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Overcoming dormancy and determining optimal temperature for slender serradella seed germination

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ABSTRACT - The objective of this study was to identify the most efficient method for overcoming coat-imposed dormancy and determine the optimal germination temperature for *Ornithopus pinnatus* seeds. Treatments to overcome dormancy were: intact seeds; immersion in hot water at 60 ºC, followed by soaking in the same water (unheated)/24 h; immersion in hot water at 90 ºC, followed by soaking in the same water (unheated)/24 h; mechanical scarification; chemical scarification, H_2SO_4/5 min; and chemical scarification, H_2SO_4/10 min. Percentages were calculated for germinated, abnormal, dormant, and dead seeds. Optimal germination temperatures were calculated using eight constant temperatures (0, 5, 10, 15, 20, 25, 30, and 35 °C), with 8 h of light and 16 h of darkness. Germination rate, frequency, and synchronization index of germination were used as parameters to establish optimum temperature for germination. A completely randomized experimental design was used, with twelve repetitions of 100 seeds per treatment. Data obtained were subjected to analysis of variance and Tukey's test at a 5% significance level. *O. pinnatus* seeds exhibit dormancy caused by the seed coat's impermeability to water. Mechanical scarification was efficient in promoting seed germination. The species is stenothermal, meaning it cannot tolerate significant temperature variations. The germination rate of seeds was linearly dependent on temperature. Synchronization of the germination process is greater in the optimum temperature range, established between 15 and 20 °C.

Key Words: germination rate, *Ornithopus pinnatus*, scarification, synchronization index of germination

Introduction

The *Ornithopus pinnatus* species is widely used among producers in the coastal region of Rio Grande do Sul State, Brazil, to enhance native pasture in areas containing wild *Pinus* sp, improve succession planting of irrigated rice crops, and recover degraded soil. Commonly known as slender serradela, it belongs to the family Fabaceae (sub-family Papilionoideae) and is a high-quality legume with good production of green fodder and dry matter from early winter to late spring (Sokoloff and Loch, 2005). When compared with other forage legumes, the species is tolerant to acidity and high aluminum contents and needs less phosphorous (Ovalle et al., 2006).

In most species of the sub-family Papilionoideae, timing and extent of seed germination are primarily controlled by physical seed dormancy induced by the development of an impermeable testa or seed coat (Taylor, 2005). This type of dormancy can be overcome through treatments that weaken or rupture the integument, allowing water absorption and the onset of germination (Mayer and Poljakoff-Mayber, 1989). Immersion in hot water for a few minutes, mechanical scarification with sandpaper, and chemical scarification with sulfuric acid (Brasil, 2009) are methods successfully used to overcome seed dormancy in species such as *Lotus subbiflorus* (Jacob Junior et al., 2004), *Trifolium riograndense* and *Desmanthus depressus* (Suñé and Franke, 2006), *Macroptilium lathyroides* (Vasconcelos et al., 2011), and *Trifolium glomeratum* (Martín and Guerrero, 2014).

Once the obstacle of dormancy is overcome, knowledge is needed of the effects of different temperatures and possible fluctuations that may occur in this period in order to determine maximum and minimum values, above and below which germination does not occur (Lopes and Franke, 2011). Temperature is particularly important in that it changes water absorption and chemical reaction rates, which trigger development, the transport of food reserves, and the re-synthesis of substances to the seedling (Marcos-Filho, 2005). Thus, temperature is responsible for both the germination rate and the final germination percentage (Martins et al., 2008; Passos et al., 2008).

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In an attempt to understand aspects related to germination, this study aimed to evaluate the effect of methods for overcoming dormancy and determine the optimum temperature for germination of *O. pinnatus* seeds.

Material and Methods

Slender serradela (*O. pinnatus*) seeds from the city of Mostardas (31º 06' 25"S and 50º 55' 16"W), Rio Grande do Sul, Brazil, were used.

Treatments to overcome dormancy were: intact seeds (this is a standard procedure, described in the Rules for Seed Testing, "RAS" (Brasil, 2009), whereby seeds are placed in a germination chamber, without treatment, to determine if they are dormant or not); immersion in water heated to 60 °C then left to cool (at 60 °C the heat source is removed, seeds are immersed, and then left to soak in the same water as it cools, for 24 h); and immersion in water heated to 90 °C then left to cool (at 90 °C the heat source is removed, seeds are immersed, and then left to soak in the same water as it cools, for 24 h). For the hot water treatments, a volume of water 10 times greater than that of the seeds was used; mechanical scarification: seeds are sanded with 180-grit sandpaper until the seed coat is visibly worn; chemical scarification: seeds are immersed in concentrated sulfuric acid (H_2SO_4) for 5 min, followed by washing under running water for 10 min; and chemical scarification: seeds are immersed in concentrated sulfuric acid (H_2SO_4) for 10 min, followed by washing under running water for 10 min.

After treatments to overcome dormancy, twelve replicates of 100 seeds were subjected to the germination test on a germitest paper moistened with distilled water 2.5 times the weight of non-hydrated paper, in transparent plastic boxes covered with a lid (11 × 11 × 3.5 cm), packed in a germinator at 15 °C. The temperature used for seed germination, date of first counting, and test duration followed recommendations for the species *O. compressus*, since *O. pinnatus* is not included in the RAS (Brasil, 2009). Determinations of total normal and abnormal seedlings, dead and tough seeds were performed using the criteria stipulated in the RAS (Brasil, 2009). Results were expressed as percentages.

For the determination of optimum temperature, eight constant temperatures were used (0, 5, 10, 15, 20, 25, 30, and 35 °C) with 8 h of light and 16 h of darkness. Light was provided by four fluorescent bulbs (20 w; 1060 lm) located inside the germinators. Germination was evaluated by scarifying twelve replicates of 100 seeds with 180-grit sandpaper on two sheets of blotting paper moistened with distilled water at 2.5 times the weight of dry paper. The germinated seeds were counted daily for 21 days, at which time they exhibited a primary root greater than or equivalent to 2 mm.

Percentage of germination, mean time, rate, and relative germination frequency were calculated using formulae proposed by Labouriau and Valadares (1976), as follows:

- Percentage of germination (%):

$$G = \left(\frac{N}{A}\right) \cdot 100,$$

in which G = percentage of germination; N = number of germinated seeds; and A = total number of seeds subjected to germination.

- Mean germination time (day^{-1}):

$$t = \frac{\left(\sum_{i=1}^{k} ni \cdot ti\right)}{\sum_{i=1}^{k} ni},$$

in which t = time from the start of the experiment to the i-th observation (day^{-1}); ni = number of seeds germinated at time i (not the accumulated number, but the number corresponding to the i-th observation), and k = last time of germination.

- Mean germination rate (days^{-1}.10^{-2}):

$$v = \frac{1}{t},$$

in which v = mean germination rate; and t = mean germination time

- Relative germination frequency (%):

$$f = \frac{ni}{\sum_{i=1}^{k} ni},$$

in which f = relative germination frequency; ni = number of seeds germinated at time i (not the accumulated number, but the number corresponding to the i-th observation); and Σni = total number of germinated seeds.

Given that seed germination is generally not perfectly synchronized, its variation over time can be quantified using a measurement known as the germination synchronization index (U). The difference between relative frequency distribution and the germination measurement is that, in the former, uneven germination can be observed (but not quantified) (Ranal and Santana, 2006).

- Synchronization index of germination (bits), according to Labouriau and Pacheco (1978):

$$U = -\sum_{i=1}^{k} fi \cdot \log_2 \cdot fi,$$

in which U = synchronization index of germination; fi = relative germination frequency; and $\log_2$ = base-2 logarithm.

The unit of the synchronization index is measured in bits. Each bit is a binary measure that counts "germinates" and "does not germinate". The transformation of germination frequencies (f_i) for each observation time (t_i) into a logarithm of the frequency at base 2 ($\log_2 f_i$) converts a binary measure (bit) into a weighted arithmetic mean. Thus, $\log_2 f_i$ is the weighted mean of each registered frequency, whose result is expressed as $f_i \log_2 f_i$ (Ranal and Santana, 2006). In interpreting the rate, the lower the U value, the more synchronized germination will be, regardless of the total number of seeds that germinate.

The experimental design was completely randomized, with twelve replicates per treatment (12 replicates of 100 seeds for the different methods to overcome dormancy and at different temperatures). The data were subjected to analysis of variance using the PROC GLM procedure from SAS software (Statistical Analysis System, version 9.1.3), and when differences were significant, the means were compared using Tukey's test at 5%.

The optimum range for maximum germination was estimated using the temperature intervals in which germination values were among the highest and overlapped the highest average germination rates and the lowest average germination time values and synchronization indices.

Results and Discussion

A significant effect (P<0.05) was observed on the treatments to overcome dormancy in *O. pinnatus* for all the parameters assessed (Table 1). Seeds not subjected to any treatment showed greater dormancy potential, demonstrating the need for a method to overcome it in this species.

Germination was higher in seeds subjected to mechanical scarification (83%) and did not differ statistically from the treatment with H_2SO_4 for 10 min (75%) (Table 1). The percentage of germinated seeds can be considered highly favorable to the application of these processes; however, mechanical scarification is a safer and more practical alternative for small farmers (Hermansen et al., 2000) in that it is a simple, low-cost, and effective method to promote rapid and uniform germination. Under laboratory conditions, several authors have demonstrated the efficacy of mechanical scarification in seeds of forage legume species (Jacob Junior et al., 2004; Patanè and Gresta, 2006; Suñe and Franke, 2006).

Treatments with sulfuric acid have been used successfully to overcome dormancy in forest seeds (Alves et al., 2006; Li et al., 2013). However, each species requires a specific seed scarification period (acid exposure time), resulting from differences in thickness and chemical composition of the seed coating (Martín and Guerrero, 2014).

As the exposure to sulfuric acid increased, the percentage of germination rose; however, the percentage of abnormal seedlings also increased (Table 1). This is likely related to the harmful effects of sulfuric acid on the embryo. Chemical scarification degrades the seed coat and increasing immersion time may rupture essential cells, favoring mechanical injuries and fungal invasion, thereby compromising emergence (Alves et al., 2006).

Studies with larger seed species showed positive results in overcoming coat dormancy with the use of sulfuric acid, as in *Leucena diversifolia* (Souza et al., 2007), *Caesalpinia leiostachya* (Biruel et al., 2010), and *Colubrina glandulosa* (Brancalion et al., 2011). These researchers observed that a successful treatment is related to the species and acid exposure time.

Even though sulfuric acid can be used successfully for the chemical scarification of seeds, primarily in laboratory

Table 1 - Means (%) of germinated, dormant, and dead seeds and abnormal seedlings of *Ornithopus pinnatus* subjected to different treatments to overcome dormancy

Treatment	Germinated	Dormant	Dead	Abnormal
	Mean (%)			
Control	58c	42a	0b	0d
Hot water (60 °C)	59c	33ab	0b	8a
Hot water (90 °C)	51c	16c	28a	5abc
Mechanical scarification	83a	17c	0b	0d
Chemical scarification (5 min)	73b	24bc	0b	3bcd
Chemical scarification (10 min)	75ab	19c	0b	6ab
Coefficient of variation (%)	6.1	17.7	18.5	20.5

Values followed by the same letter in a column do not differ statistically by Tukey's test (P<0.05).

analyses, a number of difficulties restrict its large-scale use by rural producers, such as difficulty in obtaining the acid, possibility of accidents during handling, and the proper disposal of the generated wastes (Brancolion et al., 2011).

In the studies conducted to overcome dormancy in legumes, immersion in hot water for a certain time has been used successfully to overcome dormancy in seed coats (Ferreira et al., 2014). The results of *O. pinnatus* seed immersed in water at 60 °C and 90 °C did not differ significantly from the control (Table 1). The high percentage of abnormal seedlings indicates that treatment with hot water was harmful to the seeds. Treatment with hot water at 90 °C was effective in softening the barrier imposed by the seed coat, since many seeds were turgid; however, the high temperature compromised the embryonic tissues of the seeds (Amusa, 2011; Wang et al., 2011). The use of hot water depends on imbibition time and temperature, as a function of the species treated, which, in some cases, makes this method ineffective in overcoming dormancy in a number of seeds (Perez, 2004).

In general, all the analyzed variables were significantly influenced by the tested temperatures (Table 2). The *O. pinnatus* seeds germinated between 5 and 30 °C, with cardinal temperatures between 0 and 5 °C and 30 and 35 °C, determined by the lack of germination between 0 and 35 °C. Seeds exhibited germinative capacity at well-defined temperature limits, characteristic of each species (Ramos and Varela, 2003). The optimum temperature and extent of its range determine the geographic distribution of the species (Labouriau, 1983). According to Kraemer et al. (2000), the optimum range for most of the species was between 20 and 30 °C, with germination rate and total germination declining both below and above this temperature range.

Table 2 - Mean percentage of germinated seeds (*G*), mean germination rate (*v*), and synchronization index of germination (*U*) of *Ornithopus pinnatus* seeds subjected to different temperatures

Temperature (°C)	*G* (%)	*v* (days^{-1}.10^{-2})	*U* (bits)
	Mean		
0	0e	0e	NG
5	59c	7d	3.97b
10	76b	13bc	3.81bc
15	84a	16a	2.86d
20	81ab	15ab	3.01cd
25	10d	11c	4.58ab
30	2e	8d	5.45a
35	0e	0e	NG
CV (%)	6.4	11.1	20.0

NG - no germination; CV - coefficient of variation.
Values followed by the same letter in a column do not differ statistically by Tukey's test (P<0.05).

The increase in temperature raised the germination percentage to a certain extent, since temperatures above 20 °C caused a sharp decline in the germination percentage of seeds belonging to this species (Table 2). Among the external agents capable of regulating the germination process, high temperatures are recognized as a potentially harmful factor, subjecting the seeds of many plant species to stress during the germination process (Neto et al., 2002). According to Bewley and Black (1994), the effects of temperature on germination are complex, since they affect each stage of the process in different ways and are also related to the biochemical processes that regulate the entire metabolic process.

On the other hand, low temperatures reduce metabolic rates to the extent that essential pathways for the onset of germination can no longer function and can change the cell membrane from the liquid-crystalline to the crystalline state (Hendricks and Taylorson, 1976). This may be due to chemical characteristics like proline or fat levels, or the inactivation of enzymes (Zhao et al., 1994). In the present study, this occurred at 0 °C, in which no seed germinated during the 21 days of incubation. According to Bedi and Basra (1993), low temperatures close to freezing result in poor seedling establishment and reduced biomass, particularly in tropical or subtropical species. The extent of the damage depends on the initial water content of the seed, temperature and duration of exposure and the germination period during which exposure occurred.

According to Labouriau (1983), the concept of optimum temperature should include other aspects in addition to germination percentage. There is an optimal temperature range between two intervals where germination rate increases initially and then declines (Lopes et al., 2005). For *O. pinnatus*, the highest germination rates were recorded at 15 and 20 °C (Table 2). Therefore, the optimum temperature for this species falls within this range, which provided high germination and shorter mean germination times. Suñé and Franke (2006) reported higher optimal temperatures of around 25 °C for *Trifolium riograndense* in relation to *Lotus subbiflorus*. However, in the *L. subbiflorus* species, a constant temperature of 20 ºC enabled seeds to express greater germination potential (Lopes and Franke, 2011).

The regression lines (solid ascending and descending lines) intersect at the highest point of the graph, 16.94 °C, which corresponds to the optimum theoretical temperature and is very close to the experimental result of 15°C (Figure 1).

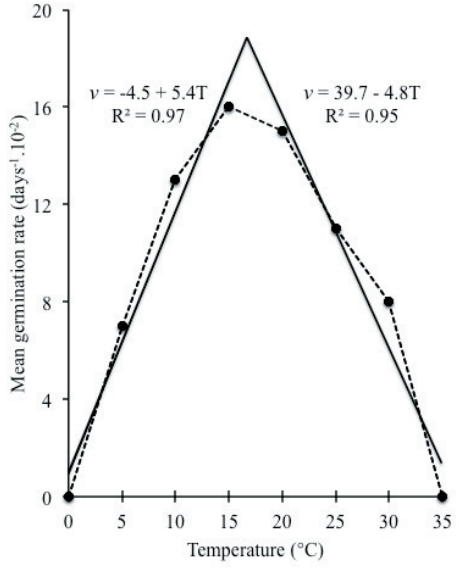
The germination rate of seeds increases linearly with temperature within the below-optimum temperature range, and subsequently declines in the above-optimum range (Perez and Moraes, 1991). However, interception is

below 0 °C, which differs from the experimental minimum (0 °C). Similarly, at the opposite end of the axis, the theoretical maximum was higher than the estimated value (35 °C). In this case, the linear model does not provide a good description of the grouped isotherm data and is not suited to estimating minimum and maximum theoretical cardinal temperatures. However, it does indicate a linear relationship for germination rate, both below and above the optimum (ascending and ascending lines, respectively). According to Labouriau (1983), determining extreme cardinal temperatures (T_m = minimum temperature and T_M = maximum temperature) and the maximum germination range is important not only in agriculture, but to help understand the geographic distribution of a seed plant species.

The distribution of seed germination varied over time for the studied temperatures (Figure 2). At 15 and 20 °C, the graphs show unimodal distribution and the isotherms for 5, 10, 25, and 30 °C are multimodal.

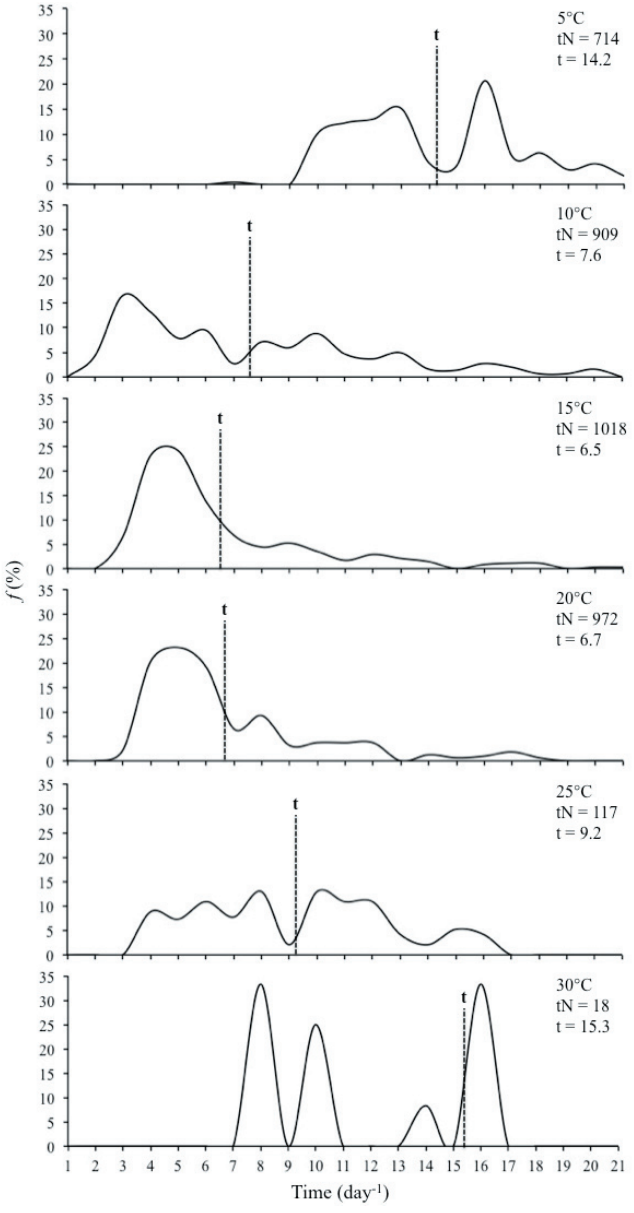
A deviation in germination time is observed to the right of the dominant frequency distribution. Asymmetrical distribution may indicate that heterogeneity occurs because most of the seeds germinate slowly or a minority germinate quickly (or both), depending on the temperature. At 25 and 30 °C, there is no longer a dominant distribution and the germination of a few seeds appears to be distributed over time. Labouriau and Agudo (1987) attributed this distribution pattern to adaptation, showing that unfavorable temperature conditions were compensated for by greater distribution of germination over time. The delay in germination may increase the likelihood of seedlings finding favorable conditions in changing environments.

Since germination rate is dependent on temperature, the distribution of germination frequencies should vary depending on the temperature. An analysis of these distributions can indicate how the thermal signal is perceived by the seed, that is, how "thermal communication" between the growth effector (embryo) and the medium occurs (Labouriau, 1983). This communication can be quantified by using germination synchronization indices (*U*).



Dotted line - experimental results; solid line - theoretical points.

Figure 1 - Influence of different temperatures on the mean germination rate (*v*) of *Ornithopus pinnatus* seeds.



tN - total number of seeds germinated; t - mean germination time.

Figure 2 - Relative germination frequency (*f*) for *Ornithopus pinnatus* seeds as a function of isothermal incubation time at different temperatures.

The lowest germination synchronization indices observed were close to the optimal temperature range (Table 2), demonstrating greater synchronization in this range and confirming unimodal distribution of the relative frequency of germination (Figure 2). Germination was less synchronized ($P<0.05$) at above- and below-optimum temperatures, with higher *U* values and a tendency to polymodal distribution of the relative frequency of germination (Table 2; Figure 2).

O. pinnatus seeds showed high germination at 15 and 20 °C, exhibiting homogeneous germination behavior and resulting in high germination synchronization (lower *U* values in Table 2). These results indicate that the germination of *O. pinnatus* tends to be heterogeneous at high or low temperatures.

The ecological implication of these results is that this species can germinate rapidly in subtropical areas where temperatures are between 15 and 20 °C, between the fall and winter. This ensures greater efficiency in the establishment of seedlings because seeds are able to produce seedlings that will find the ideal conditions for development, as suggested by Godoi and Takaki (2004).

Conclusions

Ornithopus pinnatus seeds exhibit dormancy caused by the seed coat's impermeability to water. Mechanical scarification is an efficient method for promoting germination in the seeds of this species. *O. pinnatus* is a stenothermal species, meaning it cannot tolerate significant temperature variations. The germination rate of seeds is linearly dependent on temperature. Synchronization of the germination process is greater in the optimum temperature range, established between 15 and 20 °C.

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