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Physiological performance of *Coccoloba gigantifolia* seeds subjected to desiccation and storage

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ABSTRACT: *Coccoloba gigantifolia* is recently described species, with an area of restricted occurrence and threatened with extinction, whose great ornamental potential is due to the size of its leaves and the architecture of the plant. The objective of this study was to evaluate the viability of its seeds under different water contents and storage temperatures, through two experiments. The first one used a completely randomized design (CRD) with six treatments (drying periods: 0, 1, 2, 3, 4 and 5 days) and four replications. The second experiment used a CRD in a 2 (storage temperatures: 8.0 ± 1.2 and 19.5 ± 0.7 °C) x 3 (storage periods: 1, 2 and 4 months) factorial scheme, in addition to a control treatment (without storage), with four replications. The seeds have a recalcitrant behavior, as they are sensitive to desiccation, with a marked reduction in viability with moisture content below 19.5%. Storage at temperatures of 8.0 and 19.5 °C did not maintain the physiological quality of the seeds, with 25.2% water, and the largest losses were found at a temperature of 8.0 °C.

Index terms: desiccation tolerance, recalcitrant, *ex situ* conservation.

RESUMO: *Coccoloba gigantifolia* é uma espécie recentemente descrita, com área de ocorrência restrita e ameaçada de extinção, que apresenta grande potencial ornamental devido ao tamanho de suas folhas e à arquitetura da planta. Objetivou-se avaliar a viabilidade de suas sementes submetidas a diferentes teores de água e temperaturas de armazenamento, por meio de dois experimentos. No primeiro foi adotado o delineamento inteiramente casualizado (DIC), com seis tratamentos (períodos de secagem: 0, 1, 2, 3, 4 e 5 dias) e quatro repetições. No outro foi adotado o DIC, em esquema fatorial 2 (temperaturas de armazenamento: 8,0 ± 1,2 e 19,5 ± 0,7 °C) x 3 (períodos de armazenamento: 1, 2 e 4 meses), além do tratamento controle (sem armazenamento), com quatro repetições. As sementes apresentam comportamento recalcitrante, pois são sensíveis ao dessecamento, com acentuada redução da viabilidade abaixo de 19,5% de água. O armazenamento sob temperaturas de 8,0 e 19,5 °C não manteve a qualidade fisiológica das sementes, com 25,2% de água, sendo as maiores perdas registradas na temperatura de 8,0 °C.

Termos para indexação: tolerância ao dessecamento, recalcitrante, conservação *ex situ.*

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INTRODUCTION

Coccoloba gigantifolia (Melo et al., 2019) is a recently described species of the Polygonaceae family, found only in the Rio Madeira basin, in the states of Amazonas and Rondônia, in the center and southwest of the Brazilian Amazon. It grows in open rainforests, secondary forests and scrub forests, at altitudes of 20–100 m, with flat relief and moist, sandy or clayey soils (Melo et al., 2019). It has an arboreal growth habit, with a straight trunk, reaching 10–15 m in height; the plants are polygamous-dioecious and, as a result, not all of them produce fruits and seeds; leaves are alternate, spiraled, petiolate, with elliptical blade of 0.6–2.5 x 0.5–1.4 m; anthocarps are globose, pubescent or puberulent, red, vinaceous or purple when mature, measuring 5–8 mm in length; the nucules are globose, smooth, slightly striated, with an acute or pyramidal apex; the seeds have ruminated endosperm (Melo et al., 2019).

The species has ornamental potential owing to the size of its leaves and the peculiar architecture of the plant. However, there is little information about its biology and virtually no information about its management. The scarce knowledge about the species is more worrying when taking into account the risk of extinction of the species in nature, owing to the restricted area of occurrence and deforestation for expansion of the agricultural frontier and for the construction of hydroelectric plants and highways (Melo et al., 2019). Faced with such a situation, *in situ* and *ex situ* conservation strategies should be adopted to avoid the extinction of this species.

In the long term, *ex situ* conservation of seeds can be performed in conventional germplasm banks at a temperature of -18 or -20 °C and in cryogenic banks at -156 °C (nitrogen vapor) or at -196 °C (liquid nitrogen), depending on the level of desiccation tolerance (Salomão et al., 2015). The physiological response of seeds to desiccation varies among species, owing to the presence, absence or incomplete expression of a set of cellular mechanisms to protect and repair the resulting damage (Pammenter and Berjak, 1999). As a result of this variation, the following classification was created: orthodox, when seeds tolerate a reduction in moisture content to levels of 2 to 6%, or even below; intermediate, when seeds tolerate desiccation up to a moisture content of 7 to 12%; and, finally, recalcitrant, when seeds do not tolerate desiccation at moisture contents of 15 to 20% (Hong and Ellis, 1996). Although the use of orthodox, intermediate and recalcitrant categories is convenient, Pammenter and Berjak (1999) suggested the existence of a continuous gradient, from the most tolerant to the most sensitive to desiccation, which would better represent the seeds postharvest physiology.

Orthodox seeds can be dried and stored with low moisture content, enabling storage under controlled conditions of negative temperatures and low relative humidity. It is the absence of freezing water that allows orthodox seeds to be stored at below-zero temperatures (Berjak and Pammenter, 2013). Intermediate and recalcitrant seeds do not tolerate below-zero temperatures, and cold damage occurs in tropical intermediate seeds when exposed to temperatures below 10 °C and in tropical recalcitrant seeds at temperatures equal to or below 10–15 °C, depending on the species (Hong and Ellis, 1996).

Knowledge of postharvest physiology allows establishing the most appropriate procedures for processing and storage of seeds, which are essential to maintain viability and vigor during *in vivo* conservation. In this sense, Hong and Ellis (1996) proposed a protocol to determine desiccation tolerance and seed storage behavior, through which seeds are classified on the basis of moisture content and survival rate.

As far as *Coccoloba gigantifolia* is concerned, it is still unknown whether its seeds tolerate desiccation up to 10–12% water or even up to 5% water, as well as whether they tolerate storage at positive temperatures below 10 °C or even at negative temperatures. As the species is propagated exclusively by seeds, this information represents basic knowledge that can be used to check the possibilities of storage in the short (a few days to a few months), medium (between one and ten years) or long term (over ten years) (Silva and Ferraz, 2015), aiming at *in vivo* conservation in germplasm-seed banks. Likewise, this information is useful for *in vivo* conservation in field collections, reintroduction into natural habitat for recovery of endangered populations and restoration of degraded ecosystems, which require the development of management technologies for harvesting, processing, storing and germinating seeds (Zamith and Scarano, 2004). Given the above, this study aimed to evaluate the viability of *Coccoloba gigantifolia* seeds under different moisture contents and storage temperatures.

MATERIAL AND METHODS

Origin and processing of seeds

The anthocarps (fruit + perianth) of *Coccoloba gigantifolia* came from four bunches of a plant grown at the National Institute of Amazonian Research, Campus III (3° 5’ 33.12” S; 59° 59’ 35.36” W), in Manaus, Amazonas. The region’s climate is classified as Af, with an average annual temperature of 26.7 °C and an average annual rainfall of 2,420 mm (Alvares et al., 2013). After the anthocarps were removed from the bunches, they were mixed and selected by the color of the perianth - from those that were 50% red to completely purple ones, while the others were discarded.

The nucules (pericarp + seed) were extracted manually by rubbing the anthocarps in running water. Then, the nucules were treated with sodium hypochlorite (0.5%) for fifteen minutes, and those that floated in the water were discarded. Afterwards, they were rinsed and kept in a perforated plastic bag, until the next morning. Before the beginning of the experiments, the nucules remained in a single layer inside plastic trays, for one hour, in a laboratory environment (23.2 ± 0.7 °C and RH of 60.8 ± 3.9%) for superficial drying of the pericarp. Based on this material, henceforth considering the nucules as a seed unit, two experiments were installed, as follows:

Desiccation tolerance

The experimental design was completely randomized, with six treatments (desiccation periods) and four replications of 25 seeds each. The methodology was adapted from the protocol by Hong and Ellis (1996). Thus, the seeds were divided into lots of 125 units each, placed in germination boxes (11 x 11 x 3.5 cm), on a stainless-steel screen. At the bottom of each box, 90 g of dehydrated silica gel was placed and changed daily. The seeds remained in this condition for periods of 0, 1, 2, 3, 4 and 5 days, which resulted in lots with different moisture contents, determined by the oven method at 105 ± 3 °C, for 24 hours (Brasil, 2009), using two replicates of ten seeds per lot.

Subsequently, the seeds were sown in drained plastic boxes (26 x 17 x 6 cm), containing medium textured vermiculite substrate, and kept in a greenhouse (mean minimum temperature of 26.1 ± 0.4 °C and maximum temperature of 37 ± 2.6 °C). Irrigation was performed whenever necessary, trying to keep the substrate moist and without waterlogging.

Seedling emergence was evaluated every five days for 120 days, and seedlings with elongation of the hypocotyl above the substrate (epigeal germination) were considered to have emerged. Based on these data, in percentage, emergence speed index (ESI) and mean emergence time (MET) were calculated, according to Ranal and Santana (2006).

As the treatments were quantitative (0, 1, 2, 3, 4 and 5 days of drying), regression analysis was performed for the study variables, accepting the highest level of significant adjustment, up to the third degree. Analyses were performed using the software ASSISTAT version 7.7 (Silva and Azevedo, 2016).

Viability conservation

The experiment used a completely randomized design in a 2 (storage temperatures) x 3 (storage periods) factorial scheme, in addition to a control treatment (no storage), with four replications, each containing 25 seeds. Thus, seed lots with a moisture content of 25.2% (achieved after a day of drying on silica gel, as described above) were packed in double packaging (plastic bag inside a plastic bottle, sealed separately) and stored at two temperatures [8.0 ± 1.2 °C (refrigerator) and 19.5 ± 0.7 °C (refrigerated chamber)], for different periods (1, 2 and 4 months).

After each storage period, sowing was performed and seedling emergence and other vigor variables were evaluated, in the same way as in the previous experiment. The percentage values were transformed into arcsine $\sqrt{x/100}$, while the others were transformed into $\sqrt{x}$, when there was a need to normalize them. The results were presented and discussed with the averages of the original data, without transformation. Analyses of variance and comparison of data means were performed using the software ASSISTAT version 7.7 (Silva and Azevedo, 2016).

RESULTS AND DISCUSSION

Desiccation tolerance

The different drying periods provided different moisture contents in the *Coccoloba gigantifolia* seeds (Figure 1). Before drying, the newly processed seeds had a high moisture content (49.3%), which is common in recalcitrant seeds, whose moisture content is high at the end of maturation and/or dispersion (Mayrinck et al., 2016). According to Hong and Ellis (1996), dispersed or harvested seeds with a moisture content equal to or less than 20% will probably show orthodox behavior, although no generalization can be made if the moisture content is between 25 and 55%.

After a day of drying, the *Coccoloba gigantifolia* seeds showed a marked reduction in moisture content, with a loss of about 51% of the initial moisture content, with moisture content reaching 25.2%. As drying continued, water losses occurred in a smaller proportion compared to the immediately previous period, with the seeds reaching the moisture contents of 19.5, 11.4, 8.8 and 6.8%, after 2, 3, 4 and 5 days, respectively (Figure 1). Possibly, moisture content between 49.3 and 25.2% corresponds to capillary or free water, therefore presenting faster evaporation; while the remaining water is likely bound to increasingly hydrophilic molecular sites, resulting in a slower drying rate (Bewley et al., 2013).

In response to the desiccation periods, emergence and ESI were fitted to a cubic equation while MET, to a quadratic equation (Table 1 and Figure 2). In newly-processed seeds, emergence (35%) was lower than the one achieved in seeds after one day of drying (58%). This is probably due to primary dormancy, which occurs during seed maturation,

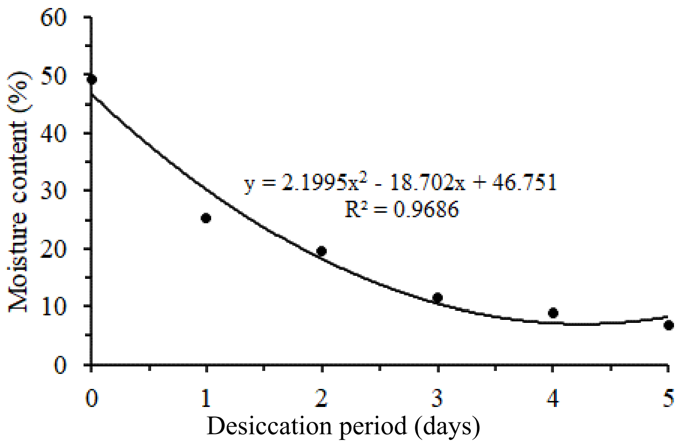

Figure 1. Moisture content of *Coccoloba gigantifolia* seeds after different desiccation periods.

Table 1. Summary of regression analysis of variance referring to emergence, emergence speed index (ESI) and mean emergence time (MET) of *Coccoloba gigantifolia* seeds under different desiccation periods.

Source of variation	DF	Mean square		
		Emergence	ESI	MET
Linear regression	1	8,558.23**	2.87**	164.67**
Quadratic regression	1	438.86 ns	0.26*	117.37*
Cubic regression	1	3,882.76**	1.60**	26.49 ns
Residual	18	103.33	0.04	15.49
CV (%)		41.49	42.68	6.82

* and **: significant at the level of 0.05 and 0.01 (p), by the F-test, respectively.
ns: non-significant at the 0.05 (p) level by the F test.
DF: degree of freedom.

preceding the dispersal of fruits and/or seeds (Bewley et al., 2013). Baskin and Baskin (2014) reported that germination studies should be carried out soon after seed harvesting, followed by a short drying period, which may favor the response to the germination test and/or dormancy breaking treatments.

After one day of drying, emergence decreased progressively until it was null, between 3 and 4 days of drying, when it reached the estimated moisture content of 8.0% (Figure 2A). ESI had a similar behavior to that of emergence; it was initially lower, rising in the period of one day of drying, after which it also decreased (Figure 2B).

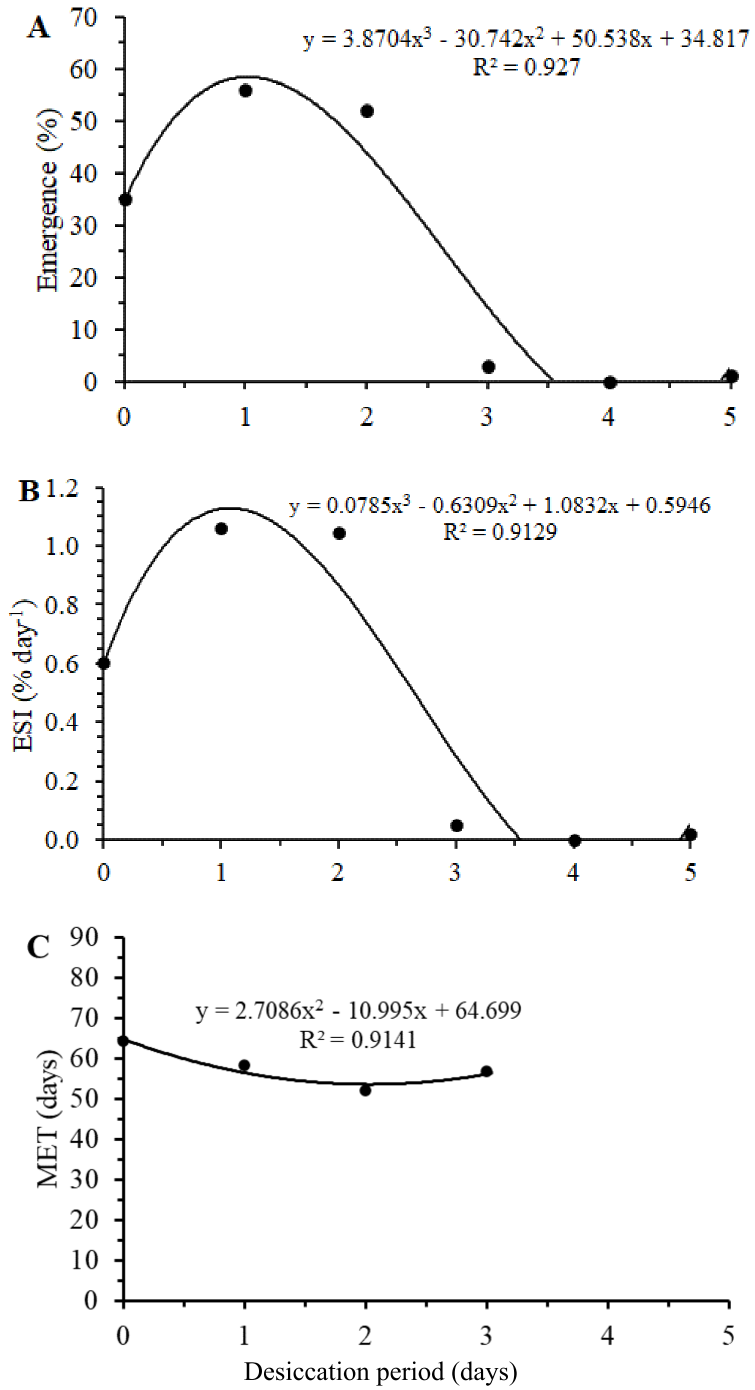

Figure 2. Emergence (A), emergence speed index (ESI) (B) and mean emergence time (MET) (C), referring to *Coccoloba gigantifolia* seeds under different periods of desiccation.

Regardless of the regression adjustment, it was found that after two days of drying (19.5% of moisture content), emergence was still satisfactory (52%), taking into account that maximum value was 56% in seeds with moisture content of 25.2% (Figures 1 and 2A). Thus, based on the classification adopted by Zamith and Scarano (2004), the germination percentage of *Coccoloba gigantifolia* seeds can be considered to be average (40% < x < 80%), a fact that has been found in species of the same genus, as in *Coccoloba arborescens* with 43% germination (Zamith and Scarano, 2004), and *C. barbadensis* with 47.7% (Soriano et al., 2011) to 69% germination (Zamora-Cornelio et al., 2010). Other species of the genus, however, have shown high germination, such as *C. diversifolia* (82%) and *C. microstachya* (98%) (Francis and Rodriguez, 1993). Thus, new studies on the germination process of *Coccoloba gigantifolia* seeds need to be conducted to discover the causes of the low initial percentage. As mentioned above, this may have been due to dormancy; in addition, there may have been incipient embryos and/or malformed seeds. In this sense, it is worth noting that, during processing, some apparently empty seeds floated in the water (data not quantified) and were discarded.

The highest MET (65 days) was found in seeds with the highest moisture content (Figure 2C). With increased desiccation time and, consequently, reduction in moisture content, MET was reduced until reaching the lowest value (54 days) after two days of drying (19.5% moisture). In seeds of *C. arborescens*, whose germination is considered to be slow, MET was 46.6 days, with a range between 30 and 61 days (Zamith and Scarano, 2004). In *C. barbadensis*, the beginning of seed germination occurred at six days and the last seed germinated at 51 days after sowing (Zamora-Cornelio et al., 2010). Given the above, considering the slow germination of *Coccoloba gigantifolia* seeds, the need for pre-germinative treatments needs to be investigated.

Coccoloba gigantifolia seeds showed a marked loss of viability when desiccated to moisture contents below 19.5%, and the lethal level corresponded to the estimated moisture content of 8% (Figure 2A), after which no germination occurred. Therefore, following the protocol of Hong and Ellis (1996), as most of the seeds died when dried to 10–12% moisture, they showed sensitivity to desiccation and, therefore, can be considered as recalcitrant. Delgado and Barbedo (2012) suggested that water potential is more reliable than seed moisture content, and that differences in desiccation tolerance between species of *Eugenia* (Myrtaceae) may be due to the degree of maturity during dispersal, which may also mean that the seeds were dispersed at a maturity stage similar to the one found in immature orthodox seeds.

The proportion of species with seeds that are sensitive to desiccation decreases as the habitat becomes drier and possibly also cooler (Tweddle et al., 2003). Therefore, seed sensitivity to desiccation is more common in species from moist habitats, such as the one where *Coccoloba gigantifolia* occurs naturally (Melo et al., 2019).

Sensitivity to desiccation is also more frequent in species whose seeds do not have dispersal dormancy, but not all species with dormant seeds are necessarily desiccation-tolerant (Tweddle et al., 2003). One species that has this rare combination is *Garcinia gardneriana* (Clusiaceae), whose seeds are dispersed with 51.7% moisture and lose viability when desiccated at moisture contents below 20.0% (Viana et al., 2020). This may also be the case for *Coccoloba gigantifolia*, although the benefits of this ecological strategy need to be clarified with detailed information on fruiting phenology, seed dispersal and specific habitat characteristics.

The first challenge for the conservation of recalcitrant seeds is to determine their response to desiccation. However, in addition to evaluating the effect of desiccation on seed viability, seed longevity response to storage environments is another practical way to determine the pattern of seed behavior (Hong and Ellis, 1996).

Viability conservation

Emergence, ESI and MET showed a significant interaction effect between the factors “storage temperature” and “storage period” (Table 2). For these variables, with the exception of MET, the control treatment (no storage) differed significantly from the other treatments, indicating that seed quality was not maintained during storage. However, the levels of the “storage temperature” factor behaved differently from the levels of the “storage period” factor (Table 3). Most of the time, although in some cases there was no significance, the temperature of 19.5 ± 0.7 °C (chamber) provided the best results compared to 8.0 ± 1.2 °C (refrigerator), which shows greater seed sensitivity to lower temperatures.

Table 2. Summary of the analysis of variance regarding emergence, emergence speed index (ESI) and mean emergence time (MET) of *Coccoloba gigantifolia* seeds stored in different environments for different periods.

Source of variation	DF	Mean square		
		Emergence	ESI	MET
Temperature (T)	1	497.34*	0.141**	545.34**
Period (P)	2	339.40*	0.100**	204.00*
T x P	2	276.98*	0.097**	586.16**
Control x Factorial Design	1	2401.44**	0.358**	120.00 ns
Residual	21	66.73	0.011	54.52
CV (%)		30.98	11.00	13.91
Average Control (%, % day^{-1} and day)		56 a	1.060 a	58.1 a
Factorial Average (%, % day^{-1} and day)		17 b	0.379 b	52.2 a

* and **: significant at the level of 0.05 and 0.01 (p), by the F-test, respectively.
ns: non-significant at the 0.05 (p) level by the F-test.
DF: degree of freedom.
When comparing the means between Control and Factorial, for each variable (emergence, ESI and MET), those with the same letter do not differ significantly from each other by Tukey's test at a level of 0.05 (p).

Table 3. Emergence, emergence speed index and mean emergence time for *Coccoloba gigantifolia* seeds stored at different temperatures for different periods.

Storage temperature	Storage period (month)		
	1	2	4
	Emergence (%)		
8.0 ± 1.2 °C (refrigerator)	17 aA	13 aB	4 aA
19.5 ± 0.7 °C (chamber)	12 bA	39 aA	15 bA
	Emergence speed index (% day^{-1})		
8.0 ± 1.2 °C (refrigerator)	0.346 aA	0.271 aB	0.067 aA
19.5 ± 0.7 °C (chamber)	0.209 bA	1.015 aA	0.364 bA
	Mean emergence time (days)		
8.0 ± 1.2 °C (refrigerator)	51.4 bA	52.9 bA	66.7 aA
19.5 ± 0.7 °C (chamber)	60.4 aA	40.1 bB	41.9 bB

Means followed by the same uppercase letter in the column and the same lowercase letter in the row, within the levels of each factor, do not differ significantly from each other by Tukey's test at the 0.05 (p) level of probability.

Unlike the findings for *Coccoloba gigantifolia* seeds, the seeds of *C. uvifera* maintained viability after fifteen months of storage at room temperature (27.2 ± 1.8 °C) and in the refrigerator (4.0 ± 1.5 °C), reaching 95% and 75% germination, respectively (Vargas-Simón and Pire, 2010).

According to Hong and Ellis (1996), desiccation tolerance can be characteristic of a particular botanical family or genus. However, differences in physiological behavior may occur between families, genera and species. In fact, although species of the same genus have related evolutionary trajectories, any difference in ecophysiological responses may indicate possible adaptations to the habitat occupied by each one, rather than phylogenetic effects (Tweddle et al., 2003). Furthermore, different physiological behaviors of seeds within the same species may be related to the genotype, the environmental conditions in regions or production seasons, differences in maturation and drying of the seeds (Barbedo, 2018).

In the first experiment, which was an adaptation of the protocol proposed by Hong and Ellis (1996), it was found that *Coccoloba gigantifolia* seeds are sensitive to desiccation. In the second experiment, the seeds showed low tolerance to refrigeration, which shows that the storage of desiccation-sensitive seeds poses a challenge to *ex situ* conservation.

According to Umarani et al. (2015), the most practical way to extend the storage period of desiccation-sensitive seeds is to store them at the lowest possible temperature, at which cell damage can be reduced while cold or freezing injuries can be avoided. Thus, studies with *Coccoloba gigantifolia* should be continued, seeking to identify the best combination between seed moisture content and storage temperature, even if it is meant for conservation for a short period of time.

CONCLUSIONS

Coccoloba gigantifolia seeds show a recalcitrant behavior, as they are sensitive to desiccation, with a marked reduction in viability when dried at moisture levels below 19.5%. Storage at temperatures of 8.0 and 19.5 °C was not favorable to maintaining seed quality with 25.2% of moisture.

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