

SCIENTIFIC ARTICLE

Nutrient uptake dynamics of Gloriosa for cut flower

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Abstract

Gloriosa superba L. is a recently introduced tropical species in Argentina, cultivated as a cut flower. It is extremely important to know the nutritional demands of the crop to provide the optimal amounts of nutrients at each stage, achieving quality and good yield in flowers, reducing production costs and environmental impact. The objective of this work was to determine the dynamics of nutrient absorption in the cultivation of *G. superba* for cut flowers, to facilitate the creation of a fertilization program, in order to avoid crop deficiencies and contribute to sustainable production. Tuber composition analyzes were carried out and, on the other hand, an essay was installed in greenhouse beds, taking samples at seven moments of the cycle. Fresh matter and dry matter of stems, leaves, flowers, tubers, roots and chemical analysis of aerial organs were measured to obtain the absorption curve. It was verified that only around 20% of each nutrient is provided by the tuber, being necessary the external contribution from initial stages of the crop. The rate of growth and accumulation of dry matter was shown as a double sigmoid, with maximum peaks in the vegetative stage of stem elongation and beginning of flowering. Nutrient amounts were absorbed in the following order: N>K>Mg>Ca>P>Fe>Mn>Zn>Cu. Fertilization rich in N, P, and Fe is recommended in the vegetative stage, balanced during the visible shoot stage, and rich in Ca, K, Mg, Mn, Zn, and Cu during flowering. **Keywords:** geophytes, *Gloriosa superba*, nutrition, phenology, tropical plant.

Resumo

Dinâmica de absorção de nutrientes de Gloriosa para flor de corte

Gloriosa superba L. é uma espécie tropical recentemente introduzida na Argentina, cultivada como flor de corte. É de extrema importância conhecer as demandas nutricionais da cultura para fornecer as quantidades ideais de nutrientes em cada fase, obtendo qualidade e bom rendimento nas flores, reduzindo os custos de produção e o impacto ambiental. O objetivo deste trabalho foi determinar a dinâmica de absorção de nutrientes no cultivo de *G. superba* para flores de corte, para facilitar a criação de um programa de adubação, a fim de evitar deficiências na cultura e contribuir para uma produção sustentável. Foram realizadas análises da composição dos tubérculos e, por outro lado, foi instalado um ensaio em canteiros de casa de vegetação, coletando amostras em sete momentos do ciclo. Foram mensuradas a massa fresca e seca de caules, folhas, flores, tubérculos, raízes e análises químicas dos órgãos aéreos para obtenção da curva de absorção. Verificou-se que apenas cerca de 20% de cada nutriente é fornecido pelo tubérculo, sendo necessária a contribuição externa desde as fases iniciais da cultura. A taxa de crescimento e acúmulo de matéria seca apresentou-se como duplo sigmóide, com picos máximos na fase vegetativa de alongamento do caule e início da floração. As quantidades de nutrientes foram absorvidas na seguinte ordem: N>K>Mg>Ca>P>Fe>Mn>Zn>Cu. Recomenda-se adubação rica em N, P e Fe na fase vegetativa, balanceada na fase de primórdio floral visível e rica em Ca, K, Mg, Mn, Zn e Cu na floração. **Palavras-chave:** geófitas, fenologia, *Gloriosa superba*, nutrição, planta tropical.

Introduction

The main species that historically participated in the world trade in cut flowers were rose, chrysanthemum, gerbera and carnation, among others. However, consumption in recent years has been very dynamic and changing, with a growing niche that demands new species and varieties (Darras, 2021; Gabellini and Scaramuzzi, 2022). *Gloriosa superba* is a tropical species, also known as climbing lily, Glory lily, or simply Gloriosa. It is native to the tropics of Asia and Africa. It belongs to the *Colchicaceae family*. (Jasmine et al., 2020). Although its main applications are

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Received Feb 10, 2023 | Accepted May 31, 2023 | Available online June 28, 2023 Licensed by CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/) Editor: José Carlos Sorgato for its medicinal properties (Birhan, 2022; Ionkova, 2022), in recent years, due to the characteristics of its flowers, it has gained importance as a cut flower (Jamal et al., 2022).

Nutrition plays a fundamental role in the quality of cut flowers, also prolonging their postharvest duration (Moghaddam and Nasir, 2020). In each crop cycle it is necessary to replenish the amounts of nutrients extracted, thus avoiding the impoverishment of the soil. These nutrients can be replaced to the system through organic fertilizers, chemical fertilizers or a combination of both in the appropriate amounts (Deivasigamani et al., 2022; Quddus et al., 2021; Kleiber et al., 2019).

If amounts of nutrients are delivered to the crop beyond what it can absorb, part is retained in the soil and is available for subsequent crops, but part is lost and contaminates the groundwater, causing problems in the future (Mistry et al., 2023). Although it is not yet possible to achieve zero contamination in agricultural activities, by making reasonable use of fertilizers, applying them in the amounts and at the times the plants need them, it is possible to achieve sustainable production over time (Ruet et al., 2020; Delgado and Rodríguez, 2018).

A nutrient uptake curve is the graphic representation of the amount of nutrients extracted by the plant during its life cycle. Once the curves are established, deficiencies can be diagnosed and corrected even before they cause symptoms that decrease yield and, above all, the quality of the product (Giraldo et al., 2020; Barahona et al., 2019). The objective of this work was to determine the dynamics of nutrient absorption in the cultivation of *G. superba* for cut flowers, to facilitate the creation of a fertilization program, in order to avoid crop deficiencies and contribute to sustainable production.

Materials and Methods

The test was carried out in the establishment of a flower producer in the town of Colonia Luján, province of Misiones, Argentina, located at 26°44'45" South latitude, 54°52'59" West longitude and an altitude of 185 masl, in a greenhouse with 150 μ m UV polyethylene. (Figure 1a). The average temperature of the period was 24.9 °C, with an absolute maximum of 37.1 °C and absolute minimum of 10.5 °C; the relative humidity of the environment between 43 and 100% and the maximum photosynthetically active radiation inside the greenhouse of 781 μ mol.m⁻².s⁻¹.

Commercial size tubers were used, with an average of 14 cm in length and fresh weight close to 50 g (Figure 1b), which were disinfected by immersion in a Carbendazim solution (2ml.L⁻¹) for 15 minutes and subsequent chamber treatment at 10 °C for 60 days prior to planting (Figure 1).



Figure 1. *Gloriosa superba* assay. a) Greenhouse; b) Plantation; c) Plantation frame; d) Data collection; e) Preparation of samples for nutrient analysis; f) Laboratory work.

A completely randomized design was used, with 3 repetitions and an n of 12. The data were processed and analyzed with the statistical software InfoStat[®] and Microsoft Excel. For the analysis of trends, presented by means of graphs based on the days elapsed since the beginning of the implantation, we worked with regressions at a significance level a = 0.05. In each particular case, the

graphs represent the mean value of the response variable together with its corresponding standard error.

Completely randomized sampling was carried out within each repetition on 7 dates throughout the cycle, the frequency of which was determined in previous trials according to phenology, resulting in three phases and six subphases (Figure 2): Vegetative phase: 1) 0-13 days from sprouting (DFS) - Expansion of leaves and elongation of stems; Shooting phase: 2) 13-17 DFS: first visible buds, 3) 17-22 DFS: 2 cm long shoots, 4) 22-27 DFS: color change of 1st shoot; Flowering Phase: 5) 27-30 DFS: opening of

1st flower or beginning of flowering, 6) 30-36 DFS: full bloom or harvest point. In this way, the requirements of each phase can be known regardless of the prevailing weather conditions.



Figure 2. Development phases of Gloriosa superba.

The existing soils in the area are represented by a complex of Kandiudultes and Kandiudalfes rhodics, known as deep red soils. In the preparation of the soil, 5 kg m⁻² of a mixture of composted sawdust with cow manure were added, carrying out the incorporation with a rotary cultivator. Beds 30 cm high and 50 cm wide were formed. Pine needle mulch was placed. It was planted in a double line at a distance of 30 cm between plants and 40 cm between lines, resulting in a density of 8.33 pl m⁻² (Figure 1c). A stake mesh was placed in a vertical position to achieve straight stems and good quality floral rods. Weed control was done manually.

Irrigation was carried out with a watering can depending on the evapotranspiration of the area, the water had a pH of 6.85, electrical conductivity of 0.07 dS m⁻¹, 6.3 ppm (parts per million) of nitrates, 4 0.4 ppm calcium, 2 ppm magnesium, 0.9 potassium, 1.5 sodium, 0 ppm carbonates, bicarbonates and chlorides. Soil samples were taken at the beginning and end of the trial.

At 4, 8, 13, 17, 22, 27 and 36 DFS, coinciding with the mentioned phenological phases, samples were taken for chemical analysis. On said dates, plant height, number of nodes, number of leaves, number of buds and number of flowers were also measured in all the plants in the trial (Figure 1d).

In the laboratory, the different organs were separated (Figure 1e), washed with distilled water, dried with absorbent paper, and fresh matter (FM) was determined. They were placed separately in wooden paper envelopes in an oven at 65-70 °C until constant weight to obtain the dry matter (DM). They were ground with an IKA[®] A11 Basic grinder. Samples of 0.25 g of dry mass were weighed in porcelain capsules of known weight, on an AND HF-200 balance with a precision of 0.001 g. They were placed in a muffle at 500°C for 4 hours and allowed to cool.

Acid digestion was carried out in a fume hood, adding 2 ml of 2M HCl to the sample and taking it to an electric heating plate, removing it before it came to a boil (Figure 1f). It was allowed to cool and brought to a volume of 50 ml with distilled water. Total nitrogen (N) was analyzed with a Pro-nitro S semiautomatic Kjeldahl distiller, JP SELECTA[®]. For the other elements, the concentrations present in a 1+5 v/v solution previously filtered with quality 0859 filter paper were analyzed. For Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Copper (Cu), Manganese (Mn) and Zinc (Zn) with a Varian 220 model atomic absorption spectrophotometer and Phosphorus (P) with a Helios Beta model UV Visible spectrophotometer.

Results and Discussion

Composition of tubers

The mean dry matter (DM) value of the analyzed tubers was 31%. The nutrient concentration data in tubers are from the first records in the species (Table 1). In addition, the total contents in tubers similar to those that were planted are shown.

		Mac	ronutrients		Micronutrients					
Ν	Р	Κ	Ca	Mg	Fe	Mn	Zn	Cu		
		Conce	entration (%)		Concentration (ppm)					
1.54	0.09	1.25	0.01	0.11	51.64	4.80	11.71	2.83		
	r	Fuber con	tent of 50 g (mg)		Tuber content of 50 g (μ g)					
239.3	13.7	194.5	1.4	17.7	800.4	74.4	181.6	43.9		

Table 1. Macro and micronutrient content in G. superba tubers.

The contents were lower than those obtained by Clark (1997), in *Sandersonia aurantiaca* bulbs, where the greatest difference occurred in Ca, which was 11 times higher than those of *G. superba*. Likewise, lower than those of *Lilium sp.* "Brunello" bulbs, with the exception of N and Mn (Barrantes and Bertsch, 2012). Analyzing the relationship between nutrients, although they have a common pattern in all, *G. superba* was characterized by having less amount of P and Ca with respect to N, compared to the other species.

Greenhouse growth parameters

The first saplings were observed after 13 days, the opening of the first flowers at 27 days and full flowering at 36 days with 4 ± 1 visible saplings and 4.6 ± 1 open flowers pl⁻¹. A total of 17 ±2 knots pl⁻¹, 26±2 leaves pl⁻¹ and a total height of 192±9 cm.

From planting to harvest, the increase in DM was observed in all the organs, except for the planted tubers that were using their reserves, decreasing at a high rate until the 2 cm seedling phase, from where it remained stable until the end of the cycle, concluding that at that moment, the contributions of the tuber were insignificant compared to the contributions of the soil (Figure 3).

Dry matter

The highest percentage of accumulation of aerial dry matter occurred at the time of the opening of the first flower, coinciding with what was cited by Barrantes and Bertsch (2012) in *Lilium sp.* The greatest accumulation in leaves coincided with that moment. However, in the stems, the highest percentage accumulation occurred in the vegetative phase and in flowers in full bloom. The total aerial dry matter was 14.06 g pl⁻¹.

Analyzing the total dry matter accumulation rate, a double sigmoid trend was observed, with maximum peaks at 8 and 27 days (Figure 4). The point of lowest growth between both peaks can be attributed to the moment in which the nutrients contained in the planted tuber were exhausted.



Figure 3. Dry matter of the different organs of *G. superba* L. cultivated in soil under cover throughout the productive cycle. a) aerial organs; b) underground organs.



Figure 4. Aerial dry matter accumulation rate, per organ and total per plant in G. superba L.

Nutrient absorption

Nitrogen

It was the element that had the highest concentrations in leaves and flowers, as well as in stems until flowering, where it was surpassed by K. The highest concentration occurred in leaves, followed by flowers and finally stems. In turn, a decreasing trend was observed as the flowering time approached. In leaves from 6.8 to 4.3%, in stems from 3.5 to 1.8% and in flowers from 3.3 to 2.8% (Figure 5a). In *S. aurantiaca* Clark (1997), obtained similar values and Clark and Burge (1999b) observed the same trend of decrease in leaves with increasing age of the plants of the same species. It was the nutrient with the highest total air absorption, reaching 478.32 mg pl⁻¹ (Table 2).

Phosphorus

Of the macronutrients, it was the nutrient absorbed in the least amount. The concentration decreased throughout the crop cycle, the lowest percentages occurred in stems, with initial values of 0.19% and final values of 0.08%. In leaves

from 0.27% to 0.11% (Figure 5b). Unlike N, it was higher in flowers than in leaves, but with a decrease in concentration when approaching the harvest point. These values were lower than those cited by Röber and Schacht (2008), for most ornamental crops, as well as those cited in gladiolus leaves (Hernández Díaz et al., 2008). However, the values in *S. aurantiaca* (0.19 to 0.21%) were close to those of *G. superba* (Clark and Burge, 1999b). In turn, (Heidari et al., 2021) found lower P contents in leaves of *Lilium spp*.

Potassium

The K percentages remained relatively stable, with slight increases in the visible shoot phase (Figure 5c). Being higher in leaves (2.31 to 2.85%), medium in stems (2.15 to 2.38%) and lower in flowers (1.72 to 2.26%). Similar values were found by Hernández et al. (2008) on gladiolus and Clark (1997) on *S. aurantiaca*. In *G. superba*, the values of N were higher than those of K, contrary to what happened for most of the crops cited by Röber and Schacht (2008).



Figure 5. Concentration of macronutrients based on DM in tissues of stems, leaves, and flowers of G. superba L.

Unlike what happened with N and P, in K, the accumulated levels absorbed by stems exceeded those absorbed by leaves. In both N and P, the peak of maximum aerial absorption was at 8 days, while in K it was at 27

days, coinciding with the opening of the first flowers.

The absorption rate curves for K somewhat resemble those for P, with the difference that the values are on average 15 times higher in K.

Table 2. A	ccumulated	1 absorp	tion of	f macro a	and micro	nutrients	on the	different	sampling date	es.
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DES	Ι	Macronutrie	nt extraction	on (mg pl ⁻¹	Micronutrient extraction (µg pl ⁻¹)				
Drs	Ν	Р	K	Ca	Mg	Fe	Mn	Zn	Cu
4	38.10	1.82	19.83	0.22	2.15	172.88	20.29	49.09	4.28
8	109.41	4.84	58.11	0.72	6.39	253.70	49.85	101.20	13.81
13	179.77	7.88	101.75	2.08	11.72	415.44	111.36	149.69	25.11
17	213.25	8.89	123.38	3.70	14.20	508.87	163.58	168.73	31.47
22	257.79	10.70	155.03	7.50	18.25	590.47	241.53	213.55	39.90
27	344.89	13.67	222.45	19.37	28.85	795.19	429.71	299.04	59.11
36	478.32	18.24	314.12	33.59	41.68	1234.44	766.63	414.04	92.29

Calcium

The concentration of Ca grew considerably throughout the cycle in leaves, going from 0.02% in the vegetative phase to 1.23% in full bloom (Figure 5d). In full bloom, Ca concentrations in leaves were 8 times higher than those in stems and 21 times higher than those in flowers. These concentrations were similar to those obtained by Clark and Burge (1999b) and Barrantes and Bertsch (2012) in *S. aurantiaca* and *Lilium sp.* Respectively and with the same tendency marked to the increase in the foliar concentration of Ca (Clark and Burge, 1999a). The absorption was low until the coloration of the saplings changed, absorbing up to that moment, only 22.33% of the total airborne Ca of the cycle.

Magnesium

The percentages of Mg were higher in leaves and lower in stems, the flowers occupied an intermediate place. The curves were similar to those of Ca, increasing considerably in leaves in shoot growth (Figure 5e). At the beginning of flowering, the values in leaves exceeded 4 times the concentrations in stems and 2.6 times the concentrations in flowers. Clark and Burge (1999a), in *S. aurantiaca* leaves expressed similar percentages and with the same tendency to increase. Barrantes and Bertsch (2012), in *Lilium sp.*, analyzing the entire aerial part without discriminating between stems, leaves and flowers mention Mg percentages of 0.2 to 0.3%, being very similar to those obtained in *G. superba*. In the concentrations of micronutrients in aerial organs, Zn had a tendency to decrease when approaching flowering, while Mn to increase. The Fe and Cu differences were less pronounced (Figure 6). Total absorption was in the following order: Fe>Mn>Zn>Cu. In *Lilium sp.*, both Barrantes and Bertsch (2012) and Ortega et al. (2006), determined the same sequence.

The average absorption rate throughout the cycle was

32.29, 21.30, 11.50 and 2.56 μ g pl⁻¹ day⁻¹ for Fe, Mn, Zn and Cu respectively. At the beginning of sprouting, the absorption rate of Fe, Mn and Zn was higher in leaves than in stems, while there were no differences with Cu. At the beginning of the blooming, in all the micronutrients it was higher in leaves than in stems. From the opening of the first flower to the moment of harvest, Fe and Mn continued to be higher in leaves, while Zn and Cu were higher in flowers



Figure 6. Concentration of micronutrients based on DM in tissues of stems, leaves, and flowers of G. superba L.

For all micronutrients, the total absorption rate was highest near the point of harvest. The Fe and Zn curves were similar, with two peaks, one at the beginning and the other at the end, while for Mn and Cu, a single peak was observed at the end of the cycle. For Barrantes and Bertsch (2012), in *Lilium sp.*, they expressed the same order of absorption, but with lower values. In N, P and Zn, 50% of the total air absorption was exceeded on day 22, while in the rest only on day 27 this percentage was exceeded. Positive correlations of Ca in leaves with Mg and Mn in leaves were observed. While the correlations were negative for Ca in leaves with P, N and Zn in leaves. The order of total absorption of the aerial part was the following: N>K>Mg>Ca>P>Fe>Mn>Zn>Cu.

Nutrient absorption by phenological stages

When analyzing the absorption of each nutrient in the different stages, Ca, only 5% was absorbed in the vegetative

stage, 32% in the seedling stage, while in the flowering stage the 63%. In Mg something similar occurred but less pronounced, with 5% in the vegetative stage and 46% in flowering. K followed with the same trend, of 30% in the vegetative stage, 28% in budding and 42% in flowering. On the contrary, the P had its maximum absorption in the vegetative stage with 40%, 25% in budding and 35% in flowering. The N behaved in a similar way, but being a little higher in flowering.

In micronutrients, although the absorption was higher in the flowering stage, it was observed that the requirements of both Fe and Zn were more constant throughout the cycle, while Mn and Cu were higher in the flowering stage.

A minimum nutrient intake of 180 mg N pl⁻¹; 8 mg P pl⁻¹; 102 mg K pl⁻¹; 2 mg Ca pl⁻¹; 12 mg Mg pl⁻¹; 415 μ g Fe pl⁻¹; 111 μ g Mn pl⁻¹; 150 μ g Zn pl⁻¹ and 25 μ g Cu pl⁻¹ in the vegetative stage; in seedlings of 78 mg N pl⁻¹; 3mg P pl⁻¹; 53 mg K pl⁻¹; 5 mg Ca pl⁻¹; 7 mg Mg pl⁻¹;175 μ g Fe

pl⁻¹; 130 μ g Mn pl⁻¹; 64 μ g Zn pl⁻¹ and 15 μ g Cu pl⁻¹, while for flowering a contribution of 221 mg N pl⁻¹; 8 mg P pl⁻¹; 159 mg K pl⁻¹; 26 mg Ca pl⁻¹; 23 mg Mg pl⁻¹; 644 μ g Fe pl⁻¹; 525 μ g Mn pl⁻¹; 200 μ g Zn pl⁻¹ and 52 μ g Cu pl⁻¹. Being necessary to add the values for the formation of the new tubercle.

Relationships between nutrients

For the relationships between nutrients by stage, the macronutrients were analyzed in relation to N and the

micronutrients in relation to Fe (Table 3). In the N-P-K-Ca-Mg relationships of the aerial part, at the beginning of the crop cycle a relationship of 1-0.05-0.52-0.01-0.06 was observed and at the end of the cycle of 1-0, 04-0.66-0.07-0.09. In the planted tubers, the relationship was 1-0.06-0.81-0.01-0.07. In macronutrients, a decrease in P and an increase in K, Ca and Mg were observed. In the micronutrients, the increase of Mn and to a lesser extent of Cu was appreciated, while Zn decreased in its relation to Fe.

Table 3. Relations between macro and micronutrients absorbed in *G. superba* L. in the three phenological phases of the crop.

DFS	Macro- nutrients	N/N	Р	Κ	Ca	Mg	Micro- nutrients	Fe/Fe	Mn	Zn	Cu
0-12		1	0.044	0.561	0.011	0.064		1	0.26	0.37	0.06
13-24		1	0.036	0.701	0.082	0.093		1	0.75	0.37	0.09
25-36		1	0.034	0.712	0.115	0.103		1	0.80	0.30	0.08
Media		1	0.038	0.657	0.070	0.087		1	0.62	0.34	0.07

Contributions of the tuber and the soil

With the absorption values of the aerial part obtained in cultivation in soil, the potential contribution of the planted tubers (assuming an efficiency and total consumption of the tuber) would correspond to 50% of the N used; 74.5% of P; 61.9 of the K; 4.1 from Ca; 42.4% of Mg; 64.8% of Fe; 9.7% of Mn; 43.9% Zn and 47.6% Cu. However, if we

consider the total accumulation of the plants, in the aerial and underground parts, with the formation of the 2 daughter tubers that on average were 42% higher than the planted tuber, the potential contribution of the tuber would only represent around 20% (Table 4). That is to say that, although the contribution of nutrients of the tuber is essential, the importance of the contribution of the soil was observed.

Table 4. Potential contribution of the tuber and estimated contributions of the soil to the growth of G. superba L.

	Absorpti	on greenhouse e	xperience	Potential contri	Soil	0/ Dotontial	% Minimum soil contribution	
Macro- nutrients	Aerial part (mg pl ⁻¹)	Underground part (mg pl ⁻¹)	Total (mg pl ⁻¹)	bution planted tuber (mg pl ⁻¹)	contribution (mg pl ⁻¹)	provided by the tuber		
Ν	478.3	679.7	1158.0	239.3	918.7	20.7	79.3	
Р	18.2	38.9	57.1	13.7	43.4	24.0	76.0	
K	314.1	552.4	866.5	194.5	672.0	22.4	77.6	
Ca	33.6	3.9	37.5	1.4	36.1	3.6	96.4	
Mg	41.7	50.3	92.0	17.7	74.3	19.2	80.8	
Micro- nutrients	$(\mu g p l^{-1})$	(µg pl ⁻¹)	(µg pl-1)	(µg pl ⁻¹)	(µg pl ⁻¹)	%	%	
Fe	1234.4	2273.1	3507.6	800.4	2707.2	22.8	77.2	
Mn	766.6	211.3	978.0	74.4	903.6	7.6	92.4	
Zn	414.0	515.6	929.7	181.6	748.1	19.5	80.5	
Cu	92.3	124.7	217.0	43.9	173.1	20.2	79.8	

Conclusions

Based on the results obtained, in *Gloriosa superba* cultivated for cut flowers, a fertilization rich in N, P and Fe is recommended in the vegetative stage, balanced during the visible shoot stage, and rich in Ca, K, Mg, Mn, Zn, and Cu during flowering.

For each crop situation, a soil/substrate analysis is recommended, in order to contribute through fertilization only the amount of nutrients that is not covered by the soil, thus avoiding an unnecessary contribution, seeking greater sustainability.

This work allows to have a starting point for the elaboration of a fertilization program for *Gloriosa superba* based on the minimum requirements in the different stages. The results obtained are an unprecedented contribution for a sustainable management in the nutrition of the species.

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Author Contribution

RDH: Field work and writing. **DIB:** Field work and corrections. **DAM:** Supervisor and coordination of study, writing. **RGV:** Supervisor of field experiments, writing; **DEM:** Supervisor of laboratory tasks, corrections.

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