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BOTANY

Seed germination of *Hesperozygis ringens* (Benth.) Epling, an endemic and threatened species

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ABSTRACT. *Hesperozygis ringens* (Lamiaceae), popularly known as *espanta-pulga*, is a threatened species endemic to rocky and sandy regions of the Pampa biome. One factor that can influence the low number of individuals of a species is a low seed germination rate influenced by temperature and/or light. Thus, the objective of this study was to evaluate the influence of light and temperature on the seed germination of *H. ringens*. The seeds of two lots were sown on a paper substrate and maintained in BOD chambers at temperatures of 15, 20, 25 and 30ºC in the presence and absence of light. The germination speed rate was evaluated every 3 days for 21 days. The experiment was completely randomized with treatments that had a 4 x 2 factorial design. The first visible sign (protrusion of the primary root) of germination was observed seven days after sowing. Germination occurred both in the presence and absence of light and the lowest temperatures significantly influenced the germination process and germination speed. For germinating the species, 15°C was the most favorable temperature compared to 20, 25 and 30°C. It can be concluded that a temperature of 15ºC favors the germination process of *H. ringens* seeds, which are insensitive to light.

Keywords: Lamiaceae; light; temperature; *espanta-pulga*.

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Introduction

Hesperozygis ringens (Benth.) Epling, popularly known as *espanta-pulga*, is a native shrub that belongs to the Lamiaceae (Figure 1a). It can be propagated from seeds (Figure 1b) or cuttings, of which the latter results in high rates of rooting, even with cuttings collected from populations *in situ* (Siqueira et al., 2020). The species is found in fields with rocky, sandy soils in Rio Grande do Sul, where it is restricted to Serra do Sudeste and the southern Missões region, in Alegrete, Caçapava do Sul, São Francisco de Assis and Santa Maria (Fracaro & Echeverrigaray, 2006; Freitas, Trevisan, Schneider, & Boldrini, 2010; Schaefer & Essi, 2017).

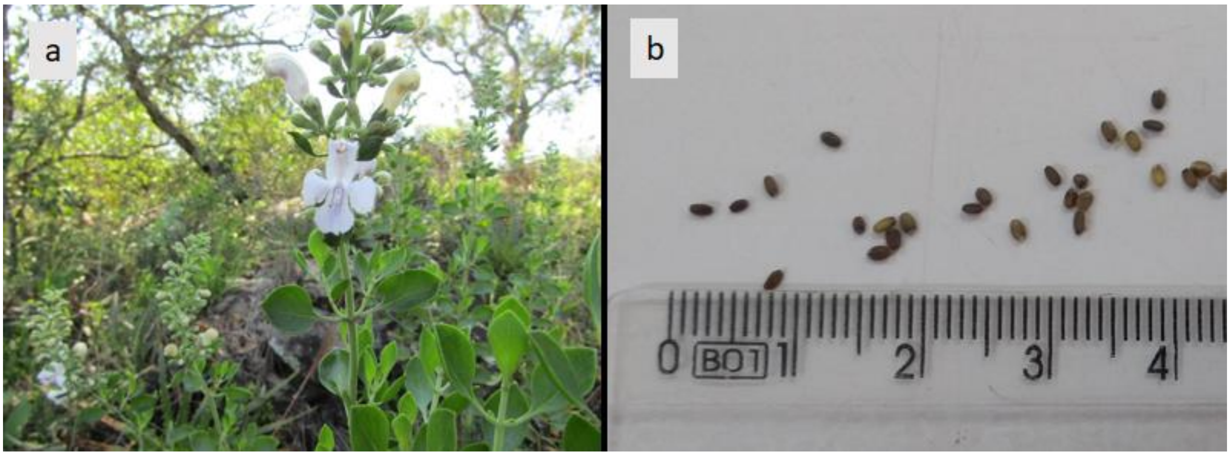

Figure 1. Adult plant (a) and seeds (b) of *Hesperozygis ringens*. Photo: L. Essi and R. Stefanello (2020).

For *Hesperozygis ringens*, some studies describe a high production of essential oil by the leaves, with pulegone (95.18%) as the principal component (Silva et al., 2014). The essential oil of this species has high antioxidant (Dolwitsch et al., 2020), antiparasitic and antimicrobial (Bandeira et al., 2017), alellopathic

(Pinheiro et al., 2016; Lima, Lima, Birck, & Stefanello, 2020), and anesthetic and larvicide action (Silva et al., 2014). Additionally, in a pioneering work about *in vitro* cytotoxicity and genotoxicity, Dolwitsch et al. (2020) demonstrated that its UAE-EtOH and SFE-CO_2 extracts are safe for human lymphocytes at the concentrations evaluated.

Due to being endemic to rocky hills and having seeds that do not germinate efficiently, the constant burning of pastures to feed cattle and the indiscriminate collecting of plants for medicinal uses, *H. ringens* has been included on the list of Brazilian species threatened with extinction (Rio Grande do Sul, 2008). Although there are taxonomic, medicinal and allelopathic studies about *H. ringens*, there are no reports about the germination process of its seeds. Thus, the objective of this study was to evaluate the influence of light and temperature on the germination of *Hesperozygis ringens* seeds.

Material and methods

The study was conducted at the Plant Genetic Laboratory in the Biology Department of the Center of Natural and Exact Sciences at the *Universidade Federal de Santa Maria* (RS). Two seed lots of *Hesperozygis ringens* (Benth.) Epling were used, which were collected (2020) in the cities of São Pedro do Sul (Lot A) and São Francisco de Assis (Lot B), Rio Grande do Sul, Brazil. The collections were made according to the authorization by SISBIO (number 60921). The plants and seeds were identified by the first author, by comparing them with specimens in the SMDB herbarium and taxonomic literature, and then confirmed by a taxonomist (third author). Vouchers from each population were deposited in SMDB. After manual separation, the seeds were stored in plastic bags in a refrigerated environment at 5-10°C for 7 days. No reports about seed dormancy of this species were found in the literature.

Before sowing, the seeds were sterilized by immersing them in 70% ethanol (10 seconds) and a solution of sodium hypochlorite with 1% chlorine (30 seconds). The seeds were then rinsed three times with distilled water.

The germination test was conducted with four repetitions of 50 seeds in plastic germination boxes (gerbox). The seeds were distributed on three sheets of paper moistened with distilled water at the proportion of 2.5 times the weight of the paper. After sowing, the plastic boxes were maintained in BOD (Biochemical Oxygen Demand) chambers at 15, 20, 25 and 30°C, in the presence and absence of light (Luz et al., 2014; Stefanello, Viana, & Neves, 2017). Counting was made every three days for 21 days.

The light condition (24 hours of light) in the germination chambers was made using four 20 W florescent bulbs with a radiant flow density at the height of the boxes of 15 $mmol.m^{-2}.s^{-1}$ (Koefender, Menezes, Buriol, Trentin, & Castilhos, 2009). For the absence of light, the seeds were sown in a room where the light was filtered with three green cellophane paper sheets. The germination boxes were maintained in the dark throughout the test by covering them with two sheets of aluminum foil (Stefanello et al., 2017). The evaluations for the condition without light were made in the room with filtered light.

The substrate was moistened once on day 10 (equal amount of water). Based on botanical criteria for germination, seeds were considered germinated when one of the parts of the embryo emerged from the seed coat, accompanied by some sign of an active metabolism, such as curvature of the radicle (Labouriau, 1983). The results were expressed as percentage of germinated seeds. The germination criterion was the protrusion of the primary root.

To evaluate the germination speed index (GSI), the germinated seeds were counted every 3 days at the same hour. The GSI calculation was adapted from the formula in Maguire (1962).

The experiment was completely randomized with treatments in a 4 x 2 factorial design (4 temperatures x presence or absence of light). A comparison between the different lots was not made because this was not the objective of the work. The data were submitted to an analysis of variance using the F test and, when a significant effect was found, the means were compared with the Scott Knott test. The analysis was made using the program SISVAR (Ferreira, 2011).

Results and discussion

The analysis of the germination data for the two lots of *Hesperozigys ringens* seeds showed there was no interaction between the light and temperature factors. Additionally, no significant differences in the two light conditions were observed; the seeds exhibited an indifferent behavior to light (Table 1). Similar results were obtained by Stefanello, Neves, Abbad, and Viana (2015), who verified that germination of chia seeds (*Salvia hispanica* L.) occurred both in the presence and absence of light.

Independent of the light environment, temperature significantly influenced germination (Table 1). The highest germination percentages were obtained at 15°C, in the presence and absence of light; although, seeds also germinated at 20 and 25°C. According to Paiva et al. (2016), the ability of seeds to germinate under a wide temperature range reflects the adaptability of the species to environmental thermal variations. Similar results were found for seed germination of other Lamiaceae species, such as *Ocimum basilicum* (Khan et al., 2014) and *Salvia hispanica* (Stefanello et al., 2015). Further, Saffariha, Jahani, and Potter (2020) evaluated the effect of five temperatures (10 to 30°C) on the germination of *Salvia limbata* seeds and verified that the greatest number of seeds germinated at 18.3°C.

Table 1. Germination (%) of *Hesperozigys ringens* seeds exposed to different temperatures, in the presence and absence of light, 21 days after sowing.

Temperature	Lot A		Lot B	
	Presence of light	Absence of light	Presence of light	Absence of light
15°C	44 a A*	40 a A	21 a A	21 a A
20°C	24 b A	20 b A	15 a A	11 b A
25°C	15 c A	4 c B	5 b A	1 c A
30°C	1 d A	0 c A	1 b A	1 c A

*Means followed by same lowercase letter in each column, and uppercase letter in each row, do not differ by the Scott Knott test at 5% probability.

For 30°C, both in the presence and absence of light, the germination results were insignificant (Table 1). High temperatures can influence enzymatic activity and restrict oxygen, which results in a rapid decrease in seed germination (Marcos Filho, 2015). Thus, high temperatures have deleterious effects on seed germination (Oliveira, Martins, Cruz, & Silva, 2014), as observed in this study, and germinating *H. ringens* seeds at 30°C is not recommended.

In general, the average germination time varied between 14 and 21 days and the first visible signs of germination, even for low percentages, were observed 7 days after sowing (Figure 2). According to Carvalho et al. (2020), the less time it takes seedlings to emerge from the soil and progress through early stages of development, the less vulnerable they are to adverse environmental conditions. Therefore, it is important to study ecophysiological conditions that influence seed germination, since the successful establishment of a species depends on seedling tolerance to unfavorable environmental conditions (Silva, Matos, Farias, Sena, & Silva, 2017).

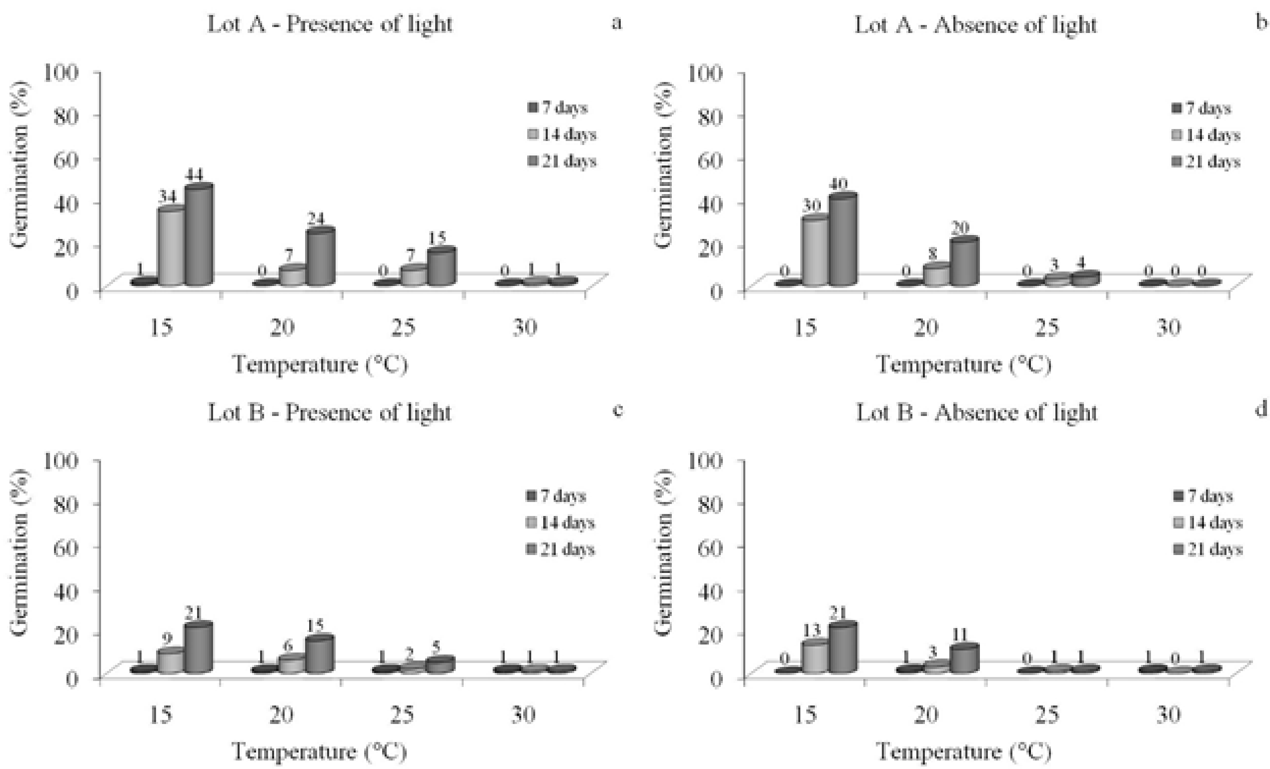

Figure 2. Average germination time of *Hesperozygis ringens* seeds.

Many aspects influence the seed germination process and can be correlated with viability, longevity and environmental conditions, such as light, temperature and oxygen (Luz et al., 2014). The germination process involves a sequence of metabolic activities where biochemical reactions occur. Each reaction has its own requirements (mainly temperature) because it depends on the activity of enzymatic systems (Marcos Filho, 2015). Knowledge about the response of seeds to temperature, as well as other climatic conditions needed to germinate and become established, is fundamental to understand the tolerance of a species (Motsa, Slabbert, Van Averbeke, & Morey, 2015).

On the other hand, although light is not an essential factor for the germination process to take place in non-dormant seeds, it can contribute to reducing problems caused by the effects of high temperatures and low water potential in the soil (Marcos Filho, 2015). The sensitivity of seeds to light varies considerably according to species, where seed germination is positively, negatively or not influenced by this factor (Carvalho & Nakagawa, 2012; Taiz, Zeiger, Moller, & Murphy, 2017). Light can induce seed germination, as found in some forage grasses, while for other species it can inhibit germination (Brasil, 2009). In the present study, *H. ringens* germinated independent of the light condition and can be considered indifferent to light.

Although this work did not have the objective of comparing the seed lots, the quality of lot B was lower than lot A. However, this does on invalidate the importance of the results, since this is a pioneering study about the species. The differences in the response between the lots could have been due to the moisture content in the seeds, the different collection localities (although the environment is very similar-rocky grasslands and sandy soil-population genetic differences may influence the response), absence of information about the occurrence of dormancy, and the presence of fungi. According to Marcos Filho (2015), germination rates are highly linked to the species and its genetic characteristics, environmental conditions during cultivation, management during and after harvest, and sanitation. In the present study the disinfection process was not successful, since there was a fungus on the seeds that could have contributed to the low germination rate. It is also notable that this study did not conduct tests to identify the fungus on the seeds.

In relation to germination speed (GSI), a behavior similar to germination was observed (Table 2). The germination speed was greater in seeds submitted to the lowest temperature (15°C), independent of the light condition. According to Carvalho and Nakagawa (2012), temperature influences both speed and germination percentage because it tends to influence the speed of water absorption and biochemical reactions.

Table 2. Germination speed index (GSI) of *Hesperozigys ringens* seeds exposed to different temperatures, in the presence and absence of light, 21 days after sowing.

Temperature	Lot A		Lot B	
	Presence of light	Absence of light	Presence of light	Absence of light
15°C	3.32 a A*	2.74 a B	1.37 a A	1.33 a A
20°C	1.37 b A	1.27 b A	0.99 b A	0.69 b A
25°C	0.91 b A	0.25 c B	0.33 c A	0.04 c A
30°C	0.08 c A	0.00 c A	0.01 c A	0.00 c A

*Means followed by same lowercase letter in each column, and uppercase letter in each row, do not differ by the Scott Knott test at 5% probability.

Understanding seed germination is fundamental to determining a plants ability to become successfully established in a specific habitat or geographic region. Seeds need to germinate at the right time and place to maximize the chance of seedlings surviving and becoming established (Cristaudo, Catara, Mingo, Restuccia, & Onofri, 2019). Thus, learning more about the ideal conditions for seed germination and the initial development of plants plays a fundamental role in scientific research and provides reliable information about how to propagate species (Stefanello et al., 2015). Although a low germination rate was observed, the results of this work are pioneering and fundamental to understanding the restricted distribution of *H. ringens*. Therefore, future studies could be conducted with new seed collections to test other sterilization times and/or products, as well as the influence of other temperatures (higher and lower) on the seed germination of this species.

Conclusion

Germination of *Hesperozygis ringens* seeds occurred both in the presence and absence of light and the lowest temperatures significantly influenced the germination process and speed. A temperature of 15°C is better to germinate the species compared to 20, 25 and 30°C. A temperature of 30°C is not recommended to germinate seeds of this species. A temperature of 15ºC, under the presence and absence of light, favors the germination process of *H. ringens* seeds.

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