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Research Article

Fertilization methods and substrate particle size differentially affect growth and macronutrient status of *Laelia anceps* subsp. *anceps*

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Abstract

The effects of three fertilization methods (FM) using the Peters Professional® 30N-10P-10K water soluble fertilizer were analyzed: fertilization in irrigation water (FIW), foliar fertilization (FF), and their combination (FIW + FF), as well as two substrate particle sizes (SuPS): larger particle size (LPS) and smaller particle size (SPS), on the growth and macronutrient status of *Laelia anceps* subsp. *anceps* (Orchidaceae). A 3 × 2 factorial experiment was established. Aerial and root growth, dry weight and concentration of macronutrients N, P, K, Ca, and Mg in leaves, pseudobulbs, and roots were all evaluated. The interaction of the study factors resulted in a greater number of leaves, while by simple effects, greater leaf area, number of pseudobulbs, root volume, and dry matter of leaves and roots were obtained, when FIW and LPS were used. With FF, greater growth in root volume was observed. There were no differences in the concentration of N in the growth organs analyzed, but due to the interaction of factors, a higher concentration of P, K, Mg, and S was observed in roots, mainly with FIW + FF and SPS; although for P, there was a higher concentration in FIW and LPS. In the case of SPS, the concentrations of K and S were higher in leaves, and in pseudobulbs P, K, Ca, Mg, and S were higher. The results showed that there are positive effects on plant growth when FIW and LPS are used, while higher concentrations of nutrients in leaves, pseudobulbs and mainly in roots are observed when using SPS.

Keywords: foliar fertilization; growth parameters; nutrient uptake; orchid nutrition; orchid substrates

Introduction

The Orchidaceae is one of the most diverse families within the plant kingdom, encompassing approximately 24,500 species (Dressler, 2005) and 736 genera (Chase *et al.*, 2015). Its wide diversity in sizes, shapes and colours has made these species one of the most valuable flower producing plants worldwide. Despite the wide diversity of species with ornamental potential, only a few genotypes dominate the global orchid market. Since megadiverse countries possess a wealth of orchids, proper protocols for the propagation and production of their species have to be carried out, which may result in economic benefits, integrate a diversity of floricultural products into the commercial chain, and discourage the looting of wild materials from their

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natural habitats. As a megadiverse country, Mexico has launched this type of initiative to propagate and produce diverse orchids in sustainable environments (Tejeda-Sartorius *et al.*, 2017).

Substrate management and nitrogen (N) fertilization in ornamental orchids are key factors determining the success of orchid production systems (Zong-min *et al.*, 2012). However, the mechanisms of nutrient uptake in wild epiphytic orchids are not entirely known, while the effects of the form and concentration of N on growth and flowering of orchids are poorly understood (Zhang *et al.*, 2018). For *Phalaenopsis*, the most studied genus of orchids, an adequate supply of N is recommended, both in vegetative and reproductive stages to achieve the best growth and flowering (Wang and Chang, 2017).

The uptake of water-soluble nutrients by the leaves is generally not very efficient and does not play a very important role in the growth of plants in their natural habitats. Nevertheless, in cultivated species, foliar application of fertilizers can provide nutrients during critical phases (Mengel, 2002). The process of nutrient absorption by the leaves is different from that of the roots because the cell walls of the leaves are covered by a cuticle, which is not found in the root structure (Fageria *et al.*, 2009). Leaf cuticles are known to be permeable to some ions in varying degrees (Kannan, 2010).

The root nutrient application method is more common and effective for nutrients required in larger amounts. However, under certain circumstances, foliar fertilization is more economical, effective, and environmentally friendly (Fageria *et al.*, 2009; Kannan, 2010; Trejo-Téllez *et al.*, 2016). Currently, there is a dire need to decrease the use of mineral fertilizers applied to the soil, such as N, phosphorus (P), and potassium (K) (Haytova, 2013), due to the high amounts of these plant nutrients leached when crops are over-fertilized (Kannan, 2010).

Both root and foliar applications of fertilizers are common agronomic practices among orchid growers, but just how efficient the processes of nutrient absorption and assimilation are with these methods is not completely known (Ruamrungsri *et al.*, 2014). This lack of information is due, at least in part, to factors such as growth conditions, environment, and genotypes of orchids tested.

Epiphytic orchids generally need a coarse growing medium, which ideally also has to be open, well-drained, moisture-and nutrient-conserving, stable, and preferably low-cost (Slump, 2004; Wang *et al.*, 2007). Plant age, development of the root system and its stability in the container are other factors affecting nutrient uptake and assimilation. Hence, large plants with thick roots need thicker mixes of substrates than those with finer roots. Moreover, young plants need a substrate mixture capable of retaining more moisture than when they become mature plants (Slump, 2004). Indeed, growing media with smaller particle sizes can promote better contact of roots with water and nutrients (Wang and Gregg, 1994). In terms of cheap and biodegradable materials, bark brings economic and environmental advantages as compared to other materials, such as rockwool and peat moss (Naasz *et al.*, 2009). Pine bark is the most widely used substrate for orchid production, and it is frequently mixed with other components such as perlite, different types of peat, coconut fiber, diatomite, rockwool, etc. (Wang *et al.*, 2007; Hwang and Jeong, 2007; Lichty *et al.*, 2015; Kim *et al.*, 2016). However, there is still little information about the size of the substrate particles and their influence on growth and nutrient retention capacity in orchids, depending on their growth stage.

Currently, we are developing models in order to establish optimal nutrient and agronomic management practices for the wild orchid species *Laelia anceps* subsp. *anceps*, with floral characteristics highly coveted in ornamental horticulture. Herein we evaluated: i) the effect of different fertilization methods: fertilization in irrigation water (FIW), foliar fertilization (FF), and the combination of both (FIW + FF), as well as two sizes of substrate particles: larger (LPS) and smaller particle size (SPS), on growth and macronutrient status of *Laelia anceps* subsp. *anceps*.

Materials and Methods

Plant material and treatments

This study was carried out in an experimental orchid nursery located at the College of Postgraduates in Agricultural Sciences, Campus Montecillo, under the following environmental conditions: (in the maximum, minimum and average order): Temperature (°C): 30.8, 11.2, 18.6; relative humidity (%): 94.4, 33.4, 70.6; and an average photosynthetic photon flux (PPF): 75.4 μmol m^{-2} s^{-1} (HOBOware Pro®).

Seedlings of *Laelia anceps* subsp. *anceps* in vegetative stage, with 3 ± 1 pseudobulbs were used. They were propagated *in vitro* by Orquídeas Río Verde® S. R. L. M. I., where they were purchased.

A 3 × 2 factorial experiment was established, where the first study factor was the fertilization method, with three levels: fertilization in irrigation water (FIW), foliar fertilization (FF), and the combination of both forms (FIW + FF); the second factor was the particle size of the substrate components (SuPS), consisting of pine bark (PB; Ecorteza®) and perlite (P), (PB 75%: P 25% v/v) with two levels: larger particle size (LPS) and smaller particle size (SPS). The sizes are described in Table 1.

Table 2 shows the physical and chemical properties of the substrates used.

Table 1. Treatments derived from different fertilization methods and particle size in the substrate for *Laelia anceps* subsp. *anceps*

Treatments	Description	
	Fertilization Methods (FM)	Substrate particle sizes (SuPS)
FIW with LPS	Fertilization in irrigation water (FIW)	Larger particle size (LPS) PB=13 mm; P=6 mm
FIW with SPS	Fertilization in irrigation water (FIW)	Smaller particle size (SPS) PB=4 mm; P=2 mm
FF with LPS	Foliar fertilization (FF)	Larger particle size (LPS) PB=13 mm; P=6 mm
FF with SPS	Foliar fertilization (FF)	Smaller particle size (SPS) PB=4 mm; P=2 mm
FIW + FF with LPS	Fertilization in irrigation water (FIW) + Foliar fertilization (FF)	Larger particle size (LPS) PB=13 mm; P=6 mm
FIW + FF with SPS	Fertilization in irrigation water (FIW) + Foliar fertilization (FF)	Smaller particle size (SPS) PB=4 mm; P=2 mm

PB: Pine bark; P: Perlite

Table 2. Physical and chemical properties of the substrates used in this research

Parameter	Substrates	
	LPS: PB 13 mm and P 6 mm	SPS: PB 4 mm and P 2 mm
RAW (%)	11.55	24.05
EAW (%)	7.55	15.35
RW (%)	4.00	8.7
Bulk density (g cm^{-3})	0.14	0.16
pH	3.41	4.17
EC (dS m^{-1})	0.53	0.26
OM (%)	58.83	58.83
N (g kg^{-1})	1.29	1.17
P (mg kg^{-1})	34.51	30.39
K ($cmol_c$ kg^{-1})	0.53	0.34
Ca ($cmol_c$ kg^{-1})	7.00	8.20
Mg ($cmol_c$ kg^{-1})	6.00	6.20
Na ($cmol_c$ kg^{-1})	0.71	0.95

CEC ($cmol_c$ kg^{-1})	60.16	48.32
Fe (mg kg^{-1})	149.89	197.65
Cu (mg kg^{-1})	27.97	25.78
Zn (mg kg^{-1})	9.96	10.20
Mn (mg kg^{-1})	39.49	39.30
B (mg kg^{-1})	1.13	0.22

LPS: Larger particle size; SPS: Smaller particle size; RAW: Readily available water; EAW: Easily available water; RW: Reserve water; EC: Electric Conductivity; OM: Organic Matter; CEC: Cation Exchange Capacity; Capacity; PB: Pine bark; P: Perlite

In all three fertilization methods, 1 g of Peters Professional® 30N-10P-10K water soluble fertilizer (equivalent to 300, 100, and 100 mg L^{-1} N, P and K, respectively) was applied. For foliar fertilization, 1 mL Aderatsa® L^{-1} as surfactant was applied to the spray solution and mixed thoroughly prior to the foliar application.

In plants treated with FIW, 100 mL per pot were applied; for FF, a fine nozzle spray pump was used to give a gentle mist, and it was sprayed on the leaves, completely covering them with the nutrient solution, until a drip from the leaves was observed. For FIW + FF the applications were made alternately, that is, one week through irrigation, and the following week to the aerial part. Both forms of fertilization were made every 7 days in the evening (between 5:00 p.m. and 6:00 p.m.). Prior to the application of the nutrient solutions, the substrate was irrigated with tap water, as well as the next day after the application, to avoid accumulation of salts, both on the substrate and on the leaves.

Studied variables and data analysis

After six months of treatment application we evaluated both aerial growth parameters: number of leaves (NL) and pseudobulbs (NPSB), leaf area (LA; LICOR® model LI-3000, Lincoln, NE, USA); as well as root growth parameters: number (NR), length (RL) and volume (RV) of roots. Subsequently, three plants per treatment were sampled and they were separated into their different organs: leaves, pseudobulbs, and roots. Once separated, the organs were dried in an oven with forced air circulation (Riossa HCF-125D, Mexico) at 70°C for 72 h, to determine their dry biomass (OHAUS Pioneer® Plus Precision PA3202, USA).

The concentrations of the macronutrients N, P, K, Ca, Mg, and S were determined in dry leaf, pseudobulb, and root tissue. Total N analysis was carried out using the semi-micro-Kjeldahl method according to Bremner (1965). The concentration of the rest of the macronutrients was determined by wet digestion of the dry tissue with a mixture of perchloric and nitric acids, described by Alcántar and Sandoval (1999); the samples were analyzed in an inductively coupled plasma optical emission spectrometer (Agilent 725 Series ICP-OES, Australia).

With the data obtained, an analysis of variance (ANOVA) was performed, and means were compared using Tukey's test ($\alpha = 5$), with the SAS statistical package (SAS, 2011).

Results

Growth parameters

Table 3 shows the significance of the study factors and their interaction in the evaluated growth variables. The interaction of the study factors significantly influenced the number of leaves, whereas the fertilization method significantly affected the number of pseudobulbs, leaf area, root volume, as well as dry biomass of both leaves and roots. The substrate particle size (SuPS) influenced number of leaves, leaf area, root volume, as well as dry biomass of both leaves and roots.

Table 3. Statistical significance (p) of the study factors, fertilization method and particle size in the substrate, as well as their interaction, on the growth parameters of *Laelia anceps* subsp. *anceps* plants

Variation source	Number of leaves	Number of pseudobulbs	Leaf area	Number of roots
Fertilization method (FM)	0.1133 ns	0.0325 *	0.0183 *	0.3198 ns
Substrate particle size (SuPS)	0.0008 *	0.1064 ns	0.0053 *	0.2401 ns
FM × SuPS	0.0122 *	0.0936 ns	0.6313 ns	0.5172 ns

Variation source	Root length	Root volume	Dry biomass		
			Leaf	Pseudobulb	Root
Fertilization method (FM)	0.4303 ns	0.0054 *	0.0085 *	0.1647 ns	0.0411*
Substrate particle size (SuPS)	0.7152 ns	0.0179 *	0.0075 *	0.2624 ns	0.0494 *
FM × SuPS	0.1910 ns	0.0766 ns	0.3135	0.2189 ns	0.0586 ns

*=p-value ≤ 0.05 is statistically significant; ns=p-value ≥ 0.05 is not statistically significant

Significant interaction effects on growth parameters. The greatest number of leaves (10.3) was observed in plants treated with the fertilization method FIW + FF established in the LPS substrate, which was statistically comparable to that observed in plants treated with FF and established in the LPS substrate. Regardless of the fertilization method, the use of substrate with SPS led to a lower number of leaves per plant (Figure 1).

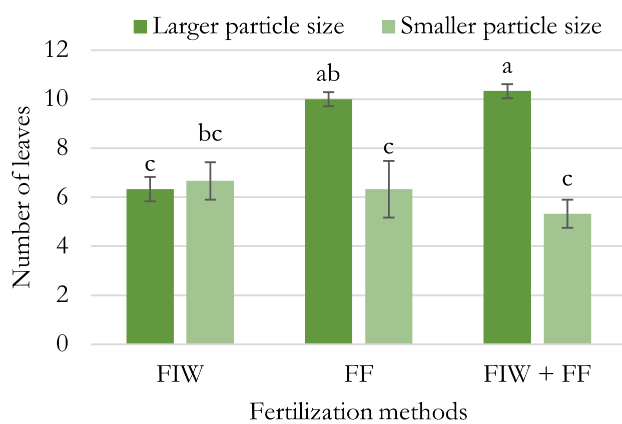

Figure 1. Number of leaves of *Laelia anceps* subsp. *anceps* plants as affected by different fertilization methods and substrate particle sizes

FIW: Fertilization in irrigation water; FF: Foliar fertilization. Bars with different letters + SD indicate significant statistical differences (Tukey, $p \leq 0.05$)

Significant main effects of fertilization methods on growth parameters. The number of pseudobulbs (6.0), leaf area (144.8 cm^2), and dry leaf biomass (3.2 g) showed the highest means in plants treated with FIW, followed by plants treated with FF, without statistical differences between these two application methods. In all three variables mentioned above, plants treated with the combination of fertilization methods (FIW + FF) displayed the lowest means, though these means were statistically similar to those observed in plants treated with FF. Root volume was higher in plants with both FIW (24.3 cm^3) and FF (22.8 cm^3) fertilization methods, which statistically surpassed FIW + FF (Figure 2). Dry biomass weight displayed no significant differences among treatments, though the lowest numerical mean was observed in plants exposed to FIW + FF.

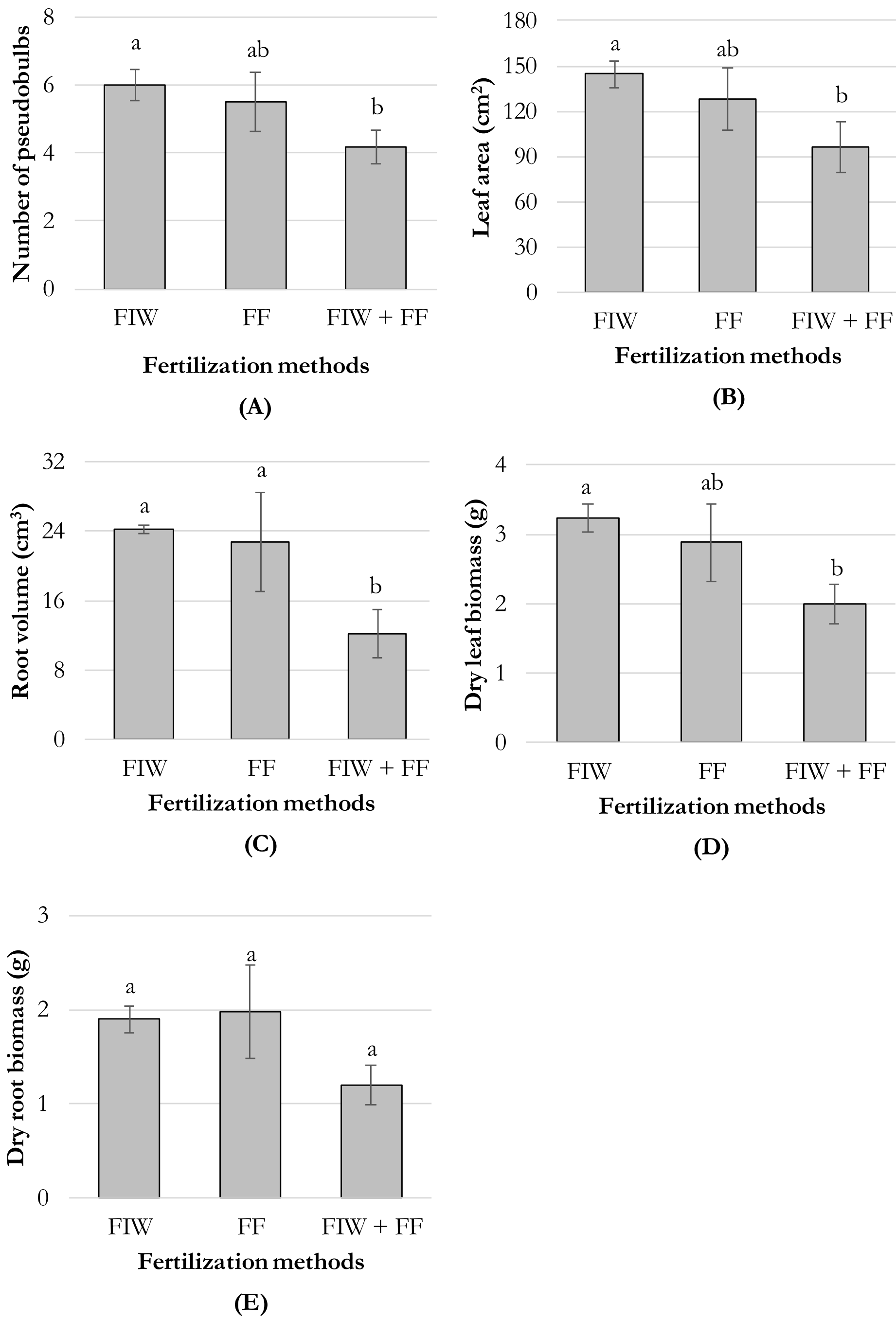

Figure 2. Number of pseudobulbs (A), leaf area (B), root volume (C), dry leaf biomass (D), and dry root biomass (E) of *Laelia anceps* subsp. *anceps* plants as affected by different fertilization methods
FIW: Fertilization in irrigation water; FF: Foliar fertilization. Bars with different letters + SD indicate significant statistical differences (Tukey, $p \leq 0.05$)

Significant main effects of particle size of the substrate on growth parameters. LPS in the substrate increased leaf area, root volume, dry leaf and root biomass by 39.7%, 44.8%, 38.7%, and 36.3%, respectively, compared to the values recorded in the substrate with SPS (Figure 3).

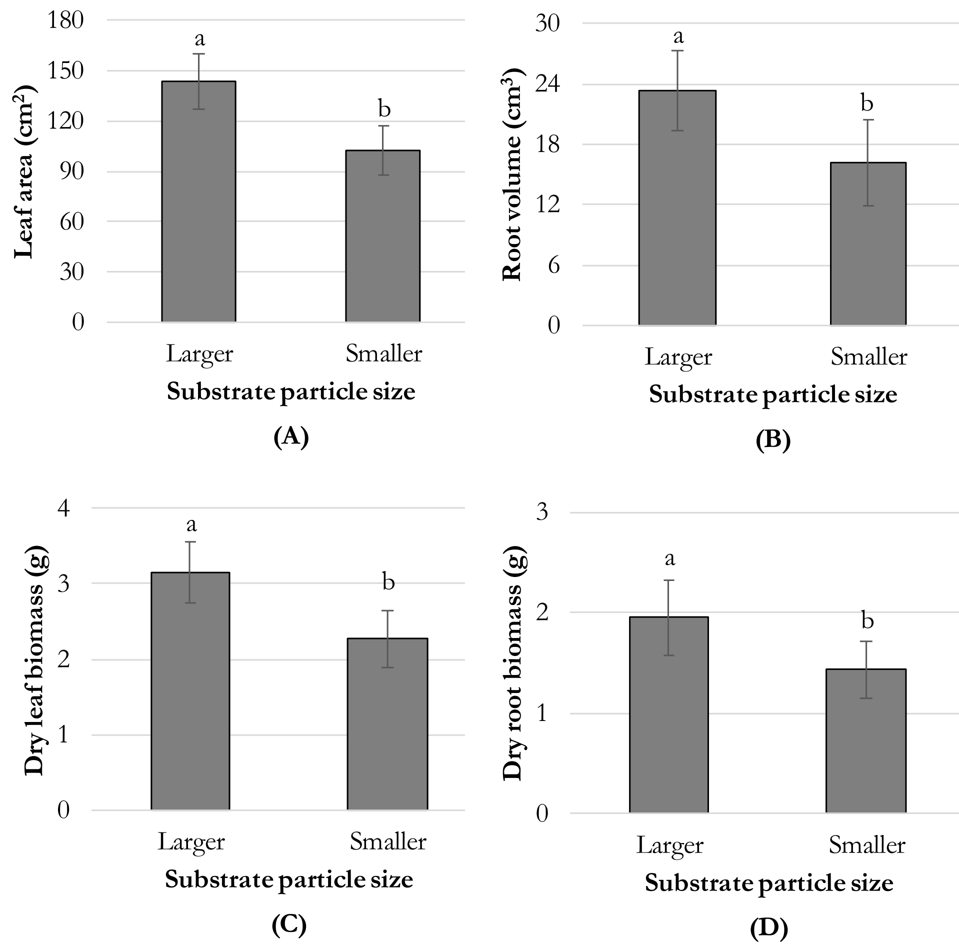

Figure 3. Leaf area (A), root volume (B), dry leaf biomass (C) and dry root biomass (D) of *Laelia anceps* subsp. *anceps* plants as affected by substrate particle size
Bars with different letters + SD indicate significant statistical differences (Tukey, $p \leq 0.05$)

Macronutrient status

Interaction of the study factors was only significant on the concentrations of P, K, Mg, and S in the roots. The fertilization method influenced the concentration of S in pseudobulbs, and that of P, K, Mg, and S in roots. The main effect of the particle size in the substrate was significant in the foliar concentrations of K and S in the leaves and in the concentrations of P, K, Ca, Mg, and S in pseudobulbs (Table 4).

Significant interaction effects on macronutrient status. In roots, regardless of the particle size in the substrate, K, Mg, and S showed higher concentrations (2.02, 5.69, and 5.16 kg^{-1}, respectively) with the combination of FIW + FF (Figures 4B-4D). P was higher in FIW and LPS (1.51 g kg^{-1}; Figure 4A). Except for this concentration of P in LPS, it was observed that, with both substrate particle sizes, FIW and FF applied individually caused the lowest concentrations of P, K, Mg, and S (Figures 4A-4D).

Table 4. Statistical significance (p) of the study factors, fertilization method and particle size in the substrate, as well as their interaction, on concentrations of macronutrients in leaves, pseudobulbs, and roots of *Laelia anceps* subsp. *anceps* plants

Leaf						
Variation source	N	P	K	Ca	Mg	S
Fertilization method (FM)	0.8460 ns	0.5536 ns	0.3024 ns	0.2036 ns	0.0505 ns	0.6239 ns
Substrate particle size (SuPS)	0.4442 ns	0.1643 ns	0.0440 *	0.1328 ns	0.0825 ns	0.0438 *
FM × SuPS	0.7844 ns	0.5026 ns	0.5464 ns	0.4130 ns	0.5605 ns	0.7418 ns
Pseudobulb						
Variation source	N	P	K	Ca	Mg	S
Fertilization method (FM)	0.6931 ns	0.0654 ns	0.0570 ns	0.1567 ns	0.1838 ns	0.0179 *
Substrate particle size (SuPS)	0.6633 ns	0.0444 *	0.0005 *	0.0299 *	0.0194 *	0.0386 *
FM × SuPS	0.7662 ns	0.7872 ns	0.9056 ns	0.2605 ns	0.1453 ns	0.0585 ns
Root						
Variation source	N	P	K	Ca	Mg	S
Fertilization method (FM)	0.0984 ns	<0.0001 *	<0.0001 *	0.5587 ns	0.0002 *	<0.0001 *
Substrate particle size (SuPS)	0.7755 ns	0.0002 *	0.0727 ns	0.0018 *	0.3052 ns	0.4973 ns
FM × SuPS	0.9790 ns	0.0005 *	0.0048 *	0.0883 ns	0.0283 *	<0.0001 *

*=p-value ≤ 0.05 is statistically significant; ns=p-value ≥ 0.05 is not statistically significant

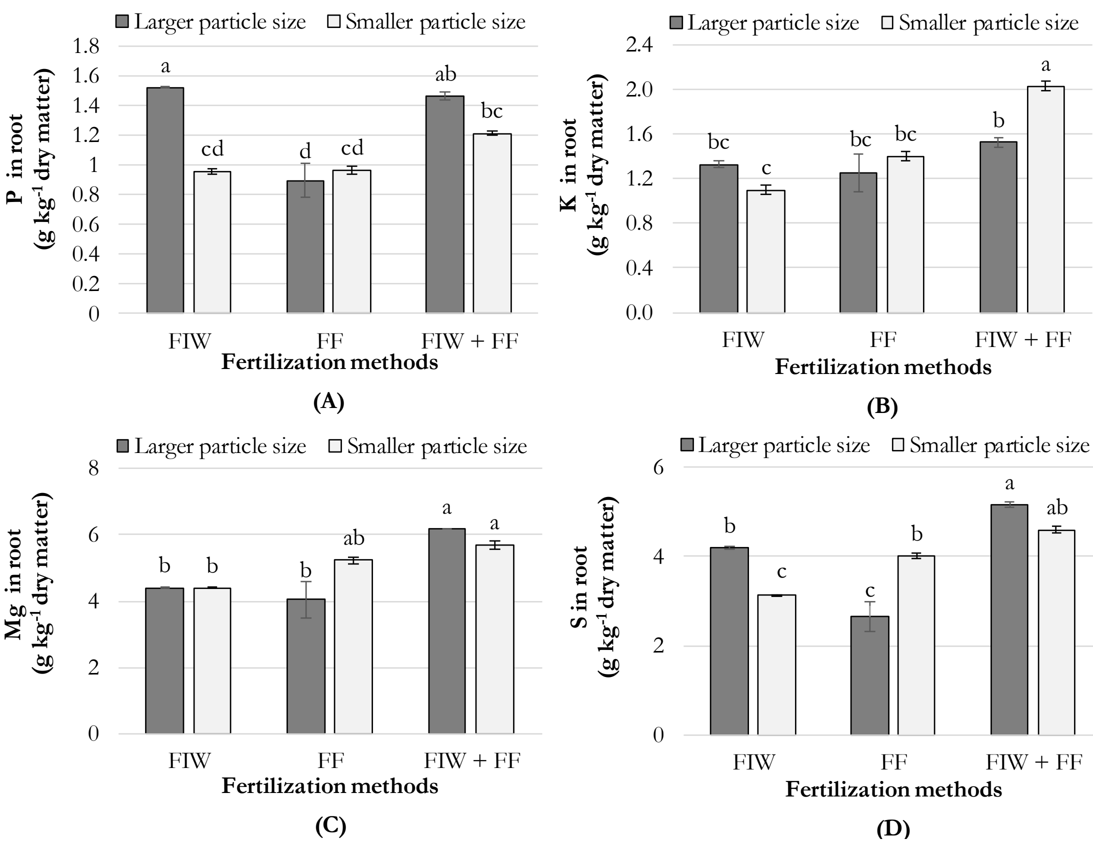

Figure 4. Concentrations of P (A), K (B), Mg (C), and S (D) in roots of *Laelia anceps* subsp. *anceps* plants as affected by different fertilization methods and substrate particle sizes

FIW: Fertilization in irrigation water; FF: Foliar fertilization. Bars with different letters + SD indicate significant statistical differences (Tukey, $p \leq 0.05$)

Significant main effects of fertilization methods on macronutrient status. The fertilization methods influenced the concentration of S in pseudobulbs (Figure 5), where FIW and FIW + FF exceed the concentration obtained with FF by 29% and 30.6%, respectively.

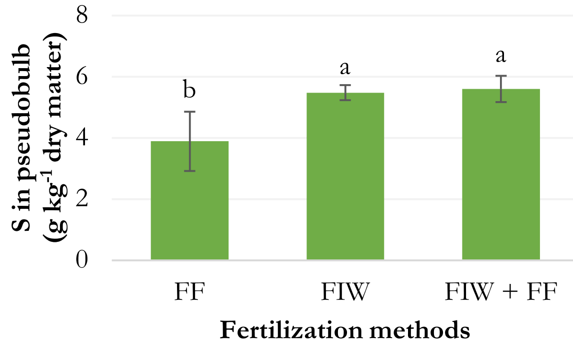

Figure 5. Concentration of S in pseudobulbs of *Laelia anceps* subsp. *anceps* plants as affected by different fertilization methods

FIW: Fertilization in irrigation water; FF: Foliar fertilization. Bars with different letters + SD indicate significant statistical differences (Tukey, $p \leq 0.05$)

Significant main effects of substrate particle size on macronutrient status. The main effect of substrate particle size was its influence on the concentrations of K and S in leaves (Figures 6A and 6B); in both, these were higher with SPS substrate by 28.5 and 26.4% respectively, compared to those obtained with LPS. This same trend was observed in the concentrations of P, K, Ca, Mg, and S in pseudobulbs (27.7, 62.4, 28.7, 31.6, and 24.3%, respectively; Figures 6C-6G).

Discussion

Aerial and root growth

The interaction effects of the FM and SuPS significantly affected the number of leaves (Table 3), where FIW + FF with LPS resulted in the highest mean for this quantitative attribute measured (Figure 1). The rest of the variables measured were not affected by this interaction (Table 3). Similarly, Wang and Gregg (1994) did not find significant differences in the interaction between substrate mixtures and fertilization rates in *Phalaenopsis* [*P. arnabilis* (L.) Blume x *P.* Mount Kaala 'Elegance']. On the contrary, Osorio *et al.* (2014) reported a highly significant interaction between substrates and fertilization used in the growth of vanilla (*Vanilla planifolia* Jacks). Therefore, the interaction effects between substrates and fertilization regimes may depend on the genotypes.

Likewise, the main effects of FM and SuPS affected variables of aerial, root and dry biomass growth (Table 3). The FIW treatment registered the highest number of pseudobulbs, leaf area, root volume, and dry biomass of leaves and roots, with statistically similar means as those observed with FF (Figures 2A-D). In *Phalaenopsis* Sogo Yukidian 'V3' plants, N uptake can be done through the leaves and roots, though they have a better absorption efficiency through the roots, and better translocation when absorbed through the leaves (Susilo *et al.*, 2013). However, according to Wang and Chang (2017), the amount of nutrients absorbed through the leaves in *Phalaenopsis* is limited and therefore it does not promote vegetative growth or flowering, the roots being the main organ responsible for nutrient uptake. When comparing different ratios of nitrogen sources (NH_4^+ and NO_3^-) in *Dendrobium* Sonia 'EarSakul', via leaves and roots, Ruamrungsri *et al.* (2014) reported that the 100:100 mg L^{-1} N combination applied to the leaves promoted growth and greater ionic absorption, which differs from our results (Figure 2). Likewise, in *Phalaenopsis* Taisuco Swan and *Phalaenopsis* Pink Chiffon, foliar fertilization was favourable, and at a concentration of 0.10 to 0.16 g L^{-1} N, plant biomass increased and optimal concentration of N (23-28 g kg^{-1}) was observed (Mantovani *et al.*, 2015). Herein, we observed that FF applied individually favours the development of roots to a greater extent (root volume; Figure

2C) than the aerial part of the plant. However, the aerial growth parameters remained statistically similar both in plants treated with FIW and FIW + FF. This species possesses coriaceous and fleshy leaves (Halbinger and Soto, 1997), and has been classified as a thick leaf orchid, characterized by thick cell walls, as well as the presence of cuticles and CAM metabolism (Hew and Yong, 2004; Zhang *et al.*, 2018). In spite of those leaf features, nutrient absorption can take place through this organ, which stimulates root growth. Conversely, foliar fertilization of *Cymbidium* sp. plants resulted in higher nutrient accumulation in pseudobulbs two hours after spraying the leaves, thus representing an efficient method of nutrient supply (García-Gaytán *et al.*, 2013).

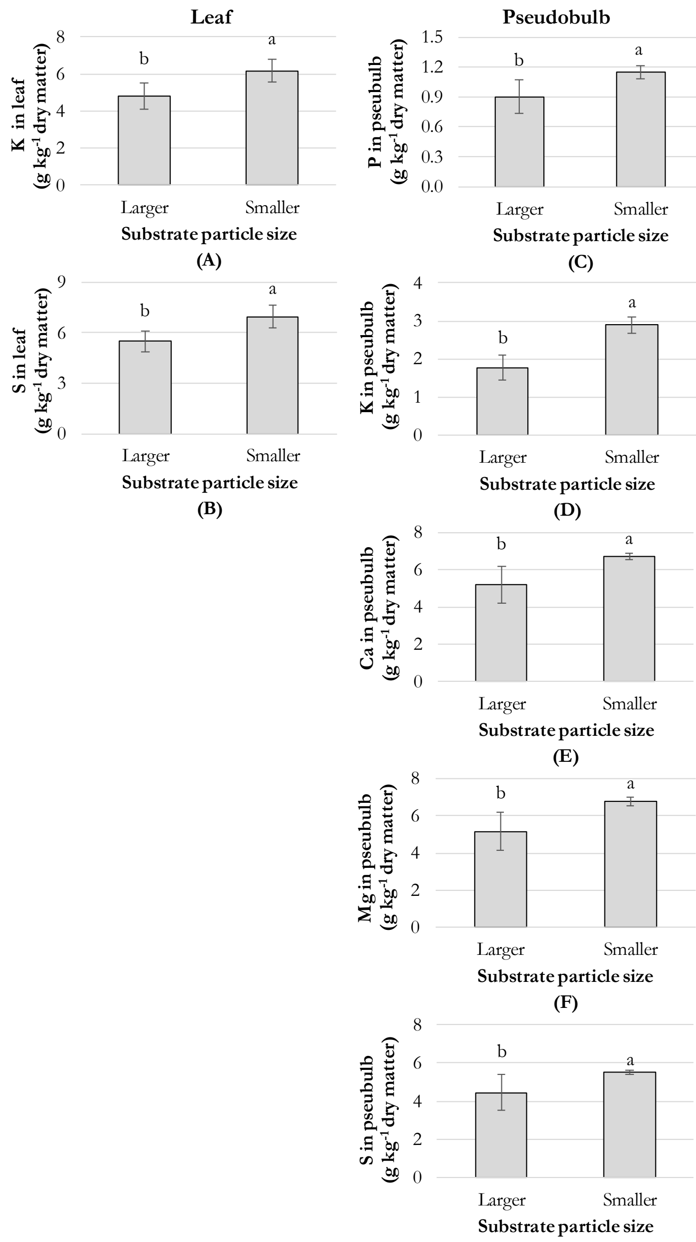

Figure 6. Concentrations of K (A) and S (B) in leaves, and of P (C), K (D), Ca (E), Mg (F), and S (G) in pseudobulbs of *Laelia anceps* subsp. *anceps* plants from the effect of substrate particle size

Bars with different letters + SD indicate significant statistical differences (Tukey, $p \leq 0.05$)

Unbalanced N supply, either in excess or deficiency, results in lower values of dry matter in *Phalaenopsis* plants (Mantovani *et al.*, 2015). Complementarily, Lichty *et al.* (2015) reported that the higher the fertigation (more than three times a week) in *Oncidium* Miltassa Shelob 'Tolkien', the lower the water holding capacity and growth of the orchids. Under our experimental conditions, the concentration, amount, and frequency of application for both types of fertilization were the same. Nevertheless, the foliar method by itself is sufficient and does not require alternating with fertilization via substrate, which results in deleterious effects such as less dry matter production.

Regarding the substrate particle size effects on growth parameters, LPS resulted in greater leaf area, root volume, and accumulation of dry leaf and root biomass (Figure 3). These results are positively associated with the highest N, P, and K concentrations and the highest CEC value in this substrate rather than in the substrate with SPS (Table 2).

Nutrient status

Under our experimental conditions, the concentration of N in both of the organs analysed was not affected by the treatments tested. In 2-year-old plants of the same species treated with 225 mg L^{-1} N, higher concentrations of N in leaves, pseudobulbs, and roots (15, 10, and 25 g kg^{-1}, respectively) were observed (Tejeda-Sartorius *et al.*, 2018), as compared to our results (9, 6, and 7 g kg^{-1} N in leaves, pseudobulbs, and roots, respectively) in 3-year-old plants. In the present study, our fertilizer (Peters® Professional 30-10-10) contained urea as the main source of N. Thus, from the 30% of total N supplied by our fertilizer, 24.8% was in the form of urea, so that the NH_4^+:NO_3^- ratio was high. In general, nitrate uptake rates in some terrestrial and epiphytic orchids are much lower than those of most crops (Hew *et al.*, 1993). Furthermore, *Laelia speciosa* preferentially absorbs more NH_4^+ than NO_3^- from the nutrient solution under higher nitrogen doses (Díaz-Álvarez *et al.*, 2015). In *Dendrobium* Sonia 'EarSakul', the absorption of NH_4^+ as the sole source of N through the roots was very high, though the translocation to the leaves and pseudobulbs was lower as compared to the application through the leaves (Ruamrungsri *et al.*, 2014). Similarly, *Phalaenopsis* Blume × Taisuco Kochdian does not grow well with 100% N-NH_4^+, requiring an adequate input of N-NO_3^- (no less than 50%, preferably 75% of the total N supply) in order to improve growth and flowering (Wang, 2008). Thus, application methods, sources and levels of N supply would result in different responses depending on the genotype tested. Moreover, developmental stages of the plants would also affect the ultimate response of the genotype to N supply (Zhang *et al.*, 2018).

The interaction of the study factors (FM and SuPS) significantly influenced the nutritional concentrations of P, K, Mg, and S in roots (Table 4), where FF had a negative effect on the concentrations of these macronutrients, regardless of the particle size of the substrate (Figure 4). The concentration of P in roots was higher in treatments with FIW and FIW + FF with LPS (Figure 4A); the concentration of K and Mg showed differences in roots and was higher when SPS and FIW + FF were used (Figures 4B and 4C). In root tissues of *Phalaenopsis* Tanigawa X Yukimai Dream 'KS 370' and 'KS 352', the concentrations of P, K, Ca, Mg, and Fe were positively correlated with the increase in the ionic strength of the nutrient solution (Hwang *et al.*, 2009). Under our experimental conditions, the root nutrient concentrations from the interaction effect may be related to the low absorption of nutrients in orchids (Zhang *et al.*, 2018) and the root structure in which the velamen plays an important role in nutrient uptake in the epiphytic habit of orchids (Zotz and Winkler, 2013). Charged ions are retained in the velamen, probably due to positive and negative charges on the cell walls, while uncharged compounds are lost to the external environment. Furthermore, nutrient absorption follows biphasic kinetics with a highly efficient active transport system at low external concentrations. However, epiphytes, including orchids, have restrictions on the physiological absorption of nutrients, including nitrogen (Silva Júnior *et al.*, 2013).

Regarding the main effect of the fertilization method, S concentration in pseudobulbs was the highest in plants treated with FIW and FIW + FF (Figure 5). Concerning the main effect of SuPS, plants established in SPS displayed higher concentrations of K and S in leaves (Figures 6A and 6B), and of P, K, Ca, Mg, and S in pseudobulbs (Figures 6C-6G). Likewise, Wang and Konow (2002) reported higher levels of N, P, and K, but similar levels of Ca and Mg for *Phalaenopsis* Blume leaves grown on fine grade Douglas fir bark as compared to the control. Similarly, in *Cymbidium* 'Baltic Glacier Mint Ice', different mixtures of growing media, nutrition and growth regulators resulted in higher concentrations of N, P, and K in leaves and pseudobulbs, which favoured the quality of the subsequent flowering of the hybrid (Barman and Naik, 2017).

As mentioned above, the highest concentrations of K and S in leaves (Figures 6A and 6B) were obtained in the substrate with SPS, where a dilution effect could be assumed given that SPS significantly decreased the dry biomass of leaves (Figure 3C). The effects on the P and K concentrations in leaves may be more evident in reproductive stages of orchids (Wang, 2010). However, in our study, SuPS significantly affected P and K in juvenile (non-reproductive) *Laelia anceps* pseudobulbs and roots, elements that could be available in the flowering stages of the species.

In our study, the substrate with SPS increased the concentrations of P, K, Ca, Mg, and S in pseudobulbs (Figure 6). This could be attributed to greater contact of the roots with a substrate with higher percentages of readily available water, easily available water, and reserve water (Table 2), which would promote greater nutrient absorption by the roots. Furthermore, a better water status in the plant also favours absorption through the leaves (Berry *et al.*, 2019), as well as the transport of nutrients (Nieves-Cordones *et al.*, 2019) to storage organs such as pseudobulbs, from where nutrients can be translocated to demand organs in the subsequent growth phases. Higher stem growth and faster flowering of *Doritaenopsis* Queen Beer 'Mantefon' were found in plants established with peat moss, attributing such result to a better contact of the roots with the substrate, which allowed an adequate supply of water and nutrients (Kim *et al.*, 2016). The composition of the medium affects the availability of water and nutrients for the growth of potted orchids. Therefore, fertigation programs must take medium composition into account (Lichty *et al.*, 2015). Under our experimental conditions, the larger particle size stimulated plant growth. Nonetheless, since there was a higher concentration of some nutrients in reserve organs such as pseudobulbs (in a medium with smaller particle size), these reserves would be useful in the further development of the plant, enabling it to achieve an even faster reproductive maturity and better flowering quality.

In commercial orchid production systems, substrate mixtures can bring better conditions for plant growth, as compared with the use of single substrates such as bark. In *Phalaenopsis* 'Stripe' and 'White Red Lip', positive results in number of roots, total fresh weight and percentage of dry matter were found when using a mixture of perlite + expanded clayball + peat moss (Hwang and Jeong, 2007). In *Cymbidium* orchids, number and length of roots, as well as leaf length increased with the use of perlite alone or in combination with sand or coconut peat (Sedaghathoor *et al.*, 2017). In *Phalaenopsis* 'Taisuco Kochdian', growth and flowering characteristics improved with the combination of pine bark with perlite and peat moss, adding a dose of 300 mg K L^{-1} (Wang, 2007). In the present study, we tested the application of 300 mg N L^{-1} N to plants established in substrate mixtures containing a combination of pine bark/perlite with different particle sizes. These treatments differentially affected plant growth and nutrient status. The substrate with LPS and FIW stimulated growth and nutrient concentrations, as compared to plants established in the substrate with SPS. Conversely, in *Phalaenopsis*, when using different proportions of pine bark with other materials of different particle size (such as peat moss, sphagnum moss, coconut husk chips) with a rate of 120 mg L^{-1} N, no effects on growth were found (Amberger-Ochsenbauer, 2010). In our study, substrate particle size had a greater impact on the nutrient concentration than the fertilization method.

Conclusions

Fertilization in irrigation water (FIW) and large particle size (LPS) of the substrate significantly increased the number of leaves of *Laelia anceps* subsp. *anceps*. In general, the individual application of either FIW or foliar fertilization (FF) stimulated growth parameters and biomass production. Conversely, the combination FIW + FF applied to plants established in the substrate with smaller particle size (SPS) negatively affected growth parameters. With all of the N application methods, there was no significant effect of its concentration on the analysed organs. The interaction of factors was significant for P, K, Mg, and S in the roots. The substrate with SPS resulted in a higher foliar concentration of K and S, as well as of P, K, Ca, Mg, and S in pseudobulbs. We are currently carrying out further studies on the impact of foliar fertilization in a broader range of developmental stages, as well as other forms and combinations of N supply in this species.

Authors' Contributions

Conceptualization: OTS, LITT; Methodology: OTS, LITT, MGPS; Validation: LITT; Formal analysis: OTS, YLFP, MGPS; Investigation; OTS, LITT; Resources; OTS, LITT; Writing-original draft: OTS; Writing-review and editing: LITT, MGPS, YLFP; Supervision: OTS, LITT; Project administration: LITT. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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