was associated with temperature variation different results were observed **(Figure 7**). Germination percentage under 20°C and 0.0MPa was statistically different when compared with control group (30°C and 0.0MPa), but when the same parameter was analyzed with the 20⁰C and -0.2MPa there is no statistical difference when compared with the control. Even when seeds were submitted under 20°C and -0.3MPa, the germination was higher than 20°C and 0.0MPa.

Seed exposed to 44°C and 0.0MPa have a considerate germination percentage (77±2%) but when seeds were submitted to 44°C at -0.2MPa and -0.3MPa the germination was suppressed **(Figure 7**).

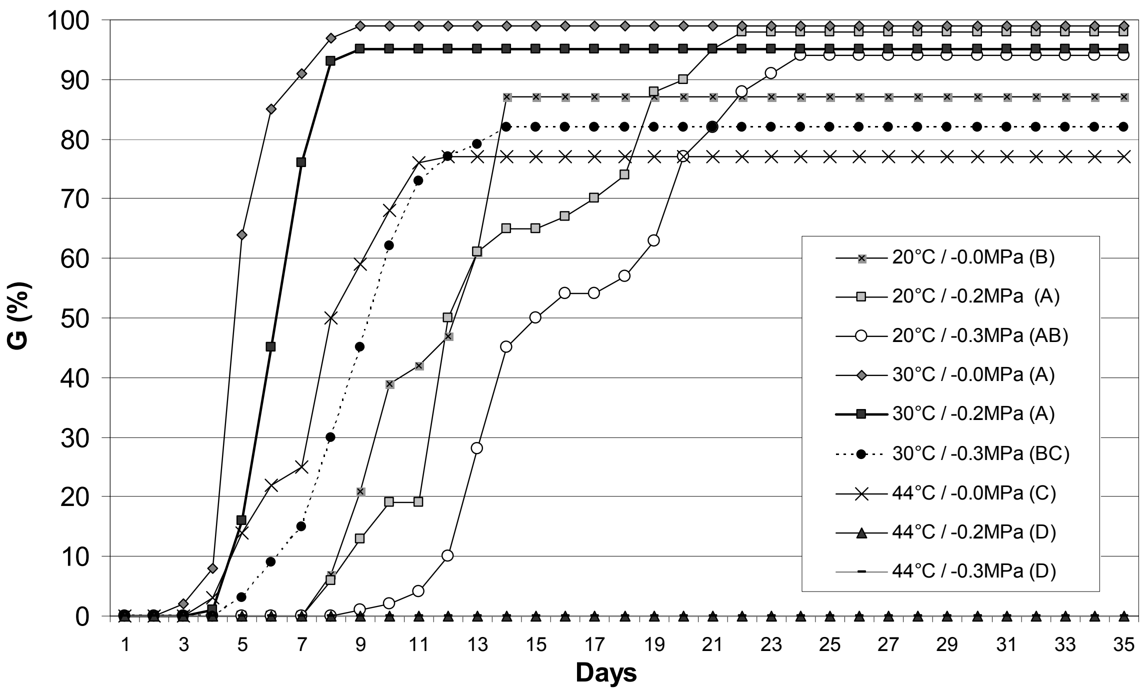

Figure 7: Seed germination under water stress in combination with thermal stress. Values on the legend followed by the same letter are not statistical different (P>0,05).

Storage and accelerated aging

The *D. miscolobium* seeds water content was 7.3 % with 1.8 % of standard deviation. The rate and germination percentage observed were the same as the control until the age of two years of storage under laboratorial conditions **(Table 4)**. After three years of storage under laboratorial condition, the rate and germination percentage show statistical differences when compared with the control and after four years no germination was observed with this way of storage. On the other hand, storage under refrigerator conditions the values of rate and percentage germination values with no statistical differences when contrast to control group, even four years after the begining of storage test **(Table 4).**

Table 4: Rate and germination percentage during storage conditions. Average ± Standard deviation. Values followed by the same letter are not statistical different (P>0,05).

Time (years)	Storage condition	Germination (%)	rate($days^{-1}$)
Control	Control	84.55 ± 8.69 a	0.209 ± 0.035 a
1	laboratory	82.16 ± 9.33 a	0.212 ± 0.033 a
1	refrigerator	83.54 ± 7.45 a	0.199 ± 0.013 a
2	laboratory	78.93 ± 7.82 a	0.212 ± 0.019 a
2	refrigerator	77.55 ± 7.19 a	0.190 ± 0.029 a
3	laboratory	54.55 ± 3.89 b	0.149 ± 0.004 b
3	refrigerator	77.55 ± 8.69 a	0.200 ± 0.018 a
4	laboratory	00.00 ± 0.00 c	0.000 ± 0.000 c
4	refrigerator	82.16 ± 9.33 a	0.217 ± 0.050 a

Seeds submitted to accelerated aging presented differences in germination percentage when compared with the control only at 50 and at 45^{o}C during 24, 48 and 72h the rate and of germination percentage were not statistically different when compared with control group. At 50^{o}C and 24h of treatment, the percentage of germination was statically different of the control, but the germination rate was not statistically different from the control. Seeds submitted to the temperature of 50^{o}C during 72h and 60^{o}C during 24, 48 and 72h lost the viability **(Table 5).**

Table 5: Rate and germination percentage of *D. miscolobium* seed submitted to the artificial aging treatment. Average± Standard deviation. Values followed by the same letter are not statistical different (P>0,05).

Treatment	*Germinability (%)*	*rate ($days^{-1}$)*
Control	99.00 ± 2.00 a	0.182 ± 0.011 a
45°C/24hs	96.00 ± 3.2 a	0.179 ± 0.008 a
45°C/48hs	97.00 ± 1.3 a	0.180 ± 0.012 a
45°C/72hs	94.00 ± 2.9 a	0.175 ± 0.058 a
50°C/24hs	72.00 ± 3.27 b	0.168 ± 0.008 a
50°C/48hs	3.00 ± 3.83 c	0.055 ± 0.064 b
50°C/72hs	0.00 ± 0.00 c	0.000 ± 0.000 c
60°C/24hs	0.00 ± 0.00 c	0.000 ± 0.000 c
60°C/48hs	0.00 ± 0.00 c	0.000 ± 0.000 c
60°C/72hs	0.00 ± 0.00 c	0.000 ± 0.000 c

Seedlings survival and growth

According to the results, seedlings could be disposed in groups, grown under open sky or shade conditions. The mortality observed on the seedlings grown up under shade condition was bigger than under open sky **(Table 6).** The mortality was not statistical different between the three different soils under shade treatment. Under open sky condition, the mortality between sand and the mixture soil was not statistically different but the cerrado soil treatment is statistical different when compared with the other two treatments.

Table 6: Percentage of survive of D. miscolobium seedling at 6^0 and 12^0 month old. Values followed by the same letter are not statistical different (P>0,05). 1- open sky / sandy, 2- open sky / cerrado soil, 3- open sky / mixture, 4- shade/ sandy, 5- shade / cerrado soil, 6- shade / mixture

Treatment	0 month	6 month	12 month
1	99±1	89±3	84±4
2	68±9	64±10	64±10
3	98±2	92±5	85±6
4	37±11	25±12	20±10
5	38±12	17±9	12±10
6	30±10	15±7	9±8

The dry weight of the seedlings submitted to the three treatments under open sky condition was higher than the other tree treatments under shade condition (**Figure 9**). When separated the weight of the root system and the above ground system the results followed the same characteristics **(Figure 9).**

Concerning to root system lengths, and light no relation was observed. There was verified that the root length was related to the substratum type. The **Figure 8** shows that in the sand substratum the root lengths were longer then in others substratum. In contrast of these results was observed that the seedlings shoot length from the under open sky conditions and sand substratum was the shorter, followed by the cerrado soil, as a media, under the same light condition .

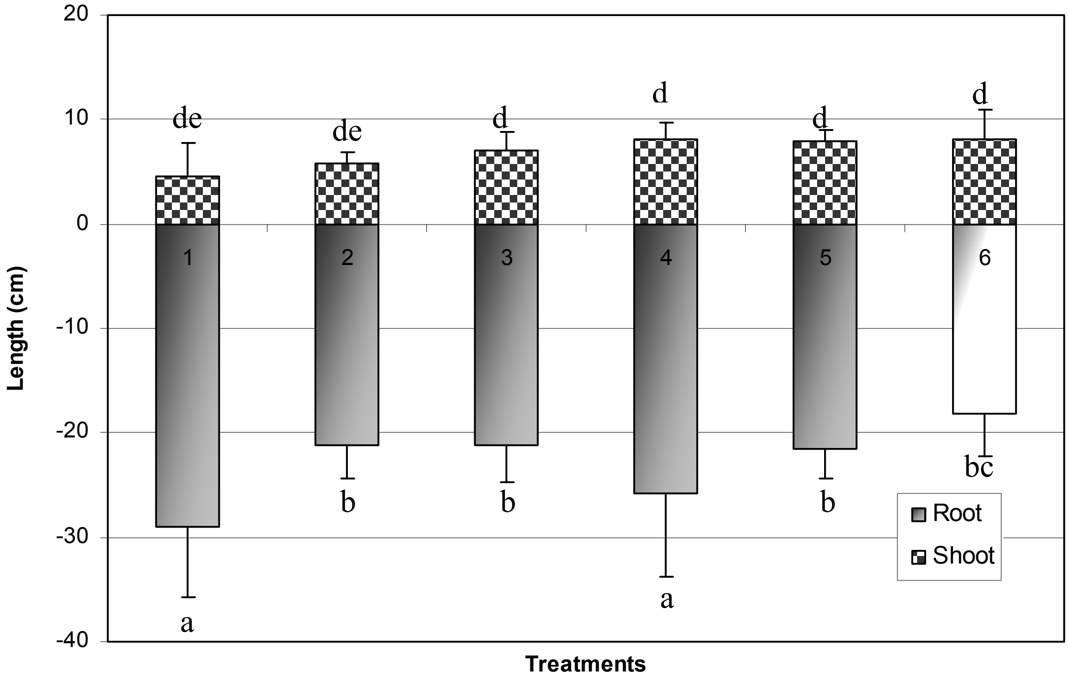

Figure 8: Root and shoot lengths in function of the treatments: 1- open sky / sandy, 2- open sky / cerrado soil, 3- open sky / mixture, 4- shade/ sandy, 5- shade / cerrado soil, 6- shade / mixture. Values followed by the same letter or number are not statistical different (P>0,05).

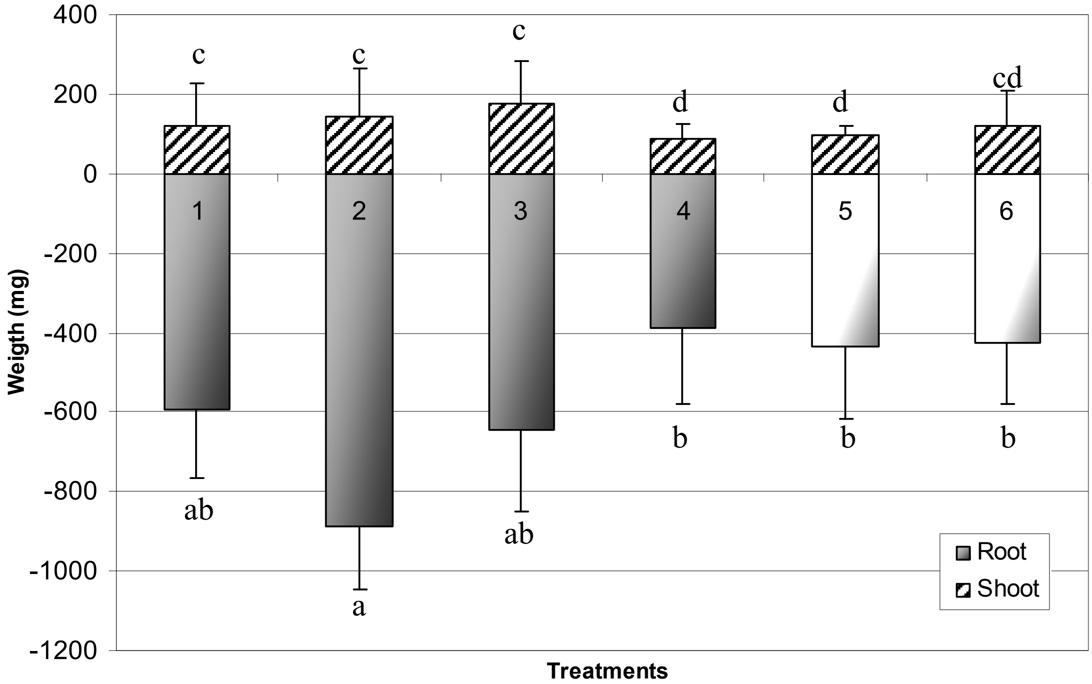

Figure 9: Root and shoot dry weights in function of the treatments: 1- open sky / sandy, 2- open sky / cerrado soil, 3- open sky / mixture, 4- shade/ sandy, 5- shade / cerrado soil, 6- shade / mixture. Values followed by the same letter or number are not statistical different (P>0,05).

Discussion

Water stress

The first step of germination is the water uptake by the seeds (Bewley & Black, 1994). The initial imbibition period is critical to germination and very low water potentials prevent the water up take, making unviable the sequential steps of germination process. The imbibition rate is determinate by the chemical composition of the seeds, tegument permeability, water availability, temperature and physiological quality of the seeds. Water absorbance promotes the tissues hydration and the increase of the respiration rate and other metabolic processes culminating with the energy supplies to the embryo growing axis, on the other hand, if the water imbibition by the seeds is very fast, result in cellular membrane disorganization, tissues ruptures and abnormal plant emergence, with low vigor (Fanti & Perez, 2004).

The results allow us to conclude that *D. miscolobium* is tolerant to water stress.

Under suboptimal temperature for germination *D. miscolobium* seeds presented a decrease on the germination rate, at the end of germination process the germinability was statistically similar at the optimal temperature and without water deficit. Water stress, at natural conditions, could be positive to the species establishment, producing a retarding on the rate and seed germination, producing a temporal distribution of the seedlings, increasing the survival probability of, adjusting to the conditions for the grow up and establishment (Bewley & Black, 1994, Fernner & Thompson, 2005).

The wide water stress tolerance provides a highest environmental adaptation capacity. This phenotype plasticity to the moisture environmental conditions provides a higher ability to plant establishment, conferring an evolutionary advantage above others species with short intervals of temperature germination, specially to seasonally stressing environments like Cerrado (Sarmiento, 1996).

Other studies have showed similar results for water stress limits with Fabaceae hard coat seed species from Cerrado (Table 7). D. miscolobium has not hard coat, what suggest that this species have great amount of structural proteins in seed, this is a interesting appointment to future research.

Table 7: Water stress limits for germination of some cerrado wood species

Species	**Water stress limits for germination**	**Authors**
Peltophorum dubium	-1.4 and -1.6MPa	Botelho and Perez, 2001
Dimorphandra mollis	–0.6 and –0.8MPa	Zpevak, 1994
S*tryphnodendron pollyphyllum*	–0.6 and –0.8MPa	Tambelini, 1994
Pterogyne nitens	–1.0 and -1.2MPa	Nassif and Perez, 1997
Enterolobium contortisiliquum	–1.6 and –1.8MPa	Hebling, 1997

Storage and artificial aged

Ageing denotes the progression of deteriorating events that take place within the seed and which ultimately lead to the death of the seed. The aging takes place during a long time, several cytological and biochemical deterioration accumulate. The length of time seeds can stay viable in the natural environment depends on the seeds themselves and the conditions around them. Some seed types do not have the ability to stay alive for a long time. These so-called recalcitrant seeds have short physiological storability, which can only be slightly extended by storing them under controlled conditions. Orthodox seeds, on the other hand, have a long storage potential and under favorable storage conditions may stay viable for decades. Soil dwelling predators, micro-organisms and a relatively high soil temperature and humidity around the seeds are the principal factors contributing to seed deterioration in the field.

The seeds longevity during their store is determined by their genetic and physiological potential and by any deteriorating events or damage, prior to or during storage, as well as by the interaction between individual factors. Seed lots with high initial viability also have a higher longevity in storage than seed with low initial viability. Seeds loss the viability progressively during the storage due the biochemical oxidative process in cellular membranes damage and lipids peroxidation. Plants have protections against these problems like the enzymes superoxid dismutase, catalase and glutathione redutase and antioxidants like ascorbate and α-tocoferol (Bailly et. al., 1997).

The progression of natural ageing with resultant loss of viability is not linear over time but typically follows a sigmoid pattern (Schmidt, 2000, Bewley & Black, 1994). Loss of viability is initially slow, followed by a period of rapid decline as showed on Table 4.

On the accelerated aging test the *D. miscolobium* seeds were submitted to height temperature and moisture conditions. These two factors are associated with the seed deterioration (Fanti and Perez, 2003). Artificial seed aging test promote metabolic alterations during the germination process including respiration process and membranes function (Basajavarajappa et al., 1991). The observation of *D. miscolobium* germination behavior under different temperatures and aging time allow to conclude, that this species is very resistant to seed deterioration, confirmed by the storage test. This is an important data for the nurseries and reforestation programs management.

Seedlings survival and growth

Savanna species are light-demanding species with large investment in roots, improving the chance of water capture from the soil water during the dry season and permitting new sprout following burning or drought (Hoffmann and Franco, 2003, Rizzini, 1965).

The results demonstrate that light condition plays an important rule on *D. miscolobium* seedling establishment and development. Independent of light condition more biomass was allocate to root system than to shoot system. This species apparently shows a preference for sand soil and consequently well drained ground, confirmed by the major dried weight of the seedlings grow up on the sand substrate. Hoffmann *et. al*. (2004) related height mortality for *D. miscolobium* seedlings submitted to gallery forest conditions (poorly drained soil and shade ambient).

Independent of the substratum, *D. miscolobium* seedlings under shade cover treatment showed a higher shoot length when compared with the open sky treatment, but the seedlings under shade demonstrate a dried weight lower than the open sky ones. Under low light intensity, the seedlings were etiolated (**Figure 8 and 9**).

Seedlings under open sky condition showed a dried weight of the root system heavier than the seedlings under shade treatment demonstrating reserve accumulation on root system. (**Figure 8**)

The most evident difference between savanna and forest species is the biomass partitioning. Savanna species allocated more biomass to coarse roots and less biomass to leaves and stems than did forest species (Hoffmann and Franco, 2003). Savanna species typically have a deep taproot that develops very quickly in seedlings (Rizzini 1965, Oliveira & Silva 1993).Coarse roots enable the carbohydrate and nutrient storage necessary for surviving frequent fire, and provide greater rooting depth, which permits access to deeper soil water during the dry season. Indeed, first-year seedlings of cerrado savanna species exhibit high survival during this dry season, even when the shoot is burned out (Oliveira & Silva, 1993; Hoffmann, 2000). The seedling is a critical stage of the life cycle and is likely the most sensitive to fire and environmental stresses.

Some cerrado species exhibited a range of responses to cover, with some ones responding positively (adapted to the conditions of close savanna) and others responding negatively (adapted to the conditions of open savanna) (Hoffmann 2000). Hoffmann (1996) reported that, *Kielmeyera* and *Zeyheria* both experienced greater mortality under cover in the establishment phase. For these species adapted to the high light environment of savanna environments, it appears that the negative effects of low light may exceed the positive effects of woody cover (Hoffmann, 2000).

The low success of savanna trees in forest is probably due to low shade tolerance. Additionally, at least some cerrado trees are intolerant of waterlogged soil (Joly and Crawford 1982), which would limit their success in poorly drained sites inner gallery forests. Some cerrado species are intolerant of basic soils rich in calcium carbonate (Haridasan 1988), which would exclude them from deciduous dry forest on calcareous substrates.

Conclusions

1- *D. miscolobium* seed germination is resistant to water stress.

2- Four years after storage refrigerator conditions inside a bottle hermetically close, the rate and germination percentage shows no alteration when compared with the control

3- *D. miscolobium* is sensible to artificial aging test only when submitted to elevated temperatures.

4- Light condition plays an important rule on *D. miscolobium* seedling establishment and development.

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CONCLUSÃO GERAL

- A partir dos resultados obtidos nos testes de germinação pode-se concluir *que D. miscolobium* é uma espécie quiescente e ortodoxa para germinação de sementes.
- Não houve relação do tamanho ou da massa da semente na velocidade de germinação e na germinabilidade. Também não houve diferença estatística entre a planta matriz e classes de tamanho das sementes na germinabilidade e na velocidade de germinação.
- A emergência de plântulas sob 50% de sombreamento foi estatisticamente menor que a ocorrida à pleno sol.
- Com estes resultados foi possível inferir que a espécie estudada tem como estratégia ecológica a germinação de sementes e o estabelecimento de plântulas na primeira estação chuvosa após a dispersão dos diásporos.
- Com respeito à germinação as sementes de *D. miscolobium* se mostraram resistentes ao estresse hídrico, e à combinação de estresse hídrico e térmico, tendo este último favorecido a germinabilidade.
- O melhor método observado para armazenamento foi vidro hermeticamente fechado dentro de refrigerador comum, onde as sementes permaneceram viáveis por no mínimo quatro anos, sem perder o vigor.
- A condição luminosa exerceu influência sobre a sobrevivência das plântulas. A maior taxa de mortalidade foi observada nos tratamentos sob 50% de sombreamento.

- Mais uma vez a condição luminosa exerceu influência sobre as plântulas. Foram observadas maiores taxas de crescimento, produção de biomassa em plântulas crescidas sob condição de pleno sol.