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Responses of Translocated *Cattleya intermedia* (Orchidaceae) to Environmental Key Features in a Vertical Gradient of Subtropical Forest, Brazil

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ABSTRACT

This study analyzed the survival and development of translocated *Cattleya intermedia* plants, aiming to understand why this threatened epiphyte is recorded growing mainly in the outermost parts of phorophytes and which are the main environmental factors related to its development, to know how to achieve conservation and restoration purposes. Plants propagated *in vitro* were translocated to a forest fragment (70 per phorophyte stratum: trunk and crown) in South Brazil and monitored for three years. The data indicated the ability of plants to grow along the vertical gradient of phorophytes. The orchids showed variations in the concentrations of photosynthetic pigments to adjust to environmental conditions, with higher concentrations in the crown and spring. Water content and nutrient concentration of translocated plants were similar to wild individuals. In the crown, plants were less affected by herbivory and some of them flowered three years after translocation. Plant survival and morphological and physiological aspects (growth, flower production and regulation of photosynthetic pigments), just as aspects of the trunk (inclination and circumference) and of the environment (light) indicated that *C. intermedia* has preference for the crown, being this the recommended stratum for its translocation aiming at conservation and environmental restoration.

Keywords: epiphyte, micropropagation, orchid, reinforcement, restoration.

Respostas de *Cattleya intermedia* Translocada (Orchidaceae) a Características-chave Ambientais em Gradiente Vertical de Floresta Subtropical, Brasil

RESUMO

Este estudo analisou a sobrevivência e o desenvolvimento de plantas de *Cattleya intermedia* translocadas, visando a compreender por que esse epífita ameaçado é registrado crescendo predominantemente nas partes mais externas dos forófitos e quais são os principais fatores ambientais relacionados ao seu desenvolvimento, de forma a saber como alcançar os propósitos de conservação e restauração. Plantas propagadas *in vitro* foram translocadas para um fragmento florestal (70 por estrato forofítico: fuste e copa) no sul do Brasil e monitoradas por três anos. Os dados indicaram a habilidade das plantas de crescer ao longo do gradiente vertical dos forófitos. As orquídeas mostraram variações nas concentrações dos pigmentos fotossintéticos para se ajustarem às condições ambientais, com maiores concentrações na copa e na primavera. O conteúdo de água e a concentração de nutrientes das plantas translocadas foram similares aos dos indivíduos selvagens.

Na copa, as plantas foram menos afetadas por herbivoria e algumas delas floresceram três anos após a translocção. A sobrevivência e os aspectos fisiológicos das plantas (crescimento, produção de flores e regulação dos pigmentos fotossintéticos), assim como aspectos do fuste (inclinação e circunferência) e do ambiente (luz) indicaram que *C. intermedia* tem preferência pela copa, sendo este o estrato recomendado para sua translocção, visando à conservação e restauração ambiental.

Palavras-chave: epífita, micropropagação, orquídea, reforço, restauração.

Introduction

Translocation of threatened species into wild is an important tool for biodiversity conservation (Armstrong and Seddon, 2008) and the re-establishment of individuals *in situ* has been described as an efficient way to improve native populations in number and diversity (Guerrant and Kaye, 2007; IUCN/SSC, 2013). The success definition in rare-plant translocation can be distinguished by biological purposes and project purposes. Biological purposes involve the establishment of new populations or the augmentation of existing populations and the project purposes evaluate means of achieving the biological ends that are pursued (Pavlik, 1996). Biological and project purposes may be achieved by the conduction of translocation projects as scientific experiments, designed to test explicit hypotheses about how to practice plant establishment (Falk et al., 1996). So, methods and protocols have to be developed improving project purposes (Guerrant and Kaye, 2007).

Orchidaceae is one of the largest families of flowering plants, and Brazil is the third most diverse country with regard to its species with 2,553 native representatives distributed among 238 genera, many of which exhibiting a high degree of endemism (64.1% of total species) (Barros et al., 2015). These plants can assume different sizes and shapes and have various ecophysiological adaptations, growing in a wide variety of habitats (see Arditti, 1967). The biology of orchids is complex, with each species possessing preferences for certain environmental factors (Adhikari et al., 2012). They can be strongly affected by anthropic disturbances (Parthibhan et al., 2015) and are threatened by a number of factors, such as habitat destruction; illegal collection and trade for ornamental, medicinal and food purposes (Benzing, 2004; Gale et al., 2018) and intensification of climate change, soil erosion and drought (Pereira et al., 2010). Environmental impacts on mycorrhizal fungi and pollinators, in addition to the specific relationships established by epiphytic species with phorophytes, constitute new challenges to orchid conservation (Reiter et al., 2016, 2017; Fay, 2018). These factors may reduce and isolate populations to a level at which they are incapable of reproducing and persisting for the long-term, and therefore influence the possibility of sustaining orchids *in situ*. Furthermore, as is discussed by Gale et al. (2018), in many cases, the status of the species may be worse than the population estimates indicate.

Micropropagation provides plants for the purpose of re-establishing orchids *in situ*, for both

reintroduction and reinforcement of natural populations and several studies can already be found in the literature providing initial data regarding the establishment of the individuals (Rubluo et al., 1993; Seeni and Latha, 2000; Decruse et al., 2003; Zeng et al., 2011; Wu et al., 2014; Soares et al., 2020). However, although orchids are linked to many factors in nature (Adhikari et al., 2012), and epiphytic species have long reproductive cycles (Larson, 1992; Zotz, 1995, 1998; Schmidt and Zotz, 2002), individual studies have emphasized only a small portion of an orchid ontogeny, evaluating one or a few aspects of re-establishment processes, with plant monitoring occurring sparsely for just a few months (Seeni and Latha, 2000; Dixon and Phillips, 2007; Dorneles and Trevelin, 2011; Wu et al., 2014; Parthibhan et al., 2015; Segovia-Rivas et al., 2018). Therefore, knowledge gaps in the processes that involve long-term maintenance of organisms and their relationships with biotic and abiotic factors of habitats hinder management aimed at survival of individuals and population establishment (Armstrong and Seddon, 2008; Ren et al., 2012).

The epiphytic orchid *Cattleya intermedia* Graham is endemic to South and Southeast Brazil (van den Berg, 2023). In the state of Rio Grande do Sul (RS), the species occurs in areas of Atlantic Forest and Brazilian Pampa phytogeographic domains and in the Pampa *sensu stricto* (Buzatto et al., 2010; Atlas Socioeconômico do Rio Grande do Sul, 2021), which are considered the two most threatened biomes of Brazil (Atlas Socioeconômico do Rio Grande do Sul, 2021). The species has high ornamental value due to the beauty of its flowers, which has led to intense collection for commercial cultivation since the 1940s (CNCFlora, 2023). Recent data indicate that populations of *C. intermedia* occur mainly in rural environments, but that they can also be found growing on trees in urban areas, some being protected in conservation units classified as municipal parks (Endres Júnior et al., 2022). Due to the significant direct and indirect anthropogenic impacts to its natural populations, *C. intermedia* is classified as Vulnerable by Lista Oficial da Flora Nativa Ameaçada de Extinção no Estado do Rio Grande do Sul (The Official List of Endangered and Threatened Native Flora of the State of Rio Grande do Sul) and the red book of Brazilian flora (Menini Neto et al., 2013; Rio Grande do Sul, 2014).

Searching for the key variables to design conservation projects of *C. intermedia*, our research group studied the appropriate concentrations of macronutrients and sucrose for *in vitro* propagation (Sasamori et al., 2015),

beneficial substrates for acclimatization (Sasamori et al., 2014). Translocation experiments with periodic, systematic and rigorously frequent evaluations contributed to first identification of factors related to survival and the first stage (Reiter et al., 2016) of *C. intermedia* development after translocation at the edge and in the interior of a forest fragment (Endres Júnior et al., 2015a, 2015b, 2018, 2022).

Considering that the occurrence and development of epiphytic species can be influenced by biotic and abiotic conditions that vary along the vertical gradient (Kersten et al., 2009), and that *C. intermedia* is observed in more abundance in the outermost parts of phorophytes (Gonçalves and Waechter, 2002; Endres Júnior et al., 2019), the aim of the present study was to evaluate and compare survival, development and physiological aspects (growth, reproduction, nutrition, photosynthetic pigment concentrations) of *C. intermedia* plants attached to host trunk and crown, and to analyze their responses to luminosity, temperature, air humidity, stem circumference and inclination, and herbivory. The data obtained in this study will promote the achievement of biological and project purposes of threatened species translocation, so that public policies can be set to improve environmental health and human well-being, considering the goals set by the UN Agenda 2030 (UN, 2023), which include protecting, restoring, and promoting the sustainable use of terrestrial ecosystems, sustainable management of forests, combating desertification, halting and reversing soil degradation, and halting biodiversity loss.

Material and methods

Habitat

The study was conducted in Área de Relevante Interesse Ecológico Henrique Luís Roessler (Area of Relevant Ecological Interest Henrique Luís Roessler - AREIHLR) (29°41'S, 51°06'W, alt. 16.4 m), a municipal conservation unit located in subtropical Brazil, where the climate is humid mesothermal with no dry season and categorized as Cfa according to Köppen's classification (Alvares et al., 2013). The 54.4-ha forest fragment is inserted in an area of transition between the Atlantic Forest and Pampa phytogeographic domains (IBGE, 2012). Accumulated annual rainfall in the region is approx. 1,650 mm and the annual average temperature is 19.5 °C (Bauer et al., 2012).

Phorophyte selection

Trees with diameter at breast height of at least 10 cm and trunk height > 4 m were chosen from among the natural hosts of epiphytes. Two distinct zones were determined for each tree: upper trunk (portion between the base of the tree and the point where the first branches originate) and inner crown (median portion between the base of the primary branches and outer crown) (Johansson, 1974; Gonçalves and Waechter, 2002). The trees were identified as *Myrcia brasiliensis* Kiaersk. (nine individuals), *Myrcia glabra* (O. Berg) D. Legrand (three individuals) (Myrtaceae), and *Myrsine coriacea* (Sw.) R. Br. Ex Roem. & Schult. (two individuals) (Primulaceae) according to Angiosperm Phylogeny Group (APG IV, 2016). Detailed information on the 14 selected phorophytes and on the epiphytic flora of the area is provided by Endres Júnior et al. (2019).

Species and biological material

In AREIHLR, individuals of *C. intermedia* can be found growing attached to the crown stems and to the trunk of phorophytes. The wild population was small, with only seven adult individuals that were tagged (Figure 1a), and with a few seedlings developing on the roots of the adult plants or nearby (Figure 1b). So, the type of translocation done was reinforcement, according to IUCN/SSC (2013).

Individuals used in the present study were propagated by asymbiotic *in vitro* sowing and grown in MS medium (Murashige and Skoog, 1962) until they reached a shoot length of approx. 5 cm and eight leaves per plant (Sasamori et al., 2015). After acclimatization in substrate for five months (Figure 1c) (Sasamori et al., 2014), each plant was tied to a piece of pine bark (5 x 10 cm), tagged and cultivated until rooting on the bark and producing new shoots with pseudobulbs (Figure 1d) (Endres Júnior et al., 2015a). The individuals selected for translocation were measured for: shoot height (SH - measured in cm from the base of the rhizome to the tip of the largest leaf), number of leaves (NL), and number of pseudobulbs (NP - minimum of 1.5 cm in length). The orchids were divided into two groups (trunk and crown, 70 individuals in each group) for which the values of the morphometric parameters did not differ significantly, according to the Student t-test for independent samples, at 5% significance (SH: $t=1.490$, $P=0.139$; NL: $t=1.262$, $P=0.209$; NP: $t=0.652$, $P=0.516$).



Figure 1. Wild and translocated individuals of *Cattleya intermedia*. (a) Mature plant naturally occurring in AREIHLR; (b) Wild seedling growing on a trunk near adult individuals; (c) Plants obtained by *in vitro* culture and acclimatized in trays with a mixed substrate; (d) Vigorous individuals grown attached to pieces of pine bark; (e) Plant in a crown with leaves that developed after translocation (lat) and growing new roots (gr); (f) Individuals after three years of development attached to a tree crown, with a floral bud (fb) growing without the protection of a floral bract – spathe (s); (g) Floral buds growing through the floral bract; (h) Orchids damaged by *Tenthecoris bicolor* (insect nymphs indicated by arrow) and (i) larvae of *Ithomiola nepos* (insect pupae indicated by arrow). Scale bars = 2.5 cm.

Translocation and plant monitoring

Each phorophyte received five individuals of *C. intermedia* tied to the trunk (at a height of 3.0 to 3.5 m) and five individuals tied to branches in the crown (at a height of 6.5 to 7.0 m). Translocation took place in October 2013 (spring), and plant survival and development were monitored every three months until September 2016 (Endres Júnior et al., 2015a).

The plants were inspected during each field campaign to record SH, NL, NP (as prior to

translocation), number of roots attached to phorophyte bark (NR - minimum 2.0 cm in length), and damage caused by herbivores, as described by Endres Júnior et al. (2018). During the 36 months of the study, each orchid was classified as: 1 - plant on trunk damaged by herbivory; 2 - plant on trunk undamaged by herbivory; 3 - plant in crown damaged by herbivory; and 4 - plant in crown undamaged by herbivory. Means were calculated for SH, NL,

NP, and NR for the plants of each category that survived until September 2016.

Water loss and nutrient concentration

To evaluate water loss and nutrient concentration of translocated individuals of *C. intermedia*, healthy shoots were selected from eight immature plants attached to the trunk and eight in the crown. For subsequent comparisons, shoots were selected from seven adult plants of a wild population at AREIHLR during the same season of the same year, and the methodology of the experiments was the same as for the reintroduced plants. Sampled shoots were always located in the same position of the plant (third-youngest shoot). The samples were excised from the rhizome with a sterile scalpel in September 2016 and were immediately transported to the laboratory where they remained in sealed plastic bags for rehydration overnight with distilled water.

Fresh weight of each shoot at saturation (FWs) was obtained with a digital balance. The cuts were sealed with parafilm and kept exposed vertically in flasks under natural light in the

laboratory (Figure 2a). Length and width were measured at the central point of each leaf. Pseudobulb length was measured (cm) from the cut to the insertion point of leaves, and width was measured at three points equally distributed throughout the length (Figure 2b). To determine rates of water loss, fresh weights (FW) were obtained periodically (every 48-72 h), and at the end of monitoring (28 d) all samples were oven-dried at 80 °C until reaching constant mass (DW). Relative water content (RWC) was calculated as $[(FW-DW)/(FWs-DW)] \times 100$ (Yang et al., 2016).

Dried biological material was used for analysis of carbon (C), nitrogen (N) (%), phosphorus (P), potassium (K), sodium (Na), and calcium (Ca) (mg kg^{-1}) concentrations. Samples from individuals recently removed from *in vitro* culture flasks were also oven-dried and analyzed in for these chemical parameters. Samples were sent to the Analytic Center of the university, where they were prepared according to Tedesco et al. (1995). Element concentrations were determined by photometry (Ca, K and Na), carbon analyzer (C), visible UV spectroscopy (P) and titration (N).

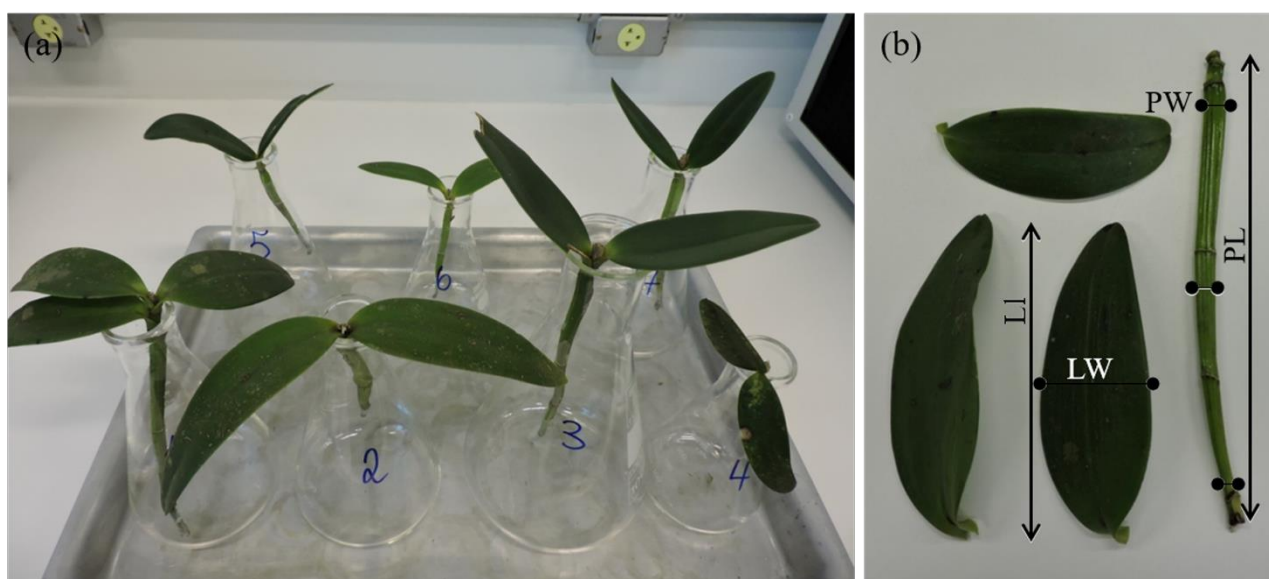


Figure 2. Samples of *Cattleya intermedia* used for the evaluation water loss and nutrient concentration. (a) Orchid shoots during dehydration; (b) Length and width measurements of pseudobulb and leaf.

Concentration of photosynthetic pigments

Chlorophyll *a* and *b* and carotenoid concentrations of plant leaves were determined in May and October of 2016 (autumn and spring). Healthy leaves were collected from three plants developing on the trunk and three in the crown. The leaves were immediately brought to the laboratory, where the samples were treated and analyzed according to the methods applied by Sasamori et al. (2018). Three replications were performed for each collected leaf, for a total of

nine replications for each stratum and season evaluated. Absorbances of each photosynthetic pigment were read using a spectrophotometer (Spectramax® M3), and the concentrations of photosynthetic pigments were calculated according to the equations proposed by Wellburn (1994).

Abiotic data acquisition

Data for luminosity, relative air humidity, and temperature were obtained quarterly on a sunny day of each season of each year from October 2013 to October 2016 (thirteen seasonal surveys). Data were collected near the trunk and the crown on the eastside of three phorophytes using a THAL 300 thermo-hygro-anemometer (Endres Júnior et al., 2015), at 09:00 h, 13:00 h and 17:00 h. Three daily means were obtained for the trunk and three for the crown for a total of 39 values each.

Phorophyte stem inclination and circumference

Stem inclination and circumference were evaluated in May 2015 for the two extremes of each tree translocation height range - 3.0 and 3.5 m for the trunk, and 6.5 and 7.0 m for the crown. Inclination was obtained using a GS-MN11-M inclinometer while circumference was obtained with a measuring tape. The two values for each tree stratum were used to calculate means.

Statistical analysis

The percentage of *C. intermedia* survival was calculated by the number of living plants in each stratum (trunk and crown, respectively) at the end of the study (2016) in relation to the initial number of translocated plants (2013), not including in the calculation those plants that were collected by park visitors. Data were submitted to the Shapiro-Wilk normality test. Shoot height (SH), number of leaves (NL), and number of pseudobulbs (NP) per plant obtained before translocation and after three years *in situ* (T1 and T2, respectively) were compared using the Student t-test for paired samples. Comparisons of the aforementioned morphometric parameters plus number of roots (NR), evaluated in herbivore damaged and undamaged orchids (T2), were performed using the Student t-test for independent samples after data transformation with the formula $(\sqrt{x})+1$. Shoot morphometric parameters, water content, and nutrient concentration were compared using ANOVA followed by the Tukey test. Phorophyte stem variables and the concentrations of photosynthetic pigments were compared using

the Student t-test for independent samples. Averages were obtained for SH, NL, NP increase (T2-T1, where T2=final value and T1=initial value) and NR (T2) for *C. intermedia* individuals per phorophyte. Pearson's rank correlation test was applied to evaluate relationships between growth parameters, inclination [data transformed by $\ln(x+1)$], and stem circumference of phorophytes. Reference values used to classify correlations were as follows: $0 > r < 0.3$ (low), $0.3 \leq r < 0.6$ (moderate), and $r \geq 0.6$ (strong) (Callegari-Jacques, 2003). Abiotic factors were compared by the Mann-Whitney test. Statistical analyses were conducted using SPSS version 27 (SPSS, Chicago, IL, USA) at 5% significance.

Results

Survival and development of reintroduced plants

Ten of the seventy trunk-translocated *C. intermedia* plants (14.3%) were collected by park visitors, while no plants were collected from the crown. Considering only the plants not collected, survival percentages were thusly 95.7% on the trunk and 100% in the crown.

Individuals of *C. intermedia* produced new shoots after translocation (Figure 1e). Plants attached to the crown showed a significant increase in shoot height (41.5%), number of leaves (49.8%), and pseudobulbs (141.2%) during the three years of monitoring. Individuals attached to the trunk had a significant increase in the number of pseudobulbs (77.0%), while shoot height decreased by 9.2% and number of leaves increased by only 0.05%, both statistically not significant (Table 1). Both groups of plants produced new roots that developed through the pine bark pieces (Figure 1e), reaching the bark of the phorophyte and fixing firmly.

Five plants attached to the crown of phorophytes produced floral bracts (spathes). In the spring of 2016, individuals had floral bud growth (Figure 1f, Figure 1g) and, although many of the produced buds were aborted, the first flowers were recorded.

Table 1. Average and standard deviation for shoot height, number of leaves, number of pseudobulbs, and number of roots of *Cattleya intermedia* plants attached to the crown and to the trunk of arboreal phorophytes, at the beginning (T1) and end (T2) of three years of monitoring.

		Shoot height (cm)	Number of leaves	Number of pseudobulbs	Number of roots
Crown	T1	8.46 ± 1.47	7.67 ± 2.55	3.13 ± 1.36	
	T2	11.97 ± 4.79*	11.49 ± 6.00*	7.55 ± 3.25*	10.12 ± 4.40
	t	-7.072	-4.708	-12.721	
	P	<0.001	<0.001	<0.001	
Trunk	T1	8.24 ± 1.47	6.83 ± 1.8	2.91 ± 1.26	
	T2	7.51 ± 4.24	7.59 ± 5.17	5.15 ± 3.47*	5.74 ± 3.24
	t	1.202	-0.014	-3.759	
	P	0.235	0.989	<0.001	

*Indicates significant differences by the Student t-test for paired samples at 5% significance.

Plant development: phorophyte strata and herbivory

Since plants were damaged by herbivores (Figure 1 h-i), and it has been previously reported that insect interference may influence survival and development of translocated *C. intermedia* under specific environmental conditions (Endres

Júnior et al., 2018), individual plants were evaluated in four different groups. Plants grown in the crown of phorophytes had higher averages for shoot height, number of pseudobulbs and roots that individuals translocated to the trunk. Only the number of leaves of damaged plants differ between trunk and crown (Table 2).

Table 2. Average and standard deviation for shoot height, number of leaves, number of pseudobulbs, and number of roots of *Cattleya intermedia* plants attached to the crown and to the trunk of arboreal phorophytes, damaged and undamaged by herbivore insects.

		Shoot height (cm)			
		Damaged	Undamaged	t	P
Crown		10.87 ± 6.32Aa*	12.46 ± 3.84Aa	1.067	0.295
Trunk		5.13 ± 3.46Bb	9.17 ± 4.06Ba	3.803	<0.001
t		-3.67	-3.643		
P		0.001	<0.001		
		Number of leaves			
		Damaged	Undamaged	t	P
Crown		9.10 ± 6.70Ab	12.59 ± 5.38Aa	2.353	0.026
Trunk		5.50 ± 5.29Ab	9.03 ± 4.65Ba	2.829	0.007
t		-1.768	-3.06		
P		0.084	0.003		
		Number of pseudobulbs			
		Damaged	Undamaged	t	P
Crown		6.76 ± 3.73Aa	7.91 ± 2.30Aa	1.379	0.179
Trunk		3.68 ± 3.64Bb	6.16 ± 2.30Ba	2.938	0.005
t		-2.807	-2.591		
P		0.008	0.011		
		Number of roots			
		Damaged	Undamaged	t	P
Crown		8.71 ± 4.23Aa	10.76 ± 4.37Aa	1.753	0.084
Trunk		4.64 ± 3.65Bb	6.50 ± 2.74Ba	2.549	0.014
t		-3.342	-4.623		
P		0.002	<0.001		

*Different uppercase letters in columns and lowercase letters in rows indicate significant differences according to Student t-test for independent samples at 5% significance.

Among plants translocated to the trunk, 40.0% were damaged by herbivorous insects, while 31.3% of those attached to the crown were damaged. A higher number of leaves was recorded for undamaged plants that were attached to the crown than damaged individuals of the same strata and from undamaged plants of the trunk. Damaged and undamaged individuals of *C. intermedia* translocated to the crown did not

differ in shoot height, number of pseudobulbs, and number of roots, while trunk-translocated plants differed for all evaluated parameters (Table 2). Three years of development of undamaged plants can be seen in Figure 3, with values increasing over the three years and being more pronounced for individuals of the crown (Figure 3).

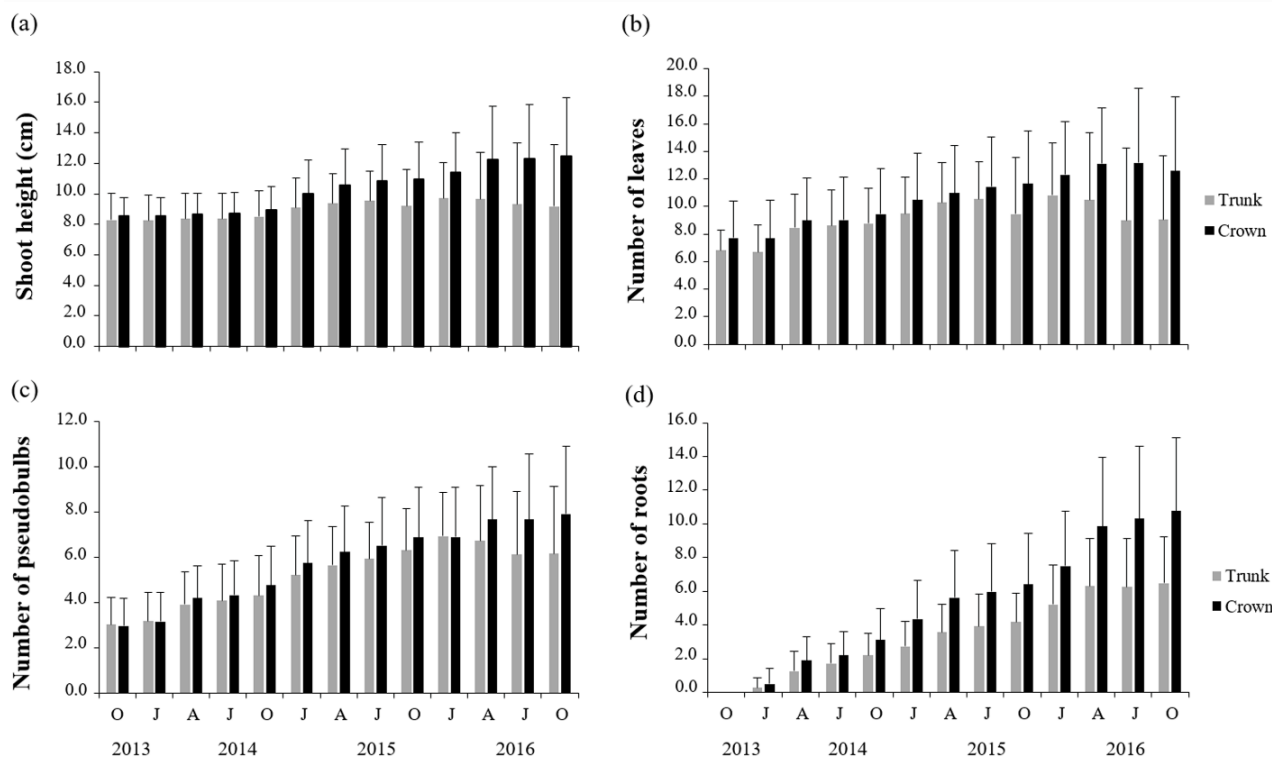


Figure 3. Average and standard deviation for undamaged *Cattleya intermedia* plants growing for three years after translocation to the trunk and the crown of arboreal phorophytes. (a) Shoot height; (b) Number of leaves; (c) Number of pseudobulbs; (d) Number of roots.

Water loss and nutrient concentration

The excised pseudobulbs with leaves obtained from the three plant groups showed equal relative water content throughout 28 d under the same laboratory conditions, in spite of significant differences for the other morphometric variables of these shoots (Figure 4, Figure 5). The shoots collected from the wild population had higher values for pseudobulb length, leaf width, fresh mass at saturation, and dry mass. Plants translocated to the crown had averages for pseudobulb width and leaf length that were intermediate between wild and the trunk translocated groups (Figure 5). No leaf abscission was recorded in any of the samples.

Carbon, phosphorus, nitrogen, and sodium concentrations did not differ between trunk and crown translocated *C. intermedia* plants. Potassium concentration was higher for plants on the trunk while calcium was higher for

plants in the crown (Figure 6). Carbon concentration in translocated plants (crown: 31.7%, trunk: 32.5%) and wild plants (26.1%) was significantly lower when compared to the concentration in individuals freshly removed from *in vitro* culture flasks. Phosphorus concentration for translocated plants was intermediate, being on average, 65.2% less than *in vitro* plants and 43.0% more than wild plants. Nitrogen concentrations for translocated and wild plants were lower than that for *in vitro* plants (81.5% in average). The highest concentration of potassium was for *in vitro* plants while the lowest was for crown and wild plants (Figure 6). All plant groups differed with regard to calcium concentration, with crown plants having the highest, followed by trunk plants, wild plants and then *in vitro* plants. Sodium concentration did not differ significantly among crown and trunk translocated plants and *in vitro* plants; these

plants had on average a 68.5% lower concentration of this nutrient compared to wild plants.

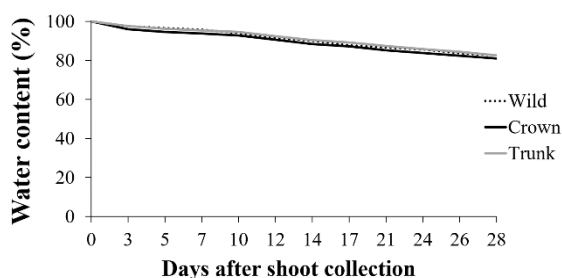


Figure 4. Relative water content of pseudobulbs with leaves collected from a wild orchid

Table 3. Chlorophyll *a*, chlorophyll *b*, and carotenoid concentration (mg g^{-1}) of *Cattleya intermedia* plants reintroduced to the trunk and to the trunk of arboreal phorophytes.

		Autumn	Spring	t	P
Chlorophyll <i>a</i>	Crown	$0.056 \pm 0.014\text{Aa}^*$	$0.055 \pm 0.021\text{Aa}$	5.010	0.797
	Trunk	$0.026 \pm 0.017\text{Bb}$	$0.049 \pm 0.014\text{Aa}$	1.015	<0.001
	t	0.856	5.111		
	P	<0.001	0.344		
Chlorophyll <i>b</i>	Crown	$0.038 \pm 0.006\text{Ab}$	$0.062 \pm 0.022\text{Aa}$	22.691	<0.001
	Trunk	$0.037 \pm 0.016\text{Ab}$	$0.058 \pm 0.023\text{Aa}$	1.939	0.003
	t	22.912	0.000		
	P	0.834	0.566		
Carotenoids	Crown	$0.009 \pm 0.002\text{Aa}$	$0.007 \pm 0.006\text{Aa}$	19.306	0.107
	Trunk	$0.004 \pm 0.002\text{Bb}$	$0.008 \pm 0.004\text{Aa}$	5.772	0.001
	t	1.186	4.190		
	P	<0.001	0.514		

*Different uppercase letters in columns and lowercase letters in rows indicate significant differences according to Student t-test for independent samples at 5% significance.

Abiotic data

Luminosity was significantly higher in the crown than on the trunk ($U=445.0$, $P=0.022$), while temperature and air humidity did not differ between the two strata ($U=634.5$, $P=0.879$ and $U=657.0$, $P=0.813$, respectively). Quarterly evaluations of luminosity over the three years of monitoring revealed spring-autumn variation from 291.7 and 373.9 Lux to 1538.33 and 1787.6 Lux, for trunk and crown, respectively (Figure 7). Temperature and air humidity had inverse winter-summer variation, with the lowest temperatures being in winter for trunk (13.04°C) and crown (13.02°C), and the highest in the summer (32.53°C and 32.19°C , respectively).

population and from translocated plants during 28 d of dehydration under laboratory conditions.

Chlorophyll and carotenoid concentrations

Trunk-translocated plants had significantly lower averages for chlorophyll *a* and carotenoids compared to crown plants in the autumn; pigment concentrations for trunk and crown plants did not differ significantly in the spring (Table 3). Concentrations of all three photosynthetic pigments were lower in autumn than in spring, and only chlorophyll *b* had a lower average in autumn than spring for crown samples (Table 3).

Both strata had similar temperature amplitudes (trunk= 19.49°C ; crown= 19.17°C), differing by only 1.66%. The lowest values for air humidity were in the summer (trunk= 44.31% ; crown= 44.02%), with trunk and crown differing by 3.99%.

Phorophyte stem inclination and circumference

Stem inclination had a greater amplitude at the trunk than in the crown (1.0 to 25.0° and 3.5 to 22.5° , respectively), with the average for the trunk being significantly lower than that for the crown (6.6 and 10.5° , respectively; $t=-2.733$;

$P=0.011$). Amplitude for circumference was also greater at the trunk (34.0 to 54.0 cm) than in the crown (22.0 to 37.0 cm), with the average for trunk being significantly higher than that for the crown (42.8 and 25.7 cm, respectively; $t=6.444$; $P<0.001$).

A moderate negative relationship was found between shoot height and stem inclination at the trunk ($r=-0.433$; $P=0.013$). Shoot height

($r=0.629$; $P<0.001$), number of leaves ($r=0.705$; $P<0.001$), and number of pseudobulbs ($r=0.706$; $P<0.001$) were positively and strongly related to trunk circumference. There was no relationship between the evaluated morphological parameters and stem inclination or stem circumference in the crown.

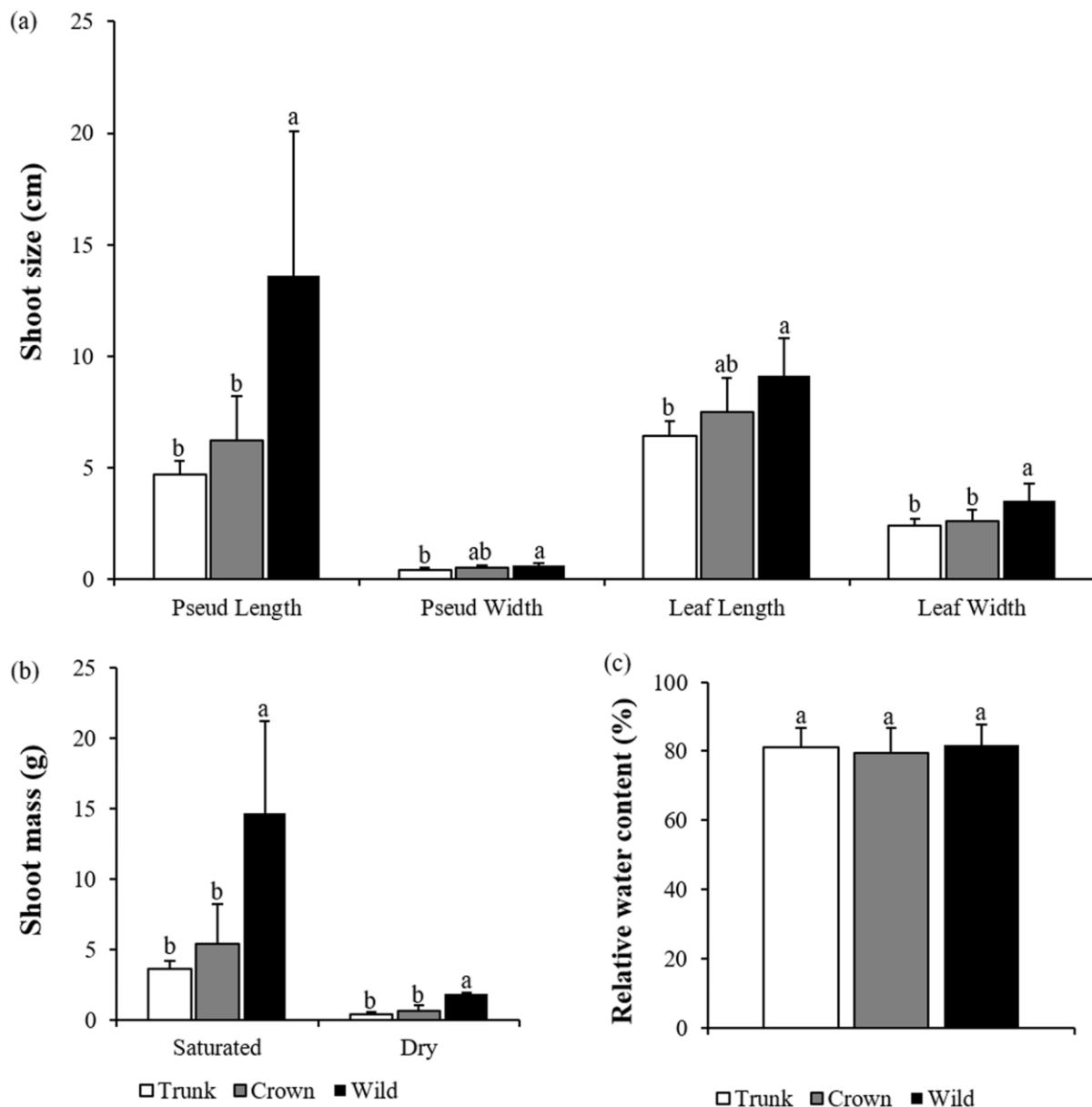


Figure 5. Average and standard deviation for shoot parameters of wild and translocated individuals of *Cattleya intermedia*. (a) Pseudobulb length and width, leaf length and width; (b) Fresh mass of tissue at saturation and dry mass; (c) Relative water content after 28 d of dehydration. Different letters indicate significant differences according to the Tukey test ($P=0.05$).

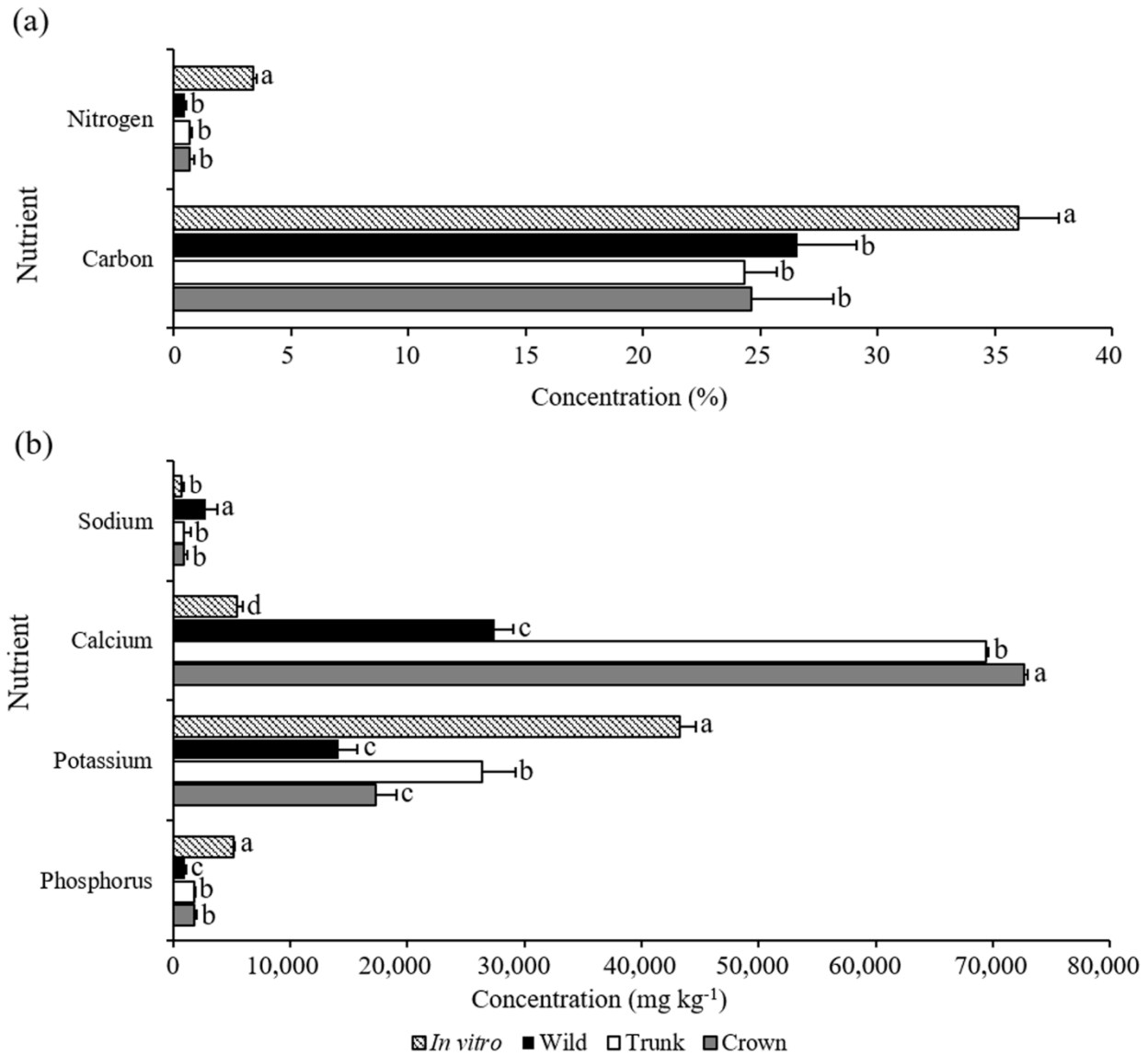


Figure 6. Average and standard deviation for nutrient concentrations of shoots of translocated, wild, and in vitro individuals of *Cattleya intermedia*. (a) Carbon and nitrogen (%); (b) Phosphorus, potassium, calcium, and sodium (mg kg⁻¹). Different letters indicate significant differences according to the Tukey test (P=0.05).

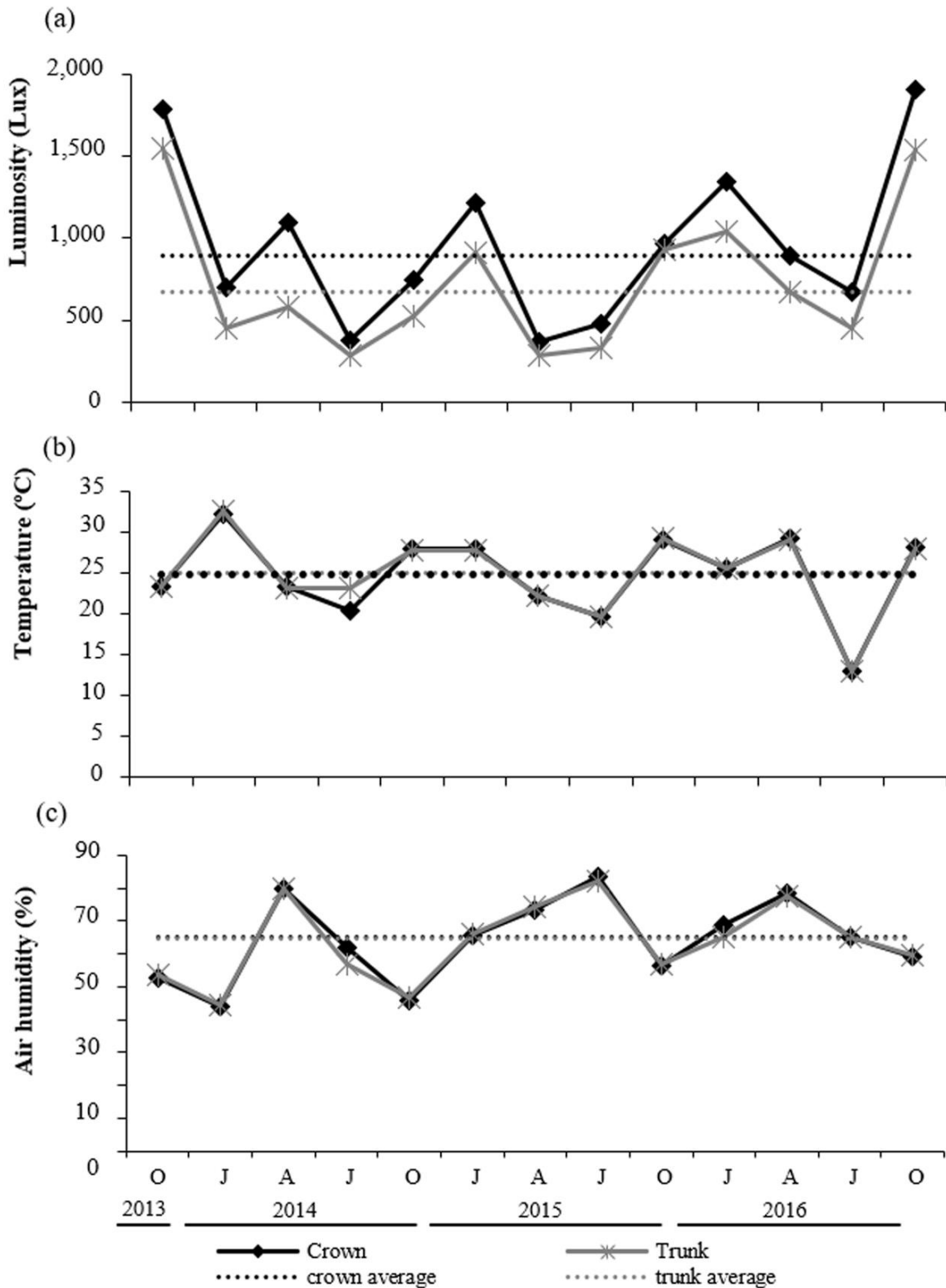


Figure 7. Abiotic data obtained throughout three years of orchid monitoring. (a) Luminosity (Lux); (b) Temperature (°C); (c) Relative air humidity (%).

Discussion

Cattleya intermedia plants had high survival rates three years after translocation for reinforcement, both when on the trunk and in the

crown. These rates were higher than those obtained for the sympodial epiphytic orchids *Barkeria whartoni* (C. Schweinf.) Soto-Arenas in Mexico (17%), *Dendrobium aqueum* Lindley in India (12%), (two years and less than

one year after transplanted to reinforce their natural populations, respectively), and *Schomburgkia crispa* Lindl. in the Brazilian Cerrado (73%) (two years and four months after reintroduction) (Parthibhan et al., 2015; Segovia-Rivas et al., 2018; Soares et al., 2020). These initiatives, unlike the present study, were developed with very young plants and did not compare different environmental conditions.

The survival recorded in the present study was also higher than that obtained with the planting of smaller and younger individuals of *C. intermedia* (sister plants to those used in the present study) that were translocated to the edge (73%) and the interior (64%) (three years and three months after translocation) of a fragment located between the Atlantic Forest and the Pampa in Southern Brazil (<9 km from AREIHLR) (Endres Júnior et al., 2018). Care for a longer period under controlled conditions, when compared to our previous studies (Endres Júnior et al., 2015a, 2018), ensured that we had larger and more resistant individuals for *in situ* establishment after translocation.

Although the two groups of *C. intermedia* - translocated to the trunk and to the crown - did not differ significantly regarding morphometric variables, they began to show differences during monitoring. The plants translocated to the crown experienced increases in all morphometric parameters during the three years *in situ*, while plants translocated to the trunk only experienced an increase in the number of pseudobulbs. The greater number of pseudobulbs allows the storage of water and nutrients (Holtum, 1955; Ng and Hew, 2000; Hoshino et al., 2023). The larger rhizomes guarantee a greater amount of renewal buds for the continuous development of the plants (Johansson, 1974). The greater number of leaves, the main organs responsible for photosynthesis in orchids (Moreira et al., 2013; Suetsugu et al., 2023), makes the crown plants fitter to light exposure (Ventre-Lespiauq et al., 2017; Kirillova et al., 2023), accentuating the differences in relation to trunk plants in their response to environmental conditions during monitoring. The presence of a healthy root system, necessary for the rapid absorption and storage of water and nutrients (Benzing et al., 1982), was guaranteed by the initial conditions of the selected plants and the maintenance of the pine bark substrate, reducing possible damage caused to the roots when they were tied to the trees. The plants, mainly in the crown, produced new roots, fixing them directly to the trunk of the phorophytes, and in the third year (2016), pine

bark pieces of some orchids were observed to be covered by avascular and vascular epiphytes, such as mosses, lichens, rhizomes of *Microgramma* C. Presl, and *Peperomia* Ruiz & Pav. Orchid roots, despite not acting in obtaining atmospheric CO₂, as well as their pseudobulbs, have chlorophyll and are important in the process of refixing carbon released during respiratory activity (Dycus and Knudson, 1957; Erickson, 1957; Ng and Hew, 2000).

A smaller percentage of plants in the crown suffered herbivory in relation to the percentage of those on the trunk, and those that had their tissues consumed by insects in the crown stratum showed greater resilience to the damage, which was evidenced by the morphometric variables. The crown plants that were damaged by herbivorous insects only experienced an impact to the number of leaves, while individuals attached to the trunk experienced lower values for all morphometric parameters compared to undamaged plants. The damage caused to the leaves of *C. intermedia* by larvae of *Ithomiola nepos* (Fabricius, 1793) (Lepidoptera) and by *Tenthecoris bicolor* J. Scott, 1886 (Hemiptera) in the present study was easily determined from visual characteristics already reported for these species (Endres Júnior et al., 2018). The impact that herbivory has on *C. intermedia* development is greater when the plants are exposed to the low luminosity of the tree trunk, corroborating records for the same species translocated into a forest interior environment (Endres Júnior et al., 2018).

Some of the damaged orchids on the trunk (36.4%) and in crown (23.8%) had the entire shoot (leaves + pseudobulb) removed from the rhizome. Such damage was probably caused by leaf-cutting ants that had nests located close to the trees on which the orchids were attached. Ants are important herbivores in subtropical regions (Leal et al., 2012), and can be a challenge in restoration programs (Renison et al., 2023) and can be a challenge in restoration programs (Renison et al., 2023). We showed that, in addition to the direct impact on these plants, the loss of leaves and pseudobulbs leads to a reduction in the total amount of nutrients and water stored by these plants, as well as a reduction in photosynthetic capacity.

Values for pseudobulb and leaf size did not differ between plants translocated to the trunk and those in the crown. However, most values were lower than those for wild plants, which were already mature and presenting flowers. Morphometric differences between samples may be associated with the maturity of individuals,

since the most developed plants attached to the crown presented an intermediate state of development of pseudobulb width and leaf length compared to trunk-attached plants and wild adult plants. It should be emphasized that some individuals growing in the crown produced floral buds in the third year of monitoring.

Despite the documented differences in shoot size between translocated and wild individuals, relative water reduction over 28 days did not differ, and the translocated plants did not even differ when comparing final water content. Biotic and abiotic conditions at the point of establishment of translocated orchids and differences in plant maturity did not influence the shoot morphometrical characteristics responsible for water retention, as lower expansion of leaves, which reduce evapotranspiration, for example (McCree and Fernández, 1989; Taiz et al., 2022). In addition, the leaves of the shoots of *C. intermedia* did not undergo senescence to avoid water loss during the 28-day dehydration period, an adaptation especially developed in some epiphytic orchids (Yang et al., 2016). *Cattleya intermedia* has succulent leaves covered by a cuticle, which increase water storage efficiency (Benzing, 1990; Gonçalves and Waechter, 2003), adaptations that by themselves seem to have guaranteed water stability for a long period without water absorption (Ulum et al., 2023).

In general, translocated plants showed lower nutrient concentrations when compared to micropropagated plants (verified for carbon, phosphorus, nitrogen and potassium) and concentrations equal to or higher than wild plants, which indicates their nutritional adaptation to the conditions provided by the environment. Potassium, calcium, and sodium concentrations were higher for *C. intermedia* (translocated and wild) than those usually required by most plant species (10,000, 5,000, and 10 mg kg⁻¹, respectively) (Epstein, 1972). The results recorded here and by other studies (Zotz and Tyree, 1996; Zotz and Hietz, 2001) indicate that that *C. intermedia* can obtain nutrients efficiently and is not experiencing nutrient scarcity.

The differences documented between plants attached to the trunk and to the crown for potassium and calcium concentrations are probably related to the conditions that these individuals experienced during the three years *in situ*, such as access to leachate nutrients by stemflow. Orchids are influenced by the availability of nutrients on the surface of their hosts (Awasthi et al., 1995), and the element concentrations in plant tissues and the substrate are both highly related (Naik et al., 2006). In spite

of not verifying a pattern indicating higher concentrations of nutrients (mg kg⁻¹) in the shoots of *C. intermedia* of the crown, we emphasize that the biomass of these plants was greater than those on the trunk, and that the accumulated total of nutrients in plants undergoing re-establishment in the crown stratum can be considered greater. Nutrient composition can differ along the vertical gradient because of structural characteristics of phorophyte stems, which determine nutrient deposition and maintenance on phorophytes (Kersten et al., 2009). Thus, plants in the crown may be benefiting from the availability of nutrients in this environment, as they have access to absorb stemflow first, and may be negatively influencing the nutrition of the plants on the trunk by reducing what leaches down to them.

The higher phosphorus and potassium concentrations (trunk) documented in translocated plant shoots, compared to wild samples, may be related to the pre-translocation treatment the individuals received during *in vitro* (MS medium for germination and growth) and *ex situ* greenhouse (Peters Professional® fertilizer) nutrition provisioning. Higher concentrations of calcium were recorded in translocated and wild plants compared to *in vitro* plants, and wild plants had higher concentrations of sodium than translocated and micropropagated plants. These differences may be related to nutrient availability of *in vitro* culture, to the individual's capacity for nutrient absorption and storage in tissues, or to the nutritional needs of *C. intermedia* under different conditions.

The concentrations of photosynthetic pigments in *C. intermedia* plants seem to respond to ambient light conditions, which differ between strata and fluctuate between seasons. Lower values of chlorophyll *a* and carotenoids were recorded for the trunk plants in autumn, when the average luminosity was 514.33 Lux, while the highest average concentrations of these pigments for crown plants occurred in the spring, when the average luminosity was 1350.58 Lux. Chlorophyll *b* responded to season, being reduced in autumn in both strata.

These differences in the concentrations of photosynthetic pigments are important for the regulation of photosynthesis and to ensure greater efficiency in production and energy savings (Lima et al., 2023). This is because the number of plastids in plant tissues is triggered by luminosity and their maintenance is dependent on the presence and quality of light (Taiz et al., 2022). Thus, the changes experienced by the plants in light availability may lead to the partial degradation of pigment molecules in the autumn, mainly for

individuals attached to the trunk. As pigment concentrations in leaves can indicate the photosynthetic potential of the plants (Curran et al., 1990; Filella et al., 1995), the highest concentrations may represent an increase in the production of photosynthates, which will be beneficial mainly for plants translocated to the crown.

The development of individuals of *C. intermedia* attached to the trunk was related to stem circumference and inclination, while such relationships were not found for individuals in the crown. According to Gowland et al. (2011), some orchid species acquire fitness benefits when established under specific conditions regarding the height and size of phorophyte branches to which they are attached, and orchids on the trunk are considered to be more dependent on the stem characteristics evaluated in the present study. Variation in shoot height on the trunk in relation to stem inclination in this stratum can be caused not only by optimal conditions; that is, it can reflect plants adapting their size to ensure that the positioning of leaves allows greater interception of sunlight (Ventre-Lespiauq et al., 2017), which, according to our results, is smaller for the trunk.

Besides, the plants on the trunk may be exposed to more pronounced hydric stress since rainwater is retained by the upper branches and some stemflow (water and nutrients) is absorbed by epiphytes growing above the translocated individuals (including *C. intermedia* fixed in the crown). Water present in tree bark may also evaporate when it runs down the stem (Hölscher et al., 2004; Brasil et al., 2018). Although the water retention capacity of phorophyte bark ($\text{mL}^{-1} \text{cm}^3$) did not differ between crown and trunk (Endres Júnior et al., 2019), a greater circumference in the trunk region of some trees may allow the retention of more water after rains, increasing the time and space for plants to absorb, store and use water for growth (Silva et al., 2023). The smaller root system of these plants can reduce their ability to obtain water, while the smaller aerial part causes a greater

surface/volume ratio, reducing their ability to store water, and imposing greater transpiration and loss of this resource (Zotz, 1998). Thus, water retention by the bark may indirectly explain the relationship between plant development and trunk circumference.

Conclusions

We conclude that plants of *Cattleya intermedia* in trunk and crown strata experience specific and distinct environmental conditions (Figure 8). Our observations allow us to infer about the reasons that lead *C. intermedia* to be found, under natural conditions, mainly in the crown of trees: adaptations of the species to the use of greater light availability, as well as the water and nutritional resources of the crown, which leads to greater development and resilience to herbivory events. Moreover, being in a higher part of phorophytes provides greater protection against irregular collections due to the ornamental characteristic of its flowers.

Monitoring multiple variables of *C. intermedia* translocated to different environments can ensure a better understanding of the success of its establishment. Although the plants used in the present study did not represent the entire genetic diversity of the species, this step of translocation was successful and provides support for future conservation projects for this and other epiphytic orchids, respecting the peculiarities of each species. Besides, translocated plants exhibited behavior similar to that of wild individuals, namely the production of vegetative organs and flowers, as well as the size of shoots and the concentrations of nutrients contained in them. Thus, for successful the establishment of translocated *C. intermedia* plants, the results of this study allow us to suggest that plants with well-developed pseudobulbs be attached to the highest parts of trees, in places with greater light availability (average of approx. 1500 Lux in the spring).

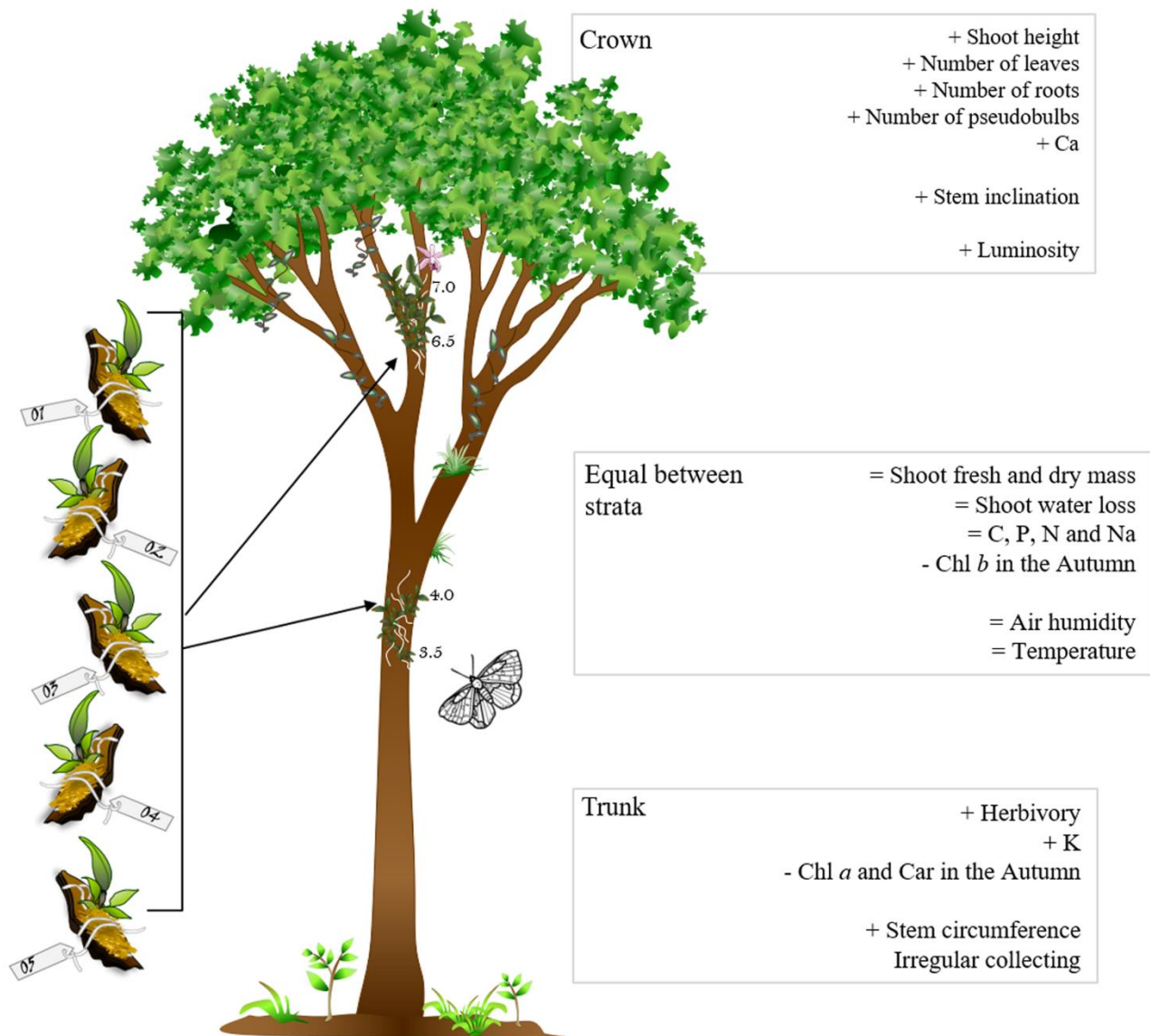


Figure 8. Schematic of translocation and phorophyte, with evaluated biotic and abiotic variables. + indicates that the value in the given stratum was higher than the respective value in the other stratum; - indicates that the value in the given stratum and/or season was lower than the respective value in the other stratum and/or season; = indicates that there were no differences between respective values of the two strata. Ca = calcium, C = carbon, P = phosphorus, N = nitrogen, Na = sodium, K = potassium, Chl a = chlorophyll a, Chl b = chlorophyll b, Car = carotenoids.

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