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## The effect of temperature on the germination of *Melocactus violaceus* Pfeiff. (Cactaceae), a threatened species in restinga sandy coastal plain of Brazil

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### ABSTRACT

*Melocactus violaceus* is an endangered species due to habitat destruction and the overcollection of this species for ornamental use. The aim of this study was to test the effect of different temperatures on the germination of *M. violaceus*. Three treatments were conducted: a constant temperature of 25°C, a 20-35°C alternating temperature, both inside germination chamber, and an alternating temperature under room temperature (mean temperature ranged from 25-37°C). The final seed germination rates at the alternating temperature treatments were not significantly different (65% in the seed germinator and 62.5% at room condition). However, both treatments with alternating temperatures had significantly higher germination rates compared to the treatment kept at the constant temperature (8%). Our study showed that alternating temperatures between 20 and 37°C provides satisfactory conditions to induce a high percentage of seed germination of *M. violaceus*, without the passage of seeds through the digestive tract of its natural disperser, the lizard *Tropidurus torquatus*. This condition contributes to efficiently producing seedlings that can be reintroduced into conservation areas or used as ornamentals that may help reduce the overcollection of the remaining native populations.

**Key words:** Cactaceae, conservation, germination, *Melocactus violaceus*, restinga, temperature.

### INTRODUCTION

Restingas and mangroves are vegetation formations associated with the Atlantic Forest (Rizzini 1979, Scarano 2002), which has been reduced to 7.5% of its original size, and is considered one of the 25 biodiversity hotspots, and one of the five most threatened regions in the world (Myers et al. 2000). Restinga is composed of sandy coastal plains of marine origin and comprises beach ridges, dunes,

inter-ridge and inter-dune depressions, swamps, marshes and lagoons (Lacerda et al. 1993). The plant communities that occur in these topographic zones can be extremely diverse.

Humans have influenced restingas since their occupation by indigenous populations 8,000 years ago (Kneip 1987). However, it was throughout the 20<sup>th</sup> century that most of the degradation of this ecosystem occurred because of urban growth, 90% of the restingas have been destroyed at the present time (WWF 2007). Even the regions that are legally

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recognized as protected areas still suffer from fire, sand mining, extraction of ornamental species, urban development, and tourism (Zamith and Scarano 2006). The failure to control these actions led to the reduction of areas within restinga, and in 1999 only 0.63% (770.65 ha) of the total area of the municipality of Rio de Janeiro was still restinga (PCRJ 2000a). The majority of the remaining habitat is in an accelerated process of degradation, putting a lot of species in threat of local extinction (PCRJ 2000b). For this reason, in 1993 the city government of Rio de Janeiro implemented the project *Flora do Litoral*, which goals are to restore the degraded restingas and to produce seedlings of native taxa, including threatened species (Zamith and Dalmaso 2000).

*Melocactus violaceus* Pfeiff. is one of the threatened species in restingas of Rio de Janeiro (PCRJ 2000b). This species is also cited as vulnerable in the states of Espírito Santo (2005) and Pernambuco (Uchoa Neto and Tabarelli 2002), and is on the Red List of the threatened flora published by the International Union for Conservation of Nature (IUCN 2009). The reproduction of *Melocactus* species in natural habitats occurs exclusively by seeds (Taylor 1991). The reproductive restrictions of *M. violaceus*, the overcollection for ornamental purposes, and the destruction of its habitat are causing this species to disappear from the restingas of the municipality of Rio de Janeiro, where individuals can rarely be found in remnants of Barra da Tijuca and Marambaia (pers. obs.). Illegal extraction has put many other cacti species at risk, and *ex situ* propagation has been suggested as an alternative for conservation of these species (Rojas-Aréchiga and Vazquez-Yanes 2000, Flores et al. 2006). However, Rojas-Aréchiga and Vazquez-Yanes (2000) pointed out that little is known about the requirements for germination, longevity, and seed viability for taxa in this family, and that there is an urgent need for more studies. Thus, the cultivation of *M. violaceus* from seeds would allow the reintroduction of individuals into less

impacted regions and restoration areas, increasing the chances of maintaining and preserving populations (Zamith and Scarano 2004).

Germination is controlled by physical factors of the environment, such as light and temperature, and the combination of these factors directly affects seedling establishment (Baskin and Baskin 1988, Ghera et al. 1992). However, the influence of these factors varies among plant species (Vázquez-Yanes and Orozco-Segovia 1993) and this information is needed for assessing the germination of seeds preserved in seed banks and to produce seedlings, both important for the conservation of threatened species (Almeida et al. 2009).

Light is an important factor that influences germination. Many cactus seeds are positively photoblastic, and complete darkness can reduce their germination capacity (Rojas-Aréchiga & Vázquez-Yanes 2000, Flores et al. 2006, 2011). Seeds of *M. violaceus* are positively photoblastic (unpublished data), which has also been observed for the seeds of *M. conoideus* (Rebouças and Santos 2007), and require light to germinate. This requirement is common among species with small seeds that have small reserves, reducing the probability of germination when the seeds are buried in the soil (Pons 2000, Zaidan and Barbedo 2004).

Among species of Cactaceae, a wide response to temperature has been observed (Rizzini 1982, Lucas and Frigeri 1990, Nolasco et al. 1996, Rojas-Aréchiga et al. 2001, Ramírez-Padilla and Valverde 2005, Méndez 2007, Simão et al. 2007), and frequently the effects of constant and alternating temperatures on cacti seed germination show contradictions between different species (Ortega-Baes et al. 2011). In a review article, Rojas-Aréchiga and Vazquez-Yanes (2000) observed that a temperature of  $20 \pm 2^\circ\text{C}$  gives a good germination rate in a wide range of genera and alternating temperatures give better germination results than constant temperatures. However, the authors mentioned that in some experiments alternating

temperatures regime did not show significant differences when compared to treatments kept at a constant temperature, and that whenever there were significant differences they were related to a lower constant temperature (around 17°C) (Godínez-Alvarez and Valiente-Banuet 1998).

The aim of this study was to test the effect of different temperature ranges on the germination of the *M. violaceus* seeds and identify the conditions that are most adequate for the production of seedlings that will be reintroduced into protected areas. Until this study, the temperature range needed for the germination of *M. violaceus* was not known.

#### MATERIALS AND METHODS

*Melocactus violaceus*, commonly known as *corôa-de-frade* in Brazil, grows in the states of Rio Grande do Norte, Paraíba, Pernambuco (Zickel et al. 2007), Sergipe, in the rupestrian fields of Chapada Diamantina in Bahia (Alves et al. 2007), Minas Gerais, Espírito Santo, and Rio de Janeiro (Taylor 1991). The subspecies that grows in the restinga from Rio de Janeiro to Rio Grande do Norte is *Melocactus violaceus* subsp. *violaceus*, and can also be found up to 1,100 m elevation in the *cerrado* of Minas Gerais (Taylor 1991). This subspecies has a depress-globose, hemispheric, or disc-shaped stem that is 6 to 16 cm in diameter, with 6 to 12 spines per areole, pinkish flowers measuring 25 × 13.5 mm, pinkish fruits measuring 12.5–19 × 5.5–7.5 mm, and seeds measuring 1.2–1.5 × 1.0–1.4 mm (Taylor 1991). In restinga, *M. violaceus* typically grows in open, non-flooded shrubland, exposed to the sun (Freitas 1990, Côrtes Figueira et al. 1994), and does not survive in heavily shaded areas (pers. obs.).

Fruits of *M. violaceus* were collected in May 2001 from 60 individuals kept at Horto Carlos Toledo Rizzini (22°59'S, 43°22'W), which houses the headquarters of project *Flora do Litoral*. The individuals came from plants that were illegally harvested from restingas in the state of Rio de

Janeiro and were seized by the *Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais (IBAMA)*. In nurseries, individuals of this species produce flowers and fruits throughout the year (Zamith and Scarano 2004).

The seeds were manually removed from the fruits using running water and were dried and stored in plastic bags for six months, at room temperature. After storage the seeds were disinfected using a sodium hypochlorite solution (15%) for 10 minutes and then washed with distilled water. The seeds were placed in plastic square boxes (11 × 11 × 3.5 cm) on top of restinga sand that had been sterilized for two hours at 150°C.

Three treatments were used to investigate the effect of temperature on seed germination: a constant temperature of 25°C; an alternating temperature of 20–35°C; and a temperature that varied based on room temperature (RT), ranging between 24–41°C with a minimum mean of 25 ± 1°C and a maximum mean of 37 ± 0.8°C. The first two treatments were conducted in germination chambers (J. Prolab Ind. e Com. de Produtos para Laboratório Ltda., Model JP 1000, Brazil and Eletrolab Ind. e Com. de Equipamentos para Laboratório Ltda., Model EL 212, Brazil), and the third treatment was kept on a lab bench, under a light intensity of 1,154 lux, produced by white light, similar to the light produced by the germination chamber. All treatments had a photoperiod of 12 hours. The experimental design was entirely random. Four replicates per treatment were made using 50 seeds per replicate. Seed germination that started with the protrusion of the radicle was observed for 63 days. Each treatment was watered, and newly germinated seeds were counted, every two to four days.

For the statistical analysis, the values for the germination percentage were transformed into arcsine  $\sqrt{\%/100}$  and submitted to variance analysis. When a significant difference was found by the F test, the mean was compared using Tukey's test at the probability level of 5% (Zar 1999). The germination speed index

(GSI) was obtained from the formula proposed by Maguire (1962):  $GSI = G_1/N_1 + G_2/N_2 + \dots + G_n/N_n$ , where:  $G_1$ ,  $G_2$ , and  $G_n$  are the numbers of germinated seeds on the first, second, and last count; and  $N_1$ ,  $N_2$ , and  $N_n$  are the numbers of days between the count and the beginning of the experiment.

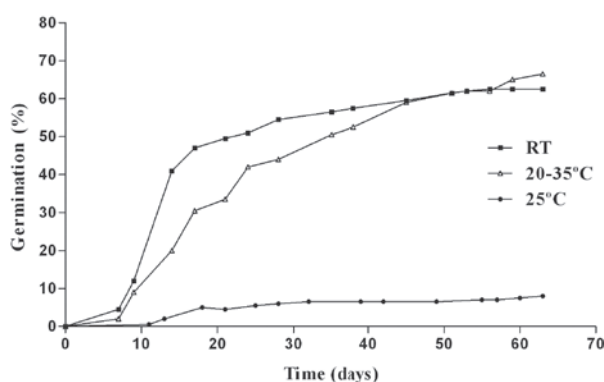
Additionally, in September 2002, based on the results obtained from the three treatments, another treatment was conducted under a constant temperature of 35°C. The aim of this treatment was to evaluate the effect of a higher constant temperature on germination. The seeds used in this treatment were stored for six months, as done for the seeds used in original treatments. Due to a lack of seeds, four replicates were made with 25 seeds each. This treatment was conducted in a germination chamber and watered every two to four days.

## RESULTS

The time course of germination at different temperatures (Fig. 1) followed a typical sigmoid curve. At the alternating temperatures (20-35°C and RT treatments), the lag time to onset of germination was ca. seven days. The constant temperature retarded germination by increasing the lag time (10 days), reducing the germination rate and lowering the final germination percentage. At the end of 63 days, no germination was observed among treatments.

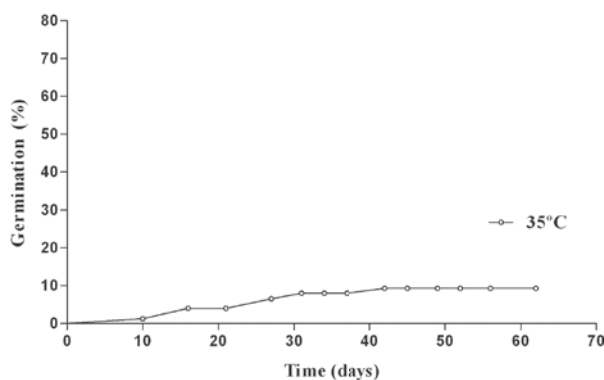
The highest percentages of germination were obtained by exposing the seeds to alternating temperatures that promoted statistically higher germination percentage ( $F=18.94$ ,  $df=2$ ,  $p<0.001$ ) than to constant temperature (Tab. I). There were no significant differences ( $p>0.05$ ) between the two treatments with alternating temperatures.

The GSI, as well as the germination percent, was significantly different ( $F=8.61$ ,  $df=2$ ,  $p<0.01$ ) between the treatment kept at a constant temperature and the treatments with alternating temperatures. The GSI was slightly higher for the RT treatment (2.21), but was not statistically different from the 20-35°C treatment (1.78).



**Figure 1** - Means of germination percentages of *Melocactus violaceus* at three different temperature treatments (alternating temperature between 20-35°C and room temperature (RT), and a constant temperature of 25°C) over 63 days.

The time course of germination at constant temperature of 35°C (Fig. 2) showed a pattern similar to germination at constant temperature of 25°C, and the GSI was  $0.11 \pm 0.09$ .



**Figure 2** - Means of germination percentages of *Melocactus violaceus* at constant temperature of 35°C over 63 days.

**TABLE I**

Means ( $\pm$  SD) of germination percentage and germination speed index (GSI) of *Melocactus violaceus* at three different temperature treatments (alternating temperature between 20-35°C and room temperature (RT), and a constant temperature of 25°C). Means were compared by ANOVA and different letters in same column indicate significant differences according Tukey test (\* =  $p<0.05$ ; \*\* =  $p<0.01$  and \*\*\* =  $p<0.001$ ).

Treatments	Germination (%)	GSI
20-35°C	66.5 $\pm$ 18.8 a ***	1.78 $\pm$ 1.14 a*
RT	62.5 $\pm$ 17.9 a **	2.21 $\pm$ 0.50 a**
25°C	8.0 $\pm$ 1.6 b	0.20 $\pm$ 0.05 b



## DISCUSSION

Alternating temperatures allowed good germination rates for seeds of *M. violaceus*. In their natural habitat, seeds are exposed to alternating temperatures, which is a more favorable condition for germination compared to a constant temperature (Baskin and Baskin 2001). However, since cactus seedling establishment generally occurs under nurse plants, where the amplitude of soil temperature fluctuations is less extreme (Godínez-Alvarez et al. 2003), Ortega-Baes et al. (2011) suggest that cacti seeds might germinate better under constant temperatures. Recent works on germination of different cacti seeds have reported that alternating temperatures induce, inhibit, or no promote different responses when compared to constant temperatures (Godínez-Alvarez and Valiente-Banuet 1998, Rojas-Aréchiga et al. 1998, Rojas-Aréchiga and Vásquez-Yanes 2000, Ramírez-Padilla and Valverde 2005, Ortega-Baes and Rojas-Aréchiga 2007, Ortega-Baes et al. 2011), and these results have been attributed to a limited number of temperature regimes tested (Rojas-Aréchiga et al. 1998, Rojas-Aréchiga and Vásquez-Yanes 2000, Ortega-Baes and Rojas-Aréchiga 2007).

In a study with four Brazilian cacti species, Almeida et al. (2009) observed that seeds of these species did not require the alternating temperature regime and constant temperatures were more favorable to germination. Constant temperatures, ranging from 25 to 30°C, also seem to be efficient to the germination of *Cereus jamacaru* in areas of *Caatinga* in the Northeast of Brazil (Meiado et al. 2010).

Variation in seed germination response can occur among species that are closely related, such as the different results found in germination studies that have used species of *Melocactus*. For example, for *M. bahiensis* (Britton & Rose) Luetzelb, which occurs in exposed areas or under or between shrubs, in the rupestrian fields and *caatingas* of Pernambuco, Bahia, and northern Minas Gerais (Taylor 1991),

it was observed that the highest germination percentage occurred when seeds were exposed to a constant temperature of 25°C (48% and GSI = 3.58), and the seeds did not germinate when the temperature varied between 20-30°C (Lone et al. 2007). For *M. conoideus* Buining & Brederoo that grows under and between shrubs in high elevation cerrados (1,050 m) in southeastern Bahia (Taylor 1991), Rebouças and Santos (2007) observed a germination index of approximately 84% under a constant temperature of 30°C. Our results for *M. violaceus*, show a significantly higher percentage and speed of germination when seeds were under alternating temperatures, suggesting that this regime is ideal for inducing seed germination of this species. This was confirmed at the end of the experiments, when the seeds used in the treatment at a constant temperature of 25°C, that did not germinate (N=184), were submitted to alternating temperatures (20-35°C). Under this new condition, these seeds began to germinate on the eleventh day, and, after 63 days, 42% of the seeds germinated (GSI =  $2.78 \pm 0.17$ ). The low percentage and speed of germination obtained under a constant temperature of 35°C corroborate that alternating temperature promotes highest germination rate for this species.

Côrtes Figueira et al. (1994) performed laboratory experiments at room temperature to study the effect of saurochory on the germination of *M. violaceus* seeds. The authors observed that germination rates of seeds that went through the digestive tract of the lizard *Tropidurus torquatus* Wied were higher than those from seeds collected directly from the fruits. Those results differ from what was found in our study because we observed good germination rates without participation of *T. torquatus*. In the present study, the germination percentages in the experiments that used variable temperatures (66.5% under 20-35°C and 62.5% at RT) were higher than the results observed by Côrtes Figueira et al., where the seeds had passed through the intestinal tract of the lizard (mean of 36%).

This difference demonstrates that *M. violaceus* seeds are able to germinate without passing through the digestive tract of this species, as long as the temperature and light conditions are adequate. However, the ingestion of fruits by this lizard is important to the ecology and conservation of *M. violaceus* because the lizards act as a seed disperser. Moreover, it is possible that germination experiments with seeds eaten by the lizard and under controlled conditions achieve a higher germination percentage than our results obtained.

The geographic distribution and the ecological preferences of many species are defined, among other factors, by the environmental conditions required for the germination of their seeds (Cuzzuol and Lucas 1999, Ramírez-Padilla and Valverde 2005). Since *M. violaceus* occurs in restinga, exclusively in the open areas among shrubs and exposed to the sun, the temperature variation during the day and night must provide the temperature variation that the seeds need to germinate.

The extraction of Cactaceae species from native habitats, because they are valued as ornamentals, has put many species at risk. Propagation studies can contribute to the conservation of these plants, since the production of seedlings can lower the demand for individuals from natural populations (Rojas-Aréchiga and Vaquez-Yanes 2000). This study showed that alternating temperatures between 20°C and 37°C provides satisfactory conditions to induce a high percentage of seed germination of *M. violaceus*. The results obtained during this study will allow us to test the viability and germination of the seeds stored in seed banks for conservation purposes, and will allow for the production of seedlings of this threatened species, making it possible to reintroduce it into protected areas. This will also contribute to reduce the number of individuals collected from the remaining natural populations.

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#### RESUMO

*Melocactus violaceus* é uma espécie ameaçada devido à destruição de seu habitat e a coleta excessiva para uso como ornamental. O objetivo deste estudo foi testar o efeito de diferentes temperaturas sobre a germinação de *M. violaceus*. Três tratamentos foram conduzidos: temperatura constante de 25°C, temperatura alternada de 20-35°C, ambos em câmaras de germinação, e temperatura alternada em condição ambiente (onde a temperatura variou entre 25-37°C). Os percentuais finais de germinação nos tratamentos com temperatura alternada não foram significativamente diferentes (65% na câmara de germinação e 62,5% na condição ambiente). Entretanto, ambos os tratamentos com temperaturas alternadas tiveram percentuais de germinação significativamente maiores comparados com o tratamento mantido em temperatura constante (8%). Nosso estudo mostrou que temperaturas alternadas entre 20 e 37°C fornecem condições satisfatórias para induzir um alto percentual de germinação de sementes de *M. violaceus*, sem a passagem das sementes pelo trato digestivo de seu dispersor natural, o lagarto *Tropidurus torquatus*. Esta condição contribui para a eficiente produção de plântulas desta espécie que podem ser reintroduzidas em unidades de conservação ou usadas como ornamentais, auxiliando na redução da coleta excessiva sobre as populações nativas remanescentes.

**Palavras-chave:** Cactaceae, conservação, germinação, *Melocactus violaceus*, restinga, temperatura.

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