



# The effect of seed size on the efficacy of dormancy breaking treatments in *Leucaena leucocephala* (lam.) de Wit

## Efecto del tamaño de la semilla en la eficacia de los tratamientos de superación de la dormancia en *Leucaena leucocephala* (lam.) de Wit

Marana-Tá Campos-Bastos<sup>1\*</sup>, Wanessa Nepomuceno-Ferreira<sup>2</sup>, Maria Amanda-Menezes-Silva<sup>1</sup>

### Abstract

Biometric seed characteristics, such as size, mass, and moisture, can significantly influence germination potential and possibly the efficiency of dormancy-breaking treatments. *Leucaena leucocephala*, an invasive exotic plant native to Central America, has dormant seeds, a mechanism that prevents immediate germination. Although this species has been extensively studied, no research has yet linked biometric seed characteristics to dormancy-breaking. Therefore, the aim of this study was to evaluate the influence of *L. leucocephala* seed size on the effectiveness of different dormancy-breaking treatments. The width, thickness, and length of seeds from both treatments were measured using a digital caliper. The moisture content was then assessed. For the germination test, small (mean width = 3.94 mm; mean length = 6.36 mm) and large (mean width = 5.23 mm; mean length = 9.59 mm) seeds were separated into four treatments (chemical scarification with 98% sulfuric acid for 15 minutes (CS), manual scarification with sandpaper 100 (MS), hot water immersion at 80 °C for 10 minutes (HWI), and a control treatment (C). In terms of germination percentage, both small and large seeds performed better in the MS and HWI treatments. The germination speed index was higher for both seed sizes when treated with MS. When MS was used, longer seeds showed higher germination percentages, and seeds with higher moisture produced shorter radicles. Thus, the efficiency of dormancy-breaking treatments may differ between large and small seeds.

**Keywords:** biometry, dormancy breaking, exotic species, seed

### Resumen

Las características biométricas de las semillas, como el tamaño, la masa y el contenido de humedad, pueden influir en el potencial de germinación y en la eficiencia de los tratamientos de superación de la dormancia. *Leucaena leucocephala*, una planta exótica invasora nativa de América Central, presenta semillas con dormancia física que impide la germinación inmediata. A pesar de su amplia distribución y estudio, los trabajos que relacionan las características biométricas de

<sup>1</sup> Instituto Federal do Ceará, Brazil.

<sup>2</sup> Universidade Federal do Cariri, Brazil.

\* Corresponding author: marana.campos.bastos60@aluno.ifce.edu.br

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las semillas con la eficacia de los tratamientos de superación de la dormancia son limitados. El objetivo de este estudio fue evaluar la influencia del tamaño de las semillas de *L. leucocephala* en la eficacia de diferentes tratamientos de superación de la dormancia. Se midieron el ancho, el grosor, la longitud y el contenido de humedad de las semillas. Posteriormente, semillas pequeñas y grandes fueron sometidas a cuatro tratamientos: escarificación química con ácido sulfúrico al 98%, escarificación mecánica con lija, inmersión en agua caliente a 80 °C y un tratamiento control. Los resultados mostraron que, en términos de porcentaje de germinación, tanto las semillas pequeñas como las grandes presentaron mejor desempeño en los tratamientos de escarificación mecánica y agua caliente. El índice de velocidad de germinación fue mayor para ambas categorías cuando se aplicó la escarificación mecánica. Además, semillas más largas presentaron mayores porcentajes de germinación bajo este tratamiento, mientras que semillas con mayor contenido de humedad produjeron radículas más cortas. Estos resultados indican que la eficiencia de los tratamientos de superación varía según el tamaño de las semillas.

**Palabras clave:** biometría, especie exótica, semilla, superación de la dormancia

## INTRODUCTION

The biometric characterization of seeds provides ecological information about plants, allowing for the identification of morphological, physiological, and anatomical patterns based on seed variation (Souza & Cavalcante, 2019). This tool enables the detection of genetic variability (Silveira et al., 2019) and provides useful information for the exploration and conservation of species, facilitating their effective and sustainable use (Gonçalves et al., 2013).

Seeds can be classified by size or mass, with large seeds and those with higher masses being potentially more vigorous (Silva, 2021), as they contain greater amounts of stored material, higher levels of hormones and larger embryos, resulting in more vigorous seedlings (Alves et al., 2005; Dubal et al., 2017). The influence of seed size has been observed in studies by Dranski et al. (2019) and Ramos Filho and Bandeira Neto (2022), focusing on *Schizolobium parahyba* [(Vell.) S.F. Blake]] and *Myrciaria dubia* (Kunth) McVaugh seeds, respectively, species characteristic of the early stages of succession. In both species it was found that large seeds were more vigorous, germinating more rapidly and with a higher percentage germinating overall.

On the other hand, other studies suggest that small seeds require less water and tend to germinate more quickly (Krzyzanowski et al., 2020; Vanzolini & Nakagawa, 2007). When evaluating size as a key factor in the germination and development

of seedlings of *Copaifera langsdorffii* Desf., Souza and Fagundes (2014) found that small seeds not only germinated faster, but also had a higher germination percentage (80%) compared to large seeds (64.4%). According to Baskin and Baskin (1998), small seeds generally germinate more rapidly, providing a competitive advantage, especially in the early stages of succession.

In general, there is a direct relationship between seed size and coat thickness, and an inverse relationship between seed size and water absorption capacity (Beninger et al., 1998). An increase in seed size results in a reduced surface-to-volume ratio, leading to lower relative water absorption capacity and delayed germination (Fowler & Bianchetti, 2000). Thus, small seeds have thinner coats and a larger surface area, a characteristic that allows for greater permeability, resulting in shorter germination periods (Dolan, 1984). Conversely, some studies conducted on legumes have identified that small seeds exhibit a greater impermeability of the seed coat (Nimer et al., 1983; Souza et al., 1996).

It has also been reported that large seeds have a higher moisture content, facilitating germination (Dresch et al., 2013). The relationship between moisture content and germination was observed in *Plathymenia reticulata* Benth and *Stryphnodendron adstringens* (Mart.) Coville, where low moisture levels decreased germination speed (Souza et al., 2021). However, in *Parapiptadenia rigida* (Benth.) Brenan, moisture content did not affect germination (Marangoni et al., 2014).

In the present study, we analyzed *Leucaena leucocephala* (Lam.) de Wit, commonly known as Leucaena, originating from Central America (Souza, 2019) but introduced to Brazil and widely distributed in the Northeast, Southeast and South regions of the country, with a higher occurrence in areas with anthropogenic land use and modified soils (Reis et al., 2022). *Leucaena leucocephala* is also quite common in resting areas in the Northeast (Fabricante et al., 2021). Currently, *L. leucocephala* is used for soil enrichment and improvement, providing shade for crops, controlling erosion, and used for animal feed and energy production, such as for firewood and charcoal (Drumond & Ribaski, 2010).

Exotic plants like *L. leucocephala* generally exhibit high plasticity, increasing their chances of adapting to new environmental conditions, allowing them to make the most of available resources (Funk, 2008; Stencil et al., 2016). Furtado (2019), in analyzing the physiological characteristics of *L. leucocephala* seeds, observed significant plasticity in response to different temperature gradients. As an invasive exotic plant, it is important to identify and understand the relationships and patterns it may have. Thus, understanding the influence of seed size on germination and dormancy-breaking treatments might aid in predicting its spread and contribute to the development of more effective control and management methods.

The *L. leucocephala* seeds are elliptical, flattened, shiny, brown in color and thrive in well-drained soils (Drumond & Ribaski, 2010). Overcoming physical dormancy is necessary for germination in this species, as it is considered a hard seed (Melo et al., 2022). According to Mendonça et al. (2020), when analyzing dormancy-breaking of *L. leucocephala* seeds using chemical and physical methods, mechanical scarification yielded the highest germination percentage. In another experiment, control seeds that underwent no dormancy-breaking methods exhibited a relatively low emergence percentage compared to those treated with mechanical and chemical scarification using concentrated sulfuric acid and hot water (Melo et al., 2022).

In general, research aimed at determining the best dormancy-breaking treatments do not assess their relationship with seed size. However, considering that germination parameters, seed

coat impermeability, and moisture content can vary according to seed size, the hypothesis was raised that the efficiency of a dormancy-breaking treatment may differ between large and small seeds. Therefore, the objective of this study was to evaluate the influence of seed size on the effectiveness of different dormancy-breaking methods.

## MATERIALS AND METHODS

### Characterization and preparation of botanical material

The *Leucaena leucocephala* seeds have a high germination potential (Dantas et al., 2021), with optimal temperatures ranging from 20 to 30 °C (Alves & Almeida, 2020) and a photoperiod of 12 hours (Ferreira et al., 2018). Seeds were collected at the Riacho Verde site in the municipality of Deputado Irapuan Pinheiro, Ceará, Brazil. The average temperature in the municipality ranges from 26 to 28 °C, featuring a Hot Semi-Arid Tropical climate with annual rainfall between 717.2 and 748.5 mm, occurring mainly from February to April (IPECE, 2017).

Seeds were collected from the ground, under different parent plants, at two different times: the first collection yielded large seeds (November 2022), and the second collection yielded small seeds (December 2022), leading to two separate treatments. Seeds were stored in closed containers in the laboratory to prevent moisture loss and contamination until experiments were conducted.

Seeds from the first collection were visually larger, whereas those from the second collection were visibly smaller. Based on this observation, the lots were classified as large and small seeds, and each dormancy treatment was applied according to this separation. In other words, treatments were performed considering the visual size of the seeds, from smaller to larger, consistent with the observed differences between the lots.

After manual counting, measurements of width, thickness and length were taken using a digital caliper. Subsequently, a pre-statistical test (independent t-test) was conducted to confirm the observed visual differences between seed lots. Follow-up moisture content testing was performed: seeds were weighed, placed in an oven at 105±3

°C for 24 hours, then weighed again (Brasil, 2009). One hundred large seeds and 100 small seeds were used, corresponding to the number of seeds for each applied treatment.

## Experimental setup

The treatments used to evaluate the effects of different techniques for overcoming dormancy and the influence of seed size and moisture on *Leucaena leucocephala* germination included chemical scarification with 98% sulfuric acid for 15 minutes (CS), manual scarification with sandpaper #100 (MS), immersion in 80 °C water for 10 minutes (HWI), and a control treatment (C) where the seeds underwent no scarification method; treatments were applied to both large and small seeds, resulting in a total of eight treatments. The experimental design was completely randomized, consisting of eight treatments with 100 seeds each, distributed into four replicates of 25 seeds.

After being washed and dried, seeds were placed in sterilized Petri dishes lined with double layers of germitest paper moistened with distilled water in a volume corresponding to 2.5 times the weight of the paper (Ministério da Agricultura, 2009). Petri dishes were transferred to a B.O.D (Biological Oxygen Demand) chamber regulated with a 12-hour photoperiod at a constant temperature of 30 °C ± 1 °C, considered optimal for the species (Felix et al., 2018). To reduce pathogen contamination, seeds were relocated to freshly prepared Petri dish every two days.

Evaluations were conducted every 24 hours, and seeds were considered germinated when the radicle reached 2 mm in length. This allowed for the determination of: Germination percentage (GP), germination speed index (GSI) and mean germination time (MGT). Germination percentage (GP) considers the total number of germinated seeds according to the previously established criterion and the total number of seeds sown ( $GP = (G/A) \times 100$ ), where G represents the number of seeds that reached the germination stage defined in the experiment and A corresponds to the total number of seeds evaluated. The Germination Speed Index (GSI) takes into account the daily number of germinated seeds and is calculated using Maguire's formula:  $GSI = \Sigma(G_i/N_i)$  where  $G_i$  is the number of seeds germinated in the i-th count and  $N_i$  is the number of days that have elapsed

since sowing. Thus, germination that occurs earlier receive greater weight in the index. The Mean Germination Time (MGT) is determined according to Baskin and Baskin's formula ( $MGT = \Sigma(n_i \cdot t_i) / \Sigma n_i$ ), considering the number of emerged seedlings between each count and the average time between the beginning of emergence and the n-th count, where  $n_i$  corresponds to the number of seedlings that emerged between two consecutive counts and  $t_i$  is the average time, in days, associated with this interval (Baskin & Baskin, 2014; Ferreira, 2011; Maguire, 1962). At the end of the final count, the length of the radicle was measured using a digital caliper with a precision of 0.01 mm (Rosa et al., 2011).

## Data analysis

To evaluate differences in biometric characteristics, moisture content, and germination data for the same dormancy-breaking method between seed lots (large and small), an independent t-test was applied. For the germination parameters from the different pre-germinative treatments, the data for large and small seeds were first subjected to the Shapiro–Wilk normality test and Bartlett's test for homogeneity of variances.

Data meeting the assumptions of normality and homoscedasticity ( $p > 0.05$ ) were analyzed using a two-way Analysis of Variance (ANOVA), and the means were compared using Tukey's test at a 5% significance level. Data that did not meet normality ( $p < 0.05$ ) were analyzed using the Kruskal–Wallis test; when significant differences were detected ( $p < 0.05$ ), group comparisons were carried out using the Mann–Whitney test. Statistical analyses were performed using PAST software (version 4.03; Hammer et al., 2001).

Graphs and visual representations of the data were created using GraphPad Prism software (version 10.6.1; GraphPad Software, San Diego, CA, USA), employing confidence interval bars and indicating the statistical differences among treatments.

## RESULTS AND DISCUSSION

### Biometric seed characterization

Statistical analysis of width, length, thickness and moisture confirmed significant differences between large and small seeds. Large seeds had an

average width of 5.23 mm while the small seeds measured 3.94 mm ( $T=28.333$ ;  $p=1.2796E-7$ ). The average length of the larger seeds was 9.59 mm compared to 6.36 mm for small seeds ( $T=24.771$ ;  $p=2.8483E-7$ ). Additionally, the average thickness of the large seeds was 1.63 mm whereas the small seeds averaged 1.25 mm ( $T=3.8732$ ;  $p=0.0082357$ ) (Table 1).

Seed polymorphism, as observed in the results for the studied species, is an ecological strategy. Seed size used by each species reflects a trade-off between the current needs of the population. If there is a need for dispersal, small seeds are favored, while large seeds are advantageous for establishing seedlings (Malavasi & Malavasi, 2001).

Moisture content was also statistically analyzed for the two seed samples, revealing that the large seeds had an average moisture content of 5.49% while 6.60% for small seeds ( $T=-1.0815$ ;  $p=0.32102$ ), showing no significant difference in moisture between the two samples (Table 1). Similar results were reported by Nagaoka et al. (2005), who noted little variation in moisture content between different *Ricinus communis* L seed sizes. Conversely, Oliveira and Bosco (2013) found that moisture content varied with different *Copernicia hospita* Martius seed sizes.

The low moisture observed might reduce metabolic activity, prolonging seed viability (Fowler &

Bianchetti, 2000) regardless of size. According to Botezelli et al. (2000), *Dipteryx alata* Vogel seeds, a legume commonly known as “baru”, exhibited moisture levels between 6.14% and 8.25%, similar to those found in the present study, yet still showed high germination percentages, concluding that moisture did not influence germination. Prolonging seed viability is beneficial for pioneering species that are effective colonizers. However, in the context of biological invasion potential, as with *Leucaena* sp., prolonged seed viability is detrimental to the environment due to its significant competitive ability and the loss of local biodiversity (Zenni, 2010).

### Seed germination under different treatments

There was a significant effect of seed size on germination percentage (%G) ( $F = 32.22$ ,  $p < 0.001$ ). Dormancy-breaking treatments also showed a significant effect ( $F = 263.29$ ,  $p < 0.001$ ). In addition, a significant interaction was observed between the factors ( $F = 28.77$ ,  $p < 0.001$ ), indicating that the effect of dormancy-breaking treatments depends on seed size (Table 2). Germination speed also showed similar results (Germination speed:  $F = 77.79$ ,  $p < 0.001$ ; dormancy-breaking treatments:  $F = 279.2$ ,  $p < 0.001$ ; interaction between the factors:  $F = 17.37$ ,  $p < 0.001$ ).

These findings confirm the hypothesis that the

**Table 1.** Descriptive statistics (mean, minimum, maximum, and 95% CI) of size, width, length, and moisture content of small (SS) and large (LS) *Leucaena leucocephala* (Lam.) de Wit seeds.

Treatment	WIDTH (mm)	LENGTH (mm)	THICKNESS (mm)	MOISTURE (%)	
LS	Mean	5.2321 <sup>a</sup>	9.5989 <sup>a</sup>	1.6361 <sup>a</sup>	5.4925 <sup>a</sup>
	Min	4.1	8	1.02	4.33
	Max	6.3	10.77	2.25	8.35
	Lower conf.	5.1621	9.4839	1.5951	3.5975
	Upper conf.	5.3013	9.7133	1.6755	6.57
SS	Mean	3.945 <sup>b</sup>	6.363 <sup>b</sup>	1.2515 <sup>b</sup>	6.6 <sup>a</sup>
	Min	3	4.4	0.8	6
	Max	4.7	9.3	1.5	7.66
	Lower conf.	3.879	6.213	1.215	5.87
	Upper conf.	4.012	6.511	1.29	7.1

SS - small seeds, LS - large seeds. Lowercase letters indicate the results of comparisons between the treatments of large and small seeds.

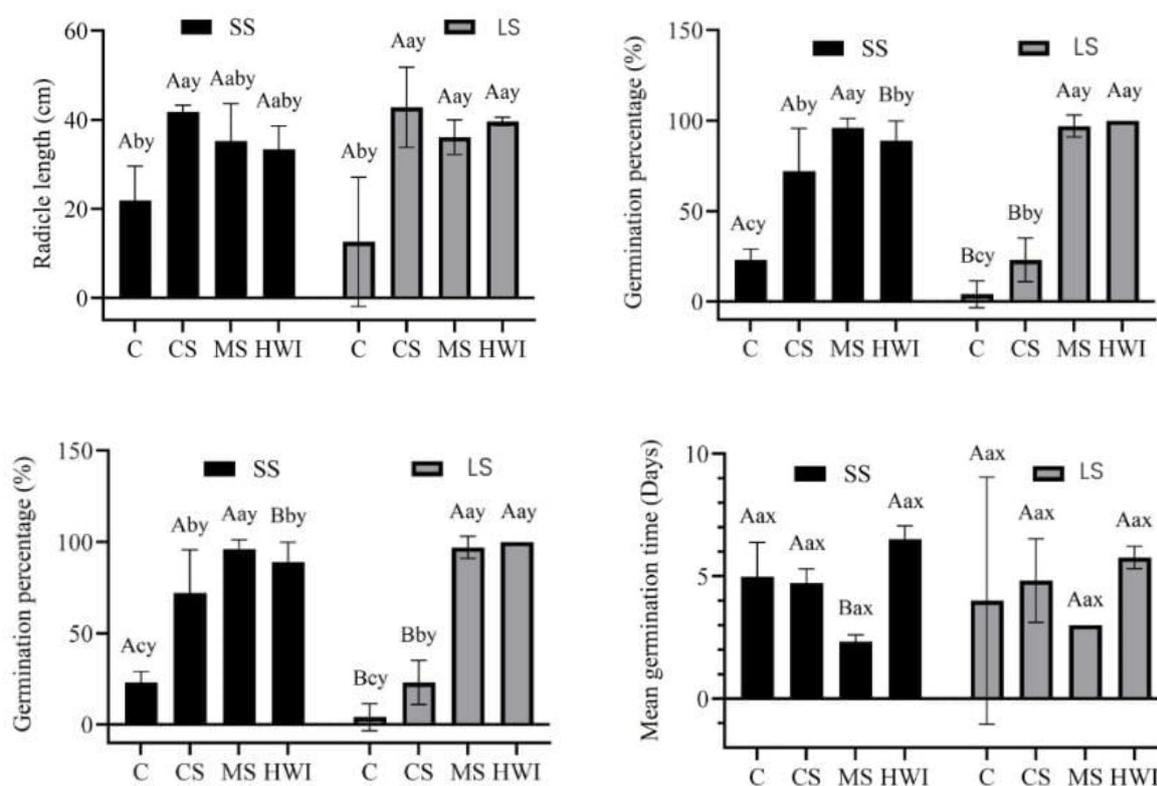


MS (Figure 1). Nevertheless, there was a statistical difference in germination rates. Treatments with the highest speed, which germinated the fastest, were those of small and large seeds with MS. The use of sandpaper leads to the seed coat being worn off, providing conditions for faster water absorption and the initiation of germination (Fowler & Bianchetti, 2000; Gugé et al., 2021).

When comparing different sizes for the same scarification method, germination speed was higher in small seeds than large seeds when subjected to control, chemical, and mechanical treatments. Thus, it is evident that small seeds were more likely to germinate and did so faster. Although some authors discuss the efficacy of large seeds, these results contradict various findings, as larger seeds have a greater amount of stored nutrients

and therefore rely less on dormancy (Mota et al., 2019; Padua et al., 2010; Rees, 1996). In general, the lowest speeds were found in small seed control treatments and in the control and CS treatments of large seeds (Figure 1), even though the species in question is resistant and does not require much to germinate.

Regarding radicle length, it was observed that, contrary to the hypothesis, treatment efficiency was not affected by seed size. However, significant differences were recorded between treatments within each size category. Treatments that resulted in the longest radicles were those with CS, for both large and small seeds. The shortest radicles were found in the control treatments, for both seed sizes (Figure 1).



**Figure 1.** Germination percentage, Mean germination time, Germination speed index and Radicle length of small (SS) and large (LS) *Leucaena leucocephala* seeds based on different dormancy breaking treatments. C: control, CS: chemical scarification in concentrated sulfuric acid, MS: mechanical scarification with sandpaper #100, HWI: immersion in hot water. Lowercase letters represent the results of comparisons between dormancy-breaking treatments for large and small seeds. Different uppercase letters indicate statistical differences for the same treatment observed for large and small seeds. Letter x indicates results subjected to the Anova test; letter y indicates results subjected to the Kruskal-Wallis test. All graphs include confidence intervals for each treatment.

## FINAL CONSIDERATIONS

It is concluded that seed size influences the efficiency of some dormancy-breaking treatments. In terms of germination percentage, small seeds showed better results in the control treatment and in chemical scarification (CS), while large seeds performed better in hot water immersion (HWI) and did not differ in mechanical scarification (MS). MS influenced mean germination time of large seeds, but no significant differences were observed in the other treatments. The germination speed index was higher for small seeds in the control, MS, and CS treatments, with no differences in hot water scarification. Radicle length showed no differences among treatments when large and small seeds were compared. In correlation analyses, longer seeds subjected to chemical scarification showed lower percentages and germination speed. Conversely, wider seeds exhibited a longer mean germination time. With manual scarification using sandpaper, longer seeds had a higher percentage of germination, while more humid seeds resulted in smaller radicles.

Based on this study, future research is suggested to evaluate the influence of seed biometrics on the germination of other *L. leucocephala* populations, and to assess the biometrics of seeds from other species. Studying seed biometrics provides valuable information for understanding species behavior, and mode of dispersal and germination. It is important to emphasize the study of seeds from exotic species, as they can harm native species and ecosystem stability.

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## AUTHOR CONTRIBUTION

Marana-Tá Campos-Bastos: Methodology, Data collection, formal analysis, and writing. Wanessa Nepomuceno-Ferreira: Conceptualization and writing. Maria Amanda-Menezes-Silva: Conceptualization, writing, supervision, and funding acquisition.

## CONFLICT OF INTEREST AND ETHICS STATEMENT

The authors declare that they have no conflict of interest.

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