INTRA-AND INTER-POPULATION VARIATION IN SEED SIZE AND DORMANCY IN
Schizolobium parahyba (Vell.) Blake IN THE ATLANTIC FOREST

VARIAÇÃO ENTRE E DENTRO DE POPULAÇÕES EM TAMANHO E DORMÊNCIA DE
SEMENTES DE Schizolobium parahyba (Vell.) Blake NA FLORESTA ATLÂNTICA

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ABSTRACT

Seed size and dormancy level were studied in 20 trees from two populations of Schizolobium parahyba to evaluate how these characteristics occur between and within populations, and whether seed germination was affected by its morphometry. These two populations are located in the coastal (Paraty) or mountain (Miguel Pereira) regions in the state of Rio de Janeiro, Brazil. The seed morphometric traits (length, width, thickness and weight) were measured. Germination with or without seedcoat dormancy treatment (mechanical scarification) was assessed by a randomized emergence test in nursery. All morphometric traits differed significantly among individuals and between populations. The trees from the mountain region showed a larger seed size and a lower dormancy level than that of trees from the coastal region. Seed size had no effect on seed germination or seedling development. Climate influence on the determination of germination behavior is discussed based on the results.

Keywords: forest seed; population diversity; seed morphometric traits; germination.

RESUMO

O tamanho da semente e seu grau de dormência foram estudados em 20 matrizes de duas populações de Schizolobium parahyba, visando avaliar como estas características se distribuem entre e dentro de populações e qual a influência da morfometria na capacidade de germinação da espécie. As populações são procedentes da região litorânea (Paraty) e montanhosa (Miguel Pereira) no Estado do Rio de Janeiro. As características morfométricas (comprimento, largura, espessura e massa) foram medidas e a germinação com e sem tratamento de quebra de dormência (escarificação mecânica) foi avaliada em um teste de emergência com delineamento inteiramente casualizado. Todas as variáveis morfométricas diferiram significativamente entre indivíduos e entre populações. As matrizes da região montanhosa apresentaram maior tamanho da semente e um menor nível de dormência do que aquelas localizadas na região litorânea. O tamanho da semente não influenciou a germinação das sementes e o desenvolvimento das plântulas. A influência do clima na seleção do comportamento germinativo da espécie foi discutida com base nos resultados obtidos.

Palavras-chave: semente florestal; diversidade populacional; morfometria da semente; germinação.

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INTRODUCTION

Tropical forest species are under natural and selective pressure. As these are not domesticated, a high variation in morphological traits is expected. Seed size is an important reproductive factor affecting predation, successful seedling establishment and dispersion of species (FOSTER and JANSON, 1985; LEISHMAN et al., 2000; NORDEN et al., 2009) and is directly related to the species ecological groups (PINA-RODRIGUES et al., 1990; MALAVASI and MALAVASI, 2001), the life form and the plant height (LEISHMAN and MURRAY, 2001). Microclimate factors, herbivory and fungal attack may affect selection by seed size during recruitment (CHRISTIE and ARMETO, 2003).

Competition between seeds of different sizes is important in the selection of seed size polymorphism, as it favors the coexistence of alternative strategies: the competitive advantage of plants with larger seeds compensates for the low number of seeds produced, and plants with smaller seeds produce more seeds to occupy sites that are not occupied by the larger seeds (GERITZ et al., 1997; FENNER and THOMPSON, 2005). Even within the same community, same species populations submitted to different disturbance levels produce seeds of distinct mean sizes (MALAVASI and MALAVASI, 2001) and seeds yielded in shaded conditions are bigger (in both size and weight) than those on full light (FOSTER and JANSON, 1985). Some studies suggest that seed size and number may determine the community structure. Seed size may be related to abundance (REES, 1995; GUO, 2003).

Large seeds show less restrictions in natural conditions and are capable of establishment in different microsites resulting in an adaptive advantage (LUSK and KELLY, 2003). This characteristic/advantage is the result of the so-called “effect of reserve size” expressed by the relationship between seed and seedling size influencing the species establishment (LEISHMAN et al., 2000). Many studies demonstrated that larger seeds show faster germination and seedling development (GERITZ et al., 1997; MALAVASI and MALAVASI, 2001).

The inter- and intra-population variation in dormancy levels has been reported and was attributed to geographical and environmental factors. SALAZAR (1986) studied the genetic variation of *Gliricidia sepium* (Jacq.) Kunth ex Walp. seeds and seedlings originating from 10 sites in Guatemala and Costa Rica, and found that seeds from sites with lower altitude and higher luminosity tend to be smaller. MEIADO and SIMABUKURO (2003) studied the biometry of fruits and seeds of two *Enterolobium contortisiliquum* (Vell.) Morong. populations originating from the state of Pernambuco (Brazil) and found that the scrub population fruits and seeds were larger, heavier and more variable than those of the marsh elevation population. Striking differences in germination traits were also found among three geographically distinct populations of the widely distributed annual arctic-alpine *Koenigias landica* L. species (WAGNER and SIMONS, 2008).

The high variation in the seed vigor and morphological characteristics according to the environment in forces the importance of using locally adapted seeds in ecological restoration. Local populations often show a home-site advantage and non-local genotypes may be maladapted to local environmental conditions (MIJNSBRUGGE et al., 2010). Populations of *Quercus suber* L. originating from sites with the driest summers were characterized by bigger acorns and exhibited the highest survival rates under dry conditions (RAMÍREZ-VALIENTE et al., 2009).

This study aimed to test and quantify intra- and inter-population seed size and dormancy level variations between two populations of *Schizolobium parahyba* (Vell.) Blake, and estimate the seed size effect on germination rate and seedling emergence. *Schizolobium parahyba*, commonly known as ‘guapuruvu’ or ‘ficheira’, is a semi-deciduous legume tree (Fabaceae), reaching up to 20-30 m in height. Individuals have long green stems and intense yellow blossoms (BACKES and IRGANG, 2004). Its natural distribution is irregular and discontinuous, and its trees may be found in the forests along the Brazilian coast, in Central America, and in the Andean region (LORENZI, 1992; AGUIAR SOBRINHO, 1996). This is a fast growing and pioneer species widely used for reclamation. It is reported to reach up to 1.7 m within two years from planting (ENGEL and PARROTA, 2001). The guapuruvu lightweight wood (density 0.32 g/cm³) can be used to make boxes, liners, boards, matches, toys, and model aircrafts (LORENZI, 1992). It is also used in the manufacturing of boats and canoes [by rural families living] along the coast of Southeastern Brazil. Its seeds are used in handcrafts, and may offer an extra income for
rural families. As the majority of tropical legumes, its seeds coat is waterproof (ROLSTON, 1978), with strong cutaneous dormancy. The seeds have orthodox behavior, and they may be refrigerated and kept viable for 22 years (CARVALHO, 2003).

MATERIAL AND METHODS

Seeds of *Schizolobium parahyba* (Vell.) Blake from two distinct populations, from the Atlantic forest area in the southeast of Brazil, were collected in July 2004 (Figure 1). The two populations are separated by 320 km in a straight line, one located in coastal region (Paraty) and the other located in the mountain region (Miguel Pereira) of the state of Rio de Janeiro, Brazil.

The first site is located in Paraty (23°13’22’’S and 44°44’04’’W), in the coastal region, with altitude ranging from 0 to 100 m, mean annual temperature of 18°C and mean annual precipitation of 2000 mm, with a short dry period in the winter (< 100 mm/month) from June to August. The soil has moderate fertility, dominated by cambissoil with typical vegetation of the Atlantic forest with a high density of Palmae and Bromeliaceae. The second site is located in Miguel Pereira (22°28’54’’S and 43°29’15’’W), with altitude ranging from 700 to 1250 m, mean annual temperature ranging from 15 to 18°C, with a dry period (< 60 mm) from May to September during the winter when temperatures drop to 16°C (INMET, 2005). The soil has low to moderate fertility with sandy loam latossoil covered by the Atlantic forest mountain vegetation, dominated by Palmae with high density of *Euterpe edulis* Mart.

The rainfall distribution over the five years before the experiment is shown in Figure 2 for the nearest stations Paraty (left) and Miguel Pereira (right). Note that Paraty shows greater variation in the amount of monthly rainfall from one year to another, considering the same month. The high variation of rainfall observed in the first months of the year can be explained by the delay in the occurrence of “veranico”, dry periods ranging from a few days to weeks, common in this rainy season, in the southern regions of Brazil. The drought that used to occur in January, can be noted in February.

Morphometric analysis

To define the minimum sample size, 500 seeds were collected from five trees. These were individually measured for width, length, thickness and weight. The minimum sample size was defined by repeatability analysis (FALCONER, 1987) applied to show the proportional variance of simple measures caused by genetic or environmental differences within and among individuals from the same population. The minimum sample size was calculated as follow:

$$R_m = \frac{\delta^2_{\text{among}}}{\delta^2_{\text{among}} + \delta^2_{\text{within}}}$$

And: $\delta^2_{\text{among}}$ = variance among classes (trees);
$\delta^2_{\text{within}}$ = variance within classes (replications);
n = number of seeds measured.
After establishing the minimum sample size, seeds were harvested from 20 trees (10 trees from each region), in September 2004, for morphological analysis and emergence test. These seeds were individually weighted and measured (length, thickness and width). These variables were considered as “seed size” parameters and seed lots from different regions were considered as different populations. From the total tree sampling, only 15 produced enough seeds for determining water content. Dry weight was obtained from two replicates of 10 seeds per tree, oven dried at 80°C for 24h. The formula for determining the moisture content of the seed was applied according to the ‘Rule for Seed Analysis’ (BRASIL, 2009).

Emergence test

The experiment was conducted in the Fernando Luis Oliveira Capellão nursery, Forest Institute, Universidade Federal do Rio de Janeiro in the municipality of Seropédica in September 2004. The emergence test was performed in an entirely random manner with 20 matrices (two origins), two treatments, four replications and 10 seeds per replicate, totaling 1,600 seeds. Seeds were submitted to the following treatments: a) no mechanical scarification and b) mechanical scarification. After being sterilized by immersion for 15 minutes in sodium hypochlorite (NaOCl 5%) and washed thoroughly with water, the scarification was performed by rubbing lightly for three seconds the side portion of the seeds on the electric emery abrasive surface.

Seeds were germinated in a nursery under 30% shade, in plastic containers of 10 cm x 25 cm, using washed and sterile sand as substrate. The following parameters were recorded: total number of germinated seeds, number of germinated seeds per stage, number of normal and abnormal seedlings, number of dead seeds, number of infected seeds, number of hard seeds (those which did not soak the water). Counts were performed initially at two day intervals up to 28 days, after which it was performed at weekly intervals until the 115 days, the test end date.

The germination speed index (GI) was calculated according to the formula proposed by Maguire (1962) using the number of germinated seeds in the numerator.

Statistical analysis

The following seed size parameters were calculated for each array: mean, standard deviation and coefficient of variation (CV). The Lilliefors, Cochran and Bartlett tests were applied to check normality and homogeneity of data (VIEIRA, 1991). Because the data did not show normal distribution and homogeneity of variance, we chose to carry out non-parametric analysis using the Kruskal-Wallis test to assess the difference in seed size among trees and between sites. The Spearman correlation index was applied to evaluate the correlation between seed morphometric variables and germination using the SAEG program (SAEG, 2001).

The number of germinated seeds, mortality, and normal and abnormal seedlings were transformed using the square root of (x +1) for each value (x) to avoid zero in the analysis. Seed vigor data by GI was transformed by the square root of (x +1 / 100). Data were then subjected to the Lilliefors, Cochran and Bartlett tests (VIEIRA, 1991) to verify normality and homogeneity of variance. A
hierarchical variance analysis was performed to estimate the effect of the site (populations), the seed treatment, the tree and their interaction on seed quality and vigor. Least Significant Difference (LSD) test was used to compare mean differences among seed quality and vigor for all variables.

RESULTS

The repeatability degree for each morphometric variable measured indicated that length and weight for samples of 20 seeds achieved 97% repeatability (Table 1). For width and thickness, samples ranging from 25 to 50 seeds were needed to obtain the same 97% of repeatability. Based on these results, seed parameters were obtained from samples of 50 seeds per tree, capturing 99% of variability for length, 98.5% for width, 97.7% for thickness and 99.1% for weight.

The number of seeds per kilogram ranged from 438 to 636, with a mean of 541 (σ = 61.92; CV = 11.4%) for Paraty and 529 (σ = 35.94; CV = 6.8%) for Miguel Pereira. Seeds from Miguel Pereira had higher means for width, thickness and weight, and both populations were similar for seed length (Table 2).

The seed water content ranged from 6.05 to 9.33% in both populations, and seeds from Miguel Pereira showed a higher water content mean (7.97% ± 0.79) when compared to Paraty (7.37% ± 1.07). There was no significant difference between populations (F = 0.299; p = 0.589), however, the water content varied significantly among trees individuals (F = 2.701; p = 0.038).

For seed size parameters, length and width indirectly affected weight as indicated by Spearman’s correlation coefficient (Table 3). Weight was strongly related to length (r = 0.727) and width (r = 0.799). Seed morphometry and water content did not correlate as indicated by Spearman’s correlation coefficient being lower than 0.40 for all variables.

In relation to seed quality, trees differed significantly for all variables, including GI (Tables 4 and 5). There were significant differences between populations and treatments for seed germination and vigor (GI). In the Paraty population, major differences in GI were observed when comparing scarified with non-scarified seeds, denoting a higher seed dormancy for this population (Table 4). Only a small percentage of germinated seeds became normal seedlings (%NS). The other part died after germination due to fungal infestation, which in a way, was favored by seed scarification.

Correlation coefficients (r) between seed morphometric variables (length, thickness, width and weight) and germination parameters varied from 0.19 to 0.55, with little or no correlation between these variables (Table 6). The highest correlation (r = 0.55) was demonstrated between weight and percentage of germinated seeds. Seed vigor parameter (GI) showed even lower values (< 0.45) when correlated with morphometric variables. The same behavior was reported for Convallaria majalis (ERIKSSON, 1999) and Uapacarkiana (NGULUBE et al., 1999). In contrast, other studies showed a significant correlation between morphometric variables and germination (POZZOBONI et al., 2003; GHODDOSI et al., 2003) or seed dormancy, as observed to Styzolobium aterrimum (BARBEDO et al., 1988).

DISCUSSION

High values of repeatability indicate that it is possible to estimate a value close to the measurement of the total population with a small number of estimations (CRUZ and REGAZI, 1994). The efficiency of this methodology was noted by NETO et al. (2004) who achieved a repeatability...
TABLE 2: Mean, standard deviation (σ) and coefficient of variance (CV) for length, width, thickness and weight from 50 seeds per mother tree collected in Paraty (tree 1 to 10) or Miguel Pereira (tree 11 to 20). Each mean seed size variable followed by the same letter did not differ among mother trees at 5% of probability by Kruskal-Wallis test. Means followed by the same capital letter did not differ at 5% of probability by Kruskal-Wallis test among populations for the same traits.

TABELA 2: Valores médios e desvio padrão (σ) de comprimento (comp), largura (larg), espessura (espes) e peso (pes) medidos em 50 sementes por matriz, coletadas em Paraty (matriz 1 a 10) e Miguel Pereira (matriz 11 a 20). Cada média de tamanho de semente seguida pela mesma letra minúscula não difere entre árvores-matrizes em 5% de probabilidade pelo Teste de Kruskal-Wallis. Médias seguidas pela mesma letra maiúscula não difere em 5% de probabilidade pelo Teste de Kruskal-Wallis.

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<th>Tree</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Thickness (mm)</th>
<th>Weight (g)</th>
<th>Tree</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Thickness (mm)</th>
<th>Weight (g)</th>
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<td>29.16</td>
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<td>4.50</td>
<td>1.87</td>
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<td>16.12</td>
<td>4.22</td>
<td>1.74</td>
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<td>0.81</td>
<td>0.17</td>
<td>0.23</td>
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<td>1.03</td>
<td>0.80</td>
<td>0.18</td>
<td>0.13</td>
</tr>
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</table>

CV(%) 5.3 4.8 3.9 12.3 CV (%) 3.5 4.7 3.9 6.8

TABLE 3: Spearman’s correlation coefficient (r) among seed morphometry parameters from 20 trees of *Schizolobium parahyba* from Miguel Pereira or Paraty populations, RJ.

TABELA 3: Resultado da análise de correlação (r) entre as variáveis morfométricas da sementes de 20 matrizes de *Schizolobium parahyba* oriundas de Miguel Pereira e Paraty - RJ.

<table>
<thead>
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<th>Parameter</th>
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<th>Weight</th>
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<tr>
<td>Thickness</td>
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<td>-0.035</td>
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<tr>
<td>Weight</td>
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<td>0.727</td>
<td>0.420</td>
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<tr>
<td>Moisture content</td>
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<td>0.295</td>
<td>0.311</td>
<td>0.378</td>
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</table>
Intra-and inter-population variation in seed size and dormancy in *Schizolobium*...  

TABLE 4: Means and standard deviations for germination (%G), germination speed index (GI), percentage of normal seedlings (%NS) and percentage of dead seed (%D) calculated for scarified (T1) and non-scarified (T2) seeds collected from 20 trees from two different regions in the state of Rio de Janeiro. In each column, means with the same letter did not differ at 5% probability by LSD test.

<table>
<thead>
<tr>
<th>Seed quality variables</th>
<th>Paraty Non-scarified</th>
<th>Scarified</th>
<th>Miguel Pereira Non-scarified</th>
<th>Scarified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>σ</td>
<td>Mean</td>
<td>σ</td>
</tr>
<tr>
<td>%G</td>
<td>64.25a</td>
<td>15.65</td>
<td>66.75a</td>
<td>11.62</td>
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<tr>
<td>GI</td>
<td>1.78a</td>
<td>1.64</td>
<td>6.51b</td>
<td>1.07</td>
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<tr>
<td>%NS</td>
<td>22.75a</td>
<td>12.17</td>
<td>14.75b</td>
<td>8.84</td>
</tr>
<tr>
<td>%D</td>
<td>47.75a</td>
<td>10.69</td>
<td>85.25b</td>
<td>8.84</td>
</tr>
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</table>

TABLE 5: F values forhierarchical variance analysis for the effect of mother tree, treatment, site and interaction among treatment and site on seed quality variables.

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<tr>
<th>GL</th>
<th>%G</th>
<th>GI</th>
<th>%NS</th>
<th>%D</th>
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<tr>
<td></td>
<td>39</td>
<td>3.946**</td>
<td>12.368**</td>
<td>3.579**</td>
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<tr>
<td>Loc</td>
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<td>0.451</td>
<td>14.177**</td>
<td>9.426**</td>
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<td>Treat</td>
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<td>8.470**</td>
<td>259.647**</td>
<td>23.166**</td>
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<td>LxT</td>
<td>1</td>
<td>16.576**</td>
<td>43.006**</td>
<td>7.137**</td>
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<tr>
<td>M/L1</td>
<td>9</td>
<td>4.431**</td>
<td>5.614**</td>
<td>4.748**</td>
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<tr>
<td>M/L2</td>
<td>9</td>
<td>7.374**</td>
<td>5.557**</td>
<td>2.650**</td>
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<td>TxM/L1</td>
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<td>1.596</td>
<td>4.073**</td>
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<tr>
<td>TxM/L2</td>
<td>9</td>
<td>0.866</td>
<td>3.147**</td>
<td>2.112*</td>
</tr>
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</table>

Within 130  
Total 159

Where in: M = mother tree; Treat = treatment; L = all sites; L1 = Paraty; L2 = Miguel Pereira; L x T = interaction among treatment and site; %G = Germination; GI = germination speed index; %NS = percentage of normal seedlings; %D = percentage of dead seed; *significant at 5% probability; **significant at 1% probability by LSD Test.

coefficient of 99% forseed weightwhen comparing fruit size variables from 13 mother trees of *Platonia insignis* Mart. in a sample of 58 seeds. The high repeatability in seed size may be attributed to the maternal origin of the seed coat and, therefore, it does not depend on the possible number of fathers. The seed coat is an important constraint in seed size because it represents a physical barrier to gene expression of the cotyledonal tissue. Maternal effect on seed size was observed in *Phaseolus vulgaris* L., with inheritance higher than 65% (MESQUITA et al., 1990), and in *Arabidopsis thaliana* (ALONSO-BLANCO et al., 1999).

The population from the mountainous region hada larger seed size when compared with the population from the coastal region. This difference...
was significant for all studied morphometric variables, except length. Length was the only morphometric variable that did not differ between populations, with greater intrapopulation variation. The intra-population effect on seed length might indicate that this variable is related to individual genetic performance of the mother tree rather than related to environmental conditions. High values of interindividual variation (> 40%) in seed size have been reported for oak species (GOMEZ 2004), *Pinus halepensis* Mill. (NATHAN et al., 1996), *Cardiocrinum cordatum* (Thunb.) Makino (SAKAI et al., 1997), and *Lobelia inflate* L. (SIMONS and JOHNSTON, 2000). Whitney and Lister (2004) noted morphological changes in the seeds and fruits of *Acacia ligulata* A. Cunn. ex Benth. along a transect of 580 km in Australia, associating these changes to climate variables such as temperature and precipitation.

Ecologically speaking, larger seeds are more likely to successfully emerge and become established as seedlings (FENNER and THOMPSON, 2005) due to the so-called “reserve size” (LEISHMAN et al., 2000). However, our results suggest that the general assumption of a positive effect of seed size on germination or vigor did not occur for *Schizolobium parahyba* based on the low correlation between seed size and quality.

For *Schizolobium parahyba*, seed quality was an attribute influenced by differences between populations. Seeds from Miguel Pereira showed more vigor and less dormancy than seeds from Paraty (Table 6). Dormancy level variation has also been found between and within populations of *Plathymenia reticulata* Benth. and *Senna multijuga* (Rich.) Irwin et Barn. (LOVATO et al., 2004), *Carex canescens* L. (SCHUTZ and MILBERG, 1997), *Bromus tectorum* L. (BECKSTEAD et al., 1996) and four weed species from Sweden (ANDERSSON and MILBERG, 1998).

The most significant environmental difference between the two studied regions, in addition temperature and altitude, is the rainfall (Figure 2). Paraty has annual average rainfall of 2158 mm, raining from September to April, while Miguel Pereira has annual average rainfall of 1137 mm, with raining from October to March. Analysis of climate data over the last five years for the two regions reveals high irregularity of rainfall in the region of Paraty (179.85 mm ± 20.33) compared with that of Miguel Pereira (94.77 mm ± 6.76). Beckstead et al. (1996) suggested that *Bromus tectorum*’s populations arising from a favorable but unpredictable environment have a higher degree of dormancy than those located in an extreme but predictable environment. This has been supported by other authors (COHEN, 1966; VENABLE and BROWN, 1988). In contrast, Harel et al. (2011) demonstrated that weight and dormancy of dominant annual species distributed within a variable gradient of rainfall and dryness in Israel decreased with increased dryness and precipitation variability.

The difference in seed dormancy between the studied regions may be partly related to environmental conditions during the period preceding the harvest. Different rates of rainfall may influence seed dormancy and germination, as reported by PHILIPPI (1993) for *Lepidium lasiocarpum* Nutt. seeds. In fact, it rained more in Miguel Pereira than in Paraty in the month of harvest (July 2004), however, no significant difference in seed water content of either region was observed (Table 6). Thus, to confirm the differentiation pattern of seed size and quality between these populations,
it is necessary to continue the study for a longer period of time.

The high variation on intra and inter-population seed dormancy levels may have the greatest consequences on the understanding of germination (ANDERSSON and MILBERG, 1998). It is essential to consider this finding for developing seed technology studies with an appropriate seed sampling design. Careful considerations should also be taken when extrapolating data findings for each species.

CONCLUSIONS

Samples of 50 seeds per mother tree allowed to capture variability of 99% for length, 98.5% for width, 97.7% for thickness and 99.1% for weight. There was a great variation of the morphometric traits among individuals of the same population.

Trees from the mountainous region showed the highest seed size and lowest level of dormancy when compared with plants from the coastal region of Rio de Janeiro.

Seed length represented 60.9% of the total morphometric variation and it could more related to maternal effect than width, thickness or weight. Seed size did not influence seed germination and seedling development.

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