

Translocation of the epiphytic bromeliad *Vriesea incurvata*: an efficient tool for biodiversity restoration in the Atlantic Forest

Translocação da bromélia epifítica *Vriesea incurvata*: uma eficiente ferramenta para a restauração da biodiversidade na Floresta Atlântica

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ABSTRACT

Micropropagation of epiphytic bromeliads associated to translocation may act as an important tool for conservation, restoration or mitigation initiatives. *Vriesea incurvata* is an epiphytic tank-forming bromeliad endemic to the Atlantic Forest, being an important species in gallery forest environments. Seeds of *V. incurvata* were germinated *in vitro*, and plants were acclimatized and translocated to each of two microhabitats (gallery forest and forest interior) of an Atlantic Forest fragment in South Brazil that harbors few individuals of the species. The 152 plants (76 per microhabitat) were monitored for survival and development, and abiotic data were recorded. There was increased development of morphometric parameters of the plants in the gallery forest, and survival rate ensured an 800% increase in the original population of *V. incurvata* in the study area. Plant survival and development parameters were positively related to light and relative air humidity. In gallery forest, plants flowered and set fruit, indicating their relationship with pollinators, since *V. incurvata* provides food for fauna. Further, the establishment of the individuals increased the availability of water in the canopy by accumulation in the rosettes, as well as the complexity of the canopy structure, providing a site for the occurrence of detritivorous, predatory and herbivorous arthropods. Thus, based on the method applied to *V. incurvata*, inserting epiphytic species into forest environments can be an efficient tool for artificial habitat regeneration, incrementing functional diversity and improving environmental quality.

Keywords: Bromeliaceae; conservation; ecosystem integrity; population reinforcement; tropical rainforest.

RESUMO

A micropropagação associada à translocação de bromélias epifíticas pode atuar como importante ferramenta para iniciativas de conservação, restauração e mitigação. *Vriesea incurvata* é uma bromélia epifítica formadora de tanque, endêmica à Floresta Atlântica, sendo uma espécie importante em ambientes de floresta de galeria. Sementes de *V. incurvata* foram germinadas *in vitro* e plantas foram aclimatizadas e translocadas para dois micro-habitats (floresta de galeria e interior florestal) de um fragmento de Floresta Atlântica no Sul do Brasil que abriga poucos indivíduos da espécie. As 152 plantas (76 por micro-habitat) foram monitoradas para sobrevivência e desenvolvimento, e dados abióticos foram registrados. Houve maior desenvolvimento dos parâmetros morfológicos das plantas na floresta de galeria, e a taxa de sobrevivência assegurou 800% de aumento da população original de *V. incurvata* na área de estudo. A sobrevivência e os parâmetros de desenvolvimento das plantas relacionaram-se positivamente com luz e umidade relativa do ar. Na floresta de galeria, as plantas floresceram e produziram frutos, indicando sua relação com polinizadores, uma vez que *V. incurvata* provê alimento para a fauna. Além disso, o estabelecimento de indivíduos aumentou a disponibilidade de água na copa por meio do acúmulo nas rosetas e a complexidade da estrutura da copa, fornecendo um sítio para a ocorrência de artrópodes detritívoros, predadores e herbívoros. Assim, com base no método aplicado em *V. incurvata*, inserir espécies epifíticas em ambientes florestais pode ser uma eficiente ferramenta para a regeneração artificial de habitats, incrementando a diversidade funcional e melhorando a qualidade ambiental.

Palavras-chave: Bromeliaceae; conservação; floresta tropical úmida; integridade do ecossistema; reforço populacional.

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Introduction

Environmental changes resulting from anthropic actions contribute to environmental degradation and significant impacts of climate change (Maschinski and Albrecht, 2017). As a consequence, biodiversity loss occurs, which in turn, interferes with environmental dynamics and causes the disruption of ecological processes that species provide and that guarantee environmental services to ecosystems (Singh et al., 2021; Pereira et al., 2022).

Plant populations are becoming increasingly fragmented and as low gene flow is one of the main threats to biodiversity conservation, about one-fifth of the world's plant species are endangered with extinction (Pimm and Raven, 2017). The Atlantic Forest comprises great heterogeneity of phytophysiognomies composed by a high diversity of plant species endemic to Brazil and which are exposed to an intense degree of degradation, characterizing the biome as a world's biodiversity hotspot (Ribeiro et al., 2009; Cantidio and Souza, 2019). This means that, despite its great importance for global biodiversity and for the livelihood of much of the Brazilian human population (Rezende et al., 2018), the Atlantic Forest has already been intensely impacted by human activities, so that what remains today are small forest fragments (ca. 83% of the total number of fragments with less than 50 ha), isolated and immersed in matrices that significantly alter their environmental conditions (edge effect) (Ribeiro et al., 2009). Such conditions place the Atlantic Forest in a high degree of vulnerability to climate change (Cantidio and Souza, 2019), and with many taxa composing lists of endangered species (Martinelli and Moraes, 2013). Though, this biome is also the target of restoration efforts (Braga et al., 2021), aiming to ensure the reconstitution of areas, increase biological diversity and biomass production (Lima et al., 2020).

Habitat restoration and translocation of individuals are important tools for biodiversity conservation (Maschinski et al., 2013). The translocation of plants is used for species conservation worldwide because it allows stabilizing and restoring declining plant populations by means of individuals grown in an *ex situ* environment that come from the same location or nearby natural populations, a practice named reinforcement (IUCN/SSC, 2013). This tool has been used mainly for the restoration of terrestrial plant populations, especially tree species (Duarte and Gandolfi, 2013; Silcock et al., 2019; Almeida et al., 2020; Braga et al., 2021; Doyle et al., 2021; Andres et al., 2022) to reestablish the phytophysiognomy (Bellotto et al., 2009). However, epiphytes do not effectively colonize these areas, due to restricted seed dispersal caused by fragmentation, and even areas restored decades ago remain with little contribution of the ecological functions of this plant group (Garcia et al., 2016).

The inclusion of epiphytic species in conservation and restoration strategies allows an ecosystem to increase in functional diversity (Garcia et al., 2016). Each functional group has morphological, physiological and phenological characteristics that define the niche to which it will belong and set its contributions to the functioning of the ecosystem (Obertegger and Flaim, 2018). Especially in a biome like the Atlantic Forest, the con-

tribution of epiphytic plants to the functioning of ecosystems is very important, given the large biomass (80% of the trees shelter epiphytes) and high diversity (more than 50% of the total species) of this plant group (Braga et al., 2021). As epiphytes have a great diversity of adaptations to survive the conditions imposed by this environment, such as water scarcity (Benzing, 1990), inserting them in an ecosystem from which they were eliminated may be crucial to restore the specific functions that were lost during degradation processes (Garcia et al., 2016). Nonetheless, there are few studies that consider epiphytic species for tropical forest restoration (Decruse et al., 2003; Aggarwal et al., 2012; Duarte and Gandolfi, 2013; Endres Júnior et al., 2015b; Endres Júnior et al., 2018).

For successful plant translocation, aiming the restoration of areas, both biotic and abiotic factors of the habitat have to be considered due to specific requirements of each focal species (Godefroid et al., 2011). This is particularly important for the epiphytic environment, where plants depend on the availability of water and atmospheric nutrients, placing them among the organisms most affected by climate change (Zotz and Bader, 2009) and by the deleterious effects of fragmentation and forest degradation (Duarte and Gandolfi, 2013). For plants whose traditional cultivation has limited success, such as epiphytic species, *in vitro* culture offers the advantage of high rates of seed germination, favoring the fast growth of propagated individuals (Sasamori et al., 2016a; Sasamori et al., 2018). Positive data were also obtained through studies on plant establishment, technique development, and biology and ecology of epiphytes (Dorneles and Trevelin, 2011; Endres Júnior et al., 2015a, 2015b; Endres Júnior et al., 2018; Endres Júnior et al., 2019; Soares et al., 2020).

Bromeliads are among the most striking elements in tropical forests and, particularly, epiphytic bromeliads play an important role in forest systems where they serve as small habitats, refuges, water accumulators, nutrient incorporators and food producers for the fauna (Benzing, 2000; Machado and Semir, 2006; Pereira et al., 2022). Of all Bromeliaceae species described for the Atlantic Forest (938), 85.7% are endemic (Flora e Funga do Brasil, 2022). *Vriesea incurvata* Gaudich. is an epiphytic tank-forming bromeliad (Negrelle et al., 2005) which is exposed to irregular collections for ornamental purposes (Negrelle et al., 2005), and so, suffers with habitat lost, as only about 12% of the original Atlantic Forest cover remains (Fundação SOS Mata Atlântica and Instituto Nacional de Pesquisas Espaciais, 2022). Therefore, this species is an important candidate for conservation and restoration studies on the Atlantic Forest. The structural complexity of the rainforest makes it critical to identify the drivers potentially involved in the success of plant survival and development, and to understand their effects on the establishment of each translocated population (Pereira et al., 2022). These drivers may be determined from the conduction of controlled scientific experiments where individuals are translocated and monitored *in situ* (Guerrant Jr. and Kaye, 2007). Though, for epiphytic tank bromeliads, as *V. incurvata*, such projects have not been reported in the literature.

Thus, the objective of this study was to evaluate the survival and development of *V. incurvata* plants propagated *in vitro* and translocated to an At-

Atlantic Forest fragment in South Brazil, analyzing their responses to luminosity, temperature, relative air humidity and precipitation. Considering that the species is abundant in gallery forest (GF) environments (Fischer and Araujo, 1995; Bonnet and Queiroz, 2006; Rocha-Uriarte et al., 2021), the hypothesis of this study was that the establishment of young plants is more effective in GF than in the forest interior (FI). This is the first experimental trial for the establishment of epiphytic tank bromeliads as a strategy for conservation and enrichment of biodiversity in degraded forest environments.

Material and methods

Study area

The study was performed in the state of Rio Grande do Sul (RS), South Brazil (Figure 1A), in the forest area of State Center for Diagnosis and Research on Family Agriculture (Centro Estadual de Diagnóstico e Pesquisa da Agricultura Familiar — CEAFA) (29°39'42.37"S, 50°12'47.60"W). The institution is linked to Department of Diagnosis and Agricultural Research (Departamento de Diagnóstico e Pesquisa Agropecuária — DDPA) of the Secretariat of Agriculture, Livestock, and Rural Development (Secretaria da Agricultura, Pecuária e Desenvolvimento Rural — SEAPDR).

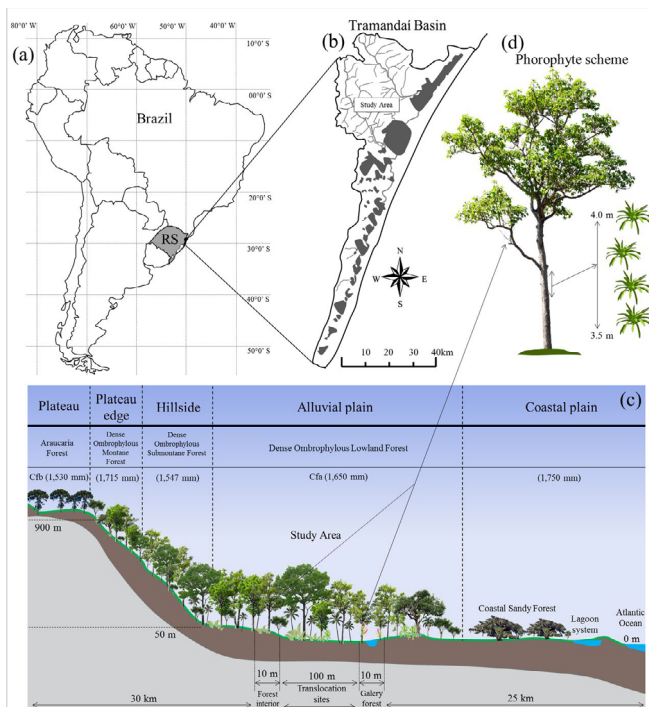


Figure 1 – (A) location of the state of Rio Grande do Sul (RS) in Brazil, (B) location of Rio Tramandaí hydrographic basin [RTHB] in RS and of the study area, (C) schematic representation of the profile of vegetation in the RTHB and the areas of *Vriesea incurvata* translocation [Gallery Forest and Forest Interior] and (D) representation of the trees used for bromeliad fixation*.

*Cfa and Cfb Köppen-Geiger climate classification [Cfa: humid temperate climate with hot summer; Cfb: humid temperate climate with temperate summer]. Values in mm indicate annual mean precipitation.

Source: adapted by the authors from Gerhardt et al. (2000), Comitê Tramandaí (2005), Alvares et al. (2013).

CEAFA is situated in the Tramandaí river hydrographic basin in the municipality of Maquiné (Figure 1B), where the vegetation is composed by Dense Ombrophilous Lowland Forest — from 50 to 400 m above sea level (IBGE, 2012) (Figure 1C) —, a phytophysognomy of Atlantic Forest *sensu stricto* in which *V. incurvata* occurs naturally (Figure 2A). According to Köppen, the climate of the study area is of Cfa type (wet temperate), with no defined dry season and hot summers (Figure 1A) (Alvares et al., 2013).

The study area has 267 ha of native forest out of a total area of 367 ha which is intended for restricted use and restoration of the native forest (about 40 years in regeneration). The forest fragment houses a small natural population of *V. incurvata* (about 15 individuals), as well as other few epiphytic bromeliad species. The recorded occurrence of a population of the species and ease of access, and the proximity between the study area and donor plants for seeds of *V. incurvata* (approximately 10 km straight line distance) were important requirements for the execution of the translocation project.

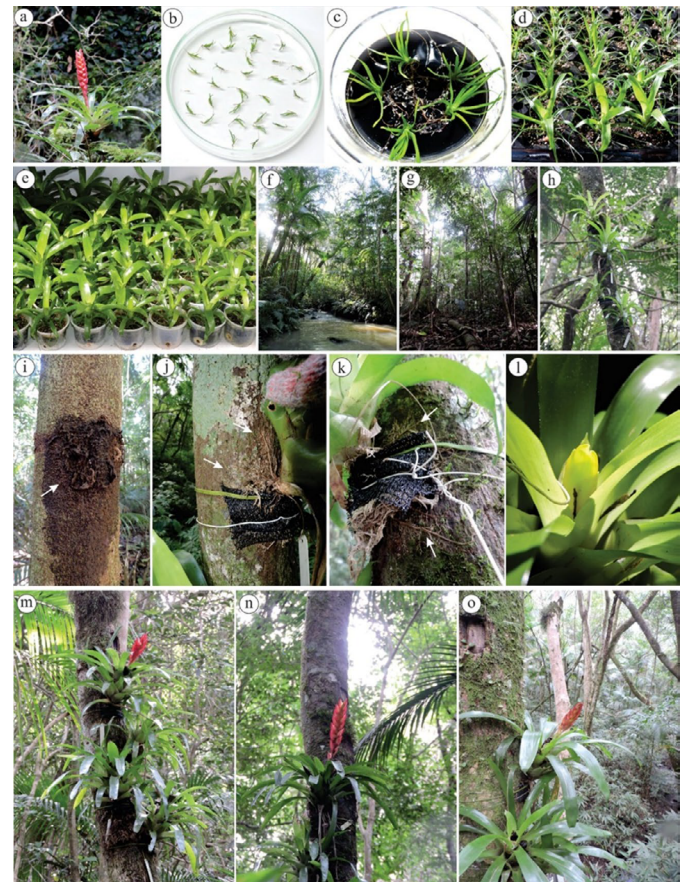


Figure 2 – (A) *Vriesea incurvata* in the natural habitat, (B) germinated seeds (60 days), (C) plants cultivated *in vitro*, (D) acclimatization of *in vitro* propagated plants, (E) acclimatized plants ready for translocation, (F) gallery forest microhabitat; (G) forest interior microhabitat, (H) plants fixed on phorophyte trunk, (I) inadequate phorophyte for fixation of plants; arrow indicates the point where the plants were fixed, (J and K) rooting of plants, (L) inflorescence emission in the 24th month after translocation and (M–O) plant flowering 30 months after translocation.

Source: prepared by the authors.

So, the type of translocation done was the reinforcement, according to IUCN/SSC (2013). One individual was collected for record at *Herbarium Anchietano* of Universidade do Vale do Rio dos Sinos, in the municipality of São Leopoldo (RS), Brazil (voucher: PACA-AGP 117997).

Cultivation of plants

Young plants of *V. incurvata*, with 1.0 ± 0.2 cm in height (Figure 2B) were obtained by *in vitro* germination of seeds collected in a fragment in the municipality of Caraá (RS) ($29^{\circ}42'25.89''\text{S}$, $50^{\circ}17'28.10''\text{W}$), where this species is one of the most abundant epiphytes (Rocha-Urriarte et al., 2015). The plants were cultured in MS medium (Murashige and Skoog, 1962) (Figure 2C) with $\frac{1}{4}$ of the original concentration of macronutrients (Sasamori et al., 2016a) and 60 g L^{-1} of sucrose (Sasamori et al., 2018). Acclimatization (Figure 2D) and growth in a greenhouse (Figure 2E) under natural light and controlled temperature ($26 \pm 1^{\circ}\text{C}$) occurred in commercial substrate (Carolina Soil[®]) composed by peat, vermiculite and carbonized rice husk (Sasamori et al., 2016b). Plants were translocated to the natural environment about 2.5 years after sowing, once they had transitioned from the atmospheric to the tank-forming stage, reaching well-formed tank growth that allowed them to store water and litter in the rosette (Zotz, 2004; Zotz et al., 2004).

Microhabitats and host trees

Two microhabitats were selected: the GF was selected because it is located along the border of a small watercourse (about 3 m wide) (Figure 2C), and FI was selected because it is at least 100 m distant from all nearest borders of the fragment (including the border of GF microhabitat) (Figure 2C). Most trees in both microhabitats were 15 to 20 meters high, with sparse larger trees (20 to 30 m high) whose crowns emerged above the forest canopy. Two 100×10 m long transects were delineated: one along the watercourse of GF, and the other 100 m from the margin of the watercourse representing FI. This allowed the evaluation of microhabitats with distinct biotic and abiotic characteristics (Figure 1). A total of 19 angiosperm trees with a diameter at breast height (DBH) of at least 10 cm were selected inside of both transects. Only phorophytes with persistent and rough bark (Hoeltgebaum et al., 2013), and the obligatory presence of epiphytes were selected since this latter condition indicates the possibility of epiphytic establishment.

Fixation of plants on phorophytes

Prior to translocation, individuals of *V. incurvata* were labeled and their morphological parameters measured:

- length of longest leaf (LLL) — distance (cm) between the base of the rhizome and the apex of the longest leaf;
- number of leaves (NL);
- leaf rosette diameter (LRD; cm).

The bromeliads were divided into two groups (GF and FI; 76 individuals in each group) for which the values of the parameters did not differ statistically (Table 1). In spring 2016, four plants were fixed between 3.5 and 4.0 meters in height (Rogalski, 2002; Bonnet and Queiroz, 2006) to the trunk of each selected tree (Figures 1D and 2H), for a total of 152 translocated individuals. The plants were fixed on the east face of the trunk so that light incidence would be lower in the greatest solar radiation (Endres Júnior et al., 2015b). The plants were fixed to the trunks with their roots protected by sphagnum and a polypropylene mesh (10×10 cm) and flexible plastic-covered ties (Figures 2J and 2K).

Monitoring of plants and abiotic data

The translocated plants were monitored for 30 months beginning 30 days after the fixation to phorophytes. Survival and leaf production were recorded every 30 days. Individuals were considered dead when there was no green tissue in leaves or firm and turgid tissue in the rhizome. The final survival percentage (30 months after translocation) was calculated by the absolute number of living plants in relation to the initial number of living plants on each phorophyte [(number of living plants after 30 months * 100)/initial number of plants]. The morphological parameters LLL, NL, LRD and number of roots fixed to the phorophyte (NRP) were surveyed every 180 days. Only roots with a minimum length of 2 cm fixed to the phorophyte were considered for NRP.

For abiotic characterization of the microhabitats, data on temperature ($^{\circ}\text{C}$), relative air humidity (%) and luminosity (lux) were collected with a portable digital thermo-hygro-anemometer luxmeter (THAL-300 Instrutherm). Measurements were made every 30 days (beginning at 30 days after the fixation of the plants to phorophytes), simultaneously at three equidistant points in each transect (one at each end and one in the middle of the transect of each environment), next to the phorophytes, at 12:00 a.m. and 4:00 p.m. Ten quarterly means were calculated for each environment based on 18 values (3 points; 2 times; 3 months). Precipitation data (mm) were obtained from the meteorological station of CEAFA, which is located beside the administrative headquarters and the area of the forest fragment.

Table 1 – Values (mean $\pm$ standard deviation) for length of the longest leaf, number of leaves and leaf rosette diameter of *Vriesea incurvata* plants prior to translocation to gallery forest and forest interior microhabitats*.

Microhabitat	LLL (cm)	NL	LRD (cm)
GF	16.8 ± 2.8	15.7 ± 2.9	16.7 ± 3.4
FI	16.8 ± 2.7	15.7 ± 2.7	17.0 ± 3.1
t	-0.02	0.00	0.00
p	0.988	1.000	1.000

*Means compared by Student's t-test at 5% probability; LLL: Length of longest leaf; NL: Number of leaves; LRD: Leaf rosette diameter; GF: Gallery forest; FI: Forest interior; t: Student's t-test; p: p-value.

Source: prepared by the authors.

Accumulated internal precipitation (drip or precipitation after rain interception by the tree canopy) was quantified by funnel-shape collectors (10 cm diameter) set at 3.75 m high (average height of the translocated plants) and 10 cm away from the tree trunk. This distance between collector and tree trunk was established to simulate rainwater uptake by the leaf rosette of the bromeliads. A silicone hose (½ inch) connected to each collector canalized the water to a reservoir (6 L), which was attached to the base of the tree. Two collectors were fixed along the transect in each microhabitat. Accumulated internal rainwater collected in the reservoir was measured every 30 days using a graduated beaker, and ten quarterly means were calculated for each environment. Data were recorded in milliliters (mL) and transformed into millimeters (mm) using the formula: $P = V/A$, in which “P” is precipitation (in mm), “V” is the volume of water in the beaker (in L) and “A” is the area of the collector (in m²).

Statistical analyses

Data were submitted to the Shapiro-Wilk normality test. Final survival data (%), after 30 months, were compared between microhabitats using the Mann-Whitney U test. The means of LLL, NL and LRD, and the data for luminosity, relative air humidity, temperature and precipitation were compared between microhabitats using Student's t-test. Data for NRP and frequency of rooted individuals (Freq.RI) were compared between microhabitats through the Mann-Whitney U test. Means of LLL, NL, and LRD were compared over time for each microhabitat by analysis of variance (ANOVA of repeated measures), followed by the Bonferroni test, while means of NRP were compared using the Friedman test. Multiple regression analysis (stepwise method) was applied to assess the relationship between plant survival and biotic parameters and abiotic variables (luminosity, relative air humidity, temperature and precipitation). Statistical analyses were performed using SPSS version 25. The probability was fixed at 5% for all analyses.

Results

Monitoring of translocated plants

The survival of *V. incurvata* plants at 30 months post-translocation was higher in GF (98.7%) than in FI (84.2%) ($U = 49.0$; $p < 0.001$). The loss of one individual in GF was directly related to a tree trunk falling upon it. Two phorophytes in FI (*Ilex brevicuspis* Reissek and *Inga marginata* Willd.) were found to be unsuitable for plant fixation because sap was released after stem perforation (Figure 2I), which resulted in the death of all individuals fixed to them, representing 67% of all dead plants for this microhabitat.

An increase in LLL was observed during the monitoring for plants in both habitats (GF: $F = 454.70$; $p < 0.001$; FI: $F = 106.95$; $p < 0.001$; Figure 3A). However, LLL was greater in GF plants, which showed a 73.4% increase after 900 days post-translocation, while FI plants showed a 50% increase. Because of the higher growth rate, LLL averages for GF plants was greater than those for FI bromeliads at each 180-day

interval of monitoring, which resulted in plants with LLL averages of 28.5 cm (GF) and 24.8 cm (FI).

LRD increased throughout monitoring in both microhabitats, being GF ($F = 305.25$; $p < 0.001$) and FI ($F = 45.64$; $p < 0.001$) (Figure 3B). In addition, LRD for the bromeliads translocated to GF was significantly greater than for those translocated to FI beginning in the first 180 days (Figure 3B; Table 2). The fixed plants experienced an 85.2% (GF) and 43.7% (FI) increase in LRD after 900 days translocation which resulted in plants with average values of 30.5 cm (GF) and 24.2 cm (FI).

The translocated bromeliads produced an average of 1.0 leaf per month. After fixation on phorophytes there was a 14% reduction in the NL of bromeliads translocated to FI, which resulted in a difference in the average between the two microhabitats from the first through the fifth monitoring period (Figure 3C; Table 2). Afterwards, the NL gradually increased, returning to the initial in 900 days (16.6; FI: $F = 4.84$; $p = 0.008$). In GF, the plant NL increased significantly each 180 days from the second year onwards, up to 22.3 (GF: $F = 50.67$; $p < 0.001$; Figure 3C).

The number of individuals with roots fixed to the phorophyte did not differ between GF and FI ($U = 114.00$; $p = 0.716$) after 900 days, with a total of 100 and 97 respectively. Higher rooting speed was observed on plants of GF ($U = 63.50$; $p = 0.021$), since 82.9% of the plants already had roots fixed to the phorophyte one year after translocation (Figures 2J and 2K), while only 44.9% of the individuals in FI fixed their roots to the trees (Figure 3D). The number of fixed roots per plant was also higher for GF bromeliads between 180 and 900 days after translocation (Table 2). Root growth per plant was progressive throughout monitoring in the GF with an average of 11.2 roots fixed after 900 days of monitoring ($Fr = 223.67$; $p < 0.001$; Figure 3D). The increase in the number of roots per plant for FI was less and gradual during monitoring, with each plant having 4.5 roots after 900 days of translocation ($Fr = 70.79$; $p < 0.001$).

Plants fixed in GF began inflorescence emission in the 24th month after translocation (October 2018; Figure 2L), with 10.5% of the individuals experiencing inflorescence emission in 900 days. In addition, the presence of still green fruits with immature seeds was observed, indicating the presence of pollinators in the forest fragment (Figures 2M-2O).

Abiotic characteristics of the translocation environment

Luminosity varied throughout each year in both microhabitats, with the highest averages being recorded in the first and last quarters of each year (November–January and August–October). Luminosity in GF ranged from 295 to 865 lux and was generally higher than in FI ($t = 8.47$; $p < 0.001$), as well as in all quarters of monitoring (Figure 4A; Table 3). Luminosity ranged from 157 to 393 lux for FI, which was about 50% lower than in GF. Relative air humidity also varied throughout each year of monitoring, with values ranging from 71 to 86% in GF and from 67 to 85% in FI ($t = 3.89$; $p < 0.001$). There was a difference when comparing the relative air humidity in each quarter, which was higher in GF in the third and fourth quarters of each year of monitoring (Figure 4B; Table 3).

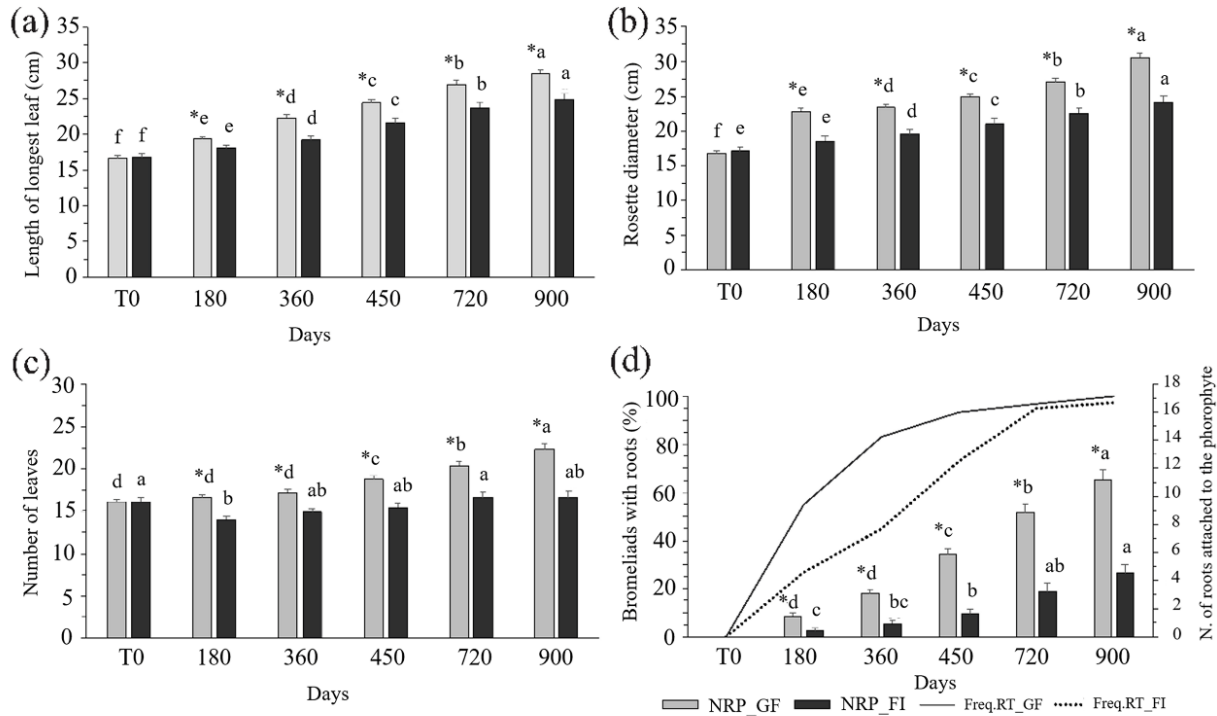


Figure 3 – Semiannual values [mean $\pm$ standard error] of morphological parameters of *Vriesea incurvata* translocated to the gallery forest [GF; light gray bars] and the forest interior [FI; black bars]: (A) length of longest leaf, (B) leaf rosette diameter, (C) number of leaves and (D) frequency of plants with roots fixed to the phorophyte [rows: Freq_RT_GF and Freq_RT_FI] and semiannual values of number of roots fixed in the phorophyte per individual [bars: NRP_GF and NRP_FI]*.

*T0 means the measurement of plant morphological parameters before translocation; *significant differences between environments (GF and FI) and by survey, according to the Student's t-test (Figures 3A–3C) and the Mann-Whitney U test (Figure 3D), at 5% probability (Table 2). Different letters indicate significant differences over time, according to the Bonferroni test (Figures 3A–3C), and the Friedman test (Figure 3D), at 5% probability (see Table 2).

Source: prepared by the authors.

Table 2 – Statistical results of the comparison of morphological parameters between gallery forest and forest interior microhabitats during the 30 months of monitoring translocated *Vriesea incurvata* plants.

Days	LLL		LRD		NL		NRP	
	t ¹	p	t	p	t	p	U ²	p
T0 ³	-0.136	0.892	-0.540	0.591	-0.424	0.673	-	-
180	2.213	0.029	5.649	< 0.001	5.509	< 0.001	528.0	0.007
360	3.962	< 0.001	4.711	< 0.001	3.618	< 0.001	369.0	< 0.001
540	3.346	0.001	4.596	< 0.001	4.500	< 0.001	224.0	< 0.001
720	3.568	0.001	5.009	< 0.001	4.324	< 0.001	235.5	< 0.001
900	3.782	< 0.001	5.156	< 0.001	4.715	< 0.001	237.0	< 0.001

LLL: Length of longest leaf; LRD: Leaf rosette diameter; NL: Number of leaves; NRP: Number of roots fixed to the phorophyte; t: Student's t-test; p: p-value; U: Mann-Whitney U test; ¹Student's t-test at 5% probability; ²Mann-Whitney U test at 5% probability; ³T0: measurement of plants before translocation; Significant values are in bold.

Source: prepared by the authors.

Temperature varied seasonally during monitoring in both microhabitats. The highest average temperatures in both microhabitats were recorded between November and January of each year (28 to 26°C), while the lowest were between May and July (20 to 16°C). Average temperatures did not differ between the two microhabitats ($t = -0.15$; $p = 0.883$), nor did the average temperatures for each quarter (Figure 4C; Table 3).

Rainfall for the first and second year of monitoring was of 1,585.3 and 1,449.2 mm, respectively. The recorded cumulative rainfall was 750.6 mm in the first six months of the third year. Rain was distributed throughout the months and did not present a defined dry season, with the highest accumulated volume being in May 2017 (297.8 mm) and the lowest in June 2017 (4.5 mm). Internal precipitation did not differ between the two microhabitats ($t = 1.03$; $p = 0.307$; Figure 4D).

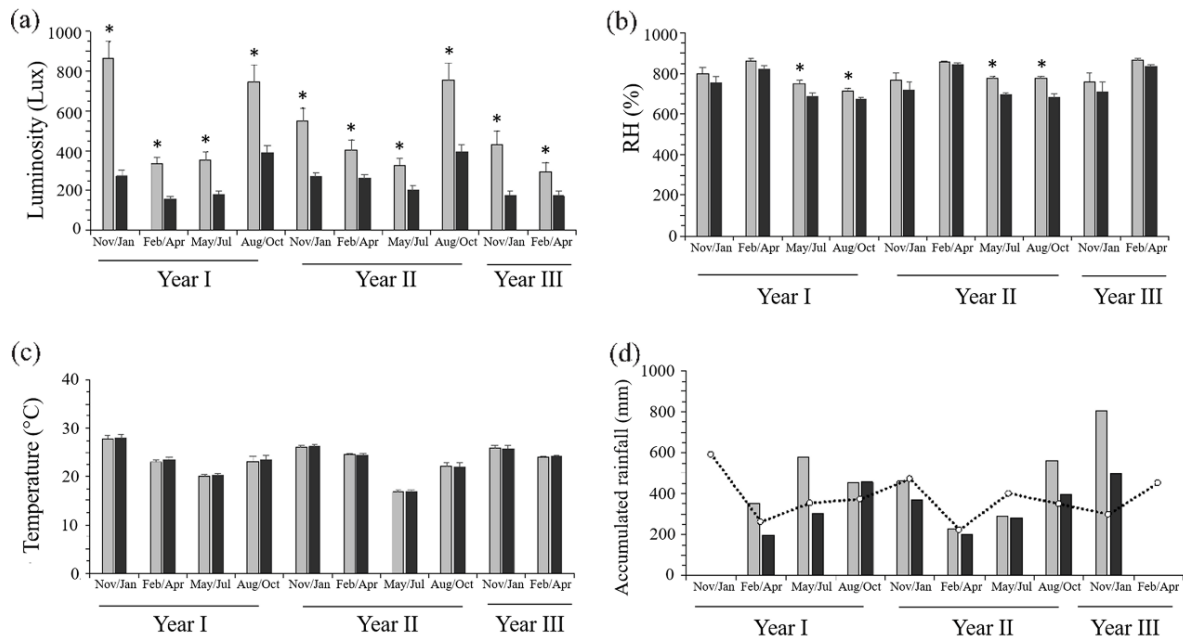


Figure 4 – Quarterly values [mean $\pm$ standard error] of abiotic factors for the gallery forest (gray bar) and forest interior (black bar) microhabitats during 30 months of monitoring translocated plants of *Vriesea incurvata*: (A) luminosity, (B) relative air humidity (RH), (C) temperature, and (D) internal accumulated precipitation (bars) and accumulated precipitation from the study area (dotted line).

* $p < 0.05$; ** $p < 0.001$ indicate significant differences according to the Student's t-test at 5% probability (see Table 3).

Source: prepared by the authors.

Table 3 – Statistical results of the comparison of abiotic factors between gallery forest and forest interior microhabitats during the 30 months of monitoring translocated *Vriesea incurvata* plants.

	Luminosity		Relative humidity		Temperature	
	t ²	p	t	p	t	p
Nov. – Jan. ¹	6.560	< 0.001	1.057	0.306	-0.143	0.888
Feb. – Apr.	5.490	< 0.001	2.139	0.050	-0.903	0.380
May. – July	3.702	0.003	2.453	0.026	-0.232	0.820
Aug. – Oct.	3.725	0.003	2.788	0.013	-0.364	0.721
Nov. – Jan.	4.318	0.002	0.933	0.365	-0.197	0.846
Feb. – Apr.	2.657	0.024	1.312	0.208	0.718	0.486
May. – July	2.921	0.011	7.777	< 0.001	0.099	0.922
Aug. – Oct.	3.767	0.002	5.304	< 0.001	0.209	0.837
Nov. – Jan.	3.737	0.004	0.783	0.445	0.028	0.978
Feb. – Apr.	2.354	0.032	2.318	0.034	-1.032	0.323

Significant values are in bold; t: Student's t-test; p: p-value; ¹Quarterly mean; ²Student's t-test at 5% probability.

Source: prepared by the authors.

GF had an average of 155.6 mm every 30 days (± 3 days), with the highest (490.4 mm) and lowest (1.4 mm) accumulated volumes also recorded in May and June 2017. Accumulated internal rainfall in FI averaged 112.3 mm every 30 days (± 3 days), with the highest and lowest accumulated volumes being recorded in January 2019 (302.5 mm) and June 2017 (1.5 mm).

Relationship between plant development and abiotic factors

Multiple linear regression showed that luminosity was an important attribute for survival and development of translocated plants. The model showed that survival was predicted by a combination of two abiotic variables (Table 4), luminosity accounting for 17.6% of the coefficient of determination, followed by relative air humidity, which added 11.5%. The rate of increase in LRD was predicted by the combination of three abiotic variables (Table 4), with luminosity again being responsible for the largest contribution to the coefficient of determination (24.3%), while relative air humidity and temperature contributed 5.2 and 4.7%, respectively. Growth rate for length of longest leaf (Rt.LLL) and growth rate for number of leaves (Rt.NL) were predicted only by luminosity, which contributed 18.4 and 11% to the coefficient of determination, respectively, of these two parameters. The NRP was predicted by relative air humidity (Table 4) which accounted for only 6.8% of the coefficient of determination. Internal precipitation was not related to survival or any of the measured morphological parameters.

Discussion

Vriesea incurvata plants had high survival rates and significant growth in both forest microhabitats studied. The parameters indicated the translocated plants were able to adjust to the habitats, which is essential and directly influences the success of translocation for reinforcement (Maschinski and Albrecht, 2017).

Table 4 – Results of multiple linear regression analysis relating the parameters of translocated plants of *Vriesea incurvata* with abiotic factors of gallery forest and forest interior microhabitats during 30 months monitoring.

Parameter	R adj	F	p	Predictor variable	B	t	p
Survival	0.291	13.123	< 0.001	Luminosity	0.457	4.164	< 0.001
				Relative humidity	0.355	3.234	0.002
Rt.LLL	0.184	14.279	< 0.001	Luminosity	0.006	3.779	< 0.001
Rt.LRD	0.342	11.202	< 0.001	Luminosity	0.481	4.476	< 0.001
				Relative humidity	0.249	2.354	0.022
				Temperature	0.240	2.243	0.029
Rt.NL	0.110	8.278	0.006	Luminosity	0.353	2.877	0.006
NRP	0.068	5.292	0.025	Relative humidity	0.289	2.300	0.025

R adj: Adjusted regression; F: Friedman test; p: p-value; β : regression; t: Student's t-test; Rt.LLL: Growth rate of length of longest leaf; Rt.LRD: Growth rate of leaf rosette diameter; Rt.NL: Growth rate of number of leaves; NRP: number of roots fixed to phorophyte; Significant values are in bold.

Source: prepared by the authors.

The fact that the plants were already in the tank stage at the time of translocation (due to the longer period of growth in the greenhouse) may have contributed to their positive performance. Tank-shaped morphological characteristics allow the accumulation of water, organic matter, and animal waste (Benzing, 2000), and thus, contribute to higher nutrient availability and photosynthetic capacity (Zotz et al., 2004). Therefore, tank epiphytic bromeliads with an already developed receptacle are most suitable for translocation because such structures allow individuals to withstand the stresses of the forest system.

The survival rates obtained here were similar to those reported for the rupicolous bromeliad *Dyckia distachya* Hassl. (20-80%) propagated in substrate through seeds and translocated to river environments in South Brazil (Zimmermann, 2011), and for adult plants of 14 species of bromeliads, three from *Vriesea* Lindl. (48-98%) translocated from an area that was flooded to a preservation area (Jasper et al., 2005). It is important to point that the studies used plants at distinct ages and proposed different conservation strategies. Plants obtained by *in vitro* propagation of the epiphytic orchid *Cattleya intermedia* Graham had a survival rate of about 60%, 24 months after translocation, and showed significant growth of aerial and radicular structures (Endres Júnior et al., 2015b), which was corroborated by the results of the present study.

Ilex brevicuspis Reissek (Aquifoliaceae) and *Inga marginata* Willd. (Fabaceae) were detrimental to the root system of translocated bromeliads due to sap and wood dust released after trunk perforation, presumably due to an insect which was not observed, thus precluding its identification. This contributed to the higher mortality recorded in FI compared to GF. Although this study did not aim to evaluate the influence of phorophytes on the establishment of *V. incurvata*, the observations made indicated that the characteristics of the trunks should be considered in the establishment of translocated bromeli-

ads. Hoeltgebaum et al. (2013) found that the frequency of epiphytic bromeliads in different tree species varied according to the successional stages of the habitats (Santa Catarina State, Southern Brazil), since they had persistent bark with wrinkles and fissures, which gave them roughness and greater capacity to retain water and nutrients (Benzing, 1995; Kersten, 2010).

The aerial system of the plants increased significantly in both microhabitats, except for the NL of FI plants. The higher luminosity in GF resulted in bromeliads with greater LLL, LRD and more leaves than FI plants, resulting from increased energy production through photosynthesis (Taiz et al., 2015). The most basal leaves of the rosette originated during the acclimatization and growth phase in controlled laboratory conditions necrosed during the first months after translocation, being progressively replaced by new leaves produced in the natural habitat. The stress caused by translocation stimulates the internal flow of nutrient elements to younger leaves (Caldeira et al., 1999) contributing to the death of the oldest leaves of the rosettes. In GF, where more light is available, the production of carbohydrates in plants to maintain metabolism and promote growth (Taiz et al., 2015) is increased in comparison to FI plants. The greater production of new leaves compensated for the loss of old leaves in GF. On the other hand, new leaves were produced more slowly in FI, evidencing the initial decrease in NL.

The protection of the root system by sphagnum and polypropylene mesh, and the use of flexible plastic-covered ties for bromeliad fixation kept individuals well fixed to the phorophytes and avoided their movement and damage to roots. Durability of the fixation material should be taken into consideration in translocation projects of epiphytes (Jasper et al., 2005) due to their low rooting speed. The ties used in the present study could be removed from some plants with the highest root numbers 2.5 years after translocation. The fact that the GF plants had 2.5 times more roots attached to the phorophyte

than did plants of FI may have been due to greater energy input from leaves. The rapid development of the root system is very important because roots are responsible for fixing and supporting the translocated plants (Benzing, 2000) besides promoting absorption of nutrients through velamen (protective structure) and absorbent root hairs (Proença and Sajo, 2008).

The restricted contribution of relative air humidity to plant survival, the growth rate of leaf rosette diameter (Rt.LRD) and the NRP can be explained by its slight variation during monitoring. The forest fragment is inserted within a Dense Ombrophilous Forest phytoecological region where the relative air humidity is constantly high and the volume of rain is well distributed throughout the seasons, as evidenced by this study. The appropriate habitat selection was important for the survival of the plants because the high precipitation of the site ensured the necessary water support for the establishment of the plants — even though the precipitation was lower than that of the seed collection area for propagation [which is close to 2,400- and 2,700-mm year⁻¹, according to the study of Rocha-Uriarte et al. (2015)]. In regions where dry and rainy seasons are well defined, translocation of individuals should be done at the beginning of the period of highest rainfall (Toledo-Aceves and Wolf, 2008), to increase the chances of plants surviving.

The greater relative air humidity in GF, especially in the third and fourth quarters of each year, is related to the presence of the watercourse, which can minimize the variation of air and soil moisture in the area (Pinto et al., 2005; Athayde et al., 2009). For epiphytes, the access to water resources occurs via precipitation or atmospheric uptake, and plant evapotranspiration increases with low levels of relative air humidity, which limits survival during periods of water deficiency (Zotz et al., 2004). Though *V. incurvata* occurs both in FI and GF, the absolute number of individuals (Fischer and Araujo, 1995) and the importance value (Bonnet and Queiroz, 2006; Rocha-Uriarte et al., 2021) of the species are higher in the latter environment, suggesting that it is conducive to the establishment of the plant. The greater diversity and abundance of bromeliads near watercourses are attributed to the high humidity of this environment (Fischer and Araujo, 1995) which, together with the availability of light, is an important factor responsible for the distribution of these plants (Pittendrigh, 1948; Benzing, 1990). Thus, epiphytic plants translocation should respect their geographic distribution and characteristics on a microhabitat scale (e.g., GF) and on a regional scale (e.g., geographic distance).

We established 139 new individuals of *V. incurvata* in the forest fragment of the study, which represents more than 800% increase of the initial population of this species in the area (that was 15 plants). This will contribute to improving environmental quality and faster regeneration of the area by increasing water availability to the ecosystem by tank formation (Negrelle e Muraro, 2006), and by provid-

ing refuges, microhabitats and nutrients for detritivorous, predatory and herbivorous arthropods (Stuntz et al., 2002; Winkler et al., 2005). Besides, it will increase the availability of food for avifauna, since *V. incurvata* is an important producer of nectar used by hummingbirds (Machado and Semir, 2006). As we observed fruit on the translocated plants, the relationship with pollinators is probably already being established.

Studies with a conservation focus, which offer concrete data that assist the understanding of the requirements for the successful establishment of translocated endemic species, contribute to the fulfillment of the goals established in the 2030 Agenda for Sustainable Development Goals (UN, 2022). Goal 15 of this agenda aims to “protect, restore, and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, halt and reverse land degradation, and halt biodiversity loss”. Among the aims of Goal 15, we highlight “restoring degraded forests and substantially increasing afforestation and reforestation globally”. The agenda also mentions the importance to improve human, technical and professional skills, as well as expertise and capabilities to effectively formulate and implement policies, plans, programs, research and projects on management, conservation and sustainable development of all types of forests and forest-based resources, and forest lands inclusive, besides other areas from which forest benefits can be derived. Investments in forest restoration are essential to address climate change and the depletion of biological diversity, in addition to strengthen environmental health, livelihoods, and the economy (UN, 2022).

The development of technologies for plant conservation meets the new demands of society. The application of these tools in the execution of conservation and restoration projects triggers a productive network that starts in species selection and obtention of biological material (seeds and seedlings), propagation of plants, and culminates in forest planters, providing employment and income for rural workers and the development of green enterprises (Adams et al., 2016; Nunes et al., 2020) assisted by a team of conservation specialists. Such enterprises are paramount agents of socio-environmental change, promoting environmental education and social inclusion.

Conclusion

The study revealed that the establishment of young individuals of *V. incurvata* is more successful in the GF than in the FI, confirming the stated hypothesis. The method applied allowed us to determine that the translocated plants responded to the environmental factors studied, mainly relative humidity and luminosity, especially in the GF environment. Since abiotic factors accounted for about 30% of the variation in the biological data, other environmental variables are also contributing to plant survival and development. Thus, it is challenging to expand the analyses to understand the influence of biotic factors typical of the translocation habitat for the establishment

of young plants of *V. incurvata* and other bromeliads with similar functional characteristics. Further investigation will help explain the frequencies of these plants in nature, focusing on the outline of conservation proposals for species that provide important services to ecosystems, thus accelerating their restoration. This work set de basis for projects on conservation of tank bromeliads and restoration of forest environments associated with them.

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