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Temperature and pre-germinative treatments for overcoming *Acacia farnesiana* (L.) Willd. (Fabaceae) seeds dormancy

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***Acacia farnesiana* L. is a species known for its uses in recovery of degraded pastures, animal feeding, medical and fungicide properties. However, it is one of the most problematic invasive species in agriculture, due to the little known dormancy aspects of its seeds that results in the propagation and dispersion to distinct areas and the establishment of the invasive plant. The knowledge on ecophysiological characteristics of invasive species seeds aiming at the dormancy process is important for the comprehension of aggressive regeneration unities, and allows the development of strategies against infestation of new areas and reduction of soil seed banks. The objective of this research was to assess how *A. farnesiana* seeds overcome dormancy using different temperatures and pre-germinative treatments. The study was conducted in Federal University of Paraiba, using seeds obtained from fruits of ten matrix trees in Paraiba State, Brazil. The completely randomized design was adopted, with treatments arranged in a 3 x 15 factorial scheme representing temperatures and pre-germinative treatments with four replicates. Parameters related to germination percentage, germination and emergency index were assessed, with best results observed in seeds scarified with sandpaper 80 followed by imbibitions of water at environmental temperature (25-30°C) for 24 h.**

Key words: Germination, invasive species, ecophysiological characteristics, dormancy process.

INTRODUCTION

Acacia farnesiana L. Willd. is a bush plant which belongs to the Fabaceae family, characterized by its height that might reach 4 m, thorny aspect and continuous flowering and fruiting, that results in an annual yield of 13 thousand

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seeds per plant (Camacho et al., 2012), which germinate in river beds and wetter regions of lowlands (Dias et al., 2008). This species is an important option for recovery of degraded pastures, whilst its fruits can be used for animal feeding (Erkovan et al., 2013) and its leaves can be explored due to its antifungal properties and for phytotherapy medicines production (Kingsley et al., 2014).

Besides its beneficial properties, this plant is considered as one of the worst invasive plant species in the world (Camacho et al., 2012) because of its seeds dormancy break by animals (endozoochory), that influences the dispersion and propagation to different areas and contributes to the formation of new populations of this invasive species (Arévalo et al., 2010). The knowledge of ecophysiological characteristics related to the seed dormancy process of invasive species is important for the understanding of aggressive regeneration unities, and might be useful for the development of strategies to control invasive populations, reduce soil seed banks and the infestation of new areas (Martins et al., 2013a).

Tropical and subtropical forestry species frequently have viable seeds that do not germinate even in favorable environmental conditions. This is explained by the dormancy process, which evolved as a surveillance mechanism that allows physical and temporal distribution of germination in different environments (Camacho et al., 2012).

The overcoming of dormancy in seeds is regulated by several factors, but temperature is one of the most important by promoting the rupture of their coat, raising their permeability to water and gases, which are essential for germination metabolism (Martins et al., 2008). For germination tests, temperature is employed under certain limits, marked by an optimal singular value or temperature intervals, in which the germination rate occurs at max efficiency (Martins et al., 2013b).

Similarly, some seeds from *Acacia* spp. also have dormancy (Rodrigues et al., 2008), specially related to the impermeability of the coat (Martins et al., 2012). However, there are available treatments to overcome this kind of dormancy in *Acacia* spp., but the proper moment of application, the recommended temperature and the most efficient pre-germinative treatments depend on the species (Martins et al., 2008). Smiderle et al. (2005) found the best germination results for *Acacia longifolia* (Andrews) Willd through thermal scarification at 100°C for 1 min followed by germination at 25°C, while Rodrigues et al (2008) noticed that *Acacia mangium* Willd. seeds presented highest germination values after chemical scarification with H_2SO_4 for 90 min; Escobar et al. (2010) and Tapia et al. (2013) reported highest values for *Acacia caven* (Mol.) Mol. after abrasion with sandpaper and germination at 30°C.

Due to the diversity of the dormancy process related to the variability of germination, invasive forestry species is hard to predict infestations. For this reason, the knowledge of specific characteristics linked to the germination process is indispensable (Erkovan et al., 2013) and there is a lack of information on *Acacia farnesiana* (L.) Willd seeds. This research aimed to evaluate the effect of different temperatures and pre-germinative treatments on the dormancy break of *A. farnesiana* seeds.

MATERIALS AND METHODS

Seed acquisition

The present work was conducted at the Seed Analysis Lab (SAL) from the Crop Production and Environmental Sciences Department of the Federal University of Paraiba, located in Areia, Paraiba state, Brazil. Seeds from *Acacia farnesiana* were obtained from mature fruits harvested from ten matrix trees in Souza City, Paraiba State, Brazil. After the harvest, the fruits were kept in polyethylene bags and taken to the laboratory, pulped manually for the removal of seeds, and then homogenized.

Seed moisture content and pre-germinative treatments

The water content was determined using an oven at 105 ± 3°C, for 24 h (BRASIL, 2009), considering four replicates of 25 seeds per matrix tree.

A. farnesiana seeds with an initial moisture content of 10% was subjected to the following pre-germination treatments: Thermal scarification – immersion in water at 60°C (T_1: Immersion water 60°C), 70°C (T_2: Immersion water 70°C), 80°C (T_3: Immersion water 80°C), 90°C (T_4: Immersion water 90°C) and 100°C (T5: Immersion water 100°C) for 1 min.

Chemical scarification – seeds were immersed in absolute sulfuric acid (H_2SO_4) for 10 (T_6: Sca. sulfuric acid 10') and 20 min (T_7: Sca. sulfuric acid 20'), with and without imbibition for 12 h (T_8: Sca. sulfuric acid 10', imb. 12 h), (T_9: Sca. sulfuric acid 10', imb. 12 h) or 24 h (T_{10}: Sca. sulfuric acid 10', imb. 24 h), (T_{11}: Sca. sulfuric acid 10', imb. 24 h) at environmental temperature (25 ± 1°C), after washing with running water for 5 min.

Mechanical scarification

Seeds were rubbed manually with sandpaper no. 80 at the opposite side of the micropile to avoid damaging the embryo, without (T_{12}: Sca. Sandp.) and with imbibition for 12 (T_{13}: Sca. Sandp., imb.12h) and 24 h (T_{14}: Sca. Sandp., imb. 24 h) at environmental temperature. In Check or control treatment (T_{15}: intact seeds), seeds were not exposed to any treatments.

Physiological treatments

Germination test was carried out in germination chambers adjusted to 20-30, 25 and 30°C with a 12 h photoperiod provided by fluorescent lamps (4 x 20 W), using four replicates of 25 seeds per treatment. Following the pre-germinative treatments, seeds were subjected to disinfection with the fungicide captan, then sown on rolls of humidified paper towel. The assessments were conducted through daily counts from the second to the eighth day after sowing, adopting the concept of normal seedlings determined by Brasil (2009).

Emergency test was performed in a greenhouse (average

temperature of 30°C and relative humidity towards 80%, by the time of the evaluations), with four subsamples of 25 seeds, previously. Before sowing in plastic seed trays (49 x 33 x 7 cm), the seeds received chemical treatment with the fungicide captan, and then disposed in sand previously autoclaved. The reposition of substrate moisture was done regularly. From the third to the twelfth day after sowing, the number of emerged seedlings with the hypocotyl above the substrate (Martins et al., 2013b) was computed daily.

At the end of the experimental setup, the first count, the germination and emergency percentage were determined. The germination and emergency speed indexes were also calculated, following the methodology suggested by Martin et al. (2013).

Experimental design and statistical analysis

It was a completely randomized design, with the treatments arranged in a 3 x 15 factorial scheme (temperatures and pre-germinative treatments, respectively), with four replicates. Data were submitted to analysis of variance, using F test for comparison of mean squares, and the means were compared by Anova and Scott-Knott test, with a 95% confidence limit.

RESULTS AND DISCUSSION

The best results relative to germination percentage and first count (Table 1) were observed at the seeds scarified with sandpaper no. 80 (T_{12}) at 30°C and scarified with sandpaper no. 80 followed by imbibition for 24 h (T_{14}) at 25 and 30°C. However, considering percentage of germinated seedlings apart from the temperatures, these treatments did not present significant difference from the seeds immersed in sulfuric acid for 10 and 20 min with 24 h of imbibition of water (T_{10} and T_{11}, respectively) and from the seeds submitted to chemical scarification and 12 h of imbibition of water (T_9: Sca. sulfuric acid 10', imb. 12h) at 30°C. It is also noticeable that thermal scarification treatments (T_1: immersion water 60°C and T_2: immersion water 70°C) implied in lower values of germination of *A. farnesiana* seeds.

The results (Table 1) prove that the treatment with sandpaper, despite being viable only for little amounts of seeds (Martins et al., 2008), promotes superior efficiency in the overcoming dormancy of *A. farnesiana* seeds as compared to the others, because it results in higher germination percentage and first count values. Similar results were found through scarification with sandpaper no. 80, with and without posterior imbibition of *Acacia mearnsii* Willd.(Roversi et al., 2002) and *Acacia caven* (Mol.) Mol. (Escobar et al., 2010; Tapia et al., 2011), with germination rates at 30°C. In the same way, *Apeiba tibourbou* Aubl. (Guedes et al., 2011) and *Cassia fistula* L. (Guedes et al., 2013) seeds submitted to mechanic scarification with sandpaper, with and without imbibition in water for 24 h and set to germinate at 30°C presented a larger number of normal seedlings at first count.

It was also noticeable that there was a significant interaction between the evaluated temperatures and the scarification with H_2SO_4 for 10 and 20 min followed by imbibition of water for 24 h. This combination was also effective to overcome *A. farnesiana* seeds dormancy probably by causing necessary ruptures at their coat, raising their permeability to water and gases, demanded factors for the germinative metabolism. These results corroborate with the ones reported for *Ormosia nitida* Vog. (Lopes et al., 2006), *Caesalpinia pyramidalis* Tul. (Alves et al., 2007), *Merremia aegyptia* L. (Pereira et al., 2007), with seeds scarified by H_2SO_4, as well observed in *Caesalpinia leiostachya* (Benth.) Ducke. (Biruel et al., 2007), *Adenanthera pavonina* L. (Rodrigues et al., 2009) and *Piptadenia moniliformis* Benth. (Azeredo et al, 2010) seeds treated for 20 min with sulfuric acid.

Analyzing the germination rates, it is possible to infer that *A. farnesiana* seeds are adapted to wide thermal amplitude, as germination succeeded both in constant and alternated temperatures. This statement is also confirmed by studies with other species of this gender, like *Acacia polyphylla* DC. (Araújo neto et al., 2003), *A. mangium* Willd. (Rodrigues et al. 2008) and *A. caven* (Mol.) Mol. (Escobar et al., 2010), where maximal germination results were reached at various temperatures (25, 30 and 20-30°C). According to Azeredo et al. (2011), these results illustrate the ability of adaptation and distribution of these species to a range of habitats, granting best chances to support adverse environmental conditions.

For germination speed index (GSI), the best results were found for the treatments with sandpaper, with and without imbibition of water (T12: Sca. Sandp. And T13: Sca. Sandp. Imb. 12h, respectively), at 25 and 30°C, not differing from the treatments with chemical scarification followed by imbibition of water for 12 (T_9: Sca. sulfuric acid 10', imb. 12h) and 24 h (T_{11}: Sca. sulfuric acid 10', imb), at 30^{O}C (Table 2). Chemical and mechanic scarification caused fissures on the seeds testa of this study, which allows the absorption of water and oxygen (Martin et al., 2008), enzymatic activation and energetic reserves hydrolysis, resulting in cell division and elongation and a faster and more constant germination (Albuquerque et al., 2009).

Such data were obtained with chemical and mechanic scarification, assessed at 25 and 30°C in *Caesalpinia ferrea* Mart. ex Tul (Medeiros filho et al., 2005), *A. mangium* Willd. (Rodrigues et al., 2008), *Adenanthera pavonina* L. (Costa et al., 2010) seeds and in *A. caven* (Mol.) Mol. propagules (Escobar et al., 2010).

The emergency results (Table 3) had the same tendency of the germination values. Higher percentages measured through the number of normal seedlings (82%) and first count (65%) were associated with mechanically scarified seeds (T_{14}: Sca. Sandp., imb. 24h). Nonetheless, the treatment with sandpaper with (T_{13}: Sca. Sandp., imb.12 h) and without (T_{12}: Sca. Sandp.) water imbibition were as well linked to greater emergency percentages in this study. T_{13} and T_{14} also contributed to the best values of emergency speed index (ESI), while the treatments with immersion in water at 60°C (T_1: immersion water 60°C)

Table 1. *A. farnesiana* seed germination percentage and first count due to different pre-germinative treatments.

Treatments	Temperatures (°C)					
	Germination (%)			First count (%)		
	20-30	25	30	20-30	25	30
T_1	0^{gA}	$0^{há}$	0^{fA}	0^{dA}	0^{dA}	0^{gA}
T_2	4^{gA}	$0^{há}$	4^{fA}	0^{dA}	0^{dA}	0^{gA}
T_3	2^{gA}	6^{gA}	0^{fA}	0^{dA}	0^{dA}	0^{gA}
T_4	5^{gA}	5^{gA}	4^{fA}	0^{dA}	0^{dA}	0^{gA}
T_5	10^{fC}	17^{eB}	24^{dA}	0^{dA}	0^{dA}	0^{gA}
T_6	9^{fC}	35^{cB}	45^{cA}	0^{dA}	0^{dA}	0^{gA}
T_7	41^{eA}	27^{dB}	17^{eC}	0^{dA}	0^{dA}	0^{gA}
T_8	83^{cA}	84^{bA}	81^{bA}	0^{dA}	0^{dA}	0^{gA}
T_9	91^{bA}	87^{bB}	95^{aA}	14^{cB}	4^{dC}	73^{cA}
T_{10}	98^{aA}	96^{aA}	96^{aA}	0^{dB}	0^{dB}	39^{eA}
T_{11}	95^{aA}	93^{aA}	95^{aA}	37^{bB}	20^{cC}	60^{dA}
T_{12}	61^{dB}	89^{bA}	93^{aA}	19^{cC}	76^{aB}	85^{aA}
T_{13}	13^{fA}	12^{fA}	15^{eA}	1^{dB}	1^{dB}	10^{fA}
T_{14}	78^{cB}	96^{aA}	97^{aA}	46^{aB}	79^{aA}	84^{aA}
T_{15}	83^{cA}	85^{bA}	80^{bA}	40^{bC}	69^{bB}	79^{bA}
CV (%)		8,04			10,16	

Means followed by the same lower case letter within a column and by the same upper case letter within a row are not significantly different by Scott-Knot test (P > 0.05). T_1: Immersion water 60°C; T_2: Immersion water 70°C; T_4: Immersion water 90°C; T5: Immersion water 100°C; T_6: Sca. Sulfuric Acid 10';T_7: Sca. Sulfuric Acid 20'; T_8: Sca. Sulfuric Acid 10', imb. 12h; T_9: Sca. Sulfuric Acid 10', imb. 12h; T_{10}: Sca. Sulfuric Acid 10', imb. 24h; T_{11}: Sca. Sulfuric Acid 10', imb. 24h; T_{12}: Sca. Sandp.; T_{13}: Sca. Sandp., imb.12h; T_{14}: Sca. Sandp., imb. 24h; T_{15}: intact seeds.

Table 2. Germination speed index of *A.farnesiana* seeds under different pre-germinative treatments.

Treatments	Temperatures (°C)		
	20-30	25	30
T_1	0.00^{fA}	0.00^{gA}	0.00^{fA}
T_2	0.22^{fA}	0.08^{gA}	0.33^{fA}
T_3	0.06^{fA}	0.38^{gA}	0.00^{fA}
T_4	0.18^{fA}	0.19^{gA}	0.33^{fA}
T_5	0.39^{fA}	0.53^{gA}	1.09^{eA}
T_6	0.33^{fB}	1.66^{fA}	1.90^{dA}
T_7	2.48^{eA}	1.70^{fB}	1.10^{eB}
T_8	6.06^{cA}	5.63^{eA}	5.21^{cA}
T_9	7.02^{bB}	6.88^{dB}	11.04^{aA}
T_{10}	5.92^{cC}	8.15^{cB}	8.87^{bA}
T_{11}	9.28^{aB}	7.84^{cC}	10.44^{aA}
T_{12}	4.43^{dB}	10.84^{aA}	11.33^{aA}
T_{13}	0.73^{fA}	0.84^{gA}	0.61^{fA}
T_{14}	7.26^{bB}	10.98^{aA}	10.87^{aA}
T_{15}	8.83^{aB}	9.91^{bA}	9.00^{bB}
CV (%) 10,91			

Means followed by the same lower case letter within a column and by the same upper case letter within a row are not significantly different by Scott-Knot test (P > 0.05). T_1: Immersion water 60°C; T_2: Immersion water 70°C; T_4: Immersion water 90°C; T5: Immersion water 100°C; T_6: Sca. Sulfuric Acid 10';T_7: Sca. Sulfuric Acid 20'; T_8: Sca. Sulfuric Acid 10', imb. 12h; T_9: Sca. Sulfuric Acid 10', imb. 12h; T_{10}: Sca. Sulfuric Acid 10', imb. 24h; T_{11}: Sca. Sulfuric Acid 10', imb. 24h; T_{12}: Sca. Sandp.; T_{13}: Sca. Sandp., imb.12h; T_{14}: Sca. Sandp., imb. 24h; T_{15}: intact seeds.

and 70°C (T_2: immersion water 70°C) for 1 min proved to be inefficient in breaking *A. farnesiana* seed dormancy. Although, the scarification with sand paper revoked the seed dormancy, it is perceptible that a longer imbibition time (24 h) was able to cause gradual increase of the emergency speed registered by the first count and ESI. This method results in small damages at the coat region contrary to the micropile of the seed, improving its permeability to oxygen and water and benefiting imbibition and metabolic reactivation with consequent growth of the embryo axis (Martins et al., 2008). These results are in agreement with the others reported for *Sterculia foetida* L. (Santos et al., 2004), *Rollinia mucosa* (Jacq.) Baill (Ferreira et al, 2009) and *Myracrodruon urundeuva* (Fr.All.) (Guedes et al., 2009) seeds scarified with sandpaper and then exposed to water imbibition for 24 h.

In nature, *A. farnesiana* seeds can be overcome by environmental factors. Scarification may occur through acidification, when seeds are eaten by seed disperser animals, like bovids, equids and some rodent species (Camacho et al., 2012), besides soil microorganisms. Additionally, the weakening of the coat of this species is common through the synergic action of fires and floodings in its native environment, caused by the high temperature amplitude (Erkovan et al., 2013).

This way, correlating the data presented in this paper with *A. farnesiana* attractiveness to animal feed, vast seed production (Camacho et al., 2012) along with the

Table 3. Emergency, first count and emergency speed index of *A. farnesiana* seedlings from seeds subjected to different pre-germinative treatments.

Treatments	Emergency(%)	Firstcount(%)	ESI
T_1	0^g	0^f	0f
T_2	5^g	0^f	0.33^f
T_3	12^f	1^f	0.53^f
T_4	45^d	0^f	1.88^e
T_5	49^d	0^f	1.25^e
T_6	32^e	1^f	1.56^e
T_7	79^b	17^d	4.80^c
T_8	15^f	0^f	0.61^f
T_9	9^f	0^f	0.50^f
T_{10}	65^c	0^f	4.14^d
T_{11}	76^b	7^e	4.78^c
T_{12}	84^a	36^c	5.88^b
T_{13}	84^a	59^b	7.00^a
T_{14}	82^a	65^a	6.95^a
T_{15}	4^g	0^f	0.22^f
CV (%)	10.84	20.58	15.08

Means followed by the same lower case letter within a column and by the same upper case letter within a row are not significantly different by Scott-Knot test ($P > 0.05$). T_1: Immersion water 60°C; T_2: Immersion water 70°C; T_4: Immersion water 90°C; T5: Immersion water 100°C; T_6: Sca. Sulfuric Acid 10';T_7: Sca. Sulfuric Acid 20'; T_8: Sca. Sulfuric Acid 10', imb. 12h; T_9: Sca. Sulfuric Acid 10', imb. 12h; T_{10}: Sca. Sulfuric Acid 10', imb. 24h; T_{11}: Sca. Sulfuric Acid 10', imb. 24h; T_{12}: Sca. Sandp.; T_{13}: Sca. Sandp., imb.12h; T_{14}: Sca. Sandp., imb. 24h; T_{15}: intact seeds.

ways to overcome seed dormancy, it is possible to conclude that *A. farnesiana* seeds have a strong capacity to germinate in several habitats, invading and changing the structure and composition of environments and native pastures.

This fact has been by researchers and is a serious problem in savannah areas in Brazil (Erkovan et al., 2013), North America (Arévalo et al., 2010) and in semiarid areas in Australia (Erkovan et al, 2013), based on studies on the probability of invasion and establishment, reproductive potential and dispersion and germination of soil seed banks, respectively. Still, more detailed researches on *A. farnesiana* dormancy and its aspects related to aggressive unities regeneration, like reproductive potential in the presence of disperser animals and long distance dispersion are required for a more precise conclusion.

Conclusion

Scarification with sandpaper 80 followed by imbibition of water at environmental temperature for 24 h, associated with temperatures of 25 and 30°C during germination, can be employed with high efficiency to overcome the mechanical resistance of the coat and to promote germination of *A. farnesiana* seeds and seedlings emergency in a lesser time.

Conflict of Interests

The authors have not declared any conflict of interests.

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