GERMINATION, SEED TRAITS, AND SEEDLING VIGOR OF *PILOSOCEREUS ROBINII* (CACTACEAE) FROM NORTHWESTERN CUBA

Duniel Barrios*
Grupo de Ecología y Conservación
Jardín Botánico Nacional
Universidad de La Habana, CUBA
**Corresponding author*
duniel.barrios@gmail.com

Jorge A. Sánchez
Instituto de Ecología y Sistemática
Ministerio de Ciencia
Tecnología y Medio Ambiente, CUBA

Luis R. González-Torres
Department of Biology
Douglas College
British Columbia, CANADA

ABSTRACT

Studies on the germination of Caribbean cacti are essential for conservation and ecological restoration programs. We evaluated the seed traits and germination response of *Pilosocereus robinii* under five temperatures and two light conditions and compared the vigor of the seedlings obtained. Seminal traits showed characteristics of orthodox seeds. *Pilosocereus robinii* seeds showed positive photoblastism and a range of optimal temperatures below 30°C. In all treatments where germination was suboptimal, the seeds demonstrated high recovery percentages when they were exposed to the optimal temperature. The vigor of the seedlings showed a behavior similar to germination. Our study shows that restoration plans for the species' populations are viable from seeds.

KEY WORDS: photoblastic response, orthodox behavior, temperatures, climatic change

RESUMEN

Los estudios sobre la germinación de cactus caribeños son esenciales para programas de conservación y restauración ecológica. Nosotros evaluamos los rasgos de las semillas y la respuesta germinativa de *Pilosocereus robinii* en cinco temperaturas y dos condiciones de luz, además, comparamos el vigor de las plántulas obtenidas. Los rasgos seminales mostraron características de semillas ortodoxas. Las semillas de *Pilosocereus robinii* mostraron fotoblastismo positivo y un rango de temperaturas óptimas inferior a 30°C. En todos los tratamientos donde la germinación fue subóptima las semillas mostraron altos porcentajes de recobro cuando fueron expuestas a la temperatura óptima. El vigor de las plántulas mostró una conducta similar a la respuesta germinativa. Nuestro estudio demuestra que los planes de restauración de las poblaciones de la especie son viables a partir de semillas.

PALABRAS CLAVES: respuesta fotoblástica, conducta ortodoxa, temperaturas, cambio climático

INTRODUCTION

Germination is one of the most critical processes in the life cycle of plants and includes two of its most susceptible phases: seed and seedling (Fenner 1985). Because of this, germination studies are vital to the success of plant species conservation programs and constitute an indispensable tool for the propagation of wild plants. Although the Caribbean is considered a biodiversity hotspot, with more than 10,000 native plants and 71% endemism (Acevedo-Rodríguez & Strong 2012), studies on the germination of its native flora represent a relatively small portion (Sánchez et al. 2019).

Prior literature on the Cactaceae family only describes germination responses for approximately 15 Caribbean species (Zimmer 1967; Dehan & Pérez 2005; Salazar et al. 2013; Barrios et al. 2015; Seal et al. 2017; Barrios et al. 2021 a,b), representing 8.6% of the 116 native species recorded for the region (Barrios unpublished data). Most of these species have optimal temperatures below 30°C, and their germination is inhibited between 35–40°C. Furthermore, all the species grow in habitats with average temperatures in the wettest quarter between 0–4°C lower than the optimal germination temperature, which represents a narrow thermal buffer capacity (Seal et al. 2017; Barrios et al. 2020). All these data are concerning when we consider the trend in the Caribbean region of decreased precipitation and increased temperature (Naranjo-Diaz & Centella 1998; Stephenson et al. 2014), which predicts increases in average temperatures higher than the optimal temperatures for the germination of most cacti in the region (Seal et al. 2017). These trends could significantly reduce the regeneration of these cacti. In fact, there is already evidence of some species in Cuba with very low regeneration (González-Torres et al., 2012; Barrios & Mancina 2017), and a *Pilosocereus* species from the US

J. Bot. Res. Inst. Texas 18(2): 359–368. 2024
https://doi.org/10.17348/jbrit.v18.i2.1372

locally extinct in the wild by the rising of sea level (Possley et al. 2024) which makes it urgent to study their germinative response, storage behavior, and seed bank conservation.

Pilosocereus robinii (Lem.) Byles & G.D. Rowley is an endemic and threatened cactus species (Salazar et al. 2013; González-Torres et al. 2016) exclusive to Florida Keys, Cuba and Bahamas (Franck et al. 2019). In both Cuba and the US, habitat loss, diseases, and hurricanes have severely affected the populations of this species, which has been under conservation for more than a decade through the efforts of the Fairchild Tropical Botanic Garden (Maschinski et al. 2023). Aspects of germination in this species have been studied with regards to its conservation. In Cuba, Quiala et al. (2009) obtained excellent results growing seeds of *P. robinii* on MS basal medium supplemented with 1.0 mg/l thiamine, 30 mg/l sucrose and 2.0 g/l Gelrite®. On the other hand, in the US, Salazar et al. (2013) stored seeds of the *P. robinii* in low humidity and temperatures for up to 28 weeks. They obtained high germinability percentages and concluded that the seeds possess orthodox storage behavior, meaning they can be stored for long periods without losing viability. However, the optimal temperature range for the species and other morphophysiological aspects of its seeds, which could aid in conservation, remain unknown.

The main objective of this study is to evaluate the germination response and vigor of its seedlings at different temperatures. In addition, the storage behavior of the species and the photoblastic response are evaluated using seminal traits. Greater germination and development of seedlings is predicted at average temperatures below 30°C, corresponding to the optimal temperatures recorded for cacti (Barrios et al. 2020). It is also expected to find positive photoblastic seeds and orthodox storage behavior like other species of *Pilosocereus* [*P. arrabidae*, *P. chrysostele*, *P. pachycladus*, *P. piauhyensis*, etc] (Flores et al. 2011; Meiado et al. 2016).

MATERIALS AND METHODS

The species

Pilosocereus robinii is a small columnar succulent tree or candelabriformis branched very close to the base up to 8.0 m high. The species has dehiscent, globose purple to reddish fruits measuring 4 cm in diameter (Alain 1953; Franck et al. 2019). The fruits can be available throughout the year, are dispersed by birds and bats and contain more than 300 shiny black seeds (Barrios, pers. obs.). *Pilosocereus robinii* inhabit coastal-subcoastal xeromorphic thickets, microphyllous evergreen forests and mesophilic semi-deciduous forest in Cuba and is only located in the provinces of Havana, Mayabeque, and Matanzas (Barrios et al. 2023).

Collection site

The fruits were collected on June 30, 2016 from 3 only accessible individuals at km 43 of the Vía Blanca [also known as Punta Jijira or Piedra Alta], Santa Cruz del Norte, Mayabeque, Cuba (23.017433° and –81.986617°). This site is a relict of coastal xeromorphic thicket of about three hectares with rocky cliffs up to 20 meters, where six species of cactus inhabit, with *Pilosocereus robinii* being one of the emerging trees (Barrios et al. 2020). The area experiences 1305 mm of annual precipitation including 489 mm in the wettest quarter (July-September) (Fick & Hijmans 2017). The average annual temperature is 26.3°C, while the average temperature reaches 28.2°C with minimums of 20.9°C and maximums of 37.8°C during the wettest quarter (Barrios unpublished data).

Morpho-physiological traits of seeds

The fruits were carefully opened in the laboratory with a knife and the seeds were cleaned with plenty of running water and dried in a cool place. Measurements of seminal traits began on the same day as the fruits were cleaned; the rest of the seeds were stored in paper bags for 4 months until the start of the germination experiments.

Seminal traits were obtained from 50 randomly selected seeds. These traits were: fresh mass, dry mass, moisture content and seed coat ratio (SCR). Mass values were evaluated on a Sartorius analytical balance (± 0.0001 g). Due to the closeness between the mass of a seed and the precision of the scale, the 50 seeds were divided into 10 replicates of five seeds. In this way, the mass obtained for a seed was determined as the mass of each replicate divided by five. For the fresh mass, the seeds were weighed three hours after cleaning. The dry mass was obtained by weighing the seeds placed in an oven for 17 hours at 103°C (± 2°C) according to FAO and ISTA (2023) standards. While the moisture content was obtained from the following formula according to FAO and ISTA (2023) standards:

Moisture content (%) = (fresh weight – dry weight)/(fresh weight) x100

The SCR was obtained by separating the coating of the dry seeds using a scalpel and tweezers on a sheet of paper.

The previous measures were used to calculate the indices proposed by Daws et al. (2006) and Pelissari et al. (2018). Both indices are based on probabilistic models that predict the sensitivity of seeds to desiccation and therefore their possible storage behavior. For both indices, *P*-values < 0.5 indicate that the seeds are tolerant to desiccation, and *P*-values > 0.5 indicate that the seeds are sensitive to desiccation (Daws et al. 2006; Pelissari et al. 2018).

Seed imbibition

Prior to the germination experiments using the seeds stored for 4 months, water imbibition was evaluated for five days at 25°C in chambers (FRIOCEL 111L, Germany) with exposure to white light (40 µmol/m^2s, length of 400–700 nm) for eight hours a day. This experiment was carried out due to the assumption by Salazar et al. (2013) that seeds could have physical dormancy. Fifty seeds were selected at random and 10 replicates of five seeds were placed in 80 × 15 mm Petri dishes with 1% agar. At the beginning of the experiment, the fresh mass of the seeds was obtained on a Sartorius analytical balance (± 0.0001 g) after which they were weighed every 24 hours until the fifth day. Before weighing surface moisture was removed from the seeds using filter paper which was immediately placed in the same medium. To prevent the seeds from losing the absorbed water, care was taken to ensure that the manipulation did not last longer than one minute. The percentage increase in mass was obtained according to the formula referred to by Dalziell et al. (2018):

Increase in mass (%)= (Wi – Wd)/(Wd) ×100

Where: Wi is the mass of the seeds after absorbing water in each time and Wd is the initial mass of the seed.

Seed germination experiments

The effects of five temperature treatments (one fixed at 25°C and four alternating at 25/30°C, 25/35°C, 25/40°C and 25/45°C) and two lighting conditions (light and darkness) were studied. The dark condition was only measured at 25°C. Each treatment consisted of seven replicates of 25 seeds that were sown in Petri dishes (80 × 15 mm) with 1% agar as substrate. Before sowing, the seeds were disinfected according to the following protocol: 1 min in 0.2% sodium lauryl sulfate (SLS) detergent solution, rinse with distilled water, 1 min in 95% alcohol, rinse with distilled water, 5 min in 1% hypochlorite, rinse with distilled water, 1 min in 95% alcohol and finally rinse with distilled water.

The dishes were incubated in growth chambers (FRIOCEL 111L, Germany) with a photoperiod of 8 h of light (40 µmol/m^2s, length 400–700 nm), except the dark condition which was obtained by covering the dishes with double aluminum foil. For the alternating temperatures (25/30°C, 25/35°C, 25/40°C and 25/45°C) the growth chambers were maintained for 12 h at 25°C and for 8 h at the highest temperature with a 4 h transition between temperatures. In this way, the average temperatures in these treatments were 27.08°C, 29.16°C, 31.25°C and 33.33°C respectively.

Germination was checked daily from the sixth day after sowing, except in the dark treatment which was checked from day 28 after sowing. Germination was considered when the radicle was visible when the testa broke. At the beginning of the fifth week (day 30), 20 completely formed seedlings were randomly selected to be weighed, from which the surface moisture was removed using filter paper before weighing on a Sartorius analytical balance.

In the fourth week, the seeds from the treatments that did not germinate were washed with plenty of distilled water and placed again for four weeks at 25°C and light (40 µmol/m^2s, length 400–700 nm). During this time, germination recovery was measured according to Baskin and Baskin (2014) as: [(a-b)/(c-b)] ×100; where (a) is the number of seeds germinated on day 56, (b) the number of seeds germinated until day 28 and (c) is the number of seeds in Petri dishes (25). Seeds that had not germinated on day 56 were subjected to a cutting test to establish whether they contained a firm white embryo (alive seeds) or a soft or gray embryo (dead seeds) according to the criteria of Baskin and Baskin (2014). Based on this, the percentage of viability was determined.

We used only Germinability (G) as response variable, because the T_{min} and T_{50} were obtained when starting to record the germination. The Germinability (G) refers to the seed germination percentage at the end of the experiment on the 28th day.

Data analysis

For all variables analyzed, normality of the data was checked using a Kolmogorov-Smirnov and Levene test for homogeneity of variance. One-way ANOVAs for Germinability were performed. Tukey's post-hoc tests were used to test for significant differences between means at $p < 0.05$. All statistical analyzes were performed using Statistica 10.0. Graphs were created using SigmaPlot 10.0.

RESULTS

Pilosocereus robinii has medium-sized seeds where the mass of the embryo is greater than that of the covers (Table 1). Furthermore, they have a low moisture content and both indices of sensitivity to desiccation predict that the seeds of the species have orthodox storage behavior. *Pilosocereus robinii* seeds imbibe between 14–31% of their initial mass in the first 24 h and up to 41% at 96 h where phase 3 is reached and germination of some seeds begins (Fig. 1).

No germination was obtained under total darkness conditions. Although the seeds were able to germinate at the 5 temperatures evaluated, different responses were found ($F= 205.4$; $p= 0.0001$). Optimal temperatures were reached at average temperatures below 30°C, between 25–29°C (Fig. 2). The recovery of germination showed differences ($F= 9.4$; $p= 0.001$) between the seeds that were kept at 25/40°C and 25/45°C, although not between the seeds subjected to darkness and 25/40°C (Fig. 3). The portion of the seeds with dormancy was less than 10% in all cases and no affectation was found by subjecting the seeds to temperatures of 40°C and 45°C (Table 2).

No fully formed seedlings were obtained on day 30 for seeds subjected to 25/45°C. The greatest vigor for the seedlings was obtained at average temperatures below 30°C (Fig. 4). Significant differences were only found between the vigor of the seedlings at 25/40°C and those of the 25/30°C and 25/35°C treatments ($F= 23.3$; $p= 0.0001$).

DISCUSSION

Pilosocereus robinii has small seeds, although relative to those reported for cacti they can be considered medium or long in size, according to the classification of Barthlott and Hunt (2000). In our study the seeds were slightly shorter (1.97 vs 2.50 mm) than those reported by Salazar et al. (2013) for the species. But in general, they are within the range [1.3–2.0 mm] of those reported in other studies for the genus (see Abud et al. 2010, 2012; Leal et al. 2007; Meiado et al. 2016). These dimensions can favor the dispersal of large quantities of seeds by birds and bats, as has been observed in several species of *Pilosocereus* from Brazil and Mexico (see Munguía-Rosas et al. 2009; Santos et al. 2019; Vásquez-Castillo et al. 2018), or even dispersal by myrmecophyly as in *P. leucocephalus* (Munguía-Rosas et al. 2009), *P. gounellei* [*Xiquexique gounellei* (F.A.C.Weber) Lavor & Calvente] and *P. pachycladus* (Leal et al. 2007).

Several seminal traits found in *Pilosocereus robinii* favor the formation of banks in the soil and predict orthodox storage behavior for the species, in accordance with the criterion of Thompson et al. (1993). These traits are: low seed mass, low moisture content and positive photoblastism of the seeds. Other evidence that supports orthodox behavior in *P. robinii* seeds are the values of the Daws et al. (2006) and Pelissari et al. (2018)

Table 1. Seed traits of *Pilosocereus robinii*.

Seed traits	**Mean ± (Min-Max)**
Length (mm)	1.97 ± (1.80–2.11)
Width (mm)	1.24 ± (1.10–1.34)
Thickness (mm)	0.82 ± (0.68–0.89)
Fresh mass (mg)	1.02 ± (0.96–1.08)
Dry mass (mg)	0.91 ± (0.84–0.96)
Seed coat ratio	0.46 ± (0.44–0.51)
Moisture content (%)	11.52 ± (8.33–14.28)
P(desiccation–sensitivity) Daws Index	0.00035 ± (0.00023–0.00044)
P(desiccation–tolerant) Pelissari Index	0.044 ± (0.026–0.066)

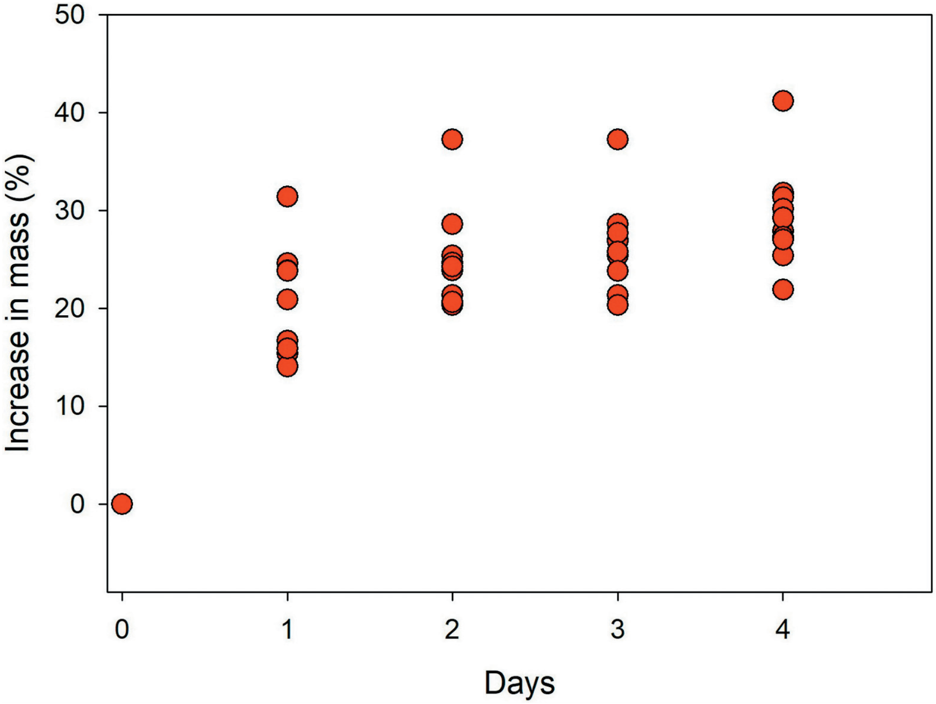

Fig. 1. Cumulative proportion of seeds from *Pilosocereus robinii* imbibed at 25°C for four days, n = 10.

indices obtained in our study. However, future studies will be necessary to corroborate the presence of soil seed banks in *P. robinii*.

Orthodox storage behavior in Cactaceae seeds is a well-documented trait, as is positive photoblastism (Barrios et al. 2020). In five species of *Pilosocereus* from Brazil (*P. albisummus*, *P. pachycladus*, *P. tuberculatus* [*Xiquexique tuberculatus* (Werderm.) Lavor & Calvente], *P. chrysostele,* and *P. gounellei* [*Xiquexique gounellei*]) the germination of their seeds has been documented in high percentages in seeds 19–30 months old stored at room temperature (dos Reis 2012) or at 7°C (Nascimento et al. 2015). Other studies have documented the viability of seeds of *Pilosocereus catingicola* subsp. *salvadorensis* (Werderm.) Zappi and *P. gounellei* subsp. *gounellei* [*Xiquexique gounellei* subsp. *gounellei*] stored at temperatures of –196, –5 and 8°C for up to 13 months, demonstrating that the seeds of these species can be artificially preserved at low temperatures (Dos Santos et al. 2018).

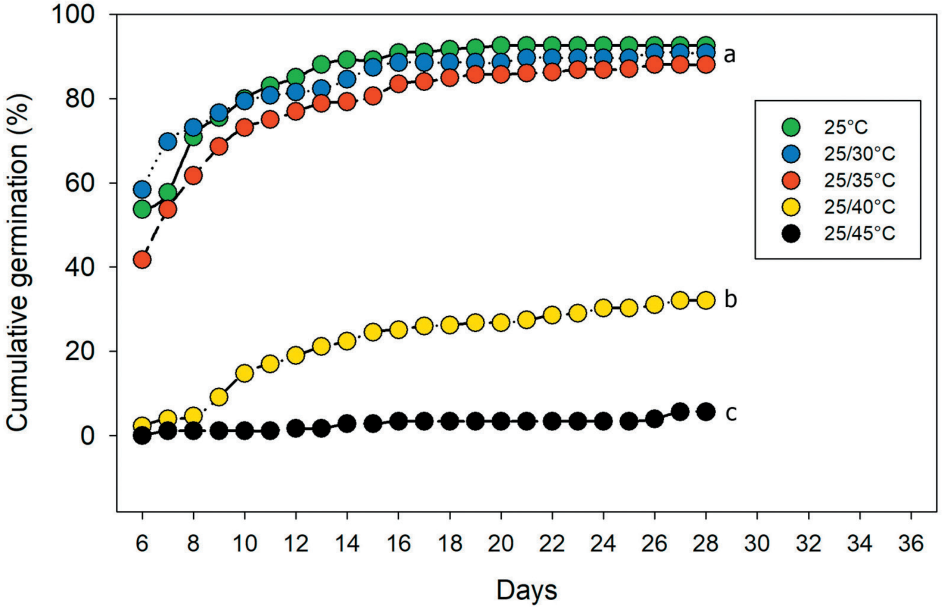

Fig. 2. Cumulative germination of *Pilosocereus robinii* at five temperatures. Alternating temperatures 25/30°C, 25/35°C, 25/40°C y 25/45°C corresponding to 27.08°C, 29.16°C, 31.25°C y 33.33°C averages temperatures respectively. Different letters indicate significant differences among species ($p \leq 0.05$).

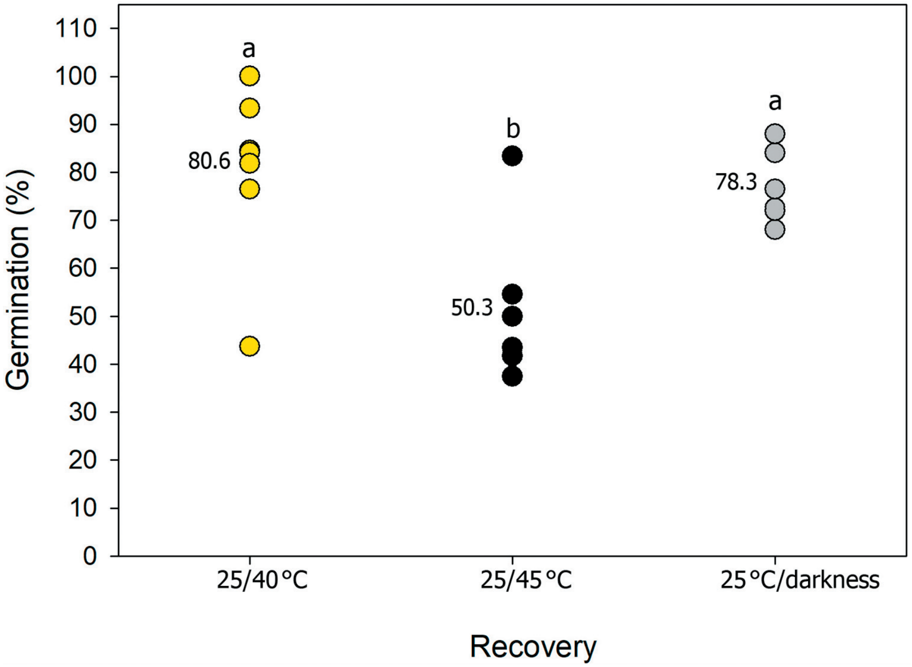

Fig. 3. Germination recovery of seeds of *Pilosocereus robinii* when temperature stresses were alleviated at 25°C after incubation for 28 days at 25/40°C (yellow circles), 25/45°C (black circles) and 25°C/darkness (grey circles). Each circles represent a replicate (n= 7) and number the mean of treatment. Different letters indicate significant differences among species at the same water potential ($p \leq 0.05$).

TABLE 2. Percentage viability of *Pilosocereus robinii* seeds that did not germinate 56 days after sowing. The temperatures correspond to the initial temperatures and lights exposition until 28th day.

Seeds	Light					darkness
	25°C	25/30°C	25/35°C	25/40°C	25/45°C	25°C
Total (%)	6.8	9.1	12	9.1	9.7	9.1
alive (%)	2.8	1.7	9.1	8.0	8.0	6.8
dead (%)	4.0	7.4	2.9	1.1	1.7	2.3

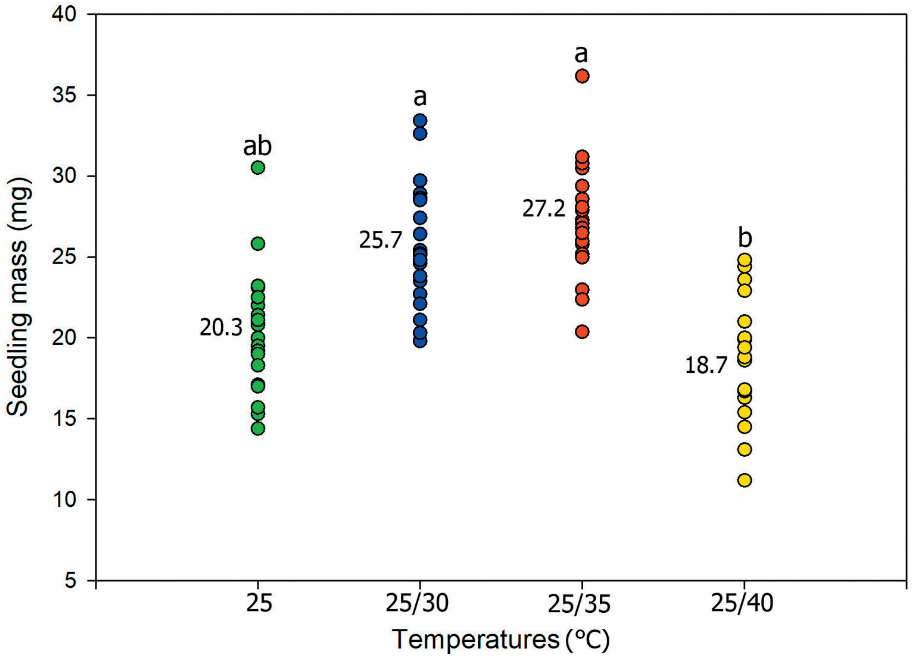

FIG. 4. Fresh seedling mass of *Pilosocereus robinii* after 4 weeks of germination treatments at different temperatures. Each circles represent a replicate (n= 20) and number the mean of treatment. Different letters indicate significant differences among species at the same water potential ($p \leq 0.05$).

In the case of *Pilosocereus* in the Caribbean region, Sentinella et al. (2020) reported germination for *Pilosocereus polygonus* (Lam.) Byles & G.D. Rowley seeds stored for 14 months. According to Salazar et al. (2013), *P. robinii* seeds collected in the ex-situ collection of the Fairchild Tropical Botanic Garden, dried and maintained for up to 28 weeks at –20°C managed to achieve more than 90% germination. By comparing all the above evidence (species from Brazil, the Caribbean and our study), we consider that there is sufficient evidence to support orthodox storage behavior in *Pilosocereus robinii* seeds.

The observation of optimal temperatures for germination below 30°C on average in *Pilosocereus robinii* agrees with the range of optimal temperatures reported for the family (Seal et al. 2017; Barrios et al. 2020). Most studies in *Pilosocereus* show optimal temperatures between 20–25°C (Martins et al. 2012; Medeiros et al. 2017; Silva 2020) or between 20–30°C (Abud 2010, 2012; Seal et al. 2017; Veiga et al. 2010).

According to records from the *Pilosocereus robinii* seed collection area, the mean temperature during the wettest quarter (28.2°C, Barrios, unpublished data) coincides with the optimal temperature range for the species. During this period, germination is estimated as most likely and the mean temperature of the wettest quarter is just the climatic variable most associated with the germination response in cacti (Seal et al. 2017). The average temperature during the wettest quarter in the collection area is 1.5°C higher than in the Fick and Hijmans (2017) models, which in turn predict increases between 1.26°C and 2.28°C by 2070. Considering these data and projecting the real average, temperatures between 29.46°C and 30.48°C would be expected for the area during the wettest quarter for 2070. The previous prediction shows that even under climate change the seeds of *Pilosocereus robinii* would be within the range of optimal temperatures, although with very little margin of thermal buffering capacity as expected in species with a tropical climate (Barrios et al. 2020; Sentinella et al. 2020).

On the other hand, the high recovery percentages in seeds subjected to high temperatures (Fig. 3) and dormant seeds (Table 2) show high resilience in *Pilosocereus robinii*. This ability in Cuban cacti has only been observed in *Leptocereus ekmanii* (Werderm.) F.M. Knuth (Barrios et al. 2021a), although several studies in Mexican species from desert habitats (Ruedas et al. 2000; Olvera-Carrillo et al. 2003; Ramírez-Padilla & Valverde 2005; Sánchez-Soto et al. 2010) have shown that exposure to high temperatures for short periods of time does not harm the germination of the seeds of these cacti (Barrios et al. 2020). This characteristic is associated with tolerance to high temperatures and may be associated with the persistence of seeds in the soil (Daws et al. 2007).

Finally, the vigor of the *Pilosocereus robinii* seedlings, as well as their germination, showed optimal behavior under the temperatures in the wettest quarter. This result shows that current climatic conditions and probably those predicted for 2070 (Fick & Hijmans 2017) do not affect the growth of seedlings of the species, particularly in rocky crevices or under bushes, in places protected from high temperatures where it is common to observe juveniles of the species. Typically, seed mass is not commonly measured in germination experiments in cacti (Barrios et al. 2020), although such data could provide valuable information about the performance of seedling establishment under different temperature regimes.

Our study shows that the propagation of *Pilosocereus robinii* by seeds is a viable and easy method to use in the conservation of the species. It also corroborates the orthodox storage behavior of its seeds. Furthermore, it shows that under current and future temperatures on the northern coast of Cuba, the reintroduction of individuals into affected areas or the establishment of populations in new areas from seeds is possible. This might also be similar for other areas within the species' distribution range in the Caribbean, which are located further north. Future studies should evaluate how the response of the seeds of the species can be affected by different conditions of water and saline stress. In addition, it would be beneficial to study the best conditions for long-term seed storage.

ACKNOWLEDGMENTS

The collecting expedition was carried out thanks to the support of Planta! The Plantlife Conservation Society, and the collaboration between the National Botanical Garden and the Botanical Garden of the Autonomous University of Mexico. We appreciate Mayté Pernús for her assistance in the laboratory. We also thank Ana González-Nayeck for her review of the English version of the manuscript. We greatly appreciate the valuable comments on our manuscript by Marcos V. Meiado and an anonymous reviewer.

REFERENCES

Abud, H.F., N.R. Gonçalves, R.D.G.E. Reis, D.D.S. Pereira, & A.M.E. Bezerra. 2010. Germinação e expressão morfológica de frutos, sementes e plântulas de *Pilosocereus Pachycladus* Ritter. Rev. Ciênc. Agron. 41(3):468–474. doi:10.1590/S1806-66902010000300021

Abud, H.F., N.R. Gonçalves, M.D.S. Pereira, D.D.S. Pereira, R.D.G.E. Reis, & A.M.E. Bezerra. 2012. Germination and morphological characterization of the fruits, seeds, and seedlings of *Pilosocereus Gounellei*. Braz. J. Bot. 35(1):11–16. doi:10.1590/S0100-84042012000100003

Acevedo-Rodríguez, P. & M.T. Strong. 2012. Catalogue of seed plants of the West Indies. Smithsonian Contr. Bot. 98:1–1192. doi: 10.5479/si.0081024X.98.1

Alain, H. 1953. Flora de Cuba. Contr. Ocas. Mus. Hist. Nat. Colegio "De La Salle 3:1–472.

Barrios, D., J. Flores, L.R. González-Torres, & A. Palmarola. 2015. The role of mucilage in the germination of *Leptocereus scopulophilus* (Cactaceae) seeds from Pan de Matanzas, Cuba. Botany 93:251–255. doi:10.1139/cjb-2014-0242

Barrios, D. & C.A. Mancina. 2017. *Dendrocereus nudiflorus* (*Cactaceae*): pasado, presente y futuro de un anacronismo en peligro de extinción según modelos de nicho climático. Revista Jard. Bot. Nac. Univ. Habana 38, 119–132.

Barrios, D. & L.R. González-Torres. 2020. Lista florística en dos parches de matorral xeromorfo costero de Santa Cruz del Norte, Mayabeque. Bissea 14(2):1–3.

Barrios, D., J.A. Sánchez, J. Flores, & E. Jurado. 2020. Seed traits and germination in the cactaceae family: a review across the Americas. Bot. Sci. 98(3):417–444. doi:10.17129/botsci.2501

Barrios, D., J. Flores, J.A. Sánchez, & L.R. González-Torres. 2021a. Combined effect of temperature and water stress on seed germination of four *Leptocereus* spp. (Cactaceae) from Cuban dry forests. Pl. Spec. Biol. 36:512–522. doi:10.1111/1442-1984.12334

Barrios, D., S. Toledo, J.A. Sánchez, & L.R. González-Torres. 2021b. Serotiny in *Melocactus matanzanu*s (Cactaceae) and cephalium role in dispersal seeds after individual's death. Seed Sci. Res. 31(4):326–332. doi:10.1017/S0960258521000283

Barrios, D., S. Arias, L.R. González-Torres, & L.C. Majure. 2023. Lista anotada de cactus nativos y naturalizados de Cuba. Bot. Sci. 101(4):1249–1300. doi:10.17129/botsci.3324

Barthlott, W. & D. Hunt. 2000. Seed diversity in the Cactaceae subfamily Cactoideae. DH Books, Milborne, Port, UK.

Baskin, C.C. & J.M. Baskin. 2014. Seeds: Ecology, biogeography, and evolution of dormancy and germination, second ed. Elsevier, UK.

Dalziell, E.L., C.C. Baskin, J.M. Baskin, R.E. Young, K.W. Dixon, & D.J. Merritt. 2018. Morphophysiological dormancy in the basal angiosperm order Nymphaeales. Ann. Bot.123(1):95–106. doi:10.1093/aob/mcy142

Daws, M.I., N.C. Garwood, & H.W. Pritchard 2006. Prediction of desiccation sensitivity in seeds of woody Species: A probabilistic model based on two seed traits and 104 species. Ann. Bot. 97:667–674. doi:10.1093/aob/mcl022

Daws, M.I., S. Bolton, D.F.R.P. Burslem, N.C. Garwood, & C.E. Mullins. 2007. Loss of desiccation tolerance during germination in neotropical pioneer seeds: implications for seed mortality and germination characteristics. Seed Sci. Res. 17:273–281. doi:10.1017/S0960258507837755

Dehgan, B. & H. Pérez. 2005. Preliminary study shows germination of Caribbean applecactus (*Harrisia fragrans*) improved with acid scarification and giberellic acid. Native Pl. J. 6: 91–96. doi:10.1353/npj.2005.0017

dos Reis, M.V., R.G. Pêgo, P.D.D.O. Paiva, F.A. Artioli-Coelho, & R. Paiva. 2012. Germinação in vitro e desenvolvimento pós-seminal de plântulas de *Pilosocereus aurisetus* (Werderm.) Byles & G.D. Rowley (Cactaceae). Rev. Ceres, Viçosa 59(6):739–744.

FAO & ISTA. 2023. Guidelines for the establishment and management of seed testing laboratories – Joint FAO and ISTA Handbook. Rome, Italy. doi:10.4060/cc6103en.

Fenner, M. 1985. Seed ecology. Chapman and Hall, London, UK.

Fick, S.E. & R.J. Hijmans. 2017. WorldClim 2: new 1-k m spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37(12):4302–4315. doi:10.1002/joc.5086

Franck, A.R. (& 11 others). 2019. Revision of *Pilosocereus* (Cactaceae) in the Caribbean and northern Andes. Phytotaxa 411(3):129–182. doi:10.11646/phytotaxa.411.3.1

González-Torres, L.R., D. Barrios, & A. Palmarola. 2012. The ecology and natural history of *Leptocereus scopulophilus* (*Cactaceae*). Cactus World 30(2):110–114.

González-Torres, L.R., A. Palmarola, L. González-Oliva, E.R. Bécquer, E. Testé, & D. Barrios. 2016. Lista Roja de la flora de Cuba. Bissea 10(número especial 1):1–352.

Leal, I.R., R. Wirth, & M. Tabarelli. 2007. Seed dispersal by ants in the semi-arid Caatinga of north-east Brazil. Ann. Bot. 99(5):885–894 doi:10.1093/aob/mcm017

Martins, L.S.T., T.S. Pereira, A.S.D.R. Carvalho, C. Barros, & A.C.S. de Andrade. 2012. Seed germination of *Pilosocereus arrabidae* (Cactaceae) from a semiarid region of southeast Brazil. Pl. Spec. Biol. 27(3):191–200. doi:10.1111/j.1442-1984.2011.00360.x

Maschinski, J., D. Coates, L. Monks, R. Dillon, S. Barrett, J. Possley, J. Lange, J. Duquesnel, J. Goodman, & L. Hermanutz. 2023. Rare and threatened plant conservation translocations: Lessons learned and future directions. In: S. Florentine, L. Broadhurst, P. Guibson-Roy, & K.W. Dixon, eds. Ecological Restoration: Moving Forward Using Lessons Learned, Springer Nature, Switzerland. Pp. 287–322. doi:10.1007/978-3-031-25412-3_8.

Medeiros, R.L.S.D., V.C.D. Souza, G.A.D. Azerêdo, M.A.B. Neto, A.D.S. Barbosa, & I.S.D.S. Oliveira. 2017. Seed vigor and germination of facheiro plants (*Pilosocereus catingicola* (Gurke) Byles & Rowley subsp. *salvadorensis* (Werderm.) Zappi (Cactaceae) at different temperatures. Ciênc. Agrár. 38(5):2873–2886. doi:10.5433/1679-0359.2017v38n5p2873

Meiado, M.V., M. Rojas-Aréchiga, J.A. Siqueira-Filhos, & I.R. Leal. 2016. Effects of light and temperature on seed germination of cacti of Brazilian ecosystems. Plant Species Biology 31:87–97. doi:10.1111/1442-1984.12087

Munguía-Rosas, M.A., M.E. Jácome-Flores, V.J. Sosa, & L.M. Quiroz-Cerón. 2009. Removal of *Pilosocereus leucocephalus* (Cactaceae, tribe Cereeae) seeds by ants and their potential role as primary seed dispersers. J. Arid Environ. 73:578–581. doi:10.1016/j.jaridenv.2008.12.017

Naranjo-Diaz, L.R. & A. Centella. 1998. Recent trends in the climate of Cuba. Weather 53(3):78–85. doi:10.1002/j.1477-8696.1998.tb03964.x

Nascimento, J.P.B., D.C.M. Vieira, & M.V. Meiado. 2015. *Ex situ* seed conservation of Brazilian cacti. Gaia Scientia 9(2):111–116.

Olvera-Carrillo, Y., J. Márquez-Guzmán, V.L. Barradas, M.E. Sánchez-Coronado, & A. Orozco-Segovia. 2003. Germination of the hard seed coated *Opuntia tomentosa* S.D., a cacti from the México valley. J. Arid Environ. 55:29–42. doi:10.1016/S0140-1963(02)00268-9

Pelissari, F., A.C. José, M.A.L. Fontes, A.C.B. Matos, W.V.S. Pereira, & J.M.R. Faria. 2018. A probabilistic model for tropical tree seed desiccation tolerance and storage classification. New forests 49:143–158. doi:10.1007/s11056-017-9610-8

Possley, J., J.J. Lange, A.R. Franck, G.D. Gan, T. Wilson, S. Kolterman, J. Duquesnel, & J. O´Brien. 2024. First U.S. vascular plant extirpation linked to sea level rise? *Pilosocereus millspaughii* (Cactaceae) in the Florida Keys, U.S.A. J. Bot. Res. Inst. Texas 18(1):211–223. doi:10.17348/jbrit.v18.i1.1350

Quiala, E., J. Matos, G. Montalvo, M.D. Feria, M. Chávez, A. Capote, N. Pérez, R. Barbón, & B. Kowalski. 2009. *In Vitro* propagation of *Pilosocereus robinii* (Lemaire) Byles et Rowley, endemic and endangered cactus. J. PACD 11:18–25. doi:10.56890/jpacd.v11i.110

Ramírez-Padilla, C.A. & T. Valverde. 2005. Germination responses of three congeneric cactus species (*Neobuxbaumia*) with differing degrees of rarity. J. Arid Environ. 61:333–343. doi:10.1016/j.jaridenv.2004.09.006

Ruedas, M., T. Valverde, & S. Castillo 2000. Respuesta germinativa y crecimiento de plántulas de *Mammillaria magnimamma* (Cactaceae) bajo diferentes condiciones ambientales. Bol. Soc. Bot. México 66:25–35. doi:10.17129/botsci.1608

Salazar, A., J. Maschinski, & D. Powell 2013. *Ex-situ* seed conservation of endangered key tree cactus (*Pilosocereus robinii*). J. Biodivers. Endang. Spec. 1(3):111–114. doi:10.4172/2332-2543.1000111

Sánchez, J.A., M. Pernús, Y. Torres-Arias, D. Barrios, & Y. Dupuig. 2019. Dormancia y germinación en semillas de árboles y arbustos de Cuba: implicaciones para la restauración ecológica. Acta Bot. Cub. 218(2):77–108.

Sánchez-Soto, B., E. García-Moya, T. Terrazas, & A. Reyes-Olivas. 2005. Efecto de la hidratación discontinua sobre la germinación de tres cactáceas del desierto costero de Topolobampo, Ahome, Sinaloa. Cact. Suc. Mex. 50:4–14.

Santos, L.D.N., I.M.S. Pereira, J.R. Ribeiro, & F.M.G. Las-Casas. 2019. Frugivoria por aves em quatro espécies de Cactaceae na Caatinga, uma floresta seca no Brasil. Iheringia, Sér. Zoologia 109:e2019034. doi:10.1590/1678-4766e2019034

Seal, C.E.(& 16 others). 2017. Thermal buffering capacity of the germination phenotype across the environmental envelope of the Cactaceae. Glob. Change Biol. 23(12):5309–5317. doi:10.1111/gcb.13796

Sentinella, A.T., D.I. Warton, W.B. Sherwin, C.A. Offord, & A.T. Moles. 2020. Tropical plants do not have narrower temperature tolerances, but are more at risk from warming because they are close to their upper thermal limits. Glob. Ecol. Biogeogr 29(8):1387–1398. doi:10.1111/geb.13117

Silva, J.H.C.S., G.A. Azerêdo, & V.A. Targino. 2020. Resposta germinativa de sementes de cactáceas colunares sob diferentes regimes de temperatura e de potencial hídrico. Scientia Plena 16(12):123101. doi:10.14808/sci.plena.2020.123101

Stephenson, T.S., L.A. Vincent, T. Allen, C.J. Van Meerbeeck, N. McLean, T.C. Peterson, & et al. 2014. Changes in extreme temperature and precipitation in the Caribbean region, 1961–2010. Int. J. Climatol. 34:2957–2971. doi:10.1002/joc.3889

Thompson, K., S.R. Band, & J.G. Hodgson. 1993. Seed size and shape predict persistence in soil. Funct. Ecol. 7:236–241. doi:10.2307/2389893

Vázquez-Castillo, S., A. Miranda-Jácome, & E. Ruelas. 2018. Patterns of frugivory in the columnar cactus *Pilosocereus leucocephalus*. Ecol. Evol. 9:268–1277. doi:10.1002/ece3.4833

Zimmer, K. 1967. Temperatur und Keimung bei verschiedenen Kakteen. Kakteen And. Sukk. 18:31–33.