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Original Paper

Propagation of two epiphytic Cactaceae for relocation to an urban protected area of the Atlantic Forest in São Paulo, Brazil

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Abstract

The relocation of epiphytes contributes to biodiversity conservation. In this sense, studies that aim to promote the success of the process are necessary. Therefore, the objective of this work was to analyze the effects of initial fresh mass and fertilization on the propagation of two species of *Rhipsalis*, thus ensuring their success after relocation to a protected area. Therefore, fertilized and unfertilized plants of *Rhipsalis puniceodiscus* and *Rhipsalis trigona* initially with one or two cladodes were propagated in a greenhouse for 25 months. After relocation to a forest area, their survival was monitored for 16 months. Survival percentage, shoot length and root length were analyzed every four months. Fresh mass, dry mass and photosynthetic pigment were analyzed before relocating to a native forest. During the protected propagation stage, for both species, individuals presenting more branches and those that were fertilized exhibited more intense overall development. The propagation of *R. puniceodiscus* and *R. trigona* in a greenhouse with two cladodes is better than with one cladode. In general, *R. puniceodiscus* displayed better development under controlled conditions than *R. trigona*. Propagation using fertilization increased fresh mass and favors growth and survival rate of *R. puniceodiscus* and *R. trigona*. It is possible to relocate both species to a preserved area of the Atlantic Forest, but *R. trigona* presented more potential.

Key words: plant fertilization; plant reintroduction; *Rhipsalis*; survival; transplantation

Resumo

A realocação de epífitas contribui para a conservação da biodiversidade. Neste sentido, estudos que visem promover o sucesso do processo são necessários. Diante disso, o objetivo deste trabalho foi analisar os efeitos da massa fresca inicial e da fertilização na propagação de duas espécies de *Rhipsalis*, garantindo assim o seu sucesso após a realocação para uma Unidade de Conservação. Para tanto, plantas fertilizadas e não fertilizadas de *Rhipsalis puniceodiscus* e *Rhipsalis trigona* inicialmente com um ou dois cladódios foram propagadas em casa de vegetação por 25 meses. Após a mudança para uma área de floresta, sua sobrevivência foi monitorada por 16 meses. A porcentagem de sobrevivência, comprimento da parte aérea e comprimento da raiz foram analisados a cada quatro meses. Massa fresca, massa seca e pigmento fotossintético foram analisados antes de realocar para uma floresta nativa. Na fase de propagação protegida, para ambas as espécies, os indivíduos com mais ramos e os que foram fecundados exibiram um desenvolvimento global mais intenso. A propagação de *R. puniceodiscus* e *R. trigona* em casa de vegetação com dois cladódios é melhor do que com apenas um cladódio. Em geral, *R. puniceodiscus* apresentou melhor desenvolvimento em condições controladas do que *R. trigona*. A propagação com fertilização aumenta a massa fresca e favorece o crescimento e a taxa de sobrevivência de *R. puniceodiscus* e *R. trigona*. É possível realocar ambas as espécies para uma área preservada da Floresta Atlântica, porém *R. trigona* se apresentou mais promissora no processo.

Palavras-chave: fertilização de plantas; reintrodução de plantas; *Rhipsalis*; sobrevivência; transplantio

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Introduction

Epiphytes are considered essential for the rainforest (Moorhead *et al.* 2010; Eskov *et al.* 2021; Taylor *et al.* 2021). Those plants serve as a source of shelter and food for fauna, such as mammals, birds, amphibians, reptiles and hundreds of insects (Nadkarni 1994; Benzing 2000; Cruz-Angón & Greenberg 2005; Méndez-Castro *et al.* 2018), thus playing a significant role in biodiversity conservation.

Epiphytic species, including the dispersed by animals, have been little studied concerning fragmented landscapes (Leitman *et al.* 2014), which highlights the importance of studies on the relocation of these plants for enriching the local biodiversity of the forest (Santos Junior & Tamaki 2014; Nievola *et al.* 2022) especially when this vegetation is severely impacted (Tremblay 2008). However, due to the scarcity of studies on this subject, species relocation on native forests, where they naturally occur, is recommended to minimize environmental impacts. Plant propagation and reintroduction of specimens to their natural environment are recommended conservation efforts (Ortiz-Rodriguez *et al.* 2020). This propagation can be favored with nitrogen fertilizers, increasing the amount of photosynthetic pigments such as chlorophylls, whose contents can be used as an estimate of the total nitrogen content of the plant (Majerowicz *et al.* 2000). The relocation of new specimens enriches the forest with more individuals and expands the genetic diversity of the specie in question, which may increase the viability of the species under study (Kohn & Lusby 2004).

The Atlantic Forest is the second-largest tropical forest in Brazil (Sugiyama 2010). Due to the high levels of endemism and being among the top 36 global biodiversity *hotspots*, conservation efforts for this forest have become a priority (Rezende *et al.* 2018). It is considered one of the areas with the most extraordinary biodiversity on the planet, with about 18,000 species of plants (Flora e Funga do Brasil 2020, continuously updated). However, due to anthropic actions, only 11 to 16% of this forest remains in Brazil (Ribeiro *et al.* 2009), thus justifying mitigation measures.

In 2009, the Pact for the Restoration of the Atlantic Forest was established to recover 15 million hectares by 2050. To date, an estimated 673,510–740,555 ha of native forests was restored from 2011 to 2015 in this phytogeographic domain (Crouzeilles *et al.* 2019). Restoration of forests is mainly executed by trees, and despite their relevant ecological roles, epiphytic richness rarely reaches values found in natural ecosystems (Duarte & Gandolfi 2017). The success of the establishing of a restored vegetation depends on forming a diversified canopy capable of creating adequate conditions for the establishment of epiphytic and climbing plants (Siqueira-Filho & Tabarelli 2006). While many Atlantic Forest recovery projects have been developed and many fragments have already been restored in the state of São Paulo/Brazil, in these forest areas few non-trees were found, such as epiphytes (Garcia *et al.* 2016). It is considered one of the areas with the most extraordinary biodiversity on the planet, with nearly 16,000 Angiosperm species (Flora e Funga do Brasil 2020, continuously updated). In the Atlantic Forest, of the 14,500 vascular plants that inhabit this place, 3,000–4,000 are epiphytes (Kersten 2010; Ramos *et al.* 2019), suggesting that these plants are relevant to the mature forest.

Among the epiphytic families more representavive in Atlantic Forest are Cactaceae (Kersten 2010; Furtado & Menini Neto 2022) and presents 53 species (Flora e Funga do Brasil 2020, continuously updated), including the genus *Rhipsalis*, which has once been abundant (Hoehne *et al.* 1941), but are rare nowadays. *Rhipsalis* are native to tropical and subtropical regions of the Americas, usually growing in phorophytes (Zappi & Taylor 2022). There are 37 species of this genus throughout Brazil and 22 in São Paulo state (Zappi & Taylor 2022).

The present study sought to analyze the effect of initial fresh mass and fertilization on the propagation of two *Rhipsalis* species with one or two cladodes and the goal of ensuring their success following relocation to a protected area.

Materials and Methods

Study area and environmental variables

The relocation occured in a native forest site located at the Parque Estadual das Fontes do Ipiranga (PEFI), a remnant of a protected area located in São Paulo, SP, Brazil. The PEFI is a protected area with 526 hectares in an extremely urbanized region of the city of São Paulo (São Paulo 2008). The predominant vegetation in the PEFI is the Atlantic Rainforest, displaying high floristic diversity (Rancura & Cerati 2020).

The selected sites were located at the coordinates 23º40'18"S and 46º38'00"W. These areas presented a light intensity that was among 40%, measured with digital luximeter (Mlm-1011

Minipa) and converted to photosynthetic active radiation (PAR).

According to the Köppen climate classification (1948), the study area, located within the Upper Tietê Hydrographic Basin, PEFI, has a temperate Cwb climate, characterized by wet summers and dry winters (Santos & Funari 2002), with an average temperature of 19.2 ºC, oscillating between a maximum of 31.2 ºC and a minimum of 10.7 ºC. The average annual rainfall is 1,515 mm, and the average humidity is 80%.

The monthly total precipitation and minimum and maximum average temperatures were obtained from the Center for Weather Forecasting and Climate Studies of the National Institute of Space Research (CPTEC/INPE 2020). Notably, in the month in which the relocation was performed (December 2017), the accumulated precipitation was 115.6 mm. During the 17-month monitoring period (December 2017 to April 2019), monthly precipitation varied between 20.6 and 469.6 mm. The lowest accumulated rainfalls were recorded in May, June and July 2018 with 20.6, 21.5, and 23 mm of precipitation, respectively. On the other hand, the highest accumulated rainfalls were recorded in January, February and March of 2019, with 268, 469.6, and 329.2 mm of precipitation, respectively. Additionally, the lowest recorded temperature was 11.7 ºC in July 2018, and the highest temperature of 32.4 ºC occurred in January 2019. In general, the climatic conditions *(i.e.*, the relocation stage) did not differ much. A notable difference occurred in 2019, during which the amount of precipitation was almost double that of the beginning of 2018 (Fig. 1).

Studied species

In the present study, two epiphytic species of Cactaceae of the genus *Rhipsalis* were used (*Rhipsalis puniceodiscus* G. Lindb and *Rhipsalis trigona* Pfeiff.), and according to a survey conducted by Hoehne *et al.* (1941), both were present in the native Atlantic Forest of the PEFI.

The *Rhipsalis puniceodiscus* and *R. trigona* specimens were obtained from trees were removed to construct the northern stretch of the Rodoanel Mário Covas highway, in the city of São Paulo, Brazil, near the coordinates 23º24'41.3"S and 46º32'24.7"W. After the rescue, plants were transported to the Instituto de Pesquisas Ambientais (IPA) of São Paulo for studies.

Propagation with fertilization

At the IPA samples of the plants, containing one cladode (n = 48) or two cladodes (n = 48), were isolated from both species. As shown in Figure 2, to facilitate the planting in the substrate, the cladodes were removed with an additional 3 cm in length. After collecting the samples, we recorded the cladode length and fresh mass. Additionally, a portion of the sample was used to analyze the photosynthetic pigments at time zero (Described in Materials and Methods section Analysis of photosynthetic pigments).

Sowing trays with square cells in the experimental assembly were filled with washed sand as substrate. The experimental was divided into four treatments for each species (n = 24 individuals each) (Figs. 3-4). Two of these were composed of one or two cladode which was fertilized weekly with 1mL per plant (or sample) of commercially available 10:52:10 N:P:K foliar fertilizer ("Plant-Prod", 1g.L^{-1}) by foliar

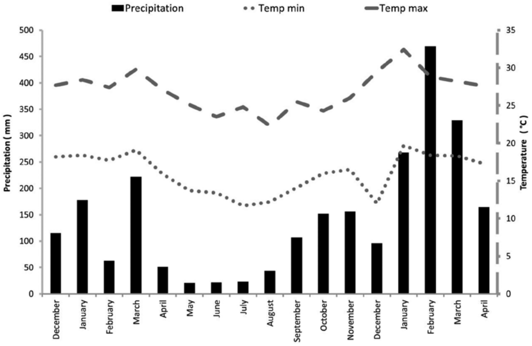

Figure 1 – Average monthly accumulated precipitation, and minimum and maximum temperature in the Santana Observatory in São Paulo, SP-Brazil. Data were obtained from National Meteorology Institute (INMET), using the Meteorological Database for Teaching and Research (BDMEP).

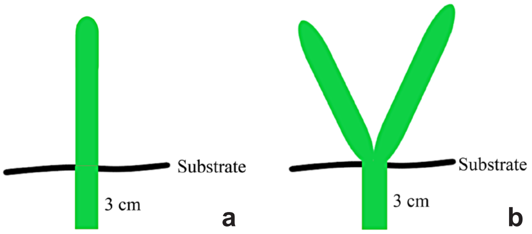

Figure 2 – a-b. Schematic representation of planting *Rhipsalis* in substrate – a. plant with one cladode; b. plant with two cladodes.

Figure 3 – a-p. Phenotypes of *Rhipsalis puniceodiscus* studied – a-d. T0 of production study in the greenhouse (2015); e-h. final phase of propagation of *R. puniceodiscus* (2017); i-l. T0 of realocation study in forest área (2017); m-p. final phase of realocation study (2019). a,e,i,m. one cladode without fertilization. b,f,j,n. one cladode with fertilization. c,g,k,o. two cladodes without fertilization. d,h,l,p. two cladodes with fertilization. Scale bar = 1cm.

cladodes. The unfertilized plants with one cladode had the shortest root lengths (Fig. 5c). The fresh mass of the fertilized plants was significantly higher than the unfertilized groups. Moreover, the fertilized plants with only one cladode had almost twice the fresh mass than the unfertilized plants with one cladode (Fig. 5d).

During the propagation phase of the present study, cultivating *Rhipsalis trigona* samples with two cladodes in a protected environment was significantly more favorable for the production of plants, and fertilization did not influence survival. As shown in Figure 6a (2015 to 2017), unfertilized and fertilized samples with two cladodes had higher survival percentages (about 90% for both conditions) than specimens with one cladode (fertilized about 72% and unfertilized about 52%). These results are further verified by the good appearance of the plants at the end of the protected propagation stage (Fig. 4e-f). Additionally, the fertilized one cladode and two cladode specimens exhibited shoot lengths that were almost 2-fold

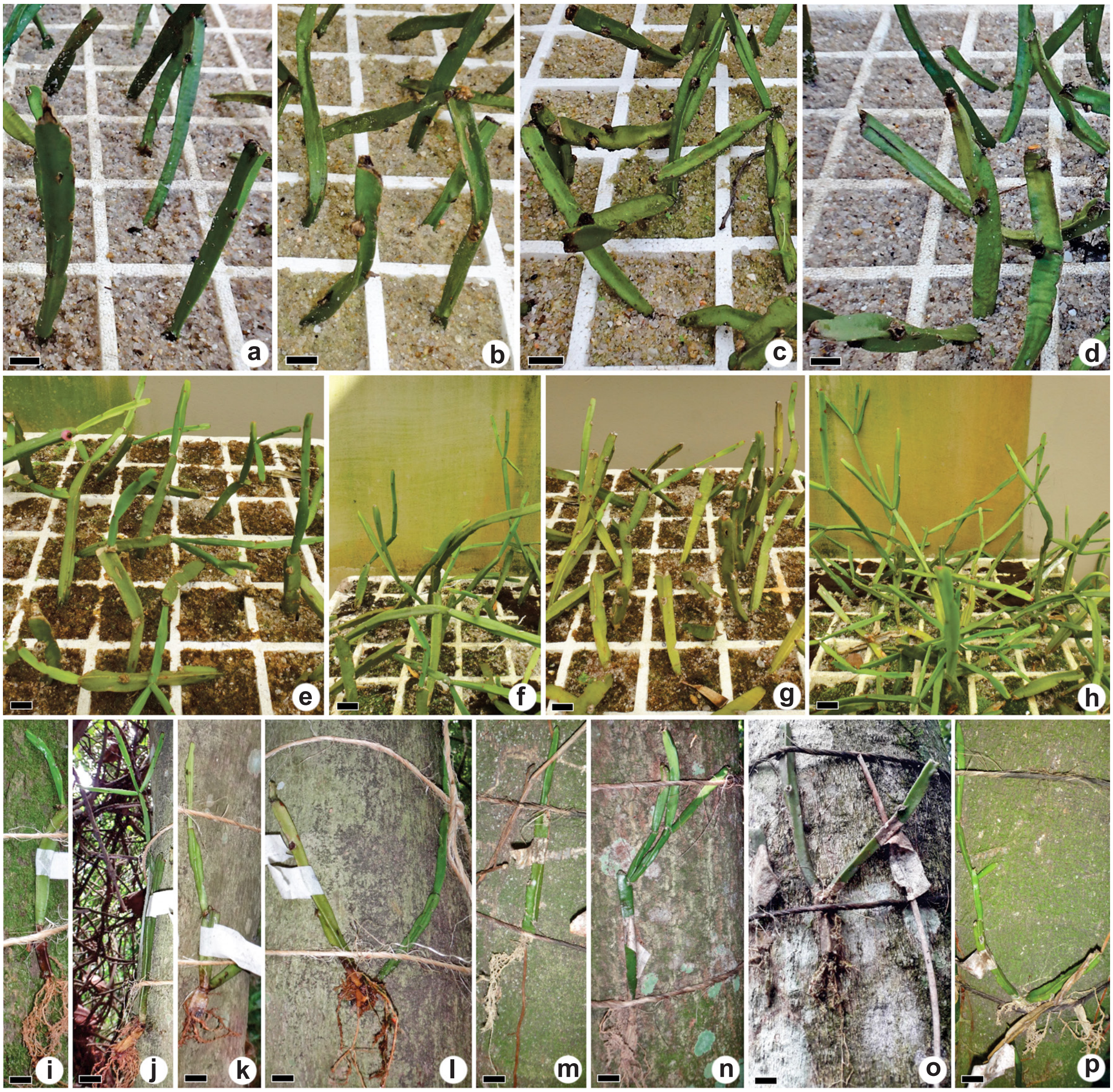

Figure 4 – a-p. Phenotypes of *Rhipsalis trigona* study – a-d. T0 of production study in the greenhouse (2015); e-h. final phase of propagation of *R. trigona* (2017); i-l. T0 of realocation study in forest área (2017); m-p. final phase of realocation study (2019). a,e,i,m. one cladode without fertilization. b,f,j,n. one cladode with fertilization. c,g,k,o. two cladodes without fertilization. d,h,l,p. two cladodes with fertilization. Scale bar = 1cm.

sprinkling. As a control, plants with one or two cladodes were only irrigated with water without supplemental nutrients. The trays were maintained in a greenhouse and automatically irrigated twice a week for 10 minutes.

The experiment was set up on November 17, 2015. Data, including survival percentage, shoot length and root length, were collected and analyzed every four months. Additionally, fresh mass, dry mass and photosynthetic pigment levels were also recorded and analyzed before relocating the material to a native forest area. The last data collection was performed on December 4, 2017, with the collection of the same variables.

Relocation in the forest

In this experimental phase, 12 individuals from the control group and 12 individuals from the fertilized group from each species with one or two cladodes were relocated to the forest study location, in fatoral design. Each plant was identified and fixed on a tree, 1m from the ground (Figs. 3i-l; 4i-l).

This experimental period lasted from December 4, 2017, to April 11, 2019. As in the "Propagation with fertilization" stage of the experiment, survival percentage, shoot length and root length were collected every four months. Sixteen months after relocating the plants, survival percentage, shoot length, root length, fresh mass, dry mass and photosynthetic pigment levels were recorded and subsequently analyzed.

Analysis of photosynthetic pigments

Photosynthetic pigments were extracted from 0.5 g of fresh cladode mass, previously stored at -20 ºC until the moment of analysis. These analyses were performed in triplicate. Briefly, the material was macerated in liquid nitrogen with a mortar and then diluted with 2 mL of iced acetone. Each sample was centrifuged at 3,500 rpm for 30 minutes. The supernatant was collected in 2 mL tubes, and the volume was adjusted to a final volume of 2 mL with cold acetone. The absorbance of the photosynthetic pigments was measured in a spectrophotometer, calibrated with pure acetone. The absorbance (A) of chlorophyll *a* was measured at a wavelength (λ) 661.6 nm, chlorophyll *b* at 644.8 nm, and carotenoids at 470 nm. The absorbance at 750 nm was subtracted from all the readings to remove any contributions from impurities present in the sample. The chlorophyll *a* (C_a), chlorophyll *b* (C_b), and carotenoid concentrations were calculated according to the following equations (Lichtenthaler 1987):

$C_a = 11.24\,A_{661.6} - 2.04\,A_{644.8}$

$C_b = 20.13\,A_{644.8} - 4.19\,A_{661.6}$

Carotenoids = $(1000 \times A_{470} - 1.90 \times C_a - 63.14\ C_b) / 214$

The pigment concentrations of the plant tissues were expressed as μg of pigment per gram of fresh mass.

Experimental design and data analysis

The experiments were designed as completely randomized (CRD), with 24 repetitions per treatment. The experiment was installed considering a 2×2 factorial scheme (number of cladodes x fertilization). The values of all the variables were subjected to analysis of variance (ANOVA). The means were compared using the Tukey test, and the level of significance was set to 5%. Data analyses were performed with the SISVAR Program (Ferreira 2011).

To verify the effect of climatic data on the variables analyzed, and the relationship of photosynthetic pigments with these same variables, Pearson's correlation coefficient was calculated. The absolute values, which ranged from 0 (zero) to 1.0 (one), were converted into categories to facilitate interpretation: very strong correlation (VSC) - over than 0.9; strong correlation (SC) - 0.7–0.9; moderate correlation (MC) - 0.5–0.7; poor correlation (PC) - 0.3–0.5; negigible correlation (NC) - 0–0.3.

Results

Propagation with fertilization

Concerning *Rhipsalis puniceodiscus*, during the propagation stage in the controlled environment, from 2015 to 2017, plant survival ranged between 76–80%, and no significant differences were detected for cladode number or fertilization (Fig. 5a). *R. puniceodiscus* plants at the end of the protected propagation stage are shown in Figure 3e-h. Concerning plant growth, fertilization significantly increased the shoot length, regardless of the number of cladodes. However, plants with one cladode exhibited a more robust response to fertilization, reaching more than 35 cm in length and being 2.1 times longer than the shoots of the unfertilized plants (Fig. 5b). While the fertilized plants with one cladode had the longest roots, this value was only slightly greater than the root lengths of the fertilized and unfertilized plants with two greater than the unfertilized plants. It should be pointed out that the initial cladode number did not affect this parameter (Fig. 6b). There was no significant difference in root length between fertilized and unfertilized plants (Fig. 6c). At the beginning of the propagation stage in 2015, it was clear that fertilization increased the fresh mass of plants with one or two cladodes when comparing them with plants that were not fertilized. In 2017, fertilized *R. trigona* specimens with two cladodes had about 50% more fresh mass than fertilized plants with one cladode. Notably, the increase in fresh mass for the fertilized, mainly with two cladode plants, was even greater than the unfertilized groups. (Fig. 6d).

Relocation in the forest

The survival steadily of *R. puniceodiscus* declined from 2017 to 2019 and did not reach a stabilization phase in any of the groups. After two years, survival percentages ranged from 50% to 56%, with no significant differences detected (Fig. 5a; Tab. 1). The images in Figure 3i-l show the plants at the end of the relocation stage. The fertilized plants presented the longest shoot lengths during the relocation stage, regardless of the number of initial cladodes (Fig. 5b; Tab. 1). Moreover, plants that initially had two cladodes presented the longest root lengths after relocation (Fig. 5c; Tab. 1). In contrast, plants that initially had one cladode and were not fertilized displayed the lowest root length, reaching only 5.8 cm on average (Tab. 1). The fertilized plants that initially had one cladode produced plants with fresh masses that were significantly higher than the other three groups (Fig. 5d; Tab. 1). We did not observe any significant accumulation of dry mass in any of the groups at the end of relocation (Tab. 1).

In the case of *Rhipsalis trigona* plants, no significant differences in the survival percentage between any of the groups were observed (Fig. 6a; Tab. 1). It should be pointed out that none of the treatment groups had less than 75% survival, two years after relocation (Fig. 4i-l). The short shoot lengths observed in the unfertilized plants during the propagation stage were maintained throughout the relocation stage, exhibiting only slight extensions. Similarly, the long shoot lengths of the fertilized plants continued to be significantly longer than the unfertilized plants, regardless of whether they initially had one or two cladodes (Fig. 6b; Tab. 1). Interestingly, the root lengths of plants that initially had two cladodes, fertilized and unfertilized, were 50% longer when compared to

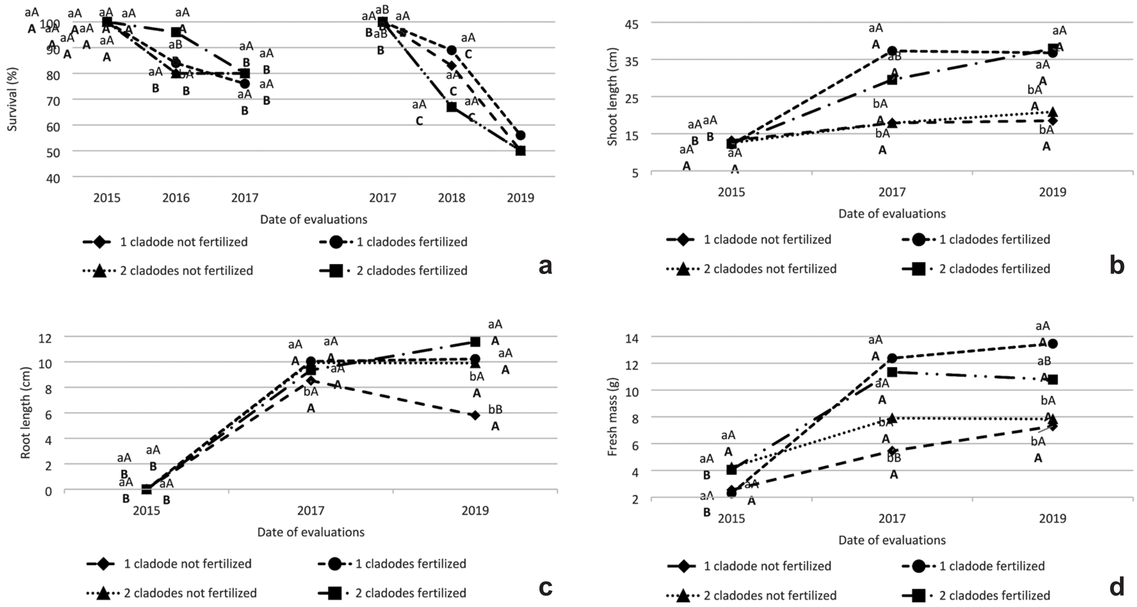

Figure 5 – a-d. Average values of *Rhipsalis puniceodiscus*, at zero time (2015), the time of relocation (2017) and the end of the experiment (2019) – a. survival; b. shoot length; c. root length; d. fresh mass. Lower case letters compare fertilizations, within the number of cladodes, upper case letters compare the number of cladodes, within the fertilization and bold upper case letters compare the dates, using the Tukey test at a 5% probability level.

the root lengths at the time of relocation in 2017. Moreover, after relocation, plants that initially had two cladodes and were fertilized had the most extended root lengths, presenting about 25% higher than in plants with two cladodes and not fertilized, thus representing the second-longest root length at the end of the experiment (Fig. 6c; Tab. 1). The plants that had one cladode, fertilized and unfertilized, displayed root lengths that were not different from the values observed at the end of the propagation stage (Fig. 6c; Tab. 1). After full evaluation period, the fresh mass remained stable in all plants, compared with the beginning of relocation in 2017 (Fig. 6d). Only unfertilized specimens with one cladode showed a significant decrease (about 22%) of fresh mass compared to 2017 (Fig. 6d). Additionally, fertilization promoted higher fresh mass when compared with plants that were not fertilized (Fig. 6d; Tab. 1). Furthermore, plants that initially had two cladodes presented significantly higher dry masses than those with only one cladode (Tab. 1).

For both species, environmental variables negatively affected plant survival (Tab. 2). Temperature and moisture showed a strong positive correlation for cladode and root lengths and fresh mass, for *R. trigona* plants, except for those with one cladode and not fertilized. With the plants of *R. puniceodiscus*, the two environmental factors affected positively and more incisively the plants, fertilized or not, but with two cladodes. Also for *R. puniceodiscus* plants, for root length and fresh mass, there was a greater positive influence of environmental variables on fertilized plants.

Photosynthetic pigments

Following relocation in 2017, the concentrations chlorophyll *a*, chlorophyll *b*, and carotenoids were significantly higher in fertilized *R. trigona* plants that initially had one cladode when compared to photosynthetic pigment concentrations of the other groups (Fig. 7a). Additionally, the final measurements in 2019 demonstrated that the plants fertilized during the protected propagation stage presented significantly higher levels of chlorophyll *a*, *b* and carotenoids, thus highlighting the influence of fertilization on photosynthetic pigment production (Fig. 7a).

The photosynthetic pigments concentrations in *Rhipsalis puniceodiscus* plants throughout

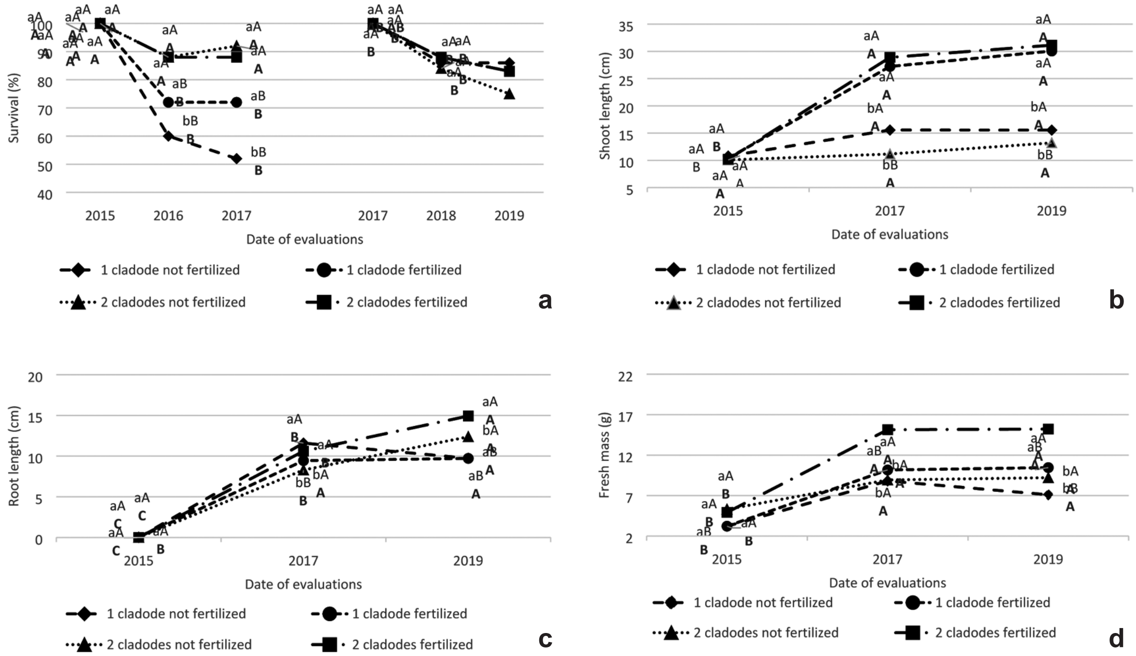

Figure 6 – a-d. Average values of *Rhipsalis trigona*, at time zero (2015), the time of relocation (2017) and the end of the experiment (2019) – a. survival percentage; b. shoot length; c. root length; d. fresh mass. Lower case letters compare fertilizations, within the number of cladodes, upper case letters compare the number of cladodes, within the fertilization and bold upper case letters compare the dates, using the Tukey test at a 5% probability level.

Table 1 – Average values of survival, shoot length and root length, fresh mass, and dry mass of *Rhipsalis puniceodiscus* and *Rhipsalis trigona*, with 1 or 2 cladodes and fertilized or not, 16 months after the relocation.

	1 cladode		**2 cladodes**	
	Not fertilized	**Fertilized**	**Not fertilized**	**Fertilized**
Rhipsalis puniceodiscus				
Survival (%)	50 aA	56 aA	50 aA	50 aA
Shoot Length (cm)	18.5 bA	36.8 aA	20.9 bA	37.9 aA
Root length (cm)	5.8 bB	10.2 aA	9.9 bA	11.6 aA
Fresh Mass (g)	7.3 bA	13.5 aA	7.8 bA	10.8 aB
Dry Mass (%)	3.0 bB	3.6 aA	3.6 aA	3.7 aA
Rhipsalis trigona				
Survival (%)	86 aA	83 aA	75 aA	83 aA
Shoot Length (cm)	15.5 bA	30 aA	13.2 bB	31.1 aA
Rooth Length (cm)	9.7 aB	9.7 aB	12.4 aA	14.9 aA
Fresh Mass (g)	7.1 bB	10.5 aB	9.2 bA	13.2 aA
Dry Mass (%)	3.6 bB	4.3 aB	4.2 bA	4.8 aA

Lower case letters compare fertilizations, within the number of cladodes and upper case letters compare the number of cladodes, within the fertilization, using the Tukey test at a 5% probability level.

the experimental period yielded different results than observed with *R. trigona*. For example, in 2017, during the protected propagation stage, fertilized plants with one or two cladodes had increased chlorophyll *a*, *b*, and carotenoid contents when compared to the unfertilized groups (Fig. 7b). This trend was also observed in the final measurement, with the fertilized plant groups presenting significantly higher levels of chlorophyll *a*, *b* and carotenoids, again providing evidence that fertilization promotes photosynthetic pigment production (Fig. 7b).

In *Rhipsalis trigona* plants, a correlation profile was not found between the amount of chlorophyll *a*, chlorophyll *b*, and carotenoids with the analyzed variables (Tab. 3). However, for *R. puniceodiscus*, there were some correlations. In the species, in general, survival was not linked to a greater amount of the three pigments in question. On the other hand, cladode and root lengths, and fresh mass, except for plants with two cladodes and fertilized, all variables showed higher values as the three pigments were found in greater quantity.

Discussion

Propagation with fertilization

During the propagation in a protected environment, the obtained results suggest that fresh mass content is more relevant than the initial number of cladodes. More vigorous samples have more reserves of water, nutrients, hormones, mineral salts, and other substances than smaller specimens, which promotes faster resumption of growth and rooting and, consequently, increased survival. In fact, a direct correlation was observed between a higher survival rate and higher mass in epiphytic bromeliads transplanted in restored areas (Duarte & Gandolfi 2013). Moreover, in a study on the rescue of Orchidaceae, Bromeliaceae, and Cactaceae, Jasper *et al.* (2005) emphasized that the transplantation of younger individuals provided greater survival. However, more mature plants fruit more quickly, which would enhance the dispersal of the species throughout the area.

In this context, fertilization during the propagation stage would endow smaller specimens with increased shoot growth when compared to unfertilized samples with more fresh mass. As expected, since nitrogen, phosphorus and potassium are the main macronutrients necessary for the plants, the resumption of the development was favored in the fertilized samples. Indeed, in a propagation study with other species of the genus *Rhipsalis*, Tavares *et al.* (2016) demonstrated that nitrogen fertilization augmented plant development.

Table 2 – Results based on Pearson's correlation coefficient between the analysed variables (survival, cladode length, root length, and fresh mass) and the environmental variables (temperature and humidity) for *Rhipsalis puniceodiscus*. and *Rhipsalis trigona*.

Rhipsalis puniceodiscus								
	Temperature				**Moisture**			
	One cladode		**Two cladodes**		**One cladode**		**Two cladodes**	
	No fertilized	**Fertilized**	**No fertilized**	**Fertilized**	**No fertilized**	**Fertilized**	**No fertilized**	**Fertilized**
Survival	SC -	SC -	SC -	SC -	MC -	MC -	NC -	NC -
Cladode length	VSC +	VSC -	VSC +	VSC +	VSC +	VSC -	VSC +	VSC +
Root length	VSC -	VSC +	VSC -	VSC +	VSC -	VSC +	VSC -	VSC +
Fresh mass	VSC +	VSC +	VSC -	VSC -	VSC +	VSC +	VSC -	VSC -
Rhipsalis trigona								
	Temperature				**Moisture**			
	One cladode		**Two cladodes**		**One cladode**		**Two cladodes**	
	No fertilized	**Fertilized**	**No fertilized**	**Fertilized**	**No fertilized**	**Fertilized**	**No fertilized**	**Fertilized**
Survival	PC -	SC -	SC -	SC -	NC -	NC -	NC -	NC -
Cladode length	VSC -	VSC +	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +
Root length	VSC -	VSC +	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +
Fresh mass	VSC -	VSC +	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +

Legend and adopted values: Very strong correlation (VSC) = over than 0,9; Strong correlation (SC) = 0,7-0,9; Moderate correlation (MC) = 0,5-0,7; Poor correlation (PC) = 0,3-0,5; Negigible correlation (NC) = 0-0,3. The signs indicate whether the correlation was positive or negative.

The photosynthetic pigment analyses justify, in part, the greater growth and survival of these plants. This interpretation is because plants with more chlorophylls display greater photosynthetic activity than those with lower levels of these pigments, resulting in more growth under the same conditions. For this reason, root formation is so important, since the photosynthetic process must be accompanied by fixation and, consequently, water absorption. Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll concentrations have a positive relationship with photosynthetic rate. Indeed, low photosynthetic pigment levels can directly reduce photosynthetic potential and primary production (Anjum *et al.* 2011). Furthermore, Bjorkman (1981) verified an increase in photosynthetic capacity owing to increased levels of chlorophyll, which was possibly associated with raised leaf thickness and a higher number of chloroplasts.

It is plausible that the observed increase in chlorophyll and carotenoid concentrations, in this work, resulted in a higher photosynthetic performance that facilitated the adaptation of relocated plants to the new environment. Additionally, since carotenoids are major components of the antenna systems of chloroplasts, the above results indicate that they play an essential role in the development of light-harvesting complexes and the dissipation of thermal energy (Jeon *et al.* 2006).

Relocation in the forest

During the relocation stage, a stabilization in the survival percentage of *Rhipsalis trigona* plants was observed after 16 months. Plants that initially had two cladodes and were not fertilized displayed lower survival. Previously, using the orchid *Cattleya intermedia* Graham *ex* Hook, Dorneles & Trevelin (2011) demonstrated that applying Hoagland fertilization solution during forest relocation improved the survival of these plants. Furthermore, in a survey on the survival of several epiphytic plants, such as bromeliads, orchids, and cacti during relocation, Santos Junior & Tamaki (2014) reported survival percentages of greater than 80% when applying various interference agents to plants.

Interestingly, in the protected propagation stage, *Rhipsalis trigona* exhibited more growth in the shoot, but increased root growth was observed after being relocated. These results indicate that the relocated plants were exposed to some stressful environmental factor(s). Thus, by diverting more resources towards root development, these plants

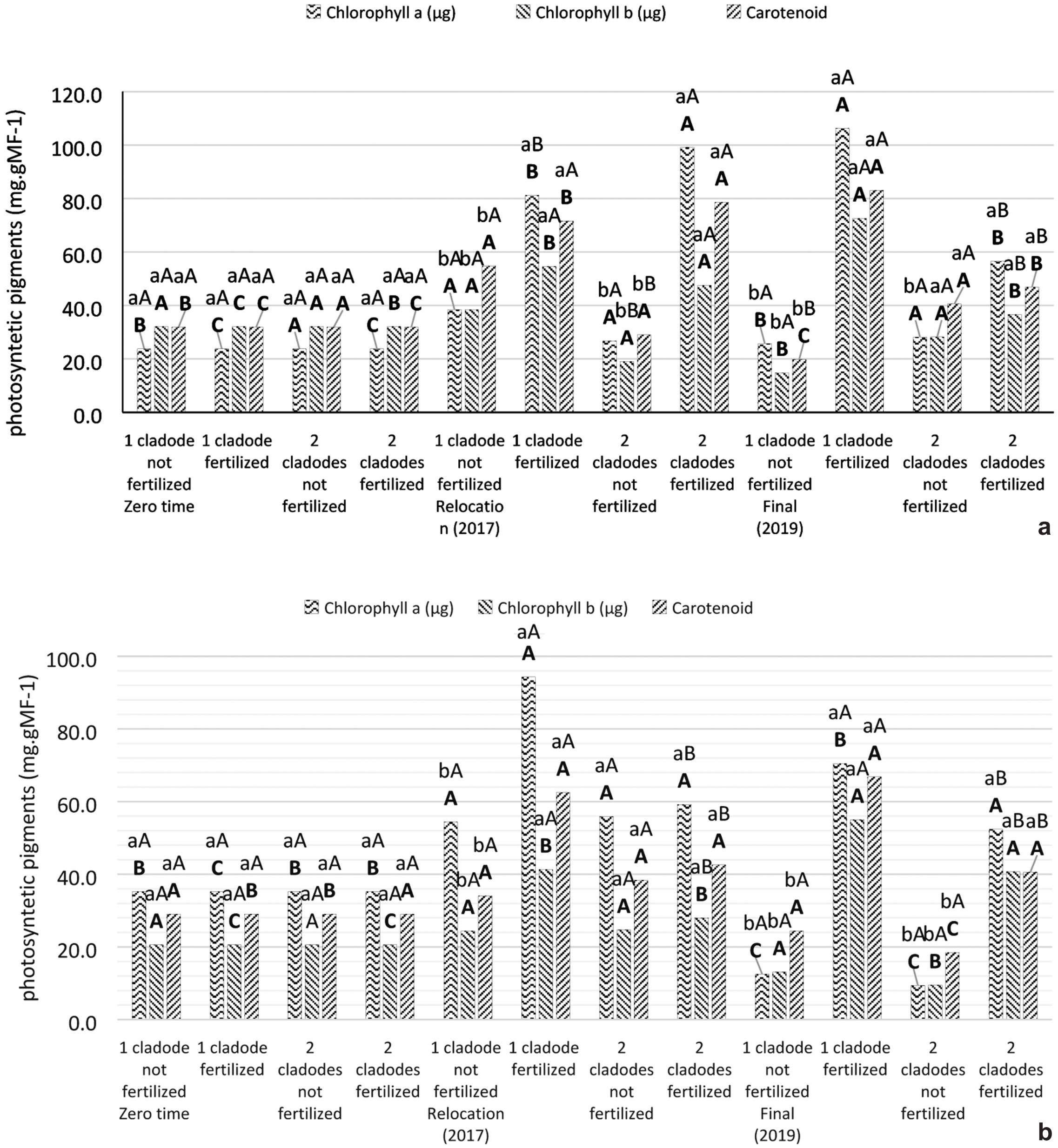

Figure 7 – a-b. Average values of chlorophyll *a*, chlorophyll *b*, and carotenoids at zero time, the time of relocation (2017), and the end of the experiment (2019) – a. *Rhipsalis puniceodiscus*; b. *Rhipsalis trigona*. Lower case letters compare fertilizations, within the number of cladodes, upper case letters compare the number of cladodes, within the fertilization, and bold upper case letters compare the dates using the Tukey test at a 5% probability level.

Table 3 – Results based on Pearson's correlation coefficient between the analysed variables (survival, cladode length, root length, and fresh mass) and the pigments (chlorophyll *a*, chlorophyll *b*, and carotenoid) for *Rhipsalis puniceodiscus* and *Rhipsalis trigona*.

Rhipsalis puniceodiscus												
	Chlorophyll a				**Chlorophyll b**				**Carotenoid**			
	One cladode		**Two cladodes**		**One cladode**		**Two cladodes**		**One cladode**		**Two cladodes**	
	No fertilized	**Fertilized**	**No fertilized**	**Fertilized**	**No fertilized**	**Fertilized**	**No fertilized**	**Fertilized**	**No fertilized**	**Fertilized**	**VSC +**	**Fertilized**
Survival	MC -	PC -	NC +	MC -	NC +	PC -	PC +	PC -	NC -	MC -	MC -	MC -
Cladode length	VSC +	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +	VSC -
Root length	VSC +	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +	VSC -
Fresh mass	VSC +	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +	VSC -

Rhipsalis trigona												
	Chlorophyll a				**Chlorophyll b**				**Carotenoid**			
	One cladode		**Two cladodes**		**One cladode**		**Two cladodes**		**One cladode**		**Two cladodes**	
	No fertilized	**Fertilized**	**No fertilized**	**Fertilized**	**No fertilized**	**Fertilized**	**No fertilized**	**Fertilized**	**No fertilized**	**Fertilized**	**No fertilized**	**Fertilized**
Survival	NC +	VSC -	VSC +	SC -	NC +	PC -	MC +	VSC -	NC -	MC -	PC +	VSC -
Cladode length	VSC +	VSC -	VSC -	VSC -	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +	VSC -	VSC -
Root length	VSC +	VSC -	VSC -	VSC -	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +	VSC -	VSC -
Fresh mass	VSC +	VSC -	VSC -	VSC -	VSC +	VSC +	VSC -	VSC +	VSC +	VSC +	VSC -	VSC -

Legend and adopted values: Very strong correlation (VSC) – over than 0,9; Strong correlation (SC) – 0,7-0,9; Moderate correlation (MC) – 0,5-0,7; Poor correlation (PC) – 0,3-0,5; Negigible correlation (NC) – 0-0,3. The signs indicate whether the correlation was positive or negative.

could absorb more water and mineral salts and fix the plant to the phorophyte. Indeed, Duarte & Gandolfi (2013) and Francisco *et al.* (2018) highlighted the importance of phorophytes as structural support for epiphyte plants, and for this, a close relationship between the parts is necessary.

However, for the relocated *Rhipsalis puniceodiscus* plants, the results were not as promising. Under a controlled and protected environment, these plants grew well. However, when transferred to the forest environment, regardless of the initial treatment or fresh mass, the survival rate dropped to 50% in 2019. Furthermore, there were no signs of stabilization, which means that these survival percentages are predicted to decline even further over time. The surviving plants displayed a considerable increase in the shoot and root lengths, which may favor their permanence in the phorophyte. Despite these low survival percentages, Duarte & Gandolfi (2017) reported successfully relocating epiphyte plants, such as cacti, bromeliads, and orchids, whose survival rates were between 55.2 % and 100 %, one year after the relocation. These authors concluded that fixing the plants well at the beginning of the rainy season appears to be more relevant to the survival of these specimens than the characteristics of the phorophytes. Additionally, McHaffie (2006) successfully reintroduced a fern in Great Britain with only 49% survival after six years.

Climatological data X relocation

Based on the collected climatological data, it appears as though rainfall may have impacted plant survival. The higher volume of rain in early 2019 seems to have no direct relationship to the fresh mass of *Rhipsalis trigona*, compared from 2017 to 2019 and seems to have little influence on the amount of fresh mass of *R. puniceodicus*. On the other hand, the higher precipitation at the beginning of 2019 may be related to the survival of *R. trigona* plants, since there was a reduction in plant mortality of three treatments from 2018 to 2019, possibly due to optimizing the processes involved in water and nutrient uptake from the phorophyte and the environment. Previous work has shown that epiphytes transplanted at the beginning of the rainy season achieved high survival rates (55.2–100% of individuals), thus enhancing overall development (Duarte & Gandolfi 2017). According to Einzmann *et al.* (2016), rain is one of the main determinants for epiphyte diversity, and drought tolerance is more important in forest fragments than intact forests. In other words, plants that are capable of enduring water shortage conditions are more likely to survive.

However, the amount of rain was not the determining factor for survival after the relocation of the two *Rhipsalis* species, due to some morphological characteristics such as succulent cladodes and the high level of photosynthetic pigments observed after the relocation. It is worth mentioning that it is common to observe a reduction in photosynthetic pigment content in plants submitted to drought (Anjum *et al.* 2011). According to Hoeber *et al.* (2020), drought resistance differs substantially among epiphyte species, and its abundance is not correlated and does not interfere with the growth of juvenile individuals.

Herein, we found that the propagation of *Rhipsalis puniceodiscus* and *R. trigona* in a greenhouse with more than 4–5 g of fresh mass (usually with two cladodes) is better than with smaller samples (usually with just one cladode). Additionally, fertilizing the plants during the propagation stage results in bigger, healthier plants. In general, *R. puniceodiscus* displayed better development under controlled conditions than *R. trigona*. However, *R. trigona* exhibited higher survival percentages during the relocation stage.

While the present study demonstrated that it is possible to relocate both of these species to a preserved area of the Atlantic Forest, more studies focusing on increasing the survival of *R. puniceodiscus* are necessary.

Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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