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Scientific Article

Treatments to prolong the postharvest life of *Heliconia wagneriana* Petersen

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Abstract

Tropical flowers have a growing market due to their exotic appearance and thus postharvest techniques are necessary to prolong their shelf life. The objective of this study was to evaluate the effectiveness of wax and salicylic acid (1 mM) in reducing chilling injury and increase the vase life in stems of *Heliconia wagneriana* stored at low temperature. For that, 120 stems were harvested in a commercial area. From these, 60 stems were waxed and air-dried; while 60 unwaxed stems served as controls. Then 2 groups of 48 stems from both treated and control were cold-stored at 13 °C and 84 % RH for 5 and 10 days. After storage, the stems were placed either in salicylic acid solution (1 mM) or tap water. The remaining stems (12 waxed and 12 control) were kept at room temperature. The variables evaluated were anatomical characteristics, fresh weight loss, solution uptake, vase life, enzymatic activity (polyphenol oxidase and peroxidase), and membrane integrity. The wax coating maintained the fresh weight and extended the vase life of the stored stems under room temperature for up to 2 more days. The vase life of the cold-stored stems of 5 and 10 days was extended by 3 and 2 more days respectively, as compared to the control. No significant effect was observed for the use of salicylic acid (1 mM). The bracts tissue of the waxed stems showed lower enzymatic activity, reflected in lower oxidative stress compared to the control. Tropical species as heliconia present a very low water absorption, then the use of wax coating is recommended to preserve the turgidity and shelf life of the stems.

Keywords: *Heliconia wagneriana*, solution uptake, enzymatic activity, waxes, vase life.

Resumo

Tratamentos para prolongar a vida pós-colheita de *Heliconia wagneriana* Petersen

As flores tropicais têm um mercado crescente, devido ao seu aspecto exótico, sendo necessárias técnicas para prolongar a sua vida útil**.** Assim, este estudo buscou avaliar a eficácia do uso de cera e ácido salicílico (1 mM) em hastes de *Heliconia wagneriana* armazenadas em baixa temperatura, a fim de reduzir as lesões por frio e prolongar a vida de vaso. Para isso, 120 hastes foram colhidas em uma área comercial. Destas, 60 hastes foram enceradas e secas ao ar; enquanto que 60 hastes não-enceradas serviram como controle. Em seguida, 2 grupos de 48 hastes, tanto enceradas quanto do controle, foram armazenadas a 13 °C e UR de 84% por 5 e 10 dias. Após o armazenamento, as hastes foram colocadas em solução de ácido salicílico (1 mM) ou água. As hastes restantes (12 enceradas e 12 controle) foram mantidas em temperatura ambiente. As variáveis avaliadas foram características anatômicas, perda de massa fresca, consumo de solução, vida de vaso, atividade enzimática (polifenol oxidase e peroxidase) e integridade de membrana. O enceramento manteve o peso fresco e prolongou a vida de vaso das hastes armazenadas em vaso em temperatura ambiente por até mais 2 dias. A vida de vaso das hastes refrigeradas de 5 e 10 dias estendeu-se por 3 e 2 dias a mais, respectivamente, em relação às hastes controle. Nenhum efeito significativo foi observado para o uso de ácido salicílico (1 mM). O tecido das hastes enceradas apresentou menor atividade enzimática, refletindo em menor estresse oxidativo em relação ao controle. Espécies tropicais como as helicônias apresentam baixa absorção de água, sendo recomendado o uso de recobrimento de cera para preservar a turgidez e a vida útil das hastes.

Palavras-chave: *Heliconia wagneriana*, absorção, atividade enzimática, ceras, vida de vaso.

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Introduction

Tropical flowers are becoming popular due to their shapes and colors, and these include different heliconia species. The genus Heliconia has about 250 species, of which 16 are native to Mexico, the most commercially known being *H. wagneriana* (Gómez-Merino et al., 2018). However, postharvest handling and storage temperatures represent a challenge to prolong their vase life, which is necessary to reach more distant markets. Postharvest techniques such as stem trimming and the application of preservative solutions are commonly used to maintain stem turgidity and prolong the vase life of cut flowers. In *Heliconia wagneriana*, trimming at the base of the stem every 48-h, combined with 10% sucrose reduced weight loss (Costa et al., 2015). Refrigeration is another commonly used technique since low temperatures reduce metabolic processes such as respiration and transpiration and consequently the loss of water. However, in tropical flowers, the temperatures used for traditional floral products - such as roses, chrysanthemums and carnations (0-2 °C) - have been associated with chilling injury occurrence, with symptoms of depressions and browning of the bracts, thereby resulting in short vase life. This susceptibility is closely related to the composition of their plasma membrane (sterols/phospholipids), and depended on genotype, tissue maturity and level and duration of cold storage (Darras, 2019).

Vase solutions are commonly used to prolong shelf life for cut flowers, generally contain sucrose, moisturizing agents, biocides and/or antioxidants; also the use of ethylene inhibitors such as nano-silver particles, 1-methylcyclopropene (1-MCP) and selenium (Scariot et al., 2014; Costa et al., 2020; Rabiza-Świder et al., 2020). The use of growth regulators in vase solutions such as salicylic acid (AS), a plant defense activator, has proven to be effective in reducing transpiration by the means of stomatal closure, stimulating the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR). For instance, stem-end dipping of SA (2 mM) in *Anthurium andraeanum* stems decreased the negative effects of chilling injury (browning index) at 4 ± 0.5 °C (Soleimani et al., 2016).

The application of wax coatings is used as a postharvest technique to improve appearance, reduce weight loss, and provide protection to the plant tissue (Carrera-Alvarado et al., 2020). The use of waxes has been frequently reported in fruits such as custard apple (Netravati et al., 2018), pineapple (Li et al., 2018), and citrus (Pereira et al., 2016), among others. In ornamental species such as *Etlingera elatior* the application of carnauba wax at 0.75%, 1.5% and 3.0% (p/v) did not affect the physiological quality of the inflorescences (Mattos et al., 2017). In *Heliconia psittachroum* cv. Golden Torch, the application of different concentrations of wax emulsions (0.25%, 0.50%, 0.75% and 1.0%) allowed the stems to have 48% higher fresh weight than the control stems and the vase life will increase from 10 to 11.78 d (Powar et al., 2014). Therefore this study aimed to evaluate the effect of wax, cold storage (13 °C and 84% RH) and SA (1 mM) in a preservative solution on the quality and vase life of *H. wagneriana* stems.

Material and methods

Stems of *H. wagneriana* were harvested in a commercial plantation and transported within 5 h to the laboratory, where 120 stems were selected, trimmed to 1.2 m from which the flowers inside the bracts were removed and divided into ten groups of 12 stems each. Five groups were sprayed with Decco wax (Vegetable Lustr® 227 F) and the other five were considered as controls. Four groups of each condition (wax and control) were stored at 13 °C and 84% RH, 2 groups for 5-d and 2 for 10-d. At the end of each period, the stems were unpacked, weighed and each group was placed in a flask with distilled water or salicylic acid (1 mM) solution (Carrera-Alvarado et al., 2020). The remaining two groups (control and waxed) were placed in flasks with 400 mL of distilled water and kept at room temperature (20 ± 2 °C). Every 5 days the solution in the flask vase was renewed and a cutout of 2 to 3 cm was made at the base of each stem. The experiment was carried out in duplicate and the following variables were evaluated:

Anatomy and frequency of stomata: In five floral stems of *H. wagneriana*, impressions of the epidermis of the bracts, the leaves and the stem were made. A layer of transparent cosmetic varnish was applied between the ribs, allowed to dry for 30 min, the epidermis was peeled off and mounted on a slide, with the printing side facing the microscope. The photographs were taken with the 10X and 40X objective of a Carl Zeiss photomicroscope with an integrated digital camera (PAXcam 3). To obtain the stomatal frequency, per mm^{-2}, the images were processed with Image J ™.

Fresh weight loss (%). On the day 2, the flower stems of the different treatments were weighed with a digital scale (Velab® ES-1000H, with 0.01 g of precision). Weight loss was calculated based on the difference between initial and final weight.

Consumption of the solution (CS): On the day 2, the volume of the solution of each experimental unit was recorded. The consumption of solution by flower stems was calculated based on the difference in weight of the solution and the weight gained by the stem.

Vase life (VL): It was determined by counting the number of days elapsed since the stems were placed in the vase solution until the inflorescences presented wilting in 50% of the bracts.

Total postharvest life (TPL): It was determined by adding to the VL the number of days elapsed since the flower stems were harvested.

Enzymatic activity (polyphenol oxidases and peroxidases): Two days after storage, 10 g of bracts were taken and macerated with liquid nitrogen and 5 mL of cold acetone (-15 °C). The macerate was vacuum filtered, acetone was added and it was filtered again until obtaining acetone powder. The peroxidase activity was determined by the method of Flurkey and Jen (1978) with the following modifications: 0.1 g of acetone powder was added 5 mL of cold Tris-HCl (0.1 M; pH 7.1) with polyvinyl pyrrolidone (PVP, 1%), the mixture was centrifuged at 10,000 g and 4 °C for 20 min. Then 0.3 mL of the supernatant was used and dissolved in 2.7 mL of catechol (60 Mm) plus 0.3 mL of Tris-HCl (0.1 M; pH 7.1).

Membrane integrity: 3 samples of 10 mm diameter of the second bract of the stem were taken. The samples were placed in 30 mL of distilled water for 24-h and the free electrical conductivity was recorded, at the beginning and 1 hour later. The membrane integrity was calculated by the following formula:

$$MI = (1-CL / CT) X 100.$$

Where MI is the absolute integrity of the membrane (%); CL, the free electrical conductivity; CT, total electrical conductivity (dS m^{-1}).

Statistical analysis. Data were processed with one- or two-way ANOVA followed by a Tukey honest significance difference (HSD) test (p-value $\leq$ 0.05). All statistical analyses were conducted using SAS® 9.0 software.

Results and Discussion

Anatomical characteristics

Generally, in heliconia cut flowers, the leaves are removed from the stem before commercialization, as they dehydrate quickly, due to the high stomatal density. In *Heliconia chartaceae*, the stomatal density was 94.73% higher in the abaxial as compared to the adaxial surface; also the abaxial stomatal density in anthurium spathe is 6.7 stomata mm^{-2} and 0.07 stomata mm^{-2} on the adaxial side (Raizer et al., 2019; Elibox and Umaharan, 2008). Our results showed that heliconia stems present 12.9 stomata mm^{-2}, and the stomatal frequency (SF) in the leaves was 123.7 and 18.9 mm^{-2} stomata on the abaxial and adaxial sides, respectively. The distribution and type of stomata indicate that the leaves are amphistomatic. The stomata are paracytic type since two subsidiary cells were observed parallel to the long axis of the occlusive cells. Metcalfe and Chalk (1979) stated that the subsidiary cells are at the same level as the epidermal cells and their size gradually increases from the center towards their ends. In the bracts, the stomata density was 34.2 and 32.1 mm^{-2} stomata corresponding to the abaxial and adaxial surfaces, respectively. The adaxial epidermis may be less responsive to environmental variations due to radiation being directly incidental upon it and transpiration occurs mainly through the epidermis of the abaxial face (Drake et al., 2019) (Figure 1). In *H. psittacorum* x *H. spathocircinata* cv. Tropics the stomatal frequency were 56.71 and 13.46 mm^{-2} stomata in the abaxial and adaxial epidermis respectively, and 31.30 µm the stomata length (Carrera-Alvarado et al., 2020).

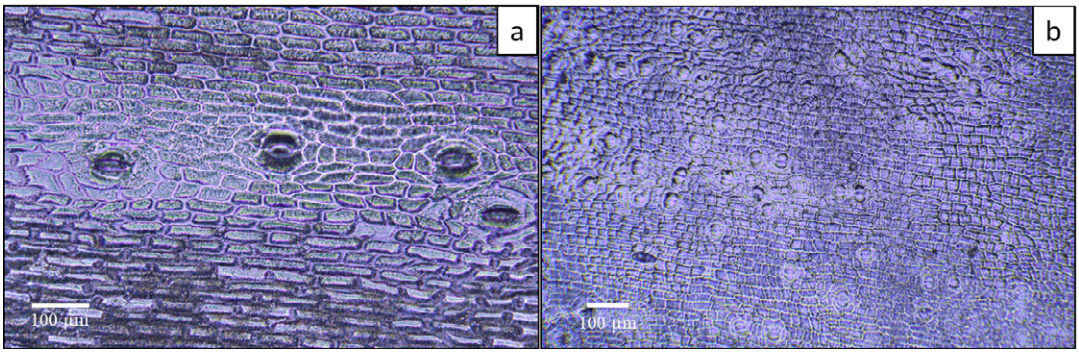

Figure 1. Presence of stomata on stems (a) and bracts (b) of *Heliconia wagneriana*

Cold storage

The application of wax in the bracts significantly reduced the solution uptake but increased the vase life (Table 1). This is attributed to the fact that the plant tissue expels the water in the form of vapor through the stomata and/or cuticle and in this case, the wax coating limits transpiration, therefore, it restricts the absorption of solution through the stems. However, it maintains stem turgor, so the shelf life of waxed stems increases by an average of 3 days. Paull and Goo (1985) reported that the application of carnauba in spathe and/or spadix in *Anthurium andraenum* cv. Ozaki, reduced water absorption from 10% to 20% but increased vase life from 17.9 to 36 d.

Table 1. Variables evaluated in stems of *H. wagneriana* for control and waxed stems.

Treatments	Solution consumption (mL g^{-1})	Vase life (d)	Total postharvest Life (d)
Control	0.075 a	9.00 b	9.00 b
Waxed	0.057 b	12.10 a	12.10 a
HSD	0.009	2.22	2.22
CV (%)	15.52	22.44	22.44

[z]Means presenting the same letters in each column are not significantly different at ($p \leq 0.05$), the level of Tukey test; HSD, honest significance difference; CV, coefficient of variation.

Low solution absorption is characteristic of heliconia stems (Jaroenkit and Paull, 2003; Carrera-Alvarado, 2020); a close relationship between the diameter of the xylem vessels and the susceptibility to embolism, then the wider vessels more vulnerable to cavitation, in contrast to narrow vessels (Arriaga-Frías et al., 2016). According to the results of both storage periods, it is observed that the water uptake of *H. wagneriana* is low since it only consumes 6% (p/v) of solution concerning its weight, like other tropical species such as *Heliconia psittacorum x H. spathocircinata* cv. Tropics with a consumption of 0.08 - 0.11 mL g^{-1}. Another factor that influences water uptake in plants are the leaves: the absorption rate is proportional to the number of leaves attached to the flower stem, however, in heliconia, there are no vascular connections between the flower stalk and the leaves as in other floral species, which contributes to the low water uptake after harvest (Carrera-Alvarado et al., 2020). Related to the vase life, cold storage only increases almost 4 d the total postharvest life compared to those stems kept at room temperature (Table 2).

Table 2. Variables evaluated in stems of *H. wagneriana* at different storage temperature.

Temperature (°C)	Solution consumption (mL g^{-1})	Vase life (d)	Total Postharvest Life (d)
20 ± 2	0.066 a	10.54 a	10.54 b
13 ± 0.5	0.034 b	6.56 b	14.16 a
HSD	0.008	1.00	1.01
CV (%)	44.83	34.57	22.27

[z]Means presenting the same letters in each column are not significantly different at ($p \leq 0.05$), the level of Tukey test; HSD, honest significance difference; CV, coefficient of variation.

The use of salicylic acid did not have a significant effect, which is probably attributed to the low rate of solution absorption, this explains the little response in prolonging the vase life of tropical species treated with pulse or preservative solutions (Table 3). In *H. psittacorum* cv. Golden Torch the postharvest application of gibberellic acid (1 mM) (Souza et al., 2015a) and hydroxyquinoline citrate (300 mg L^{-1}) did not increase the vase life (Souza et al., 2015 b). Lessa et al. (2015) report that in stems of *Zingiber spectabile* treated with tap water, Hidrosan™ (0.8 g L^{-1}), aspirin™ (1 tablet L^{-1}), sodium hypochlorite (50 mg L^{-1}), 5% coconut water + Hidrosan ™ (0.8 g L^{-1}), no significant differences were found in the absorption rate and vase life. Although the foliar application of SA (0.5 mM) in gladiolus flowers pre-transport increased vase life 3.5 days and improved the membrane stability index, compared to the control (Rahmani et al., 2015).

Table 3. Variables evaluated in stems of *H. wagneriana* with or without wax coating bracts and stored, at 13 °C, 84% RH, for 5 or 10 d and placed in water or salicylic acid.

Factors	Fresh weight loss (%)*	Solution consumption (mL g^{-1})	Vase life (d)	Total postharvest life (d)
Storage time (d)		**Unwaxed**		
5	3.52 a	0.051 a	6.10 a	11.10 b
10	2.56 b	0.022 b	4.75 b	14.75 a
HSD	0.043	0.005	0.67	0.067
		Waxed		
5	1.79 b	0.050 a	9.80 a	14.8 a
10	2.57 a	0.012 b	5.85 b	16.0 b
HSD	0.28	0.004	0.82	0.88

[z]Means presenting the same letters in each factor or combination are not significantly different at ($p \leq 0.05$), the level of Tukey test; HSD, honest significance difference; * Day 2 after storage.

The storage for 10 d showed a lower VL (2-3 d) than the ones stored for 5 d, in a manner that the symptoms that limited VL were characterized by necrotic spots and depressions generally in the basal part of the bract on the second or third day (Figure 2). Regarding the total postharvest life (TVL), although it is higher in both storage periods, it was not significantly prolonged, especially when it was done for a period greater than 5 d, which is not beneficial for its subsequent commercialization. According to these results, Bañuelos-Hernández et al. (2016) reported that after 10 d of storage at 12 °C (90% RH; 26 µmol m^{-2} s^{-1}) the increase of vase life of *H. psittacorumL. f.* cv. Trópica was only 4.5 d.

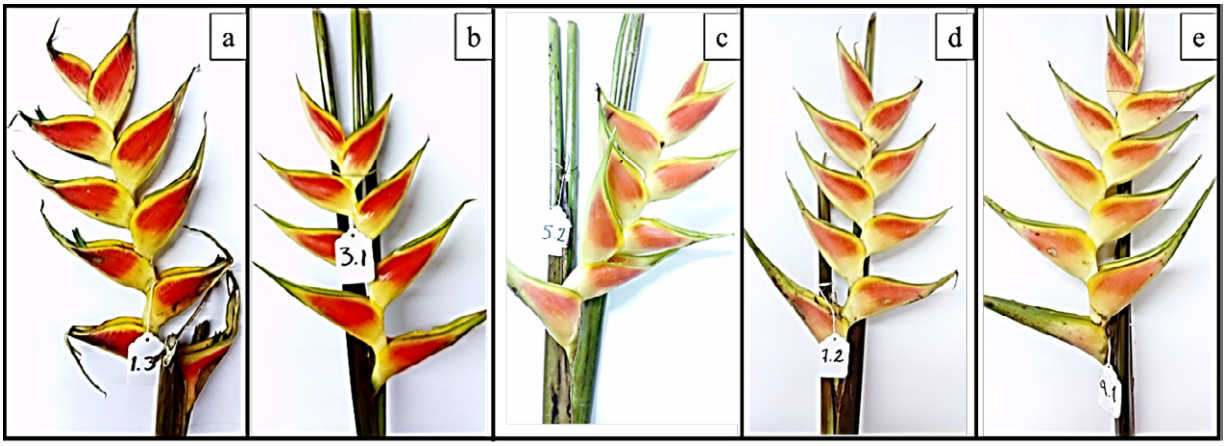

Figure 2. Flower stems of *H. wagneriana* after storage: a) Control (22 °C, 68 % RH); b) Waxed and cold storage (5 days); c) Waxed and cold storage (10 days); d) Cold storage (5 days); e) Cold storage (10 days). Cold storage (13 °C/84% RH).

Schouten et al. (2018) mention that the end of vase life is a consequence of negative water balance, which leads to rapid leaf wilting, then there are two ways to reduce wilting: maintain water absorption or reduce the rate of transpiration. Therefore, since *H. wagneriana* stems have low water absorption it is essential to reduce their water loss, because it has been proven that hydration did not ensure greater vase days (Linares-Gabriel et al., 2019) so the application of wax improves their VL by maintaining the turgor of the bracts.

Enzymatic activity (PPO and POX) and membranes integrity (MI)

The PPO and POX activity of the waxed bracts was as significantly lower ($p \leq 0.05$) as those kept at room temperature (Table 4). Huang et al. (2019) stated that a higher POX enzymatic activity during postharvest is a defense route to resist oxidative damage. In a study carried out with *Alpinia purpurata*, the control treatment compared with the pulse treatment (sucrose, 40% + ascorbic acid, 10 mM) showed high enzymatic

activity, highlighting the oxidative stress characteristic of senescence (Morais et al., 2015). In plant tissues, there is a balance between reactive oxygen species (ROS): superoxide radicals (O_2^{-}.), singlet oxygen ($^1O_2$), hydroxyl radicals ($^{-}$OH) and hydrogen peroxide (H_2O_2); and the enzymatic (superoxide dismutase, catalase, peroxidase) or non-enzymatic antioxidant system (phenols, ascorbate, glutathione, α-tocopherol and β-carotene), but under stress, reactive molecules ROS oxidize and modify some cellular components and affect the normal functions provoking cellular damage (Huang et al., 2019).

Table 4. Enzymatic activity of stem bracts of *H. wagneriana* affected by storage time and wax application

Factors	Polyphenols (mg g^{-1} min^{-1})	Peroxidases (mg g^{-1} min^{-1})
Storage time (d)		
0	1.03 b[z]	20.42 b
5	1.79 a	25.55 a
10	1.90 a	28.78 a
HSD	0.37	3.97
Bracts		
Unwaxed	1.71 a	27.26 a
Waxed	1.43 b	22.57 b
HSD	0.25	2.65
CV	15.39	10.35
S*B	*	ns

[z]Means presenting the same letters in each factor are not significantly different at ($p \leq 0.05$), the level of Tukey test; HSD, honest significance difference; CV, coefficient of variation; S*B, interaction between storage times with waxes in bracts; ns, not significant; *, significant ($p \leq 0.05$). n=3.

POX and PPO are antioxidant enzymes associated with ROS scavenging in plants and linked to the enzymatic browning of tissues. PPO catalyzes two different reactions in the presence of O_2: the o-hydroxylation of phenolic substrates to o-diphenols, and the oxidation of o-diphenols to quinones. These quinones can spontaneously polymerize through non-enzymatic routes generating brown pigments (Huang et al., 2019).

The initial MI was 87.04% and by day five the stems of the control treatment had a decrease of ~ 27%, while in the stems that were waxed it only decreased by 10%. According to these results, Zhao et al. (2021) reported the alleviation of chilling injury in peach fruit treated with salicylic acid (1 µmol L^{-1}) before cold storage (4 °C), with an internal browning index of 25% lower compared to the control. Salicylic acid treatment improves the resistance to chilling injury of cold-stored peach fruit in part by increasing the expression of particular cold tolerance genes. But in our experiment, the low absorption of SA did not protect the stems from cold damage, because the bracts showed depressions and necrotic spots 2 d after storage, which led to the end of the VL (Figure 3). Browning tissue due to stress conditions in plants is generated by the activity of PPO and POX. However, when the accumulation of reactive oxygen species (ROS) exceeds the antioxidant enzymatic activity, the peroxidation of the lipids of the membranes and the interruption of cell compartmentalization can be triggered, thus generating a decrease in the integrity of the cell membranes (Darras et al., 2019; Aghdam et al., 2016). In *H. bihai* Lobster Claw and Halloween cultivars, stored at 6.5 °C for 6 and 8 d, there was a decrease in MI, which coincided with a worsening of the symptoms of cold damage: browning and/or necrotic spots (Costa et al., 2011). In anthurium stems stored at low temperature (4 °C), cold damage was characterized by wilting of the spadix and/or browning of the spathe (Aghdam et al., 2016).

Figure 3. Chilling injury in *H. wagneriana* stored 10 d at 13 °C and 84% RH.

Conclusions

The results showed the beneficial impact of the wax treatment on the conservation of postharvest quality in cut flowers of *H. wagneriana* during their storage at room temperature and refrigeration. Moreover, due to the low absorption capacity of the stems, the use of vase solutions has little or no benefit. The results suggest that the reduction in cold damage is a function of storage time and may be due to decreases in PPO and POX enzymatic activity, parallel to better preservation of the membrane integrity, which led to a decreased fresh weight loss.

Author Contribution

GCA: Field work and data capture, literature review. **MLAG**: Research project coordinator, processing and data analysis, literature review and writing. **JVV:** Experiment design and data analysis. **GCG:** Histological analysis and interpretation. **JSR:** Literature review. **OBB:** Field work-heliconia production.

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