

## Methods to promote *Borreria latifolia* seed germination<sup>1</sup>

### Métodos para promoção da germinação em sementes de *Borreria latifolia*

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**ABSTRACT** - Specific knowledge on dormancy and germination patterns of weed species aids the development of integrated management strategies. This study aimed to evaluate and select effective methods for overcoming dormancy in *B. latifolia* seeds, as well as determining their influence on the germination process. A completely randomized experimental design was used, with four replications and 20 seeds per repetition. Seven tests were conducted, with the corresponding *B. latifolia* seed treatments: Test 1 - mechanical scarification, sulfuric acid, hot water and running water; Test 2 - pre-cooling at 4 °C and dry heat at 60 °C. Test 3 - gibberellic acid at concentrations of 0, 50, 100, 200 and 400 ppm; Test 4 - acetic acid and potassium nitrate 2%; Test 5 - dual combinations of the three best treatments observed in the first four tests. Test 6 - pre-soaking in a sandbox and in germination paper and; Test 7 - imbibition curve. The seeds were submitted to the germination test in BOD at 25 °C with a photoperiod of 12h, in transparent polypropylene boxes. Germination percentage (GP), mean germination time and relative frequency of germination were determined and the weight of the seeds after soaking in Test 7 was measured. The best treatments for overcoming seed dormancy were dry heat, gibberellic acid 283 ppm and KNO<sub>3</sub> 3h. The association of dry heat + potassium nitrate 3 h and potassium nitrate 3 h +gibberellic acid increased germination percentage by 25% compared to isolated treatments.

**Key words:** Broadleaf buttonweed. Overcoming. Dormancy. Soaking. Rubiaceae.

**RESUMO** - O conhecimento específico sobre os padrões de dormência e germinação de plantas daninhas auxilia o desenvolvimento de estratégias integradas de manejo. Objetivou-se avaliar e selecionar métodos eficazes de superação de dormência de sementes da espécie *B. latifolia*, bem como determinar sua influência no processo germinativo. Foram conduzidos sete ensaios com tratamentos correspondentes: Ensaio 1 - escarificação mecânica, ácido sulfúrico, água quente e água corrente; Ensaio 2 - pré-esfriamento a 4 °C e calor seco 60 °C; Ensaio 3 - ácido giberélico nas concentrações de 0; 50; 100; 200 e 400 ppm; Ensaio 4 - ácido acético e nitrato de potássio 2% (3 e 6 h); Ensaio 5 - três associações duplas dos melhores tratamentos dos quatro primeiros ensaios; Ensaio 6 - estratificação em caixa de areia e pré-embebição em papel de germinação e; Ensaio 7 - curva de embebição. Utilizou-se o delineamento inteiramente casualizado, com quatro repetições de 20 sementes. As sementes foram submetidas ao teste de germinação em B.O.D. a 25 °C e fotoperíodo de 12 h, em caixas de polipropileno transparente. Foram avaliados a porcentagem de germinação, tempo médio de germinação e a frequência relativa de germinação e mensurou-se o peso das sementes após embebição no ensaio 7. Os tratamentos mais eficientes em superar a dormência das sementes foram os de calor seco, ácido giberélico 283 ppm e nitrato de potássio 3 h. As associações de calor seco + nitrato de potássio e nitrato de potássio + ácido giberélico incrementaram em 25% a porcentagem de germinação em relação aos tratamentos isolados.

**Palavras-chave:** Erva quente. Superação. Dormência. Embebição. Rubiaceae.

DOI: 10.5935/1806-6690.20180054

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Received for publication on 22/03/2017; approved 14/06/2017

<sup>1</sup>Parte da Dissertação de Mestrado do primeiro autor apresentada na Universidade Tecnológica Federal do Paraná

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## INTRODUCTION

There has been a significant increase in the infestation of *Borreria latifolia* (Aubl.) K. Schum. (GONON *et al.*, 2013) among Brazilian crops as a result of tolerance to the Glyphosate herbicide, which is the most used product in chemical weed control (GALON *et al.*, 2013). *Borreria latifolia* is an herb species endemic to Brazil, having an annual cycle and reproduction through small, light seeds (KISSMANN; GROTH, 2000), produced in abundance by the plant.

Seed germination results from the correct balance between favorable environmental conditions and inherent characteristics, and consists of an ordered sequence of metabolic processes, resulting in resumption of embryo development, by which a seedling is produced (TAIZ; ZEIGER, 2009). However, it is common for weed diaspores to be endowed with dormancy mechanisms, even under all favorable environmental conditions. There is a strong relationship between seed dormancy and the ability of a plant to succeed as a weed (ALI; TANVEER; NADEEM, 2012). Discontinuity in the germination process of a species allows survival in a seed bank over time, although this has a negative impact on seed management (FINCH-SAVAGE; LEUBNER-METZGER, 2006). Practices such as soil tillage may promote dormancy in some species, while it may provoke an interruption to dormancy in others (MONQUERO; SILVA, 2005). Therefore, knowledge of the factors affecting weed germination will enable the definition of more effective management strategies and may also promote greater efficiency in weed control, avoiding the undesirable practice of herbicide application during pre- or post-emergence. This type of scientific investigation is also highly valuable in seed technology when seeking standardization of methods with a high degree of uniformity in the germination process (PAZUCH *et al.*, 2015).

Dormancy in plants is attributed to various mechanisms, which are well documented in the literature (FERREIRA; BORGUETTI, 2004). Among the methods employed to overcome dormancy, those that promote disintegration of the integument present advantages (DIAS; ALVES, 2008), which, besides increasing permeability to water and gases, may also promote increased sensitivity to light and temperature (VIVIAN *et al.*, 2008). Other methods, such as immersion or germination in potassium nitrate-soaked substrates, may be favorable to overcoming dormancy for many species, its action being related to its role in the seeds' hormonal balance, which results in the reduction of germination inhibitors, such as abscisic acid (GASHI *et al.*, 2009; VASCONCELOS *et al.*, 2010). Exposing the seeds to high temperatures (between 40 and 50 °C) may also promote germination through oxidation and removal

of the short-chain saturated fatty acids, which may be linked to dormancy control (DIAS; ALVES, 2008). However, few methods for overcoming dormancy have been standardized and optimized for the seeds of weed species (ALBUQUERQUE *et al.*, 2007; VIVIAN *et al.*, 2008).

Some studies have demonstrated that certain species of the *Borreria* genus present high germination levels, even in the absence of techniques for overcoming dormancy. Germination values of around 75% in *B. densiflora* var. *latifolia* were obtained through temperature alternation between 20 and 30 °C and a 12-hour photoperiod (MARTINS *et al.*, 2010). However, preliminary experiments to the present study demonstrated that, even under ideal conditions, *B. latifolia* seed germination remained below 20%.

This study aimed to evaluate and select effective methods for overcoming dormancy in *B. latifolia* seeds, as well as determining their influence on the germination process.

## MATERIAL AND METHODS

To assess the methods for overcoming dormancy, six tests were carried out along with an additional seventh, which evaluated the seed imbibition process. All the experiments were conducted in the Weed Science Laboratory of the Federal Technological University of Paraná - Pato Branco Campus, utilizing *B. latifolia* seeds gathered from the soybean plantations in the municipality of São João, PR (25°50'40.5" S, 52°43'8.256" W at 675 m altitude) during April 2013. After collection, the seeds were manually processed and placed in the shade for three days (at an average temperature of 27 °C and 65% relative humidity) to standardize water content.

A completely randomized experimental design, with four replications was adopted. For the first five tests the experimental units consisted of transparent plastic boxes (11 x 11 x 3 cm) (Gerbox®) each containing 20 seeds. In each box, two sheets of paper were overlaid after being moistened with distilled water, at a ratio of three times the original paper weight (except for the gibberellic acid treatments, in which the substrate was moistened with regulator solution). In the sixth test, which involved stratification, unwashed sand in wooden boxes and rolls of paper (Germitest®) were used as the substrates, according to the treatment applied. The experimental units were then placed in a Biochemical Oxygen Demand (BOD) germination chamber and maintained at a temperature of 25 °C for a 12-hour photoperiod, conditions considered favorable for germination of the plant species under study (PARREIRA *et al.*, 2011).

Initially, the seed sample was homogenized and reduced to obtain the test samples, following the procedures prescribed in the Rules for Seed Analysis (RAS) (BRASIL, 2009). During the testing period, the seeds were stored in a cold dry chamber (10 °C and 20% RH). Prior to testing, the seeds were disinfested using 1% sodium hypochlorite for 5 minutes, and 70% alcohol for 1 minute. Subsequently, the following tests were conducted:

**Test 1** - scarification and washing: mechanical scarification using sandpaper No. 80, in an electric motor (EM) driven, hand-held rotary drum, for 5 (a) and 10 minutes (b); immersion in 98% H<sub>2</sub>SO<sub>4</sub> solution for 30 sec (c) and 1 minute (d), with one part seed to two parts acid in m / v, maintaining constant agitation; the seeds were then washed under running water and immersed in hot water (98 °C) for 60 min (without temperature maintenance during this period) (e); washed under running water for 10 min (f); control with substrate moistened with distilled water (g).

**Test 2** - heat treatments: pre-cooling at 4 °C in the refrigerator for 3 h (a) and 24 h (b); dry heat at 60 °C in a drying oven with air circulation for 20 (c) and (d) 30 mins; control (e).

**Test 3** - gibberellic acid: substrate previously moistened with gibberellic acid solutions + distilled water, at concentrations of 0 (a); 50 (b); 100 (c); 200 (d) and 400 ppm (e). During the tests, distilled water was added to replace moisture;

**Test 4** - acetic acid and nitrato de potássio: immersion in commercial vinegar containing 5% v / v acetic acid for 5 (a) and 10 minutes (b); immersion in 2% m / v potassium nitrate solution (KNO<sub>3</sub>) for 3 (c) and 6 h (d); control (e).

**Test 5** - best treatment combinations: the three best treatments from those described above were selected and combined in pairs, as follows: (a) 60 °C dry heat in a drying oven with circulating air for 30 min + immersion in KNO<sub>3</sub> (2 % m / v) for 3 h; (b) 60 °C dry heat in a drying oven with circulating air for 30 min + immersion in gibberellic acid 400 ppm (solution added to the substrate); (c) immersion in 2% KNO<sub>3</sub> for 3 h + gibberellic acid 400 ppm (applied to the substrate) and (d) control.

**Test 6** - stratification and pre-soaking: During stratification, the seeds were packed between layers of unwashed sand in wooden boxes of 20 x 10 x 10 cm (length x height x width) and moistened. Sand was moistened following the procedures prescribed in RAS (BRASIL, 2009). Pre-soaking was conducted in rolls of paper towel (Germitest®), with four repetitions, seeding was carried out on two sheets of Germitest® paper subsequently covered

with a third. The paper rolls were previously moistened with three times their weight of distilled water. Next, both the boxes and paper rolls were wrapped in plastic bags, sealed with adhesive tape and placed in a BOD-type germination chamber, at a temperature of 10 °C and kept in darkness for 60 days. The humidity of the boxes and rolls was monitored periodically, with further moistening when required. After the incubation / imbibition period, the seeds were removed and then separated from the sand using plastic sieves and washing under running water. The control treatment used seeds stored without treatment to overcome dormancy.

The procedures followed in the treatment combination, stratification, and pre-soaking tests were identical to those implemented in the four individual treatment trials described above. Germination was assessed on a daily basis, for 46 days, and seeds were considered germinated when the radicle length exceeded 2 mm. Germination percentage, mean germination time and relative germination frequency were calculated according to the formulas described by RAS (BRAZIL, 2009), and Labouriau and Valadares (1976). The responses of the dependent variables to the different gibberellic acid concentrations were adjusted through the quadratic polynomial model; the optimal gibberellic acid concentration needed to maximize the value of the variables was based on the formula  $y = -b/2c$ .

**Test 7** - imbibition curve: six *B. latifolia* seeds, per repetition, without application of a treatment to overcome dormancy, were placed in Petri dishes on a double layer of Germitest® paper, moistened at a ratio of three times its dry weight. The dishes were placed in a BOD-type germination chamber, adjusted to 25 °C for a photoperiod of 12 h. The seed imbibition curve was obtained through weighing on a precision scale at one-hour intervals (starting at zero hour, prior to the beginning of imbibition) until the sixth hour, and subsequently at 24 h intervals for six days.

The data were submitted to the Lilliefors test to test the null hypothesis. The data without a normal distribution underwent the arc sine  $\sqrt{X}/100$  transformation. Subsequently, the results were submitted to analysis of variance and the hypotheses tested by the F test ( $p = 0.05$ ). The Duncan test, along with the Winstat computer program (MACHADO; CONCEIÇÃO, 2005), was used to complement the analysis for qualitative treatments. Graph elaboration and regression adjustments were performed using the SigmaPlot program, version 11.0.

## RESULTS AND DISCUSSION

It can be observed from the values obtained in test 1 of the germination tests (Figure 1A) that the

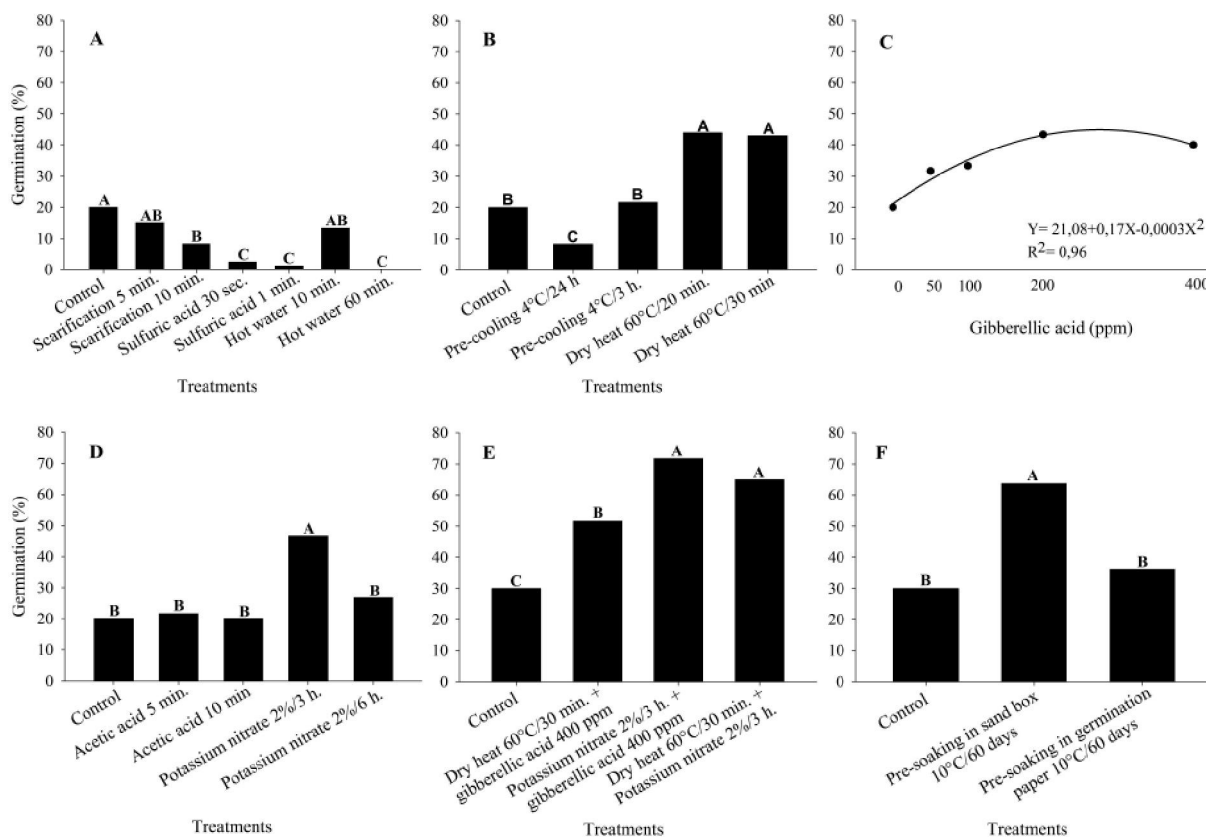
treatments performed to overcome *B. latifolia* seed dormancy, both in the control and after scarification with sandpaper for 5 minutes and washing under running water for 10 minutes, showed values significantly equal to or higher than those obtained from the other treatments ( $p < 0.05$ ). It can also be observed that the treatment involving hot water immersion completely inhibited germination, whereas in the treatment using  $H_2SO_4$  for 30 and 60 seconds, germination was reduced to levels between 3 and 1%, respectively (Figure 1A). Sulfuric acid is observed to corrode the integument of the seed, which is favorable for many species, as it allows water to be absorbed (DOUSSEAU *et al.*, 2007). However, this effect may also damage the embryo, impairing germination, as observed in this test.

The results from Test 1 (Figure 1A) corroborated those reported by Parreira *et al.* (2011), for the same species, in which the seeds immersed in concentrated  $H_2SO_4$  for 3 min and diluted  $H_2SO_4$  (50%) for 3 min provided a germination percentage below 5 and 10%, respectively. However, for these same authors, mechanical scarification with abrasive sandpaper

resulted in 64% germination, which is much higher than the values found in the current work. This may have occurred as a result of methodological differences between the two experiments. In the present study, the seeds were scarified for 10 min in a rotating drum with sandpaper inside, the efficiency of which not having been previously assessed, whereas in the test mentioned above, the seeds were manually rubbed between two sheets of sandpaper for 2 min.

Friction with abrasive sandpaper reduces thickness of the integument facilitating its break down, thereby raising water absorption efficiency (HU *et al.*, 2009; MENDES *et al.*, 2009). However, efficiency of the mechanical scarification process depends on scarification time, intensity of the force applied to the seeds and sample homogeneity. Friction exerted on smaller seeds or those with a less lignified integument can induce crack formations that may injure the embryo and, consequently, result in germination loss (PAZUCH *et al.*, 2015). For the equipment used in the present study (rotary drum), it is necessary to assess other lengths of scarification time, as well as the granulometry of the sandpaper.

**Figure 1** - Effect of the treatment to overcome dormancy on germination percentage in *Borreria latifolia* seeds. Test 1 (A), Test 2 (B) Test 3 (C), Test 4 (D), Test 5 (E) and Test 6 (F)



In the second experiment, the dry heat treatments performed at 60 °C for 20 and 30 minutes promoted germination of approximately 45% of the *B. latifolia* seeds in both treatments (Figure 1B); which differed statistically ( $p < 0.05$ ) from the other treatments, such as the pre-cooling at 4 °C / 1 day, which presented the lowest performance (8% germination). The poor germination reported in treatments with pre-cooling may have occurred because, in general, seed metabolism is decreased at low temperatures (MATOS; BORGES; SILVA, 2015). Parreira *et al.* (2011) reported 42% germination with only 15 min dry heat applied at 60 °C, demonstrating that a very long heat exposure period is not required to induce germination of *B. latifolia* seeds. The increase in temperature, to a certain degree, promotes faster, more efficient germination, albeit varying according to the species (FINCH-SAVAGE; LEUBNER-METZGER, 2006).

In test 3, the investigation (Figure 1C) showed that it was at a concentration of 283 ppm that the gibberellic acid promoted the highest germination percentage in the seeds (45%). Gibberellic acid acts on hydrolysis control of the reserve tissue to provide the embryo with energy, weakening the structures surrounding it and boosting cellular elongation and radicle development, thus triggering the germinative process in both dormant and non-dormant seeds (TAIZ; ZEIGER, 2009). Stimulation to seed germination in several cultivated species through gibberellic acid application has been reported in various studies (FEITOSA *et al.*, 2015, SILVA *et al.*, 2013). Nevertheless, despite being considered a promotor of weed germination (VIVIAN *et al.*, 2008), few studies support this claim.

For the acetic acid and potassium nitrate ( $\text{KNO}_3$ ) treatment groups, the treatment using 2%  $\text{KNO}_3$  for 3 h resulted in the highest percentage of *B. latifolia* seed germination (47%), (Figure 1D). All the other treatments showed no effect on *B. latifolia* seed germination and were similar to the control ( $p < 0.05$ ), recording values close to 20% germination. Parreira *et al.* (2011) reported that 2%  $\text{KNO}_3$  applied for 3 h resulted in 51% germination; however, the same treatment with 6-hour seed immersion achieved 55%, which differs to the findings of the present study, in which extended exposure to  $\text{KNO}_3$  caused a reduction in germination.  $\text{KNO}_3$  is a powerful oxidant and electron acceptor that stimulates the pentose-phosphate pathway, an alternative pathway for glucose oxidation, which thus neutralizes or decreases seed dormancy (ELLIS *et al.*, 1983). Its action may also be linked to its role in hormone balance within the seeds, resulting in the reduction of germination inhibitors like abscisic acid (GASHI *et al.*, 2012; GOLMOHAMMADZADEH; ZAEFARIAN; REZVANI, 2015). The use of  $\text{KNO}_3$  in treatments to overcome dormancy is widely recommended,

as it has been shown to interrupt dormancy in around 20% of the species on the RAS list (BRASIL, 2009).

Among the first four experiments, the most efficient treatments for increasing germination (statistically superior to the control) of *B. latifolia* seeds within each test (except test 1, in which no treatment was greater than the control), were those involving the application of dry heat at 60 °C for both 20 and 30 minutes, gibberellic acid at 283 ppm (however, for the combined test the 400 ppm concentration was used because of a means comparison analysis of the means during the experiment, which showed no difference for the 200 ppm treatment) and  $\text{KNO}_3$  for 3 h, which achieved an average of 45% germination. These treatments were selected for performance of Test 5. In comparison to the isolated treatments, all the treatment combinations carried out to overcome dormancy in *B. latifolia* seeds (Figure 1E) demonstrated an increase in germination levels, especially the  $\text{KNO}_3$  + gibberellic acid and the dry heat +  $\text{KNO}_3$  combinations, which produced 71 and 66% germination, respectively, representing increases of 20 to 25%, in relation to individually applied treatments.

The above results indicate that the *B. latifolia* seeds exhibit morpho-physiological type dormancy. Besides a poorly developed embryo (morphological dormancy), morpho-physiological dormancy presents a physiological aspect that requires treatments or conditions to overcome dormancy. Depending on the species, growth is either preceded by breaking down physiological dormancy, or both processes occur simultaneously (BASKIN; BASKIN, 2004). This type of dormancy is rarely observed in an individualized manner, it normally being associated with other causes, which explains the better results found in the treatment combinations. Generally, thermal treatments and gibberellic acid are connected with overcoming this kind of dormancy (CARDOSO, 2009). Dormancy can be conceptually subdivided into six different mechanisms (VIVIAN *et al.*, 2008), and therefore the overlapping of its effects may add confusion to the definition of the study results and the subsequent conclusions.

The treatment which involved pre-soaking on germination paper (Figure 1F) was found to be ineffective in overcoming seed dormancy, and was similar to the control. Moreover, at the time of seed removal in order to perform the tests, most of the seeds revealed contamination from unidentified pathogens. The stratification treatment in the sandbox at 10 °C for 60 days resulted in 64% germination. However, it was considered non-viable, as it requires more than 100 days from the time of implantation to its completion.

It can be observed that for the values obtained for mean germination time (TMG), when the seeds are

subjected to  $H_2SO_4$  for 30 seconds and 1 minute, as well as being immersed in hot water for 1 hour, very poor results were achieved (less than 5 days) in comparison to the other treatments (Figure 2A). The treatments in Test 2 (Figure 2B) showed no significant statistical differences in terms of the TMG, taking an average of 25 days for germination to occur. In the treatments in Test 3 (Figure 2C) the TMG varied from 25 to 30 days, with the shortest TMG occurring at approximately 26 days, at a gibberellic acid concentration of 229 ppm, which is a little lower than the optimum germination concentration of 283 ppm. The 2%  $KNO_3$  treatment for 6 h showed a positive effect in Test 5, with a mean of 25 days for germination, while the acetic acid treatment for 10 min, gave a value of 33 days, the highest TMG (Figure 2D). As in the germination results, the combinations of  $KNO_3$  + gibberellic acid and dry heat +  $KNO_3$  stood out for the lowest TMG, between 26 and 30 days, respectively, similar to the control (Figure 2E). In regard to stratification and pre-soaking (Figure 2F), no significant difference was observed between the treatments used.

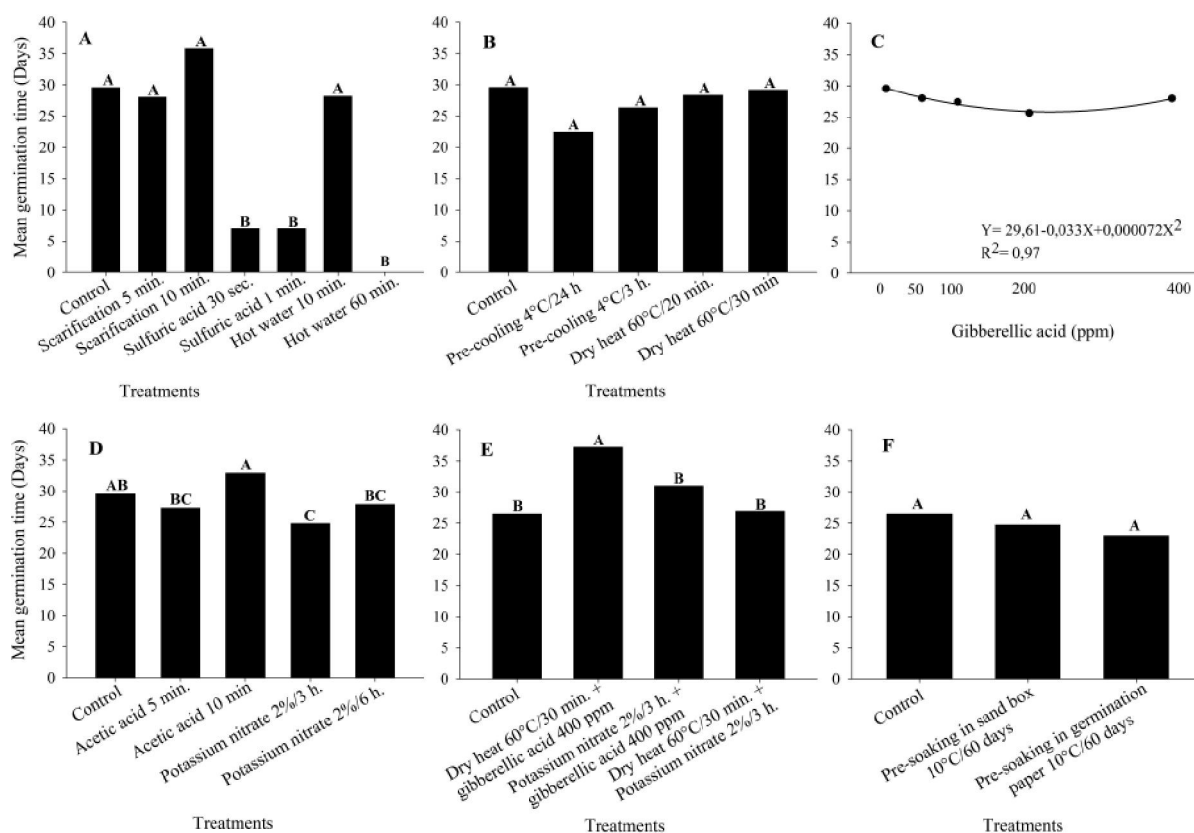
In relation to the relative germination frequencies (FRG's) (Figure 3), differences were observed within

each treatment group. For Test 1 (Figure 3A), in which the treatments presented germination similar to or less than the control, the FRG of the treatments were similar to those of the control, being well distributed along the assessment period. A platicurtic distribution was observed, characterized by low germination synchronism compared with the more responsive treatments for overcoming dormancy (FERREIRA; BORGUETTI, 2004).

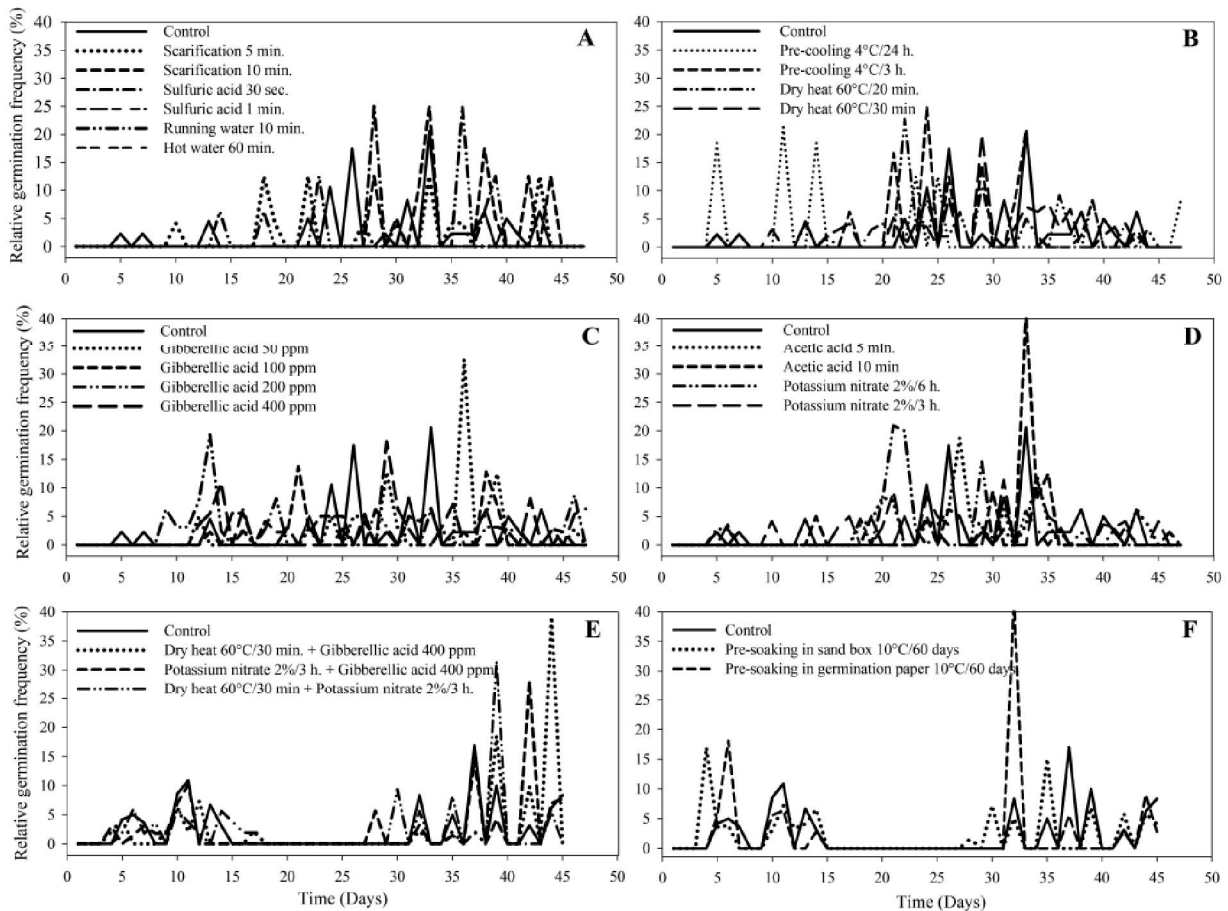
Germination was found to be more concentrated during the period between the 20th and 25th day, for the treatments using dry heat, in Test 2 (Figure 3B), demonstrating a leptokurtic germination distribution, which occurs when a large proportion of the seeds germinate in a concentrated manner within a short period of time. This type of curve implies that germination does not take place randomly, but in fact responds to a specific stimulus or mechanism, resulting in synchronization (FERREIRA; BORGUETTI, 2004).

In Test 3, the treatments showed greater germination synchronization compared to the control, (Figure 3C), particularly with the treatment using 100 ppm gibberellic acid. In Test 4 (Figure 3D), the highest germination peak

**Figure 2** - Effects of the different treatments to overcome dormancy on mean germination time in *Borreria latifolia* seeds. Test 1 (A), Test 2 (B) Test 3 (C), Test 4 (D), Test 5 (E) and Test 6 (F)



**Figure 3** - Influence of different treatments to overcome dormancy on the relative germination frequency of *Borreria latifolia* seeds. Test 1 (A), Test 2 (B) Test 3 (C), Test 4 (D), Test 5 (E) and Test 6 (F)



occurred in the 10-minute acetic acid treatment, at around 33 days, achieving 40% germination. The 5-minute acetic acid treatment and 6-hour treatment with  $KNO_3$  stood out for concentrated germination in a short period of time, demonstrating a leptokurtic distribution.

For the best treatment combinations and pre-soaking (Figures 3E and 3F), it can be observed that germination was separated into two distinct periods, with an interval of approximately 10 days, which had not been observed during the first four tests. This difference in the germination pattern may be related to the storage period of the seeds, given that the last two tests were conducted approximately two months after the installation of the first four tests. Storage is an important factor for seeds that present dormancy, and variations in conditions such as relative humidity and temperature influence their longevity over time (MORAIS; ROSSETTO, 2013; PARIHAR *et al.*, 2016). Even though the seeds had been stored correctly and for a short period, they continued the deterioration process, which is unavoidable, continuous and irreversible. The germination interval may also be

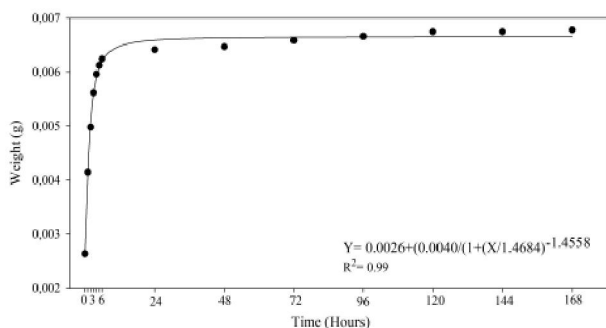
related to its distribution over time, a defining feature in most weeds, which ensures survival and perpetuation of the species (MONQUERO; SILVA, 2005). In the treatment combinations (Figure 3E), higher germination frequencies were observed during the second period described earlier, whereas the control showed a more even germination distribution over the two periods, although both presented a pause in germination.

The two pre-soaking treatments presented an initial germination peak of approximately 15% of the total at around day five (Figure 3F). However, the treatment involving pre-soaking with paper demonstrated the highest germination peak, on around day 32, during the second germination cycle of the seeds. Although the germination was higher than that of the control, the germination distribution was homogeneous along the assessment period. The sand stratification treatment was used so as to simulate the real-life situation, in which the seeds are deposited in the soil and remain in a humid environment, but with cooler temperatures (winter period) that impede germination. As in the field, when the test seeds were

exposed to an ideal temperature range, the germination process began, although this remained governed by dormancy, promoting distribution over time, which increases the chances of survival of the species.

According to the imbibition curve (Figure 4), 92% of the total water imbibed by the seeds in the seven-day period occurred within the first six hours. Generally, seeds need to absorb 50% of their weight in water, in order to trigger the remobilization process of the stored reserves and the consequent continuation of the growth process of the embryo, until completing the germinative process (MARCOS-FILHO, 2015). This phenomenon is also regulated or influenced by the integument of the seeds, besides other factors.

**Figure 4** - Water imbibition curve of *Borreria latifolia* seeds



The imbibition curve is an important technical procedure that facilitates identification of the type of dormancy mechanism presented by the seed and is particularly related to the integumental hardness and impermeability (BRASILEIRO *et al.*, 2009). The results indicate that dormancy in *B. latifolia* seeds is not caused by impermeability of the integument. It is important to emphasize that during the imbibition phase there can be a great variation in the water potential between the seed interior and the environment in which it is found, which can range from -1 MPa to -400 MPa. This can result in water entering the seed interior in large quantities and at great speed, which can be detrimental to germination or even lead to seed death, as it damages the cellular membranes (GORDIN; SCALON; MASETTO, 2015). The results obtained in all the tests with *B. latifolia* indicate that the dormancy present in the seeds exhibits a morpho-physiological mechanism. This morpho-physiological dormancy occurs in species possessing a rudimentary or immature embryo. For these species, the embryo may appear undifferentiated, appearing as a homogeneous cell mass. Generally, dormancy may be overcome through favorable conditions of humidity and temperature, with

specific light and darkness phases possibly also being required. Morphological dormancy rarely occurs in an individualized manner, and is normally related to other causes of dormancy, which explains the better results found in the treatments involving combinations.

## CONCLUSIONS

1. Treatments involving the use of dry heat at 60 °C / 30 min, gibberellic acid at 283 ppm and seed immersed in KNO<sub>3</sub> for 3 hours are, in isolation, the most effective in overcoming dormancy in *B. latifolia* seeds;
2. The dual combinations of these three methods for overcoming dormancy stood out for the 25% increase in efficiency of the germinative process, in comparison to application in isolation.

## REFERENCES

- ALBUQUERQUE, K. S. *et al.* Métodos para a superação da dormência em sementes de sucupira-preta (*Bowdichia virgilioides*). **Ciência e Agrotecnologia**, v. 31, n. 6, p. 1716-1721, 2007.
- ALI, H. H.; TANVEER, A.; NADEEM, M. A. Evolution of some seed dormancy breaking methods on germination of *Rhynchosia capitata* (Roth DC). **Pakistan Journal of Weed Science Research**, v. 18, n. 4, p. 423-432, 2012.
- BASKIN, C. C.; BASKIN, J. M. A classification system for seed dormancy. **Seed Science Research**, v. 14, n. 1, p. 1-16, 2004.
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria Nacional de Defesa Agropecuária. **Regras para análise de sementes**. Brasília: ACS, 2009. 399 p.
- BRASILEIRO, B. G. *et al.* Caracterização morfológica e germinação de sementes de jalapa (*Operculina macrocarpa* L.). **Revista Brasileira de Sementes**, v. 31, n. 3, p. 78-86, 2009.
- CARDOSO, V. J. M. Conceito e classificação da dormência em sementes. **Oecologia Brasiliensis**, v. 13, n. 4, p. 619-631, 2009.
- DIAS, M. C. L. L.; ALVES, S. J. Avaliação da viabilidade de sementes de *Panicum maximum* Jacq. pelo teste de tetrazólio. **Revista Brasileira de Sementes**, v. 30, n. 3, p. 152-158, 2008.
- DOUSSEAU, S. *et al.* Superação de dormência em sementes de *Zeyheria montana* Mart. **Ciência e Agrotecnologia**, v. 31, n. 6, p. 1744-1748, 2007.
- ELLIS, R. H. *et al.* Procedures for the safe removal of dormancy from rice seed. **Seed Science Technology**, v. 11, n. 1, p. 77-112, 1983.
- FEITOSA, M. *et al.* Efeito dos reguladores giberelina e citocinina na quebra de dormência de sementes de capim-andropogon. **Revista de Ciências Agrárias**, v. 38, n. 1, p. 34-40, 2015.



- FERREIRA, A. G.; BORGUETTI, F. **Germinação**: do básico ao aplicado. Porto Alegre: Artmed, 2004. 323 p.
- FINCH-SAVAGE, W. E.; LEUBNER-METZGER, G. L. Seed dormancy and the control of germination. **New Phytologist**, v. 171, n. 3, p. 501-523, 2006.
- GALON, L. *et al.* Glyphosate translocation in herbicide tolerant plants. **Planta Daninha**, v. 31, n. 1, p. 193-201, 2013.
- GASHI, B. *et al.* Effect of gibberellic acid and potassium nitrate on seed germination of the resurrection plants *Ramonda serbica* and *Ramonda nathaliae*. **African Journal of Biotechnology**, v. 11, n. 20, p. 4537-4542, 2012.
- GOLMOHAMMADZADEH, S.; ZAEFARIAN, F.; REZVANI, M. Effects of some chemical factors, prechilling treatments and interactions on the seed dormancy-breaking of two Papaver species. **Weed Biology and Management**, v. 15, n. 1, p. 11-19, 2015.
- GORDIN, C. R. B.; SCALON, S. P. Q.; MASETTO, T. E. Disponibilidade hídrica do substrato e teor de água da semente na germinação de niger. **Pesquisa Agropecuária Tropical**, v. 45, n. 3, p. 312-318, 2015.
- HU, X. W. *et al.* Role of the lens in controlling water uptake in seeds of two Fabaceae species treated with sulphuric acid and hot water. **Seed Science Research**, v. 19, n. 2, p. 73-80, 2009.
- KISSMANN, K. G.; GROTH, D. **Plantas infestantes e nocivas**. 2. ed. São Paulo: BASF, 2000. t. 3, 726 p.
- LABOURIAU, L. G.; VALADARES, M. E. B. On the germination of seeds *Calotropis procera* Ait.f. **Anais da Academia Brasileira de Ciências**, v. 48, n. 2, p. 263-284, 1976.
- MACHADO, A. A.; CONCEIÇÃO, A. R. **WinStat**: sistema de análise estatística para Windows. Versão Beta. Pelotas: Universidade Federal de Pelotas, 2005. Software.
- MARCOS-FILHO, J. **Fisiologia de sementes de plantas cultivadas**. Londrina: ABRATES, 2015. 660 p.
- MARTINS, B. A. B. *et al.* Germinação de *Borreria densiflora* var. *latifolia* sob condições controladas de luz e temperatura. **Planta Daninha**, v. 28, n. 2, p. 301-307, 2010.
- MATOS, A. C. B.; BORGES, E. E. L.; SILVA, L. J. Fisiologia da germinação de sementes de *Dalbergia nigra* (Vell.) Allemão ex Benth. sob diferentes temperaturas e tempos de exposição. **Revista Árvore**, v. 39, n. 1, p. 115-125, 2015.
- MENDES, R. C. *et al.* Tratamentos pré-germinativos em sementes de mamona (*Ricinus communis* L.). **Revista Brasileira de Sementes**, v. 31, n. 1, p. 187-194, 2009.
- MONQUERO, P. A.; SILVA, A. C. Banco de sementes de plantas daninhas e herbicidas como fator de seleção. **Pesquisa & Tecnologia**, v. 2, n. 2, p. 203-209, 2005.
- MORAIS, C. S. B.; ROSSETTO, C. A. V. Testes de deterioração controlada e envelhecimento acelerado para avaliação do vigor em nabo forrageiro. **Revista Ciência Agrônômica**, v. 44, n. 4, p. 703-713, 2013.
- PARIHAR, S. S. *et al.* Seed dormancy, germination and seed storage in henna (*Lawsonia inermis*). **The Indian Journal of Agricultural Sciences**, v. 86, n. 9, p. 1201-1207, 2016.
- PARREIRA, M. C. *et al.* Superação de dormência e influência dos fatores ambientais na germinação de sementes de *Spermacoce latifolia*. **Revista Brasileira de Ciências Agrárias**, v. 6, n. 3, p. 427-431, 2011.
- PAZUCH, D. *et al.* Superação de dormência em três espécies de *Ipomoea* spp. **Ciência Rural**, v. 45, n. 2, p. 192-199, 2015.
- SILVA, A. B. *et al.* Germinação de sementes de braquiária sob diferentes concentrações de giberelina. **Semina: Ciências Agrárias**, v. 34, n. 2, p. 657-662, 2013.
- TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. 4. ed. Porto Alegre: Artmed, 2009. 819 p.
- VASCONCELOS, J. M. *et al.* Métodos de superação de dormência em sementes de croada (*Mouriri elliptica* Mart). **Ciência e Agrotecnologia**, v. 34, n. 5, p. 1199-1204, 2010.
- VIVIAN, R. *et al.* Dormência em sementes de plantas daninhas como mecanismo de sobrevivência - breve revisão. **Planta Daninha**, v. 26, n. 3, p. 695-706, 2008.



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