Acta Botanica Brasilica 32(4): 555-566. Oct-Dec 2018.
doi: 10.1590/0102-33062018abb0009

Survival and development of reintroduced *Cattleya intermedia* plants related to abiotic factors and herbivory at the edge and in the interior of a forest fragment in South Brazil

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Received: January 11, 2018
Accepted: April 9, 2018

ABSTRACT

Biotic and abiotic factors, such as luminosity, temperature, air humidity, and herbivory, can affect the establishment of reintroduced plants in natural habitats. This study evaluated the effects of these factors on the survival and growth of *Cattleya intermedia* plants reintroduced into a forest fragment in South Brazil. Plants of *C. intermedia* were obtained from *in vitro* seed germination in asymbiotic culture. Eighty-eight plants were reintroduced at both the forest edge and forest interior. Plants with greater shoot heights and number of leaves and pseudobulbs suffered more damage from herbivores at the edge. There were no significant differences in morphometric parameters between damaged and non-damaged plants in the interior. *Tenthecoris bicolor*, *Helionothrips errans*, *Ithomiola nepos*, *Molomea magna* and Coleoptera larvae damaged *C. intermedia*. Luminosity was higher at the edge, while air humidity and temperature were the same in both environments. Herbivory associated with abiotic factors increased plant mortality in the interior, while abiotic factors were determinative of plant survival at the edge. Luminosity is important to the survival of reintroduced epiphytic orchids, and herbivory affects the success of reintroduction.

Keywords: conservation, micropropagation, monitoring, Orchidaceae, reintroduction

Introduction

The Atlantic Forest of tropical South America boasts 20,000 plant species, 40 % of which are endemic, yet only 12.5 % percent of the original forest area remains (Fundação SOS Mata Atlântica 2018). As an increasingly fragmented biome, the remaining patches of Atlantic Forest habitat are small, highly impacted and possess a high degree of heterogeneity in abiotic conditions (Viana & Pinheiro 1998). This habitat fragmentation leads to edge effects, which include many microclimatic changes, like increased light and wind exposure and reduced humidity (Silva & Schmitt 2015). Also, changes in forest structure and composition can especially affect the diversity, abundance and distribution of epiphyte plants (Laurance & Yensen 1991; Bernardi & Budke 2010; Bianchi & Kersten 2014).

Orchidaceae is one of the most diverse and abundant families of Atlantic Forest epiphytes (Kersten & Silva 2001; Borgo & Silva 2003; Hefler & Faustioni 2004; Staudt *et al*. 2012). The plants of this family are particularly vulnerable to anthropogenic disturbances, such as habitat loss, predatory collection and exotic species introduction (Martinelli & Moraes 2013; Endres Júnior *et al*. 2015a; Parthibhan *et al*. 2015). Their restricted distributions and high environmental specificities and endemism make orchids particularly vulnerable to habitat loss (Adhikari *et al*. 2012; Martinelli

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& Moraes 2013). Nevertheless, studies on ephiphyte community dynamics have not shown an ecological pattern influenced by edge effects, probably due to the low-density of orchid populations (Bernardi & Budke 2010; Bataghin *et al*. 2012; Bianchi & Kersten 2014).

Cattleya is a neotropic orchid genus widely distributed in Brazil, occurring in almost all phytogeographical domains. The richness of *Cattleya* is currently estimated at 102 species in Brazil, of which 95 are endemic to the country (Flora do Brasil 2018). These plants hold great economic importance among orchid species due to the beauty of their flowers (Martinelli & Moraes 2013). *Cattleya intermedia* is an epiphytic plant that occurs in the South and Southeast regions of Brazil (CNC Flora 2018). In Rio Grande do Sul, this species has been found in areas of transition between the Atlantic Forest and the Brazilian Pampa biomes, as well as in the Pampa biome *sensu stricto* (IBGE & MMA 2004; Buzatto *et al*. 2010). *C. intermedia* can be found near watercourses and roadsides, on trunks and crowns of trees in forests or isolated in open areas, such as large *Ficus organensis*, a protected tree in the region. Due to significant direct and indirect anthropogenic impacts, *C. intermedia* is listed in The Red Book of Brazilian Flora (Martinelli & Moraes 2013) and in the Lista Oficial da Flora Nativa Ameaçada de Extinção no Estado do Rio Grande do Sul (Rio Grande do Sul 2014) (The List of Endangered and Threatened Flora of Rio Grande do Sul) and its populations have declined by more than 30 % over the last 50 years (CNC Flora 2018).

Studies about the relevance of epiphytic plants to global biodiversity and ecosystems, plus their ornamental and economic importance, are essential to provide justification for ongoing conservation efforts (Benzing 1990), especially regarding *C. intermedia* (CNC Flora 2018). The propagation of *in vitro* plants associated with their reintroduction into natural habitats is an important tool for the recovery of endangered orchid populations (Rubluo *et al*. 1993; Brasil 1994; Decruse *et al*. 2003). However, endangered species have a low capacity for plasticity in response to environmental changes compared to those that are not threatened (Guerrant & Kaye 2007). Nevertheless, *C. intermedia* has a set of adaptations to dry environments and high luminosity (Gonçalves & Waechter 2003).

Despite its ecological importance and unfavorable conservation status, studies on the habitat requirements and reestablishment in nature after *in vitro* propagation of *C. intermedia* are still scarce (Dorneles & Trevelin 2011; Endres Júnior *et al*. 2015a; b). The same is true for epiphytic species in general (Seeni & Latha 2000; Zettler *et al*. 2007; Aggarwal *et al*. 2012; Wu *et al*. 2014). The limited number of successful reintroduction initiatives can be attributed to the complexity of the relationships established between these plants and other organisms and the environment (Peng 2003). This emphasizes the relevance of basic studies focusing on improving threatened plant reintroduction (Hai *et al*. 2014). As a result of a project for the reintroduction of *C. intermedia*, we have obtained initial information on variability in survival and development among individuals. The reintroduced orchids showed differences of the morphometric parameters when comparing data from edge and interior of a forest fragment. A higher number of herbivore insects were observed at the edge, but the study of its relation with plant survival and development and with abiotic factors has yet to be undertaken (Endres Júnior *et al*. 2015b). In this work the authors hypothesized that there is a difference (I) of herbivory on *C. intermedia* plants, (II) of abiotic parameters (luminosity, temperature and relativity humity), (III) of plant survival and development in different environments (forest edge and interior), and that (IV) this possible difference between plants is expected to be related to herbivory and abiotic factors in both environments.

Materials and methods

Study environment

The study was carried out in Campo Bom (29°40’06”S, 50°00’36”W; 29°40’44”S, 50°01’05”W), Rio Grande do Sul, Brazil. The study area is a seasonal semideciduous forest fragment of 60 hectares inserted in an agricultural matrix with cattle, maize, pasture and exotic plant species production. The different types of environments present in the fragment provide conditions favoring the occurrence of a rich epiphytic flora (aproximately 40 orchid species) (D Endres Júnior unpubl. res.): e.g., *Cattleya intermedia* Graham and *C. tigrina* A. Rich. ex Beer, endangered species of orchids from Rio Grande do Sul; and *Trichocentrum pumilum* (Lindl.) M.W. Chase & N.H. Williams, *Prosthechea vespa* (Vell.) W.E. Higgins, *Gomesa flexuosa* (Lodd.) M.W. Chase & N.H. Williams and *Spathiger strobilifer* (Rchb. f.) Small, widely distributed in the study area. *C. intemedia* was observed in low abundance both at the edge and in the interior of the forest, which prevented any comparisons between native and reintroduced orchids (see *Plant Reintroduction*).

The southwest margin of the forest fragment, bordered by animal pasture and pine plantation, was selected for the study. A 100-meter line transect was used to select 22 phorophytes at the edge of the fragment (between 5 and 15 meters from the fragment border). The trees selected had a trunk of at least four meters long and with a diameter at breast height of 10 centimeters. Another 100-meter line transect was used to select another 22 trees with the same requisites in the interior of the fragment (between 160 and 175 meters from the fragment border). Previous records showed that between October 2011 and October 2013, luminosity was higher and relative air humidity was lower at the edge. Temperature did not differ between the two environments (Endres Júnior *et al*. 2015b).

Plant reintroduction

Individual plants of *C. intermedia* (Fig. 1A) were obtained from *in vitro* seed germination in asymbiotic culture. The seed capsules (Fig. 1B) were collected in the Sinos River basin, which is within the range of the natural occurrence of this species in the state of Rio Grande do Sul. After superficial sterilization, the capsules were cut open inside a laminar flow bench and the seeds were sowed in flasks containing MS medium (Murashige & Skoog 1962) in March 2010. Each germinated seed gave rise to a plant. After three months, the plants were transferred into fresh medium as described by Sasamori *et al*. (2015). At the end of six months of *in vitro* culture, the plants were 4.0 ± 0.2 centimeters

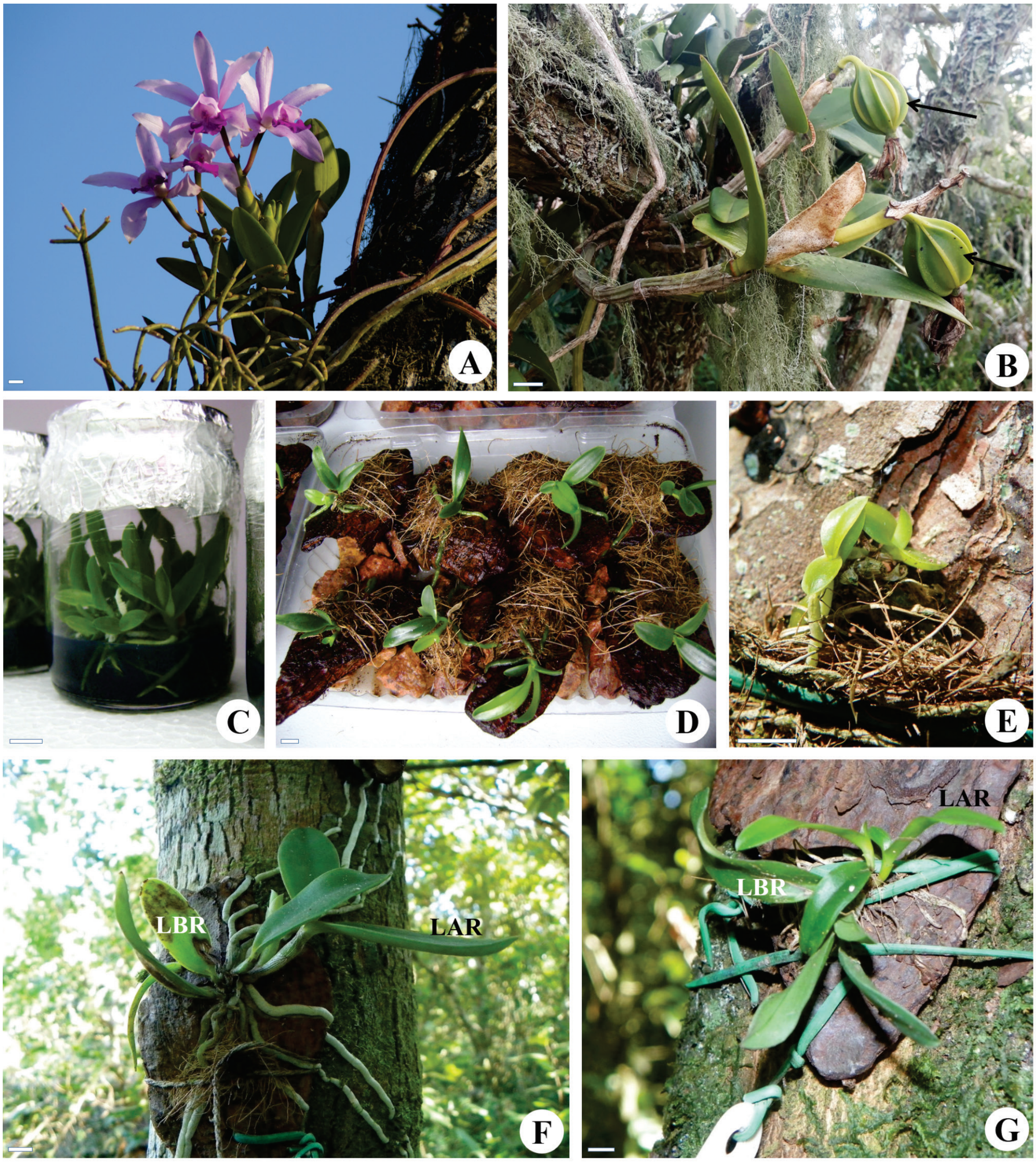

Figure 1. Stages of propagation, reintroduction and monitoring of *Cattleya intermedia* plants. Scale bar = 1 cm. Flowering adult individual recorded during the identification of species present in areas of the Sinos River basin (**A**); capsule (arrow) at the end of summer (March), the period of the year in which it ripens and releases the seeds (**B**); plants grown *in vitro* after six months (**C**); tray for plant acclimatization and substrate fixation (**D**); individuals reintroduced at the forest edge that experienced leaf abscission (of leaves formed prior to reintroduction) and the formation of new shoots (**E**); individuals reintroduced at the forest edge wich maintained leaves formed before reintroduction (LBR), and had continuous growth and increment of the morphometric parameters from new larger shoots (LAR) (**F**); reintroduced plants in the forest interior, exhibiting little growth (LAR), besides being slightly shorter than those that already existed (LBR) when reintroduced (**G**).

high (Fig. 1C). Individuals were removed from the flasks and their roots were washed in tap water to remove culture medium residue. Each plant was tied individually to a pine bark plate (10 cm x 5 cm) and kept in trays (Fig. 1D) under controlled conditions (Sasamori *et al*. 2014; Endres Júnior *et al*. 2015b) for 10 months.

Eighty-eight plants were reintroduced at the edge and the interior of the forest fragment in October 2011. For reintroduction, the plants were divided into two groups with statistically equal shoot height (4.82 cm ± 0.85 cm) – considering the rhizome base and the largest leaf tip – and number of leaves (3.45 cm ± 0.91 cm). This species exhibited slow growth, which was expected, and so plants did not possess pseudobulbs at reintroduction. Four individuals were tied to the east face of the trunk of each host tree at a height between 3.5 and 4.0 meters.

Plant monitoring

For the first months after reintroduction (October 2011 to January 2012), a qualitative analysis of the plants revealed morphometric differences (Fig. 1E-G) and organ damage on *C. intermedia*. Herbivore insects were observed on the plants. Thus, from February 2012 to January 2013, the plants were analyzed for the type of damage they experienced based on the descriptions of plant and animal relationships by Del-Claro & Torezan-Silingardi (2012). These authors classify herbivores as parasites or predators, depending on whether they consume parts of the plant or the entire individual, respectively; endo- or ectoparasites, when they are internally or externally to the plant tissues; chewing and sucking insects, when the invertebrates use green tissue or intracellular content. Since the *C. intermedia* plants were still quite small, all their leaves, shoots and roots were inspected for up to five minutes per plant. Photographic records of the different types of damage were made and, when present, specimens of herbivore insects were collected using tweezers and placed in Eppendorf tubes containing 70 % ethyl alcohol. Immature individuals and eggs were deposited in glass flasks and taken to the laboratory where they were kept at 26 °C in bottles covered with absorbent paper for aeration until they reached adulthood and could be identified. Identification of insects was performed using a specific bibliography for each group, and in consultation with taxonomic specialists.

From February 2012 to January 2015, shoot height (SH), number of leaves (NL), number of pseudobulbs (NP – considered as caulinar structure, between the rhizome and the leaf, of more than 1.5 cm) and the number of roots attached to the phorophyte trunk (NR - root longer than 2.0 cm) were demined for each plant. The survival rate and mean SH, NL, NP and NR per plant were calculated. Individuals were considered dead when there was no green tissue in the stem or in the leaves, and no budding for a period of more than three months.

To determine the frequency of damage to plants and the impact of insect damage on plant development, each individual was inspected monthly for damage from February 2013 to January 2015. Plants that suffered herbivory prior to February 2013 were not included in the evaluation. During these 24 months, each orchid was classified into one of four categories according to its damage: 1 - plant damaged by herbivory at the edge; 2 - plant not damaged by herbivory at the edge; 3 - plant damaged by herbivory at the interior; and 4 - plant not damaged by herbivory at the interior. The survival rate (based on the number of live plants in February 2013), the frequency of damaged plants and mean SH, NL, NP and NR per plant were calculated for each category. The plants that survived until the end of the study (February 2015) were considered for estimating the morphometric parameters.

Abiotic data acquistion

Abiotic data (luminosity, relative air humidity and temperature) were obtained quarterly, from February 2013 to January 2015, at the edge and interior of the forest fragment using a thermo-hygro-anemometer THAL 300 (Endres Júnior *et al*. 2015b). Data were collected on a sunny day of each season of the year at five equally distributed points along the line transects at two-hour intervals from 8 a.m. to 6 p.m. Daily data were used to calculate five means for each environment, for a total of eight seasonal surveys with five means for the edge and five means for the interior. Rainfall data were obtained for the same period from Campo Bom Meteorological Station n. 83961 (29°41’11”S, 51°02’51”W), while photoperiod data were obtained from the National Observatory (ON), which were used for the calculation of monthly means.

Data analysis

A Shapiro-Wilk test was used to assess normality of the plant and meteorological data, which were found to not meet the assumptions of normality, even after transformation. A chi-square (χ^2) statistic was used to investigate if the distribution of survival rates of damaged and non-damaged plants in each environment differed. Biological data (shoot height, number of leaves, number of roots and number of pseudobulbs) were compared using Wilcoxon and Mann-Whitney tests. Abiotic data (luminosity, relative air humidity and temperature) were compared using the Mann-Whitney test. The analyses were performed using SPSS (v. 22) with a confidence level of 95 %. A Principal Component Analysis (PCA) was performed to integrate data regarding plants damaged by herbivores, mortality, luminosity, relative humidity and temperature of each environment, as well as rainfall and photoperiod. Only eigenvalues greater than 1 were used as criteria for the extraction of principal components. The PCA was performed using PAST (v. 3.02) (Hammer *et al*. 2001).

Results

Plant monitoring

At 15 months post-reintroduction, 64 plants (72.7 %) survived at the edge of the fragment and 56 (63.6 %) at the interior. Plants at the edge showed a decrease in shoot height ($Z = 6.4993$, $p < 0.001$) and number of leaves ($Z = 5.1546$, $p < 0.001$). At the interior, the shoot height decreased ($Z = 5.5881$, $p < 0.001$) and the number of leaves increased ($Z = 4.4912$, $p < 0.001$). However, especially in February 2013, these individuals showed a SH higher than the ones at the edge ($U = 1081.5$, $p < 0.001$) and NL ($U = 1021.0$, $p < 0.001$). Orchids formed pseudobulbs and attached their roots more intensely to the host tree at the edge ($U = 1140.0$, $p < 0.001$) (Tab. 1). The results revealed that the plants at the edge of the fragment formed a more heterogeneous group than those of the interior, which exhibited less variation, as demonstrated by the higher standard deviations for the means from the edge (Tab. 1).

For the 24 months of monitoring, from February 2013 to January 2015, the survival rate for plants at the edge was 79.7 %. Thirty-seven of the 64 edge plants suffered herbivory damage (Tab. 2). The survival rate was higher in damaged than non-damaged plants ($\chi^2 = 4.814$, $p = 0.0282$). The first mortality was recorded at the edge, in June 2013, when the greatest number of plants (nine) were damaged, corresponding to 27.3 % of all plants damaged in this environment. Eight plants (29.6 %) were damaged between February 2014 and November 2014, but no dead plants were recorded during this period (Fig. 2A).

The survival rate for plants at the interior was 64.3 %. Twenty-one of the first 56 individuals were damaged by herbivores (Tab. 2). There was high mortality recorded between November 2013 and May 2014, representating 50 % of the total seedlings lost. Of all the plant damage in the interior, 19.1 % occurred between July 2014 and December 2014, but no dead plants were recorded during this period (Fig. 2B). The survival rate did not differ between damaged and non-damaged plants ($\chi^2 = 3.175$, $p = 0.0748$).

Table 1. Survival, shoot height (SH), and number of leaves (NL), roots (NR) and pseudobulbs (NP) (mean ± standard deviation) of *Cattleya intermedia* reintroduced at the edge and the interior of the forest.

		Oct/11	Feb/12	Jan/13
Survival (%)	Edge	100	96.6	72.7
	Interior	100	83.0	63.6
SH	Edge	4.8 ± 0.8 a*	4.1 ± 1.6 b	3.4 ± 2.0 b
	Interior	4.8 ± 0.9 a*	4.7 ± 1.1 a	4.7 ± 1.1 a
NL	Edge	3.3 ± 0.9 a*	2.4 ± 1.7 b	2.5 ± 1.8 b
	Interior	3.6 ± 0.9 a*	3.4 ± 1.2 a	3.7 ± 1.2 a
NR	Edge	-	0.2 ± 0.6	1.0 ± 1.8 a
	Interior	-	0.0 ± 0.0	0.1 ± 0.3 b
NP	Edge	-	0.0 ± 0.2 a	0.4 ± 0.8 a
	Interior	-	0.1 ± 0.3 a	0.1 ± 0.4 a

Means from October 2011 followed by an asterisk (*) are significantly different from the means from January 2013, according to the Wilcoxon test at 5 % probability. Means followed by the same letter in the column, for each variable, indicate no significant difference, according to the Mann-Whitney test at 5 % probability.

Table 2. Number of *Cattleya intermedia* plants with and without damage caused by herbivore insects at the forest edge and interior, and survival (%), shoot height (SH), number of leaves (NL), number of roots (NR) and number of pseudobulbs (NP) (mean ± standard deviation) between February 2013 and January 2015.

		Edge		Interior	
		Damaged plants	Undamaged plants	Damaged plants	Undamaged plants
Number of plants		37	27	21	35
Survival 2015 (%)		86.5	63.0	47.6	71.4
SH	2013	4.5 ± 1.7 Ab	2.6 ± 1.8 Ba	4.7 ± 0.9 Aa	4.7 ± 1.1 Aa*
	2015	5.8 ± 4.2 Aa	3.2 ± 3.5 Ba	4.0 ± 1.3 Aa	4.1 ± 1.5 Ab*
NL	2013	3.5 ± 1.8 Aa	1.4 ± 1.3 Bb	4.0 ± 0.7 Aa	3.8 ± 1.3 Aa*
	2015	3.5 ± 2.5 Aa	2.9 ± 2.0 Aa	2.6 ± 1.0 Ab	3.4 ± 1.7Aa
NR	2013	1.7 ± 3.1 Ab*	0.5 ± 0.8 Aa	0.1 ± 0.3 Aa	0.1 ± 0.3 Aa
	2015	3.4 ± 3.6 Aa*	0.5 ± 1.5 Ba	0.3 ± 1.0 Aa	0.2 ± 0.7 Aa
NP	2013	0.7 ± 1.0 Ab*	0.1 ± 0.2 Ba	0.2 ± 0.4 Aa	0.2 ± 0.5 Aa
	2015	2.2 ± 2.0 Aa*	0.5 ± 1.3 Aa	0.4 ± 1.0 Aa	0.4 ± 1.0 Aa

Means followed by the same uppercase letter in the rows for each environment and means followed by the same lowercase letter in the columns for each variable indicate no significant difference according to Wilcoxon and Mann-Whitney tests, respectively, at 5 % probability. Asterisk (*) indicates a significant difference between means from the edge and the interior, for the same group of plants, according to the Mann-Whitney test at 5 % probability.

At the edge, plants that suffered damage by herbivores had greater shoot height ($U = 109.0$, $p < 0.001$), number of leaves ($U = 97.0$, $p < 0.001$) and number of pseudobulbs ($U = 175.0$, $p = 0.0416$), when compared to non-damaged plants. The damaged plants experienced increases in SH ($Z = 2.0849$, $p = 0.0371$), NP ($Z = 4.0145$, $p < 0.001$), and NR ($Z = 3.5839$, $p < 0.001$), over the period of 24 months. For non-damaged plants, only the number of leaves increased significantly until the end of the study ($Z = 2.7308$, $p = 0.0063$. Tab. 2). In the interior, there were no significant differences in morphometric parameters between damaged and non-damaged plants. Damaged plants showed a significant reduction in the number of leaves ($Z = 2.3805$, $p = 0.0173$), while non-damaged plants experienced a reduction in shoot heigh ($Z = 1.9649$, $p = 0.0495$). The number of roots and pseudobulbs did not differ significantly between edge plants and interior plants (Tab. 2).

Damage caused by exophytic parasites was verified at the edge. The herbivore insects fed on shoot sap and mature leaves of reintroduced plants, causing chlorotic spots, necrosis, and leaf loss (Fig. 3A-D). Young and adult individuals of *Tenthecoris bicolor* J. Scott, 1886 (Hemiptera) were responsible for multiple whitish spots on leaf surfaces (Fig. 3A). Some of the leaves were blackened and easily detached from the body of the plant. Characteristic leaf marks (Fig. 3B) were also recorded in order to identify consumption by *T. bicolor*. Chlorotic spots with another pattern, caused by *Helionothrips errans* (Williams, 1916) (Thysanoptera), were also recorded for the *C. intermedia* plants at the edge. This kind of mark is characteristic of fecal pellet deposition by *H. errans* and is usually detected on the abaxial surface of leaves (Fig. 3D).

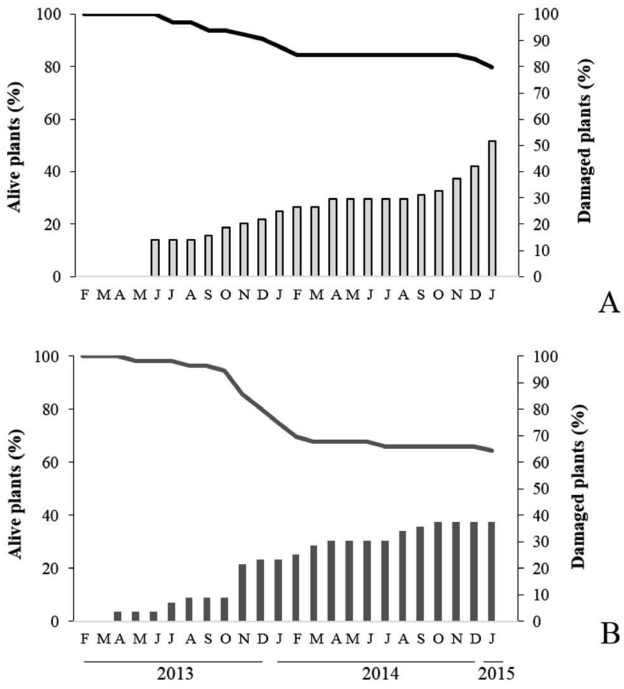

Figure 2. Percentage of live plants (line) and accumulated percentage of plants damaged by herbivores (columns) in relation to the initial number of live plants at the forest edge (**A**) and interior (**B**).

The consumption of leaf tissue by the leaf-chewing larvae of *Ithomiola nepos* (Fabricius, 1793) (Lepidoptera) was recorded for plants of both environments evaluated, which causes the formation of "windows" (Fig. 3E), or even the loss of whole leaves (Fig. 3F). The pupae (Fig. 3G) were collected and kept in the laboratory on seedlings of *C. intermedia* until species identification was possible (Fig. 3H).

The *C. intermedia* plants reintroduced into the forest interior exhibited damage caused by leaf-mining insect larvae (Coleoptera) (Fig. 3I). In some plants, the feeding tunnels created by these larvae were in their early stage and restricted to the leaves, while in others damage extended to the caulinar tissues and led to plant death; 83.3 % of plants damaged by the leaf-mining insects died.

Molomea magna (Walker, 1851) (Hemiptera) was also recorded causing damage to the orchids. Although leafhoppers do not feed on *C. intermedia*, they usually select reintroduced plants as hosts for oviposition. Leafhoppers leave scars on the abaxial side of leaves and secret a white substance prior to egg-laying (Fig. 3J). The eggs were collected and kept in the laboratory on scarred leaves of *C. intermedia* (Fig. 3K) until identification of the *M. magna* nymphs was possible (Fig. 3L). Since these leaves were collected, the damaged caused by leafhoppers was not considered in the analyses of mortality and morphometric parameters.

Abiotic data analysis

Rainfall between February 2013 and January 2014 exceeded 1800 milimeters, with a monthly mean of 158.12 milimeters (min. 60.2 mm and max. 370.9 mm). From February 2014 to January 2015, rainfall exceeded 2200 milimeters with a monthly mean of 189.92 milimeters (min. 44.0 and max. 381.8 mm). Higher values of luminosity were observed at the edge than in the interior of the forest in the first ($U = 83.50$, $p = 0.0028$) and second year of the quantitative analysis ($U = 122.00$, $p = 0.0349$). There were no statistically significant differences in mean relative air humidity and temperature between the two environments (Tab. 3).

Multivariate data analysis

The first principal component of the PCA explained 57.09 % of the data obtained for the edge of the fragment, with mortality, temperature and photoperiod being positively correlated with each other, and relative air humidity being negatively correlated with them. The second component explained 19.39% of the variance, and included the number of damaged plants, luminosity and rainfall, with the first being negatively correlated with the second two (Fig. 4A). The two axes explained 76.48 % of the total variance of the data. The first principal component of the PCA explained 52.10 % of the data obtained for the interior of the fragment, including mortality, number of damaged plants, temperature, luminosity and photoperiod, which were positively correlated with each other. The second component explained 22.37 % of the variance, and included relative air humidity and rainfall, which were positively correlated (Fig. 4B). The two axes explained 74.47 % of the total variance of the data.

Discussion

The plants at the edge of the forest fragment exhibited more variation in the growth parameters than the plants at the interior. This difference is due to the leaf loss that many of the young plants experienced during the process of adapting to the new meteorological conditions with reintroduction into the environment (Endres Júnior *et*

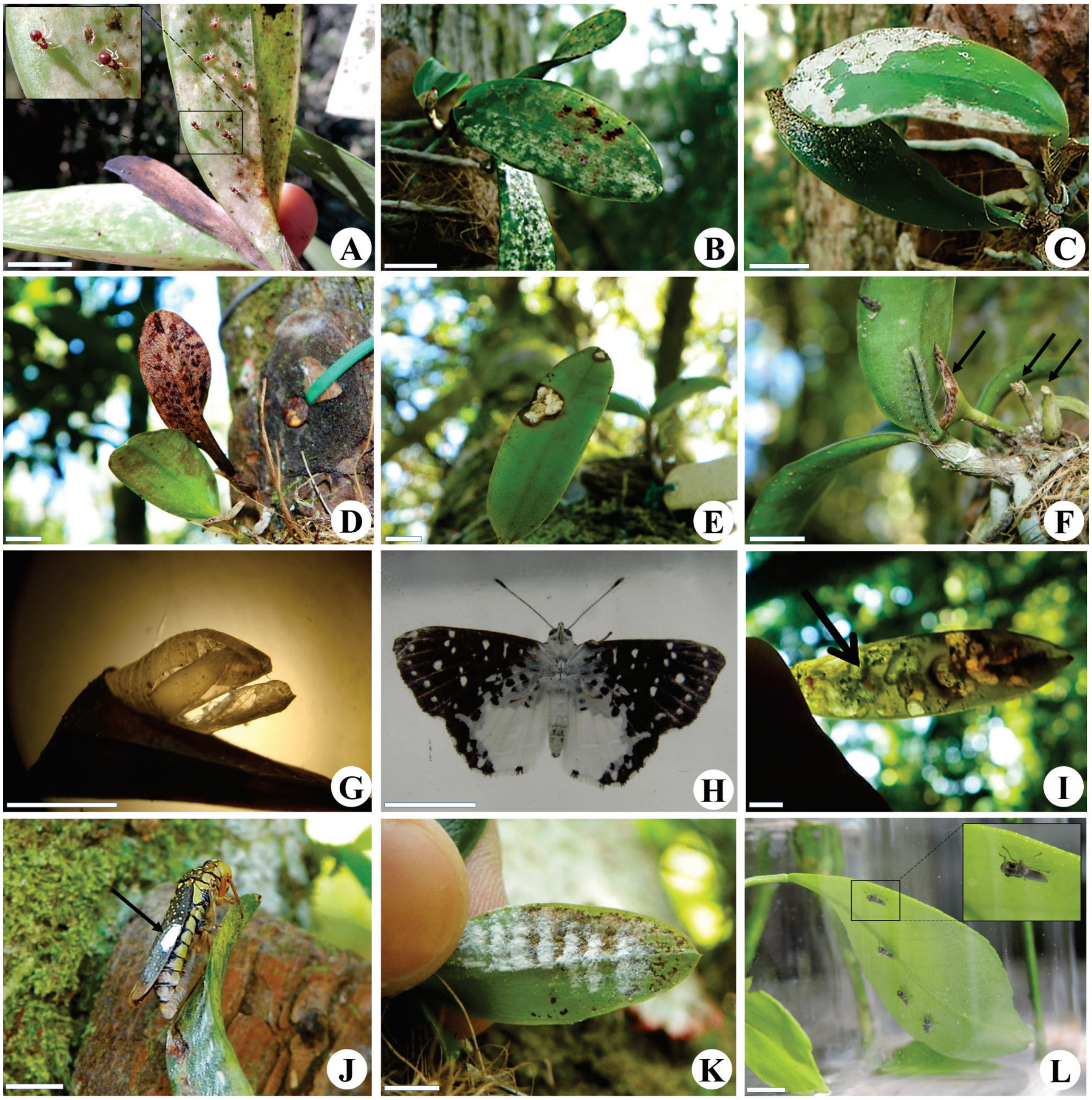

Figure 3. Records of herbivory damage to reintroduced *Cattleya intermedia* plants and the insects associated with the damage. Leaves damaged by *Tenthecoris bicolor* (insect in evidence) on abaxial side (**A**); leaf adaxial side with chlorotic spots caused by the sap-sucker *T. bicolor*, where it is also possible to observe necrotic regions (**B**); leaf adaxial side with chlorotic spots caused by the sap-sucker *Helionothrips errans* and (**C**); necrotic leaf after insect action with characteristic fecal pellets on the abaxial side (**D**); "window" formed by consumption by *Ithomiola nepos* larvae in its earliest stage of development (**E**); *I. nepos* larvae in the last stage before the pupae; arrows indicate the remaining caulinar regions after the consumption of three leaves of the same plant (**F**); *I. nepos* pupae found on a *C. intermedia* leaf after imago ecdysis (**G**); *I. nepos* after metamorphosis in the laboratory (**H**); view of abaxial side of a leaf with ovoposition structure of *Molomea magna* (**I**); leaf damaged by leaf-mining Coleoptera larvae (insect in evidence) (**J**); scarred leaf after hatching of *M. magna* eggs (**K**); *M. magna* nymphs in the laboratory (**L**).

al. 2015b). Shoot height and the number of leaves are not independent variables, since the former is measured as the distance from the base of the rhizome to the tip of the longest leaf of the plant, and both parameters experienced a reduction. At the same time, some of individuals at the edge did not exhibit a decrease in morphometric parameters after reintroduction, and formed new shoots with their first pseudobulbs and roots attached directly to the trunk of the host phorophytes.

The rate of herbivory damage was higher at the forest edge when compared to the interior. In several forest types, edge formations appear to act positively on herbivore activity; Wirth *et al*. (2008) verified that approximately 80 % of such studies indicated this tendency. This result may be related to a preference by adult insects to oviposite on plants that are more exposed to the sun (Moore *et al*. 1988), and thus a faster development of insects near the edge (Cappuccino & Martin 1997). It also may be due to the physiological conditions of the plants, such as higher nutritional value of the leaves due to the disponibility of nitrogen and soluble sugars, thus increasing the quality of the food and its palatability (Fortin & Maufette 2001). Furthermore, plants under stressful conditions at the edge can increase nutrient availability to herbivores, which is mainly exploited by larvae and may contribute to population growth (White 1984).

The most frequent herbivores recorded at the edge of the forest fragment were *H. errans* and *T. bicolor*. *H. errans* is considered an important herbivore of Orchidaceae in other countries (Gillespie 2008) and was recorded for the first time in South America feeding on *C. intermedia* by Endres Júnior *et al*. (2015a). *T. bicolor* was observed damaging native individuals of *C. intermedia* and also of *C. tigrina*. These records are considered highly important, since they allow the knowledge about animal-plant relationships.

Insects feed on the epidermis and neighbouring adjacent cells, causing chlorosis to leaf tissues and leaf abscission (Gillespie 2008; Light & MacConaill 2011). These intense and/or continuous leaf injuries can negatively affect plant development due to the loss of mesophyll content and the consequent loss of available energy for vegetative growth (Pellegrino & Musacchio 2006; Endres Júnior *et al*. 2015b). Leaf loss had no such a significant impact on the survival and development of the plants at the edge, since the means of this variable were equal at the beginning and at the end of the quantitative study period, even though there was an increase of other morphometric parameters.

Table 3. Luminosity, relative air humidity and temperature (mean ± standard deviation) from 2013 to 2015 at the edge and the interior of the forest fragment.

	2013-2014	2014-2015
	Luminosity (Lux)	
Edge	776.7 ± 341.0*	644.3 ± 248.2*
Interior	232.0 ± 97.8	208.9 ± 109.8
	Relative air humidity (%)	
Edge	65.4 ± 8.1	72.1 ± 9.5
Interior	68.0 ± 7.7	73.8 ± 7.8
	Temperature (°C)	
Edge	22.9 ± 3.9	23.1 ± 6.0
Interior	22.8 ± 3.6	23.0 ± 6.0

Asterisk (*) indicates a significant difference between edge and interior in the same year, according to the Mann-Whitney test at 5 % probability.

This can be occurring due to the higher size of the damaged orchids and its great morphologic resilience capacity. *C. intermedia* has important regrowth strategies, such as the presence of multiple buds in its rhizome (Stieha *et al*. 2016). At the edge of the fragment, these plants developed more roots and pseudobulbs, also very important nutrient storage organs in Orchidaceae (Zotz 1999). Other compensatory growth resources can be adopted by reintroduced plants (Robert *et al*. 2014), like nutrient translocation from leaves to roots, since there were just a few evidences of damage in roots and pseudobulbs, mainly restricted to Coleoptera herbivory in the interior.

At the edge, plants damaged by herbivores exhibited a higher survival rate than non-damaged plants. The production of new tissues and organs by plants, as well as the appearance of new previously non-existent components, such as roots, shoots and leaves, can alter "plant appearance", which is related to susceptibility to herbivores (Feeny 1976). Rate of herbivory is related to plant phenotype, which may vary among individuals of the same population due to genetic characteristics or the environmental factors to which they are exposed (Feeny 1976). Thus, since plants at the beginning of the quantitative study period already varied, herbivores were directed to the plants most susceptible to being discovered, which were those that were most developed. The survival time and the number of live individuals after reintroduction in each environment can also inffluence positively herbivore discovery rates, as demonstrated by the results obtained for edge and interior.

Considering that all the insects recorded in the present study used *C. intermedia* plants for reproduction, the choice of larger individuals is an interesting strategy that may ensure that the larvae or nymphs have their survival and development guaranteed, since the total consumption of host tissues, which could happen with smaller plants, would cause the resource to become unavailable (Schmidt & Zotz 2000). So, the use of larger plants of *C. intermedia* for reintroduction may be beneficial. Schmidt & Zotz (2000) observed that damage caused by larvae of *Napaea eucharilla* (Riodinidae, Lepidoptera) on *Werauhia sanguinolenta* was higher in larger individuals, with a reduction of 25-30 % of leaf area per year, although, mortality related to herbivory was low, between 1.8 and 2.6 %. In our study, the intense damage to leaves and shoots of the reintroduced plants did not lead them to death at the edge, as the individuals quickly formed new shoots after damage. After the leaf damage, the edge plants immediately formed new buds, which seems to be a circular cause-and-effect relationship between "plant appearance" and "compensatory growth."

In contrast, smaller plants experienced little development after the number of leaves and shoot height were reduced, leading to their death a few months later, which was positively related to temperature and photoperiod. These results corroborate the fact that epiphytes, when at an early stage of development, are more vulnerable to adverse abiotic conditions of the environment and have higher mortality rates (Zotz & Schmidt 2006). Moreover, it is known that the edge effect acts on the dynamics of certain factors in forest fragments, such as birth rate and mortality of plants (Viana & Pinheiro 1998), but these aspects are scarcely described for epiphytic flora. The multivariate data analysis demonstrated that temperature and photoperiod are more important to mortality of reintroduced *C. intermedia* plants at the edge than herbivory damage.

In the interior of the fragment, plants shown a higher shoot formation and leaf production in periods with higher luminosity (D Endres Júnior unpubl. res.), and this may have caused changes in the plant appearance and increased rates of herbivory. It may happen due to the low luminosity, as recorded here, and other abiotic factors, such as wind speed, which contribute to plant desiccation and leaf abscission (Murcia 1995; Silva & Schmitt 2015). Since there were no significant development differences between individuals, the herbivory rates may have been more related to the seasonal variations of developmental parameters, such as the intensity of shoot formation. Budding increases the availability of plant tissues to herbivores, so most leaf damage occurs during leaf expansion (Coley & Aide 1991). Damage caused by herbivorous insects had a strong impact on plant development and were related to many cases of mortality. Although herbivory is not usually directly associated with mortality of epiphytic plants (Winkler *et al*. 2005), it was, in fact, as important as abiotic factors to the mortality of reintroduced *C. intermedia* plants in the interior, contrary to what was observed at the edge of the forest fragment. The seasonal increase of luminosity at the forest interior may not keep the rate of photosynthesis, despite providing an increase of budding in the reintroduced plants. Thus, the lower luminosity in the colder seasons of the year lead the plants to have low recovery capacity after damage caused by herbivore insects (Reichman & Smith 1991; Strauss & Agrawal 1999).

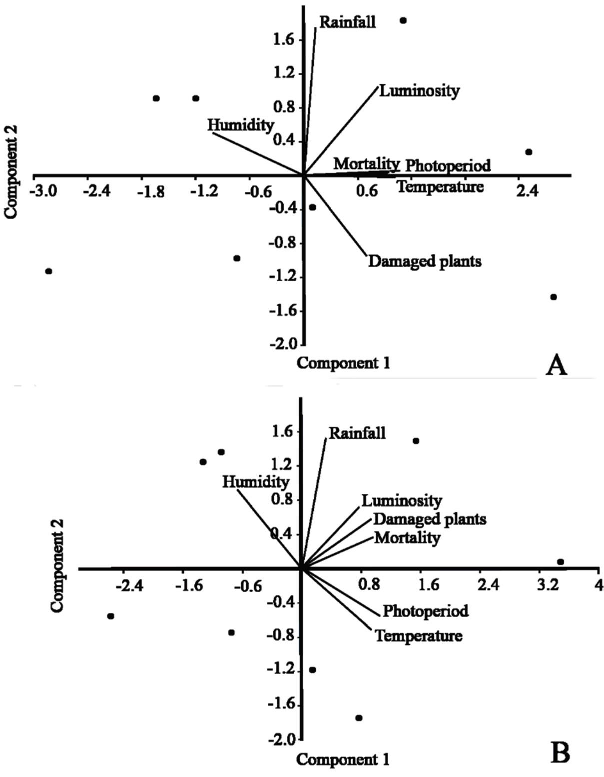

Figure 4. Principal component analysis biplot graph of scores for the two components of the edge (**A**) and the interior (**B**) of the forest fragment.

Coleoptera larvae were only recorded in the reintroduced orchids in the forest interior. These cases resulted in plant death in a short period of time, and thus are quite dangerous for *C. intermedia* plants, which may be due to the type of tissue that these insects were consuming. In contrast to the other insect species of the present study, these larvae were deposited in the inner part of the leaves, but also reached the rhizomes, which eventually lead to the loss of meristematic tissue and, ultimately, death (Winkler *et al*. 2005).

The larvae of *I. nepos* have been prevously cited by Endres Júnior *et al*. (2015b) as an herbivore of *C. intermedia* at forest edge and interior. This species belongs to Riodinidae, a family known for epiphyte herbivory. This species was also verified as an herbivore of *C. intermedia* in the present study. *Napaea eucharilla* was recorded on *Werauhia sanguinolenta* (Schmidt & Zotz 2000) and other species of Bromeliaceae (DeVries 1997). *Cremna thasus* was cited by Zotz (1998) as an herbivore of *Dimerandra emarginata*, and by DeVries (1997) on *Caularthron bilamellatum*. Although this species has not been recorded on other species of orchids until the end of the study, this species can probably use other plants of this family as food resources, according to Callaghan (1991).

Even though it was not observed consuming *C. intermedia*, the leafhopper *M. magna* caused damage to leaf tissues of the reintroduced plants in both environments. *M. magna* is a sap-sucking phytophage associated with *Citrus sinensis* (Azevedo-Filho & Carvalho 2005) in Rio Grande do Sul, Brazil. So, the record of the animal-plant relationship established in this study is of great importance, since there is no detailed information on leafhopper reproductive behavior in the literature. In general, leafhoppers reproduce by selecting leaves, depositing their eggs on the abaxial face (DeLong 1971) and covering them with brocosomes, which are lipoprotein structures for egg protection (Rakitov

2002; Azevedo-Filho & Carvalho 2005). This species seems to be the most selective in relation to the size of the plants it uses for oviposition, using only mature leaves of *C. intermedia* plants with an average shoot height of 8.0 cm. The grooves made by these insects on the abaxial surface of leaves were very characteristic and easily identified during the inspections, when oviposition events were also observed on adult individuals of *Lophiaris pumila*, a native orchid. When leaves of *C. intermedia* and *L. pumila* possessing eggs of *M. magna* were collected and maintained at a constant temperature of 26 °C, incubation occurred for between six and 14 days after collection. Eight to 28 nymphs emerged from each oviposition, which died in about 48 hours remaining with the leaves of the two orchid species, suggesting that this species feeds on another plant species present in the forest fragment (Azevedo-Filho & Carvalho 2005).

C. intermedia proved to be an important resource for insects, since all the insects collected used these plants for reproduction. In addition, it is expected that when individual plants of *C. intermedia* reach reproductive maturity, new relationships will be established given that they will possess increased architectural complexity (Lawton 1983). Also, the development of this orchid increases the availability of resources, such as flowers, fruits and seeds. Epiphytes are important in maintaining the diversity of arthropods in the tropical forest canopy (Benzing 1990; Stork 1987a; b), especially given their high diversity (Benzing 1990). This emphasizes the importance of epiphytic plants for the maintenance of biological diversity. The use of epiphytic species in forest restoration programs may contribute to the colonization and maintenance of insect species specialized on this plant group, due to increased species diversity and the availability of resources.

The relationships established between the reintroduced plants and the herbivorous insects differed between the two environments evaluated, as did survival and developmental responses to damage. While herbivory was as important as abiotic factors regarding mortality in the interior of the forest, abiotic factors were more important than herbivory regarding mortality at the edge. However, herbivory is an important factor to be considered in the design of conservation studies of endangered orchid species. In association with abiotic factors, herbivory can impact the performance of plants reestablished *in situ*.

The outstanding representativeness and importance of orchids among tropical and subtropical epiphytes is made explicit by several studies performed in Brazil (Kersten & Silva 2001; Borgo & Silva 2003; Hefler & Faustioni 2004; Staudt *et al*. 2012). Orchid diversity is related to the specificity of many species. Native *Cattleya* sp. are normally endemic to the country or have a restricted distribution (Flora do Brasil 2018) and are undeniable representatives of this group diversity. Thus, these plants are more vulnerable to extinction, such as the case with *C. intermedia*, than those with greater plasticity (Martinelli & Moraes 2013).

The high specificity of orchids and the difficulty of finding protected forest areas restrict the choice of fragments that could allow detailed studies about the relationship between reintroduced plants and environmental variables. Even within a single area, it is important to compare environments with different characteristics when studying orchids. We demonstrated that some of the interactions between biotic and abiotic factors of each environment affected the survival and the development of plants reintroduced to their natural habitat. Luminosity was a factor that positively influenced the development of *C. intermedia*. Other factors can also contribute to the success of the reintroduction of these plants, which may also be determinant for other species. Growing under controlled conditions after acclimatization can provide plants with greater resistance to the impact suffered by moving them to the natural environment.

The main contribution of this study is detailed information about monitoring reintroduced plants and their relationship with biotic and abiotic factors, which is usually not considered in reintroduction studies. Herbivory on epiphytic orchids, for example, was demonstrated to be an important factor for the success of plant reintroduction in forest fragments.

Acknowledgements

The authors would like to thank CAPES for providing research grants for the first and second authors; Dr. Ricardo R. Siewert, Dr. Adriano Cavalleri and Dr. Gervásio Silva Carvalho for identifying the insect species; and the anonymous reviewers for their valuable comments that improved the manuscript.

References

Adhikari YP, Fischer A, Fischer HS. 2012. Micro-site conditions of epiphytic orchids in a human impact gradient in Kathmandu Valley, Nepal. Journal of Mountain Science 9: 331-342.

Aggarwal S, Nirmala C, Beri S, Rastogi S, Adholeya A. 2012. *In vitro* symbiotic seed germination and molecular characterization of associated endophytic fungi in a commercially important and endangered indian orchid *Vanda coerulea* Griff. Ex Lindl. European Journal of Environmental Sciences 2: 33-42.

Azevedo-Filho WS, Carvalho GS. 2005. Brochosomes-for-eggs of the Proconiini (Hemiptera: Cicadellidae, Cicadellinae) species associated with orchards of *Citrus sinensis* (L.) Osbeck in Rio Grande do Sul, Brazil. Neotropical Entomology 34: 387-394.

Bataghin FA, Pires JSR, Barros F. 2012. Epifitismo vascular em sítios de borda e interior em Floresta Estacional Semidecidual no Sudeste do Brasil. Hoehnea 39: 235-245.

Benzing DH. 1990. Vascular epiphytes. New York, Cambridge University Press.

Bernardi S, Budke JC. 2010. Estrutura da sinúsia epifítica e efeito de borda em uma área de transição entre Floresta Estacional Semidecídua e Floresta Ombrófila Mista. Floresta 40: 81-92.

Bianchi JS, Kersten RA. 2014. Edge effect on vascular epiphytes in a subtropical Atlantic Forest. Acta Botanica Brasilica 28: 120-126.

Borgo M, Silva SM. 2003. Epífitos vasculares em fragmentos de Floresta Ombrófila Mista, Curitiba, Paraná, Brasil. Revista Brasileira de Botânica 26: 391-401.

Brasil. 1994. Decreto n° 2, de 03 de fevereiro de 1994. Aprova o texto da convenção sobre diversidade biológica, assinada durante a Conferência das Nações Unidas sobre Meio Ambiente e Desenvolvimento, realizada na Cidade do Rio de Janeiro, no período de 5 a 14 de junho de 1992. Brasília, LexML - Diário Oficial da União, n. 25, seção 1.

Buzatto CR, Ferreira PPA, Welker CAD, Seger GDS, Hertzog A, Singer RB. 2010. O gênero *Cattleya* Lindl. (Orchidaceae: Laeliinae) no Rio Grande do Sul, Brasil. Revista Brasileira de Biociências 8: 388-398.

Callaghan CJ. 1991. Notes on the immature biology of two Riodinine butterflies: *Metacharis ptolomaeus* and *Napaea nepos Orpheus* (Lycaenidae). Journal of Research on the Lepidoptera 30: 221-224.

Cappuccino N, Martin MA. 1997. The birch tube-maker Acrobasis betulella in a fragmented habitat: the importance of patch isolation and edges. Oecologia 110: 69-76.

CNC Flora. 2018. *Cattleya intermedia*. In: Lista Vermelha da flora brasileira versão 2012.2 Rio de Janeiro, Centro Nacional de Conservação da Flora. http://cncflora.jbrj.gov.br/portal/pt-br/profile/Cattleya%20 intermedia. 09 Jan. 2018.

Coley PD, Aide TM. 1991. Comparison of herbivory and plant defenses in temperate and tropical broad-leaved forests. In: Price PW, Lewinsohn TMW, Fernandes W, Benson WW. (eds.) Plant-animal interactions: evolutionary ecology in tropical and temperate regions. New York, John Wiley and Sons. p. 25-49.

Decruse SW, Gangaprasad A, Seeni S, Menon S. 2003. Micropropagation and ecorestoration of *Vanda spathulata*, an exquisite orchid. Plant Cell, Tissue and Organ Culture 72: 199-202.

Del-Claro K, Torezan-Silingardi HM. 2012. Ecologia das interações plantas-animais. Rio de Janeiro, Technical Books.

DeLong DM. 1971. The bionomics of leafhoppers. Annual Review of Entomology 16: 179-210.

DeVries PJ. 1997. The butterflies of Costa Rica and their natural history. Vol. II. Princeton, Princeton University Press.

Dorneles LT, Trevelin V. 2011. Aclimatação e reintrodução de *Cattleya intermedia* Graham ex Hook (Orchidaceae) obtidas por propagação *in vitro*. Iheringia, Série Botânica 66: 167-174.

Endres Júnior D, Sasamori MH, Cavalleri A, Droste A. 2015a. *Helionothrips errans* (Thysanoptera: Thripidae): a new threat to native orchids in Brazil. Florida Entomologist 98: 1247-1249.

Endres Júnior D, Sasamori MH, Silveira T, Schmitt JL, Droste A. 2015b. Reintrodução de *Cattleya intermedia* Graham (Orchidaceae) em borda e interior de um fragmento de Floresta Estacional Semidecidual no sul do Brasil. Revista Brasileira de Biociências 13: 33-40.

Feeny P. 1976. Plant apparency and chemical defense. In: Wallace JW, Mansell RL. (eds.) Biochemical interaction between plants and insects. New York, Plenum Press. p. 1-40.

Flora do Brasil. 2018. *Cattleya*. http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB11329. 10 Jan. 2018.

Fortin M, Maufette Y. 2001. Forest edge effects on the biological performance of the forest tent caterpillar. Ecoscience 8: 164-172.

Fundação SOS Mata Atlântica. 2018. Nossa causa: Floresta. https://www.sosma.org.br/nossa-causa/a-mata-atlantica/. 09 Jan. 2018.

Gillespie P. 2008. New thrips pest of orchids. NSW Department of Primary Industries. Primefact 817: 1-3.

Gonçalves CN, Waechter JL. 2003. Aspectos florísticos e ecológicos de epífitos vasculares sobre figueiras isoladas no norte da planície costeira do Rio Grande do Sul. Acta Botanica Brasilica 17: 89-100.

Guerrant EO, Kaye TN. 2007. Reintroduction of rare and endangered plants: common factors, questions and approaches. Australian Journal of Botany 55: 362–370.

Hai R, ShuGuang J, HongXiao L, QianMei Z, HongFang L. 2014. Advances in the reintroduction of rare and endangered wild plant species. Science China, Life Scineces 57: 603-609.

Hammer O, Harper DAT, Ryan PD. 2001. PAST: Paleontological Statistics package for education and data analysis. Paleontologia Eletrônica 4: 1-9.

Hefler SM, Faustioni P. 2004. Levantamento florístico de epífitos vasculares do bosque São Cristovão –Curitiba – Paraná – Brasil. Revista Estudos de Biologia 26: 11-19.

IBGE – Instituto Brasileiro de Geografia e Estatística, MMA – Ministério do Meio Ambiente. 2004. Biomas do Rio Grande do Sul. http://www.atlassocioeconomico.rs.gov.br/biomas. 10 Jan. 2018.

Kersten RA, Silva SM. 2001. Composição florística e estrutura do componente epifítico vascular em floresta de planície litorânea na Ilha do Mel, Paraná, Brasil. Revista Brasileira de Botânica 24: 213-226.

Laurance WF, Yensen E. 1991. Predicting the impacts of edge effects in fragmented habitats. Biological Conservation 55: 77-92.

Lawton JH. 1983. Plant architecture and the diversity of phytophagous insects. Annual Review of Entomology 28: 23-29.

Light MHS, MacConaill M. 2011. Potential impact of insect herbivories on orchid conservation. European Journal of Environmental Sciences 1: 115-124.

Martinelli G, Moraes MA. 2013. Livro Vermelho da Flora do Brasil. Rio de Janeiro, Instituto de Pesquisas Jardim Botânico do Rio de Janeiro.

Moore LV, Myers JH, Eng R. 1988. Western tent caterpillars prefer the sunny side of the tree, but why? Oikos 51: 321-326.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497.

Murcia C. 1995. Edge effects in fragmented forests: implications for conservation. Tree 10: 58-62.

Parthibhan S, Kumar T, Rao MV. 2015. Phenology and reintroduction strategies for *Dendrobium aqueum* Lindley – An endemic, near threatened orchid. Journal for Nature Conservation 24: 68-71.

Pellegrino G, Musacchio A. 2006. Effects of defoliation on reproductive success in two orchids, *Serapias vomeracea* and *Dactylorhiza sambucina*. Annales Botanici Fennici 43: 123-128.

Peng SL. 2003. Studies and practices of restoration ecology in tropical and subtropical area. Beijing, Science Press.

Rakitov RA. 2002. What are brochosomes for? An enigma of leafhoppers (Hemiptera, Cicadellidae). Denisia 4: 411-432.

Reichman OJ, Smith SC. 1991. Responses to simulated leaf and root herbivory by a biennal, *Tragopogon dubius*. Ecology 72: 116-124.

Rio Grande do Sul. 2014. Decreto n° 52.109, de 01 de dezembro de 2014. Declara as espécies da flora nativa ameaçadas de extinção no Estado do Rio Grande do Sul. Porto Alegre, Lex-Diário Oficial do Rio Grande do Sul, ano LXXII, nº 233, 2-11.

Robert CAM, Ferrieri RA, Schirmer S, *et al*. 2014. Induced carbon reallocation and compensatory growth as root herbivore tolerance mechanisms. Plant, Cell and Environment 37: 2613-2622.

Rubluo A, Chávez V, Martínez AP, Martínez-Vázquez O. 1993. Strategies for the recovery of endangered orchids and cacti through *in vitro* culture. Biological Conservation 63: 163-169.

Sasamori MH, Endres Júnior D, Droste A. 2014. Sobrevivência e desenvolvimento de plântulas de *Cattleya intermedia* Graham (Orchidaceae) micropropagadas e aclimatadas em substratos com fibra de coco. Pesquisas, Botânica 65: 293-303.

Sasamori MH, Endres Júnior D, Droste A. 2015. Asymbiotic culture of *Cattleya intermedia* Graham (Orchidaceae): the influence of macronutrient salts and sucrose concentrations on survival and development of plantlets. Acta Botanica Brasilica 29: 292-298.

Schmidt G, Zotz G. 2000. Herbivory in the epiphyte, *Vriesea sanguinolenta* Cogn. & Marchal (Bromeliaceae). Journal of Tropical Ecology 16: 829-839.

Seeni S, Latha PG. 2000. *In vitro* multiplication and ecorehabilitation of the endangered Blue Vanda. Plant Cell, Tissue and Organ Culture 61: 1-8.

Silva VL, Schmitt JL. 2015. The effects of fragmentation on Araucaria forest: analysis of the fern and lycophyte communities at sites subject to different edge conditions. Acta Botanica Brasilica 29: 223-230.

Staudt MG, Lippert APU, Cunha S, Becker DFP, Marchioretto MS, Schmitt JL. 2012. Composição florística de epífitos vasculares do Parque Natural Municipal Tupancy, Arroio do Sal, RS – Brasil. Pesquisas, Botânica 63: 177-188.

Stieha CR, Abbott KC, Poveda K. 2016. The effects of plant compensatory regrowth and induced resistence on herbivore population dynamics. The American Naturalist 187: 167-181.

Stork NE. 1987a. Arthropod faunal similarity of Bornean rain forest trees. Ecological Entomology 12: 219-226.

Stork NE. 1987b. Guild structure of arthropods from Bornean rain forest trees. Ecological Entomology 12: 69-80.

Strauss SY, Agrawal AA. 1999. The ecology and evolution of plant tolerance to herbivory. Tree 14: 179-185.

Viana VM, Pinheiro LAFV. 1998. Conservação da biodiversidade em fragmentos florestais. Série Técnica IPEF 12: 25-42.

White TCR. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia 63: 90-105.

Winkler M, Hülber K, Mehltreter K, Franco JG, Hietz P. 2005. Herbivory in epiphytic bromeliads, orchids and ferns in a Mexican montane forest. Journal of Tropical Ecology 21: 147-154.

Wirth R, Meyer ST, Leal IR, Tabarelli M. 2008. Plant herbivore interations at the forest edge. In: Lüttge U, Beyschlag W, Murata J. (eds.) Progress in Botany 69. Berlin/ Heidelberg, Springer-Verlag. p. 423-448.

Wu K, Zeng S, Lin D, *et al*. 2014. *In vitro* propagation and reintroduction of the endangered *Renanthera imschootiana* Rolfe. PLOS ONE 9: 1-12. doi:10.1371/journal.pone.0110033

Zettler LW, Poulter SB, McDonald KI. 2007. Conservation-driven propagation of an epiphytic orchid (*Epidendrum nocturnum*) with a mycorrhyzal fungus. HortScience 42: 135-139.

Zotz G. 1998. Demography of the epiphytic orchid, *Dimerandra emarginata*. Journal of Tropical Ecology 14: 725-741.

Zotz G. 1999. What are Backshoots Good For? Seasonal Changes in Mineral, Carbohydrate and Water Content of Different Organs of the Epiphytic Orchid, *Dimerandra emarginata*. Annals of Botany 84: 791-798.

Zotz G, Schmidt G. 2006. Population decline in the epiphytic orchid *Aspasia principissa*. Biological Conservation 129: 82-90.