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3 **Effect of priming on physiological quality of**
4 ***Handroanthus serratifolius* (Vahl.) seeds**

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9 **ABSTRACT**

10 This work aimed to evaluate the effect of different priming treatments in the longevity of *H. serratifolius* seeds. Seeds were osmoconditioned in PEG -1.0 MPa at 10, 15 and 20 °C or hydroprimed at 5, 10 and 15 °C. Final germination, speed and uniformity of germination were assessed. Priming did not affect the final percentage nor uniformity of germination; however, the germination speed was increased after hydropriming at 15 °C and osmoconditioning at 15 °C compared to the control. In order to evaluate priming effect on seed longevity, it was tested hydroprimed (15 °C). Primed and not primed seeds were placed into an incubator (25 °C, dark, 100% RH) until they reach 15% moisture content. Then, seeds were incubated in a container at 40 °C for 0 to 144 hours, so, samples were taken in each period for determination of viability. The results suggest that priming increases longevity of *H. serratifolius* seeds.

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12 *Keywords: Seed longevity; controlled deterioration; hydropriming; forest seeds; germination.*

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15 **1. INTRODUCTION**

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17 The use of high physiological quality seeds is one of the critical aspects to improve the
18 performance of the plants in the field. Several factors can affect the quality of a seed lot,
19 which range from the genetic characteristics of the specie, environmental factors affecting
20 the development of seeds, methodology of collection and cleaning, storage and use of
21 techniques such as priming [1].

22 Priming is a technique used for seed invigoration that aims the increase in germination rate
23 and uniformity, especially in seed lots with low vigor. It was proposed by [2] and constitutes
24 basically in a controlled hydration of the seeds, preventing the radicle protrusion. After the
25 treatment, seeds can be dried back before use.

26 The main effects of priming are the increase in speed and uniformity of germination, and in
27 some cases increases in tolerance to environmental stresses on the seeds and seedlings
28 [3]. However, in some conditions, the priming effect cannot be positive, especially when it is
29 followed by drying before germination. This inconsistent response is also observed in seed
30 longevity. However, besides negative effects have been reported [4-6], increase in seed
31 longevity has also been observed [7].

32 Considering the factors that determine the improvement of seed quality with respect to
33 speed and uniformity of germination, it is expected an increase in the longevity of the seed
34 lots and, thus, it has been found in some cases the increased storage potential of the seeds
35 after priming [7-8].

36 The *H. serratifolius*, is an arboreal species of Bignoniaceae family. It is widely used in urban
37 greening projects [9]. Seeds of some species of this genus show significant variation in
38 quality during storage, which can hinder the conservation and propagation practices [10].
39 According to [11] the low longevity of seeds of *H. serratifolius* associated with the
40 seasonality of production is a challenge for the production of seedlings of this specie. Thus,
41 techniques that increase the storage potential of seeds of this species should be studied as
42 a way to benefit the conservation and reforestation programs. In this context, the study
43 aimed to evaluate the effect of different priming treatments in the longevity of *H. serratifolius*
44 seeds.

45 46 **2. MATERIAL AND METHODS**

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48 The experiment was conducted at the Forest Seed Laboratory (Department of Forest
49 Science - Federal University of Lavras, Brazil).

50 Seeds of *Handroanthus serratifolius* were collected from trees located in Lavras - MG, in
51 September 2013. After cleaning, seeds were placed in a drying room (20 °C/50% RH) for
52 two weeks and stored in a cold chamber (5 °C) in a semi-permeable container (plastic bag).

53 Before use, the seed coat was removed from the seeds in order to enable identification of
54 damaged and deteriorated seeds and to reduce fungi infestation during the priming
55 treatments and germination tests.

56 **2.1 Determination of water content**

57 Water content of the seeds was assessed according to [12] using oven drying method (105 ±
58 3 °C for 24 hours).

59 **2.2 Priming treatments**

60 Seeds were first submitted to different priming methods in order to determine the best
61 method of conditioning. It was tested two priming methods: osmopriming using a
62 polyethylene glycol (PEG) at -1.0 MPa, and hydropriming (in distilled water). For each
63 treatment, 100 seeds were soaked in 10 ml distilled water or 10 ml of PEG solution over filter
64 paper in Petri dishes. Priming was conducted at three temperatures: 10, 15 and 20 °C
65 (osmopriming) and 5, 10 and 15 °C (hydropriming). After priming, seeds were rinsed in tap
66 water, blotted dry and placed in a dry room (20 °C and 50% RH) for up to one week (until
67 reach the equilibrium moisture content).

68 After drying seeds were germinated in Petri dishes at 25 °C under constant light. In order to
69 determine the best priming treatment, germination was scored daily for determination of final
70 percentage of germination, germination rate (t50) and uniformity (u7525).

71 **2.3 Germination tests**

72 Seeds were germinated in 90 mm Petri dishes at 25 °C under constant light over two
73 moistened germination paper towels using four replicate samples of 25 seeds for each
74 treatment. Before germination, seeds were surface-sterilised in 1% sodium hypochlorite
75 solution for 10 minutes and then rinsed for one minute with tap water. Germination was
76 assessed daily by counting the numbers of seeds presenting radicle protrusion of at least 2
77 mm.

78 **2.4 Priming effect on seed vigor**

79 After determination of the best priming method (hydropriming at 15 °C), seeds were primed
80 as describe above and submitted to controlled deterioration. Dried seeds (not primed seeds)
81 were used as control.

82 For controlled deterioration, primed and control seeds were placed in a moist chamber (25
83 °C and 100%RH) for about five hours, when seed water content reached 15% (wet basis).
84 Seeds (primed and control) were then transferred to a sealed container and incubated at 40
85 °C for 0, 6, 12, 24, 36, 48, 60, 72, 96, 120 and 144 hours. After each period, a seed sample
86 was taken for determination of viability by germination test.

87 **2.5 Statistical analysis**

88 The effect of priming methods on the quality of seeds was analyzed using a completely
89 randomized design with four replications of 25 seeds and six treatments.

90 The estimation of t50 and u7525 values was performed using GERMINATOR [13]. After the
91 adjustment of curves and determination of the indices t50 (time required for 50% germination
92 of seed germination, i.e. germination speed) and u7525 (time comprised between 25 and
93 75% of total germination, i.e. germination uniformity) mean values obtained were compared
94 by the Student t test at 5% probability.

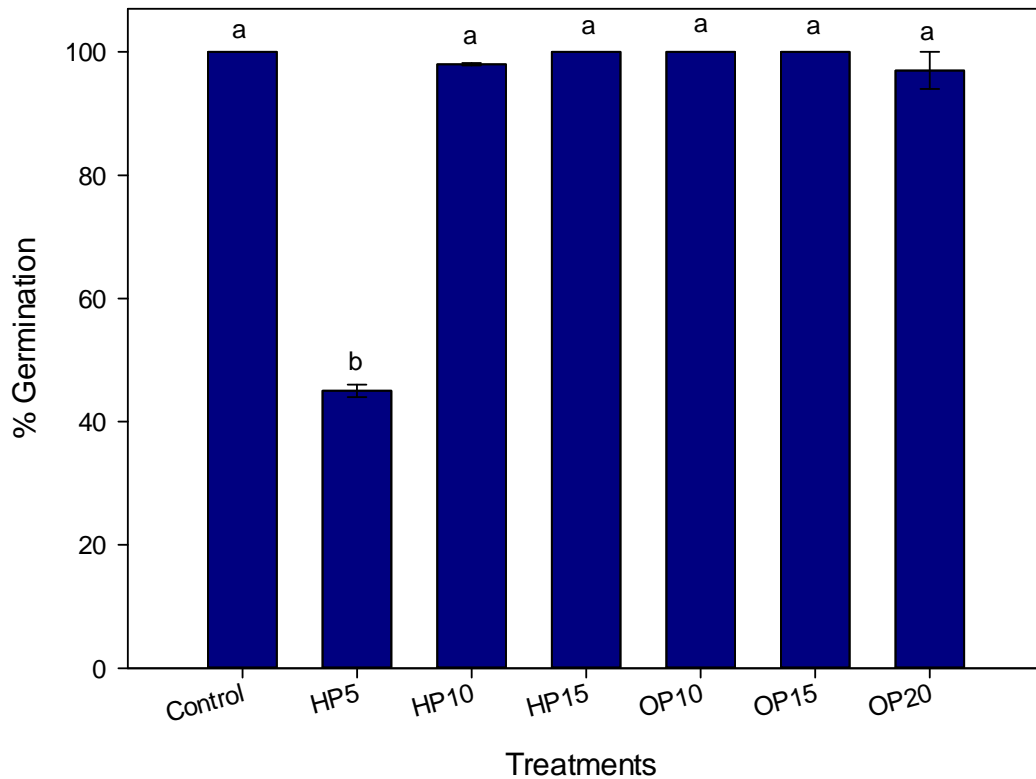
95 For analysis of the effect of priming on seed germination, it was used a completely
96 randomized design with four replications of 25 seeds, with treatments in a factorial scheme
97 2x11 (primed and not primed seeds x 11 periods of exposure to 40 °C). Comparisons were
98 performed by regression analysis using the software ASSISTAT v7.7 [14].

99 **3. RESULTS AND DISCUSSION**

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101 **3.1 Effect of priming on seed germination**

102 *H. serratifolius* seeds showed little variation in response to priming treatments concerning to
103 the maximum germination. In general, the osmopriming and hydropriming did not provide a
104 statistically significant increase compared to the control during germination (Fig. 1).
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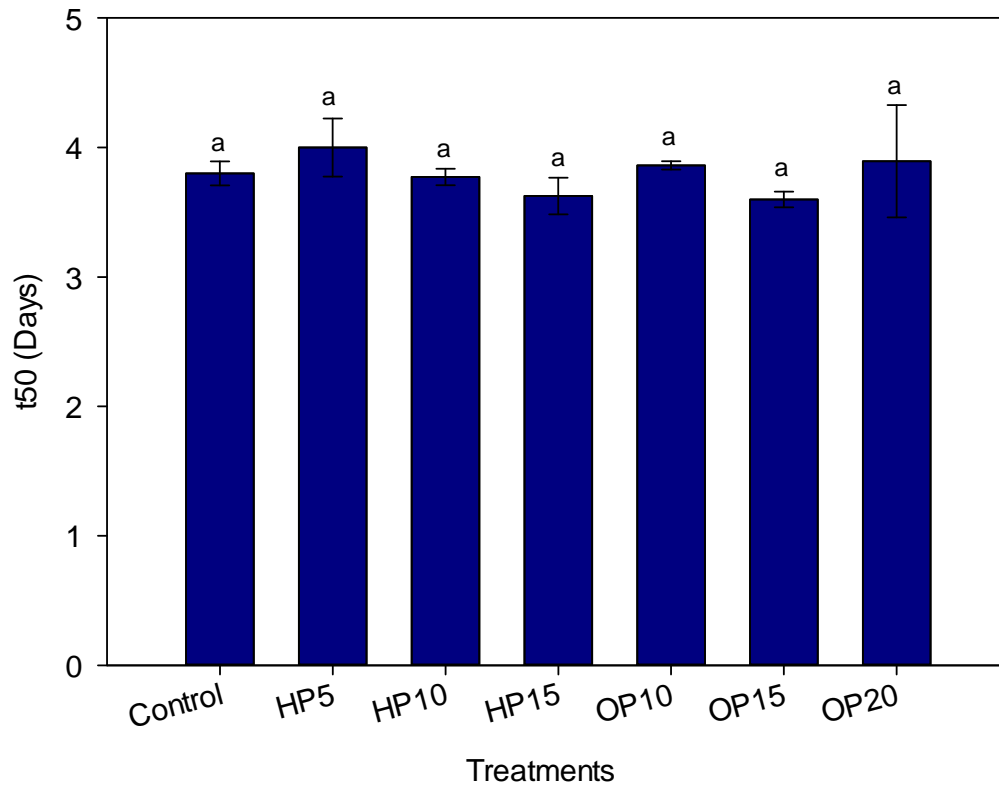


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Fig. 1. Effect of priming on germination of *H. serratifolius* seeds. Control, HP (Hydropriming), OP (Osmopriming). Bars represent standard deviation.

It was observed that seeds submitted to hydropriming showed 100% germination at temperatures of 10 to 15 °C, however, low percentage of germination was observed when seeds were hydroprimed at 5 °C. Osmoprimes seeds (PEG at -1.0 MPa at 10 and 15 °C) presented 100% germination, with a slight reduction (not statistically significant) in germination (97%) when osmoprimes was performed at 20 °C.

Regarding the speed of germination as measured by t50 (time required for 50% of the seeds germinate), there was a significant effect (Fig. 2) among the treatments. Osmoprimes and hydroprimed seeds at 15 °C had lower t50 (higher speed of germination) when compared to the control.

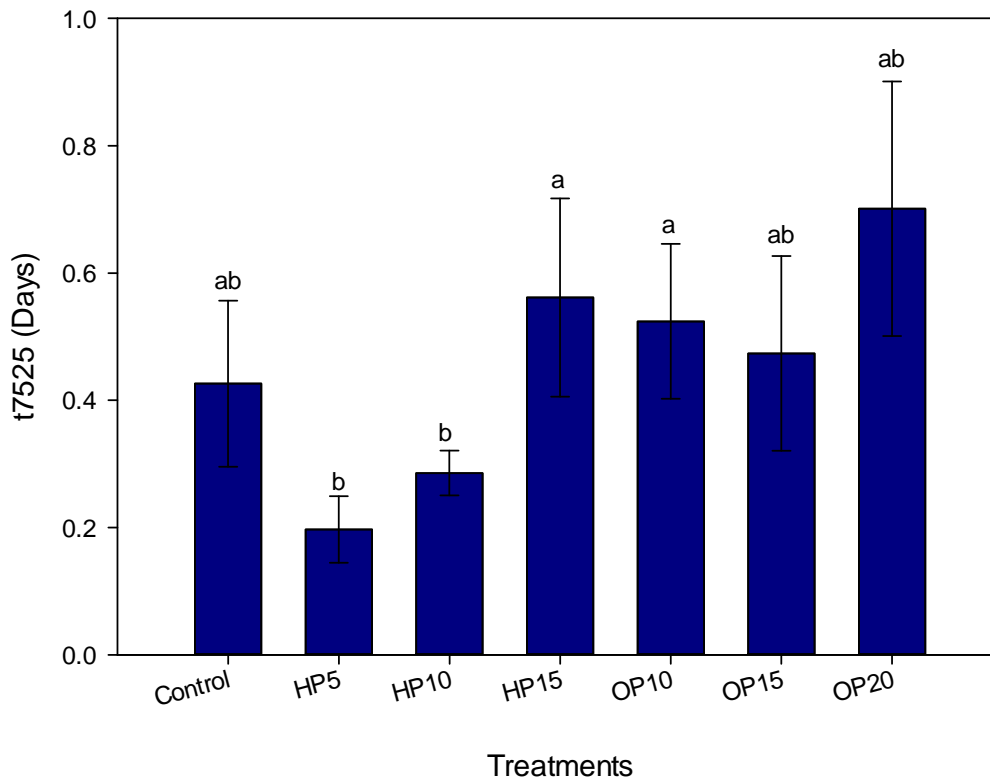


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Fig. 2. Effect of priming on speed of germination (t50) of *H. serratifolius* seeds. Control, HP (Hydropriming), OP (Osmopriming).

Mean ± S.E.M = Mean values ± Standard error of means of four replicates.

Although *H. serratifolius* seeds showed a positive response to priming regarding to the speed of germination (t50), there was no significant effect for most of the treatments with respect to the germination uniformity (u7525) (Fig. 3). The majority of the treatments did not differ in the uniformity of germination. Hydroprimed seeds at 5 °C and 10 °C showed a significant reduction in u7525, however, final percentage of germination of seeds hydroprimed at 5 °C was very low. The best results were observed in the following treatments: HP15, OP10, OP15 and OP20. (Fig. 3).



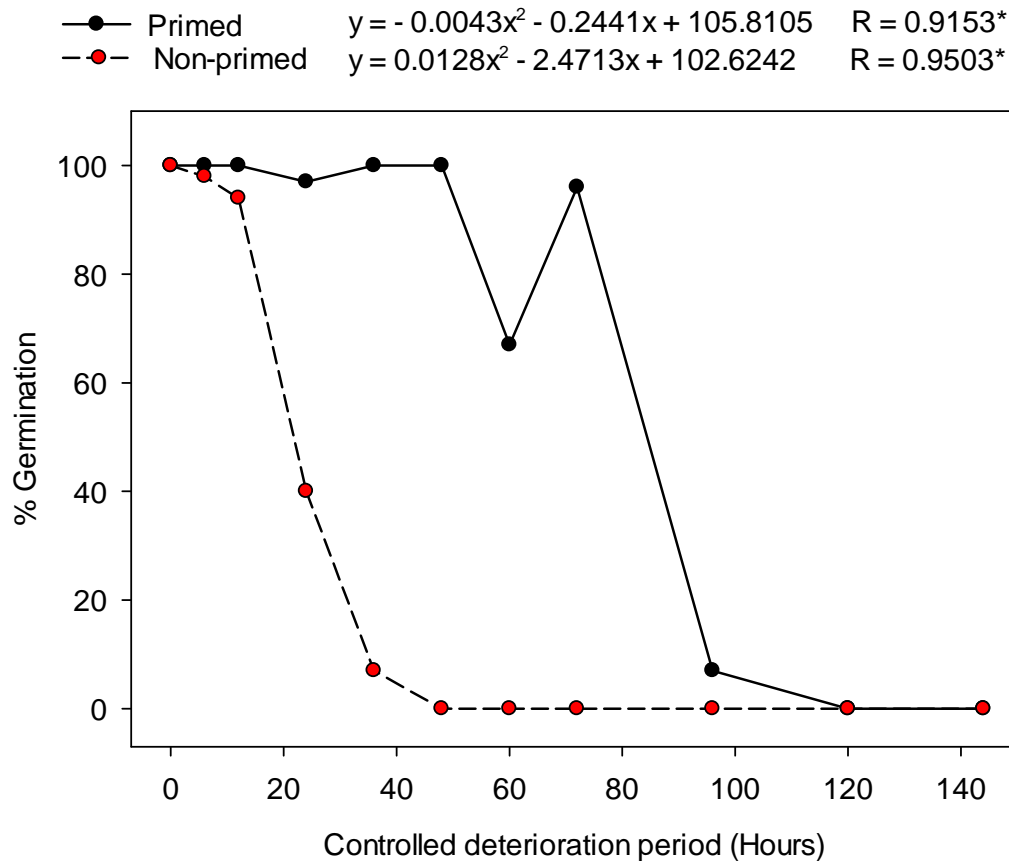
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Fig. 3. Effect of priming on germination uniformity (u_{7525}) of (*H. serratifolius*) seeds. Control, HP (Hydropriming), OP (Osmopriming).

Mean \pm S.E.M = Mean values \pm Standard error of means of four replicates.

3.2 Priming effect on seed longevity

151 Once seeds did not present huge variations in response to priming, hidroprimed seeds at 15
152 °C were selected because of high germination, low t_{50} , u_{7525} and simplicity of the protocol.
153 However, despite the low response of seeds to priming, probably due to high quality of the
154 seed batch, hidroprimed seeds showed different behavior when compared to the control (not
155 primed) after controlled deterioration. There were significant differences ($P < .0001$) between
156 treatments. Hidroprimed seeds showed higher tolerance to stress conditions during
157 controlled deterioration (Fig. 4).



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160 **Fig. 4. Effect of priming on seed longevity after controlled deterioration on *H.***
161 ***serratifolius*).**

162 After incubation for up to 12 hours at 40 °C, *H. serratifolius* seeds did not show changes in
163 viability, which remained above 90%. However, after 24 hours of controlled deterioration, not
164 primed seeds showed a reduction in germination to 40% and the total mortality after 36
165 hours of controlled deterioration. On the other hand, primed seeds only showed significant
166 reduction in viability after 60 hours (67%) of controlled deterioration.

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4. DISCUSSION

169 4.1 Effect of priming on seed germination

170 Some studies have found positive effects of priming on seed germination [15-17]. However,
171 the effects of priming in other species has not shown significant changes in the final
172 percentage of germination [18-21], as observed in this study.

173 One explanation for the absence of effect of treatments on the response of seeds in relation
174 to germination is the initial quality of the seed batch used. Seeds used in the control
175 treatment (not primed), in this work, showed 100% germination, which reduces the chances
176 of improvement on seed quality. Only priming at 5 °C adversely affect germination. This
177 negative response may be related to the low temperature limits tolerated by this specie,

178 indicating that temperatures around 5 °C can cause damages to *H. serratifolius* during
179 germination. According to [22], low temperatures slow down metabolic activity, causing a
180 reduction in the percentage of germination and therefore resulting in delayed germination
181 process.

182 Bering in mind the high seed quality used in this work, was not observed no positive effect
183 on germination speed. These results are not similar to that found by [23] and [24] that
184 observed positive effects of hydropriming in parsley and carrots seeds, which an increase in
185 germination rate were found.

186 The germination uniformity, is equally important to be considered. In general, after priming
187 there is an increase in the uniformity of germination of seeds compared to the control [25-
188 27). In this study, germination uniformity measured by u7525 was not affected by priming, as
189 observed for [28] in *Senna spectabilis* seeds.

190 **4.2 Priming effect on seed longevity**

191 According to [11] *H. serratifolius* presents problems during storage, which makes it
192 necessary studies to increase the longevity of seeds of this specie when stored.

193 It was observed that not primed *H. serratifolius* seeds where severely affected by controlled
194 deterioration when compared to primed ones. This result shows that hydropriming probably
195 induced physiological changes in the seeds leading to a higher tolerance to stress conditions
196 (high humidity and temperature). [17] after priming sorghum seeds in PEG solution at -0.6
197 and -1.2 MPa, and distilled water (hydropriming), observed increase the germination
198 percentage and longevity in hydroprimed sorghum seeds. Similarly, [24] and [29] found that
199 the priming contributed to the increase of vigor in carrot and gherkin seeds. The authors
200 report the importance of hydropriming treatment for seeds that will be exposed to adverse
201 environmental conditions after sowing.

202 The mechanisms associated with improvement in the quality of seeds after priming are not
203 yet fully known [30]. Some authors attribute this effect to the increase in activity of the
204 antioxidant system and membrane repair processes [31-34]. The greater tolerance of primed
205 seeds under controlled deterioration can be correlated with the gain of longevity after
206 priming. [7] analyzing seeds of *Digitalis purpurea* after priming found that the treatment
207 increased the longevity of seeds. Priming treatments used in this study have been shown to
208 increase the longevity of seeds *H. serratifolius*, however, more studies are needed using
209 different priming conditions in order to optimize a protocol for this species, bearing in mind
210 the potential of the priming technique in changing answers of *H. serratifolius* seeds for
211 tolerance to stresses. In the same way, studies to determine the factors associated with
212 better performance of *H. serratifolius* seeds after priming should be performed.

213 **5. CONCLUSION**

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216 Hydropriming at 15 °C increases germination speed and longevity of *Handroanthus*
217 *serratifolius* seeds, however other studies should test this same treatment to avaluate the
218 seed longevity along the storage.
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220 **COMPETING INTERESTS**

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222 Authors have declared that no competing interests exist.

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UNDER PEER REVIEW