Growing Media and Nutrient Solution Concentration Affect Vegetative Growth and Nutrition of *Laelia anceps* Lindl.

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Abstract. The domestication of wild orchids for commercial production is a new endeavor, which may represent a sustainable alternative to the collection/harvest from natural populations of threatened or endangered orchid species. In the present study, the growth and nutrition of vegetative plants of *Laelia anceps* Lindl. as affected by three components of the growing medium (peat, volcanic rock, and/or horticultural grade charcoal) and the nutrient solution concentration, measured as osmotic potential (ψ_s), were assessed using mixture experiments methodology. Leaf dry mass (DM) was the highest when plants were irrigated with nutrient solutions of -0.076 MPa. The lower leaf DM at lower or higher ψ_s was influenced by the medium because plants grown in 100% volcanic rock exhibited no effect, whereas plants grown in either 100% charcoal or 100% peat had a marked reduction. Regardless of the ψ_s of the nutrient solution, the highest leaf DM was observed in mixtures of two components containing charcoal and peat at high proportions. Dry mass of pseudobulbs and roots was highest in plants irrigated with solutions of -0.051 MPa, especially in mixtures with charcoal or 100% peat. Decreasing the ψ_s of the nutrient solution resulted in increased shoot nitrogen (N) and potassium (K) concentrations and decreased concentration of phosphorus (P), calcium (Ca), magnesium (Mg), boron (B), manganese (Mn), zinc (Zn), and copper (Cu). Increasing charcoal proportion in the growing media resulted in increased plant iron (Fe) and Cu concentration. However, increasing volcanic rock reduced plant P and K and increased Mn concentration. A higher proportion of peat was correlated with a decrease in plant Fe concentration. Leaf DM fit models on which macronutrient:micronutrient or micronutrient:micronutrient ratios were calculated, suggesting that nutrient imbalance may be responsible for a plant's responses. The coefficients with the higher values included a micronutrient:micronutrient ratio, suggesting that an extremely fine balance in the uptake of a given micronutrient in relation to other micro- or macronutrient is of major importance for adequate growth of *Laelia*.

The Orchidaceae family contains more than 20,000 species distributed around the world (Molgo and De Dijn, 2007), ≈ 1200 of

which are found in México (Espejo-Serna and López-Ferrari, 1998; Soto, 1998), including 11 species of *Laelia* Lindl. *Laelia anceps* Lindl. is an epiphytic orchid that naturally occurs in the oak forests of the central and southern regions of the country. In the fall and winter, *L. anceps* produce flower stalks bearing white or pink flowers (8 to 10 cm in diameter) of exceptional beauty

and fragrance. When in season, large numbers of *L. anceps* flower stalks and those of other *Laelia* species are harvested from the wild for a variety of festivals, including weddings and other religious ceremonies (Ávila-Díaz and Oyama, 2007). Over time, collection/harvesting of flowers and plants from the wild has threatened the sustainability of *L. anceps* to the point of near extinction in certain regions. Ortega-Loeza et al. (2011) reported that *L. speciosa* (H.B.K.) Schltr. is threatened as a result of overcollection and loss of natural habitat through deforestation. Environmental agencies in México have been, with limited success, trying to protect *L. anceps* and other orchid species against illegal collection/harvesting (Ávila-Díaz et al., 2009). Unfortunately, harvesting of flowers and plants from natural populations continues in certain regions.

In México, the domestication of wild orchids for commercial production, a relatively new endeavor, may represent a sustainable alternative to the collection/harvest of these flowers from natural populations. However, commercial cultivation of newly introduced orchids can be tricky and requires sound management practices to attain economic success. The interaction between nutrients in the media solution and the physical and chemical properties of the growing media may affect the response of plants to a specific substrate and nutrient solution formulation.

Tree bark is a traditionally used media for orchid cultivation in México as well as in the United States (Wang and Konow, 2002). However, when bark is mixed with other substrate components of higher water-holding capacity, for example peat and vermiculite, better growth and flower quality are attained (Wang, 1998). In addition, the nutrient requirements of orchids were thought to be relatively low because they respond slowly to fertilization. However, studies have demonstrated that *Phalaenopsis* Blume and *Dendrobium* Sw. respond well to fertilization as indicated by increased growth rate and plant quality (Wang, 1995, 1996; Wang and Konow, 2002). Thus, nutrition and growing media selection are critical factors for commercial production of orchids. Unfortunately, information on fertilization and substrate preparation for the cultivation of orchids is limited and not necessarily supported by the literature (Wang and Konow, 2002).

The objective of the present study was to investigate the response of *L. anceps* to media composition (charcoal, volcanic rock, and peat) and nutrient solution concentration (in terms of ψ_s) to serve as a basis for the commercial production of this threatened species.

Materials and Methods

Cultural conditions. The experiment was conducted under glasshouse conditions in México (lat. $19^{\circ}29'05''$ N, long. and $98^{\circ}53'11''$ W) from 1 May to 15 Dec. 2007. The glasshouse was equipped with a fog system for relative humidity control and a heater for temperature control. Average temperature and relative humidity for the

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duration of the experiment were 22.5 ± 7.5 °C (maximum 30 °C, minimum 15 °C) and $80\% \pm 20\%$ (maximum 100%, minimum 55%), respectively. The experimental site was shaded with a 60% shade cloth, rendering an average photosynthetically active radiation of $227 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Micropropagated 15-month-old *L. anceps* liners, 9 to 10 cm in height, with four to six pseudobulbs and four to six leaves, were planted in 15-cm (2.04-L) standard pots. Pots were filled with 1.80 L of the media and plants were allowed to establish for 7 d before experiment initiation.

Growing media. The growing media selected for the study were a mixture of three components: horticultural-grade charcoal (1 to 2 cm in diameter), volcanic rock (1 to 2 cm in diameter), and medium-length fiber peat, which were mixed at varying proportions (v/v). The proportion of each component present in the growing media was designed using mixture experiments methodology and are expressed as a fraction in Figure 1; the total volume of the mixed components was maintained constant, and the sum of all the fractions must be equal to one. Thus, the response of plants to the mixtures depends only on the proportions of the components (Cornell, 2002). Physical and chemical properties of the three components are shown in Table 1. Before transplanting, the pH of the 13 resulting mixtures of charcoal:volcanic rock:peat was adjusted to 6.3 to 6.5 with dolomitic limestone or agricultural-grade sulfur.

The response to the mixtures is represented in an equilateral triangle (Fig. 1) on which each vertex corresponds to one component. On the sides of the triangle, there are the binary mixtures that contain two of the mixture components, whereas in the interior, there are the tertiary blends with mixtures containing the three components at varying proportions.

Nutrient solutions. The nutrient solutions were a modified complete Hoagland's formulation (in $\text{meq}\cdot\text{L}^{-1}$: 12 NO_3^- , 3 NH_4^+ , 1 H_2PO_4^- , 8 Ca^{2+} , 6 K^+ , 4 Mg^{2+} , 8 SO_4^{2-} , and 5 $\text{mg}\cdot\text{L}^{-1}$ Fe) with increasing concentrations of macronutrients and Fe: 66%, 100%, and 133% of the complete Hoagland's solution, which rendered an ψ_s of -0.051 , -0.076 , and -0.101 MPa, respectively. Electrical conductivity (EC) of the nutrient solutions was 1.42, 2.11, and $2.81 \text{ dS}\cdot\text{m}^{-1}$, respectively. Boron, Zn, Cu, and Mn were maintained constant in the nutrient solutions at 0.50, 0.50, 0.02, and $0.65 \text{ mg}\cdot\text{L}^{-1}$, respectively. Municipal water (EC = $0.76 \text{ dS}\cdot\text{m}^{-1}$, $\psi_s = -0.027$ MPa) was used to prepare the nutrient solutions. Fertilizer salts were added to the irrigation water to reach the selected ψ_s . The pH of the nutrient solutions was adjusted to 6.0 with sulfuric acid 0.1 N. Throughout the experiment, plants were manually irrigated as needed with one of the three nutrient solutions.

The experiment fulfilled mixture experiments methodology with a simplex-centroid design augmented with six interior points

(Cornell, 2002). Each point in the triangle of Figure 1 corresponds to one of the 13 media mixtures, whereas the ψ_s of the nutrient solution was used as a process variable with the three levels mentioned.

Assessment of plant growth. Plants were harvested 180 d after experiment initiation and separated into leaves, pseudobulbs, and roots. Plant parts were washed twice in distilled water, placed in paper bags, dried in an oven at 60 to 65 °C for 5 d, and the DM of leaves, pseudobulbs, and roots was determined. Plants parts were ground to pass a 40-mesh screen and for shoot tissue analysis, a mixture composed of the dry tissues from the leaves and pseudobulbs was prepared. The analysis included: N, P, K, Ca, Mg, Fe, B, Cu, and Zn. Nitrogen was determined by the Kjeldahl procedure and the remaining elements by inductively coupled plasma mass spectrometry (model Liberty; VARIAN, Santa Clara, CA).

Statistical design and analysis. Plant responses to the growing media and ψ_s of the nutrient solutions were analyzed using Design Expert® Version 6.0.4. Statistical models were selected using adjusted R^2 and the model with fewer and significant terms ($P < 0.05$) to estimate optimum mixture of medium components and ψ_s of the nutrient solution. The mixtures combined with the nutrient solutions resulted in 39 treatments, which, along with the four one-plant replications, were arranged in a randomized block design. Pairwise shoot nutrient concentration ratios were regressed to estimate the linear models that best explained leaf DM. These models were selected using backward elimination of model terms with SAS Version 8.0 (SAS Institute, Inc., 2001). Nutrient concentrations were statistically analyzed using analysis of variance and Tukey's multiple comparison test with $P < 0.05$.

Results

Leaf DM was affected by the ψ_s of the nutrient solution and the growing media (Table 2). According to the models, leaf DM was the highest when plants were irrigated with a solution of -0.076 MPa. Lower or higher ψ_s had a deleterious effect on leaf DM (Fig. 2A–C). Furthermore, smaller leaf DM at the lower or higher ψ_s was influenced by the medium mixtures as plants grown in 100% volcanic rock exhibited no effect, whereas plants grown in either 100% charcoal or 100% peat had a lower leaf DM. Regardless of the ψ_s of the nutrient solution, the highest leaf DM was observed in mixtures of two components containing higher proportions charcoal and peat. The media in which plants produced greater than 80% of maximum leaf DM are shown in the shaded area of the counterplot in Figure 2B.

The ψ_s of the nutrient solution and the substrate mixes affected the growth of pseudobulbs (Table 2). Higher ψ_s of the nutrient solution resulted in lower pseudobulbs DM but it was more affected when plants were grown in media containing charcoal or peat.

Fig. 1. Design points corresponding to the mixtures of charcoal: volcanic rock: peat evaluated in terms of their proportion in the growing media. The growing media were assessed at three increasing fertilizer ratios measured as osmotic potential (ψ_s) -0.051 , -0.076 , and -0.101 MPa.

Table 1. Physical and chemical properties of the growing media components evaluated.

	Charcoal	Volcanic rock	Peat
Solid space (%)	31.5	31.1	22.3
Total pore space (%)	68.5	68.9	77.7
Water-holding capacity (%) (by volume)	21.7	20.1	70.0
Air capacity (%)	46.9	48.8	7.90
Apparent density ($\text{g}\cdot\text{cm}^{-3}$)	0.35	0.96	0.15
Bulk density ($\text{g}\cdot\text{cm}^{-3}$)	1.12	3.08	0.73
Electrical conductivity ($\text{dS}\cdot\text{m}^{-1}$)	1.59	0.27	0.56
Cation exchange capacity [$\text{cmol}(+)/\text{kg}$]	12.9	4.9	51.3

Table 2. Models that estimate² *Laelia anceps* Lindl. leaf, pseudobulb, and root dry mass (DM) in response to mixtures of charcoal (Ch), volcanic rock (Vr), and peat (Pm) for growing media formulation at three nutrient solution concentrations measured as osmotic potential (ψ_s).

Growth parameter	ψ_s (MPa)	Model	P	Adjusted R^2
Leaf DM (g)	-0.051	$1.830\text{Ch} + 1.925\text{Vr} + 2.074\text{Pm} - 1.195\text{Vr}*\text{Pm}$	<0.0001	0.650
	-0.076	$2.494\text{Ch} + 1.925\text{Vr} + 2.398\text{Pm} - 1.195\text{Vr}*\text{Pm}$		
	-0.101	$1.244\text{Ch} + 1.925\text{Vr} + 1.758\text{Pm} - 1.195\text{Vr}*\text{Pm}$		
Pseudobulb DM (g)	-0.051	$2.735\text{Ch} + 2.159\text{Vr} + 2.774\text{Pm} + 2.163\text{Ch}*\text{Pm}$	<0.0001	0.704
	-0.076	$2.346\text{Ch} + 2.159\text{Vr} + 2.676\text{Pm} - 1.511\text{Ch}*\text{Pm}$		
	-0.101	$1.001\text{Ch} + 2.159\text{Vr} + 1.900\text{Pm} + 2.939\text{Ch}*\text{Pm}$		
Root DM (g)	-0.051	$2.591\text{Ch} + 2.503\text{Vr} + 2.030\text{Pm} - 3.645\text{Ch}*\text{Vr} - 1.830\text{Ch}*\text{Pm} + 4.308\text{Ch}*\text{Vr}*(\text{Ch}-\text{Vr})$	<0.0001	0.840
	-0.076	$1.708\text{Ch} + 1.791\text{Vr} + 2.111\text{Pm} + 0.008\text{Ch}*\text{Vr} - 2.880\text{Ch}*\text{Pm} + 4.308\text{Ch}*\text{Vr}*(\text{Ch}-\text{Vr})$		
	-0.101	$1.053\text{Ch} + 1.692\text{Vr} + 0.946\text{Pm} - 0.360\text{Ch}*\text{Vr} + 1.594\text{Ch}*\text{Pm} + 4.308\text{Ch}*\text{Vr}*(\text{Ch}-\text{Vr})$		

²To estimate the growth parameter, select the model for the respective ψ_s , substitute the proportion of each component in the media (charcoal, volcanic rock, or peat) as a fraction of 1 on the respective term (Ch, Vr, or Pm), and multiply it by the respective coefficient. For example, to estimate leaf DM of plants irrigated with a solution of -0.076 MPa and grown in a substrate formulated with 50% charcoal, 25% volcanic rock, and 25% peat, the model will be in the form: leaf DM = $2.494(0.50) + 1.925(0.25) + 2.398(0.25) - 1.195(0.25)(0.25) = 2.25$ g.

Furthermore, DM of pseudobulbs was highest in plants irrigated with solutions of -0.051 MPa, especially in mixes with charcoal or 100% peat (Figs. 3A). Regardless of the ψ_s of the nutrient solution, *Laelia* grown in volcanic rock exhibited the lowest pseudobulb DM. Irrigation with solutions of -0.076 MPa resulted in decreased pseudobulb DM when plants were grown in a substrate that contained a mix of charcoal and peat or high proportions of charcoal (Fig. 3B). However, pseudobulb DM decreased when the ψ_s was decreased to -0.101 MPa and the medium was 100% peat (Fig. 3C). The media at which the plants produced greater than 80% of maximum pseudobulb DM are shown in the shaded area of the counterplot in Figure 3A when ψ_s was -0.051 MPa and in substrate mixtures of high proportions of either charcoal or peat, mixtures of charcoal and peat, and low proportions of volcanic rock.

Fig. 2. Response surface exhibiting the effect of the proportion of charcoal: volcanic rock: peat in the growing media as affected by increasing fertilizer ratios in the nutrient solution measured as osmotic potential (ψ_s) -0.051 (A), -0.076 (B), and -0.101 MPa (C) in *Laelia anceps* Lindl. leaf dry mass (DM). The shaded area in B indicates the media predicted with the highest 20% of leaf DM in accordance with the estimated models.

Fig. 3. Response surface exhibiting the effect of the proportion of charcoal: volcanic rock: peat in the growing media as affected by increasing fertilizer ratios in the nutrient solution measured as osmotic potential (ψ_s) -0.051 (A), -0.076 (B), and -0.101 MPa (C) in *Laelia anceps* Lindl. pseudobulb dry mass (DM). The shaded area in A indicates the media predicted with the highest 20% of pseudobulb DM in accordance with the estimated models.

Growing media and ψ_s affected root DM (Table 2). In general, root growth was promoted when nutrient solution ψ_s was -0.051 MPa and the medium contained higher proportions of charcoal (Fig. 4A). Furthermore, decreasing the ψ_s of the nutrient solution to -0.076 (Fig. 4B) and -0.101 MPa (Fig. 4C) resulted in decreased root DM in all the substrate mixtures assessed, particularly when grown in media containing high proportions of charcoal. The media in which the plants produced greater than 80% of maximum root DM are shown in the shaded area of the counterplot in Figure 4A when ψ_s is -0.051 MPa and in mixes with high proportions of charcoal.

Analysis of nutrient concentration in the shoot (pseudobulb + leaves) following mixture experiments methodology rendered non-significant models. However, when the data were analyzed as a factorial experiment, a significant substrate, ψ_s , and interaction effect were detected. Averaged across the growing media, decreasing the ψ_s of the nutrient solution resulted in increased shoot N and K concentrations (Table 3) and decreased concentration of P, Ca, Mg (Table 3), B, Mn, Zn, and Cu (Table 4). Osmotic potential had no effect on Fe concentration (Table 4).

Table 5 shows the positive or negative sign of significant correlations ($P < 0.05$) between the media components or ψ_s with shoot nutrient concentration. Increasing charcoal proportion in the growing media resulted in increased shoot Fe and Cu concentration; however, increasing volcanic rock reduced P and K and increased Mn concentration. Increasing the proportion of peat was correlated with a decrease in plant Fe concentration. Decreasing the ψ_s of the nutrient solution resulted in increased shoot N and K concentration and reduced Ca, Mg, and micronutrients.

Leaf DM fit the following model ($R^2 = 0.970$, adjusted $R^2 = 0.925$, $P < 0.0001$, $n = 117$), on which macronutrient:micronutrient

or micronutrient:micronutrient ratios were calculated, regardless of the growing medium used or the ψ_s of the nutrient solution.

$$\begin{aligned} \text{LDM(g)} = & -19.06 - 1.19 \times 10^{-3} \text{N/Fe} \\ & + 1.33 \times 10^{-3} \text{N/Zn} - 1.64 \text{P/B} \\ & + 2.28 \times 10^{-2} \text{P/Cu} - 7.68 \times 10^{-2} \text{P/Fe} \\ & - 2.24 \times 10^{-1} \text{P/Mn} + 3.37 \times 10^{-1} \text{P/Zn} \\ & - 2.83 \times 10^{-1} \text{K/B} + 3.53 \times 10^{-3} \text{K/Cu} \\ & + 3.28 \times 10^{-2} \text{K/Fe} - 1.98 \times 10^{-2} \text{K/Mn} \\ & - 2.13 \times 10^{-2} \text{Ca/Fe} + 1.93 \times 10^{-2} \text{Ca/Zn} \\ & + 1.05 \text{Mg/B} - 1.12 \times 10^{-2} \text{Mg/Cu} \\ & - 3.66 \times 10^{-2} \text{Mg/Fe} - 7.71 \times 10^{-2} \text{Mg/Zn} \\ & + 2.58 \text{B/Fe} + 4.74 \text{B/Mn} \\ & - 3.16 \text{B/Zn} - 2.57 \text{Cu/Fe} \\ & + 4.00 \text{Fe/Zn} + 3.65 \text{Mn/Zn} \end{aligned}$$

Discussion

Our results demonstrate that *L. anceps* responded to fertilization rate, because the nutrient solution of -0.076 MPa resulted in the highest leaf DM. Thus, the nutrient concentration of solutions at -0.051 MPa was not sufficient to maintain growth of *Laelia*. However, N, P, K, Mg, B, Fe, Mn, Zn, and Cu of these plants were comparable to those irrigated with -0.076 MPa solutions, suggesting a higher level uptake of minerals by plants receiving the -0.076 MPa nutrient solution. The highest leaf Ca concentration was found in plants irrigated with solutions of high ψ_s , -0.051 MPa, probably as a result of its lower fertilizer rate. Solutions of high ψ_s have comparatively more energy than solutions of low ψ_s and in turn allow a higher water uptake rate by roots and a mass flow of water and Ca to the roots (Yang et al., 2012). Calcium concentration exhibited by *Laelia* was within the sufficiency range reported for

other orchid species such as *Cattleya* Lindl. (Mills and Benton Jones, 1996) and *Phalaenopsis* (Wang and Konow, 2002). Wang (1998) reported contrasting results in *Phalaenopsis* in that increases in nutrient concentration of the nutrient solution were related with increased leaf Ca concentration in plants grown in Douglas fir bark. However, the range of nutrient concentrations assessed by Wang (1998) was lower than those used in our study.

Decreased leaf DM in plants irrigated with solutions of high fertilizer rate (-0.101 MPa) indicates that high nutrient solution concentration resulted in a detrimental effect, probably associated with the lower ψ_s of the nutrient solution. This may reflect the sensitivity of epiphytic orchids to salinity because in their natural habitat they are not exposed to high salinity (Wang, 1998). A number of floricultural species are reported as sensitive to high salinity in irrigation water (Grieve, 2011).

The low ψ_s of irrigation water is one of the components of salinity that causes growth reduction in plants (Munns and Tester, 2008) as a result of slower water uptake and decreased enlargement rate of cells and/or cell division (Munns and Tester, 2008; Zhang et al., 2010). In our study, we detected a marked reduction in leaf area of plants irrigated with solutions at the lowest ψ_s (data not shown), suggesting that either cell elongation or cell division, or both, were affected. The decreased leaf DM of plants irrigated with solutions of high nutrient concentration may also be the result of a nutrient imbalance, because plants with increased N and K (directly associated with the highest N and K concentrations of the irrigation solution) exhibited a decrease in P, Ca, Mg, B, Mn, Zn, and Cu concentration, suggesting a lower uptake rate and/or translocation from roots to leaves.

Plants of *Phalaenopsis* have been reported to exhibit enhanced growth and flower count when grown in a mixture of bark with media

Fig. 4. Response surface exhibiting the effect of the proportion of charcoal: volcanic rock: peat in the growing media as affected by increasing fertilizer ratios in the nutrient solution measured as osmotic potential (ψ_s) -0.051 (A), -0.076 (B), and -0.101 MPa (C) in *Laelia anceps* Lindl. root dry mass (DM). The shaded area in A–B indicates the media predicted with the highest 20% of root DM in accordance with the estimated models.

Table 3. *Laelia anceps* Lindl. macronutrient concentration (mmol·kg⁻¹) in shoot as affected by the proportion of media components: charcoal (Ch), volcanic rock (Vr), and peat (Pm), at three nutrient solution osmotic potential (ψ_s) (-0.051, -0.076, and -0.101 MPa).

	Nitrogen			Phosphorus			Potassium			Calcium			Magnesium		
	-0.051	-0.076	-0.101	-0.051	-0.076	-0.101	-0.051	-0.076	-0.101	-0.051	-0.076	-0.101	-0.051	-0.076	-0.101
Ch															
1	3436	3588	2779	54.8	42.1	48.6	247	270	264	297	279	254	223	227	180
0	2476	4346	3083	47.0	37.9	39.0	224	225	289	298	248	199	265	234	172
0	2830	3335	1	57.8	43.6	39.3	261	270	270	313	230	194	258	218	168
0.5	3436	2830	4952	55.3	35.7	39.3	226	254	318	284	167	229	197	173	189
0.5	3032	2880	3689	47.6	39.2	38.5	299	270	313	314	188	213	202	154	173
0	2476	2628	4194	39.6	45.9	38.5	198	242	301	209	226	247	166	174	193
0.67	3133	2678	2880	60.2	37.9	39.1	293	291	223	345	222	181	253	179	182
0.17	3537	3032	4548	42.9	38.9	47.2	259	286	257	286	225	196	200	201	199
0.17	3335	2804	3184	52.2	64.8	44.2	253	296	290	200	278	215	178	227	180
0.42	3083	2325	2931	37.5	56.2	40.3	213	284	249	176	279	250	176	229	195
0.42	2577	3436	3487	43.8	41.2	38.8	289	290	305	271	219	234	215	222	203
0.17	2122	3487	3335	44.2	50.6	49.1	262	220	272	287	262	263	219	174	218
0.33	2779	3588	4194	47.3	54.8	50.5	237	302	317	245	345	254	185	271	229
Least significant difference ^z	1219	1563	1870	22.0	21.1	10.6	100	112 NS	122 NS	132	113	71	87	80	65 NS
Average of ψ_s	2943 b ^y	3151 b	3572 a	48.5 a	45.3 ab	42.5 b	251 b	269 ab	282 a	271 a	244 b	225 b	209 a	205 a	189 b
Analysis of variance															
Substrate mixture		0.003			<0.001			0.045			0.011			<0.001	
Interaction		<0.001			<0.001			0.002			<0.001			0.003	
		<0.001			<0.001			0.023			<0.001			<0.001	

^zLeast significant difference according to Tukey's mean comparison test ($P < 0.05$).

^yMean comparison across the ψ_s of the nutrient solution, means followed by different letters are significant at $P < 0.05$.

NS Nonsignificant according to Tukey's mean comparison test ($P > 0.05$).

components of high water-holding capacity such as peat and vermiculite compared with plants grown in a medium composed exclusively of bark (Wang and Konow, 2002). Likewise, in our study, the response of *Laelia* to the nutrient solution concentration was influenced by the composition of the growing media. At optimum ψ_s (-0.076 MPa), the highest leaf DM was projected in mixtures containing either charcoal or peat or a combination of both plus a low proportion of volcanic rock.

The optimum growth of *Laelia* when cultivated in media containing high proportions of peat and charcoal suggests that this orchid demands a combination of components that provide a high water-holding capacity (peat), high aeration (charcoal), high cation exchange capacity (peat), and low apparent density (charcoal and peat). The volcanic rock used did not meet these standards because it had a low cation exchange capacity, a low water-holding capacity, and a high bulk density compared with peat and charcoal.

Charcoal has been successfully used as a component of growing media for orchid cultivation (Barman et al., 2012; Wang and Gregg, 1994) and other epiphytic species such as *Anthurium andraeanum* Linden ex André (Singh et al., 2009). Charcoal has been attributed with several beneficial effects when applied to soil, which may be effective when used for production purposes as a growing media component. According to Glaser et al. (2002) and Laird (2008), charcoal: 1) increases the capacity to absorb plant nutrients and reduces nutrient leaching; 2) may retain and slowly release most nutrients required for plant growth; 3) improves the bulk density of soils because it has a very low density, thus, root penetration is facilitated while water drainage and soil aeration are increased; and 4) it is a liming agent that reduces substrate acidification. Coarse materials are recommended when combined with a water-absorptive material for growing *Phalaenopsis* because it provides excellent air movement through the medium (Wang et al., 2007); such properties were provided by charcoal and peat in our study.

Shoot growth (leaf DM + pseudobulb DM) of *Laelia* was enhanced over root growth at -0.076 MPa, whereas the lowest and highest ψ_s resulted in enhanced root growth over shoot growth (data not shown), corroborating that fertilization rate affected DM allocation. Similar results were reported by Wang (1998) in *Phalaenopsis* irrigated with water of increasing salinity. The decreased root growth under high fertilization rates may be the result of the lower ψ_s of the nutrient solution because it has been reported to exert an immediate effect on plants, including *Phalaenopsis*, by limiting root tip cell expansion (Munns and Tester, 2008; Wang, 1998). Apparently, our results are in contrast to those reported by Wang and Gregg (1994) because *Phalaenopsis* root DM increased dramatically when fertilized with increasing concentrations of 20N-8.6P-16.6K from 0.25 to 1.0 g·L⁻¹; we suggest that

Table 4. *Laelia anceps* Lindl. micronutrient concentration ($\mu\text{mol}\cdot\text{kg}^{-1}$) in shoot as affected by the proportion of media components: charcoal (Ch), volcanic rock (Vr), and peat (Pm), at three nutrient solution osmotic potential (ψ_s) (–0.051, –0.076, and –0.101 MPa).

		Boron			Iron			Manganese			Zinc			Copper		
Ch	Vr	Pm	–0.051	–0.076	–0.101	–0.051	–0.076	–0.101	–0.051	–0.076	–0.101	–0.051	–0.076	–0.101	–0.051	–0.076
1	0	0	7.54	6.36	5.98	1.27	0.94	0.84	2.28	2.06	1.92	1.10	0.98	0.93	0.30	0.39
0	1	0	7.67	6.29	5.04	0.94	0.64	0.71	2.92	2.45	1.62	1.16	0.96	0.74	0.26	0.23
0	0	1	7.58	6.20	4.50	0.80	0.61	0.62	2.09	2.14	1.66	1.19	0.92	0.71	0.34	0.14
0.5	0.5	0	6.79	5.39	4.91	0.91	1.46	0.93	2.02	1.56	1.76	1.06	0.84	0.76	0.37	0.26
0.5	0	0.5	6.65	5.14	5.47	0.74	0.57	0.79	2.00	1.31	1.33	0.81	0.64	0.72	0.34	0.26
0	0.5	0.5	5.97	5.71	5.71	0.86	0.75	0.96	1.81	1.28	1.65	0.85	0.80	0.69	0.18	0.29
0.67	0.17	0.17	8.00	5.72	4.87	1.01	0.71	0.88	2.67	1.77	1.44	1.03	0.77	0.71	0.26	0.22
0.17	0.67	0.17	6.05	6.41	5.38	0.73	0.82	0.90	1.92	2.28	1.68	0.86	0.87	0.88	0.24	0.21
0.17	0.17	0.67	5.25	7.33	5.15	0.59	0.71	0.77	1.60	2.16	2.57	0.77	0.96	1.01	0.21	0.16
0.42	0.42	0.17	4.64	7.93	5.17	0.58	0.91	0.72	1.49	2.45	1.97	0.66	1.16	0.76	0.32	0.34
0.42	0.17	0.42	6.24	6.81	6.13	0.81	0.70	0.89	1.68	1.63	1.87	0.97	0.79	0.82	0.48	0.25
0.17	0.42	0.42	5.09	5.72	4.91	0.69	0.83	0.85	1.97	1.26	1.71	0.88	0.82	0.90	0.31	0.19
0.33	0.33	0.33	4.83	8.04	5.67	0.99	1.20	0.74	1.64	1.86	1.71	0.85	1.13	0.81	0.30	0.41
Least significant difference ^a			3.12	2.94 NS	2.25 NS	0.44	0.55	0.52 NS	0.96	1.04	0.70	0.39	0.42	0.25	0.37 NS	0.11
Average of ψ_s ^b			6.33 a	6.39 a	5.30 b	0.84	0.84	0.81	2.01 a	1.86 ab	1.76 b	0.94 a	0.90 a	0.80 b	0.30 a	0.27 a
Analysis of variance																
Substrate mixture			0.204			<0.001				<0.001			<0.001		0.002	
ψ_s			<0.001			0.777				0.003			<0.001		<0.001	
Interaction			<0.001			<0.001				<0.001			<0.001		<0.001	

^aLeast significant difference according to Tukey's mean comparison test ($P < 0.05$).

^bMean comparison across the ψ_s of the nutrient solution, means followed by different letters are significant at $P < 0.05$.

NS Nonsignificant according to Tukey's mean comparison test ($P > 0.05$).

the fertilizer formula at the highest concentration used by Wang and Gregg (1994) rendered isosmotic solutions like in our study at the highest and, suggesting that our results with *Laelia* are comparable to those cited for *Phalaenopsis*. A differential fertility requirement by the two orchid species may also explain the contrasting results.

According to Wang (1998), increasing salinity of irrigation water linearly decreased *Phalaenopsis* root fresh weight regardless of the growing media, whereas shoot mass increased when grown in Douglas fir bark. However, when grown in a mixture of Douglas fir bark and peat, both shoot and root DM decreased as irrigation salinity increased. The contrasting response to salinity in irrigation water in orchids grown in different media may have been the result of the high cation exchange capacity of peat, which may increase the retention of sodium, chlorine, and other ions, as demonstrated by the higher EC of the leachate reported, thus rendering the toxicity effects of such ions.

The interaction between the growing media and the nutrient solution ψ_s indicates that shoot nutrient concentration was differentially affected by the combination of both factors. However, a model to explain the association between plant growth and nutrient concentration could not be estimated using mixture experiments methodology. Wang (1998) reported that increasing EC of irrigation water had no effect on the concentration of most macronutrients and micronutrients in leaves of *Phalaenopsis*, except for Ca, when plants were grown in a media composed exclusively of Douglas fir bark. However, when plants were grown in a mixture of Douglas fir bark and peat, there was a reduction in P, K, Fe, and Cu concentration and an increase in Ca, Mn, and Zn.

In the present study, leaf DM of *Laelia* fit models on which macronutrient:micronutrient or micronutrient:micronutrient ratios were calculated, regardless of the growing media or the ψ_s of the nutrient solutions, suggesting that nutrient imbalance may be responsible for how plants respond to the growing media and fertilizer rate interaction. Diagnosing the nutrient status of a crop based on nutrient ratios, instead of using the critical sufficiency ranges, has been shown to be superior for the prediction of the response of plants to nutrient acquisition. The Diagnosis and Recommendation Integrated System is a method proposed by Beaufils (1973) that takes into account pairwise-calculated nutrient ratios and currently is a widely used tool for defining the nutritional status of crops. According to our results, the growth of orchid plants such as *Laelia* may also be predicted using the nutrient ratios approach rather than solely nutrient concentration.

All the terms of the model that explained leaf DM of *Laelia* estimated with our results included micronutrients in the ratios; however, the most influential coefficients (coefficients with the highest values) were those that included a micronutrient:micronutrient ratio, suggesting that an extremely fine balance in

Table 5. Sign of the simple linear correlations that resulted significant ($P < 0.05$) among the proportion of the components of the growing media or the osmotic potential (ψ_s) of the nutrient solution and shoot concentration of macro- and micronutrients in *Laelia anceps* Lindl.

	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Boron	Iron	Manganese	Copper	Zinc
Charcoal							+		+	
Volcanic rock		—	—					+		
Peat							—			
ψ_s	+	+	+	—	—	—		—	—	—

the uptake of a given micronutrient in relation to other micronutrients was of major importance for adequate growth of *Laelia*.

The model suggests that (considering the negative coefficients as detrimental to leaf DM, whereas the positive ones have an enhancing effect) an excess of B uptake compared with Fe, Mn, and Zn reduced leaf DM, whereas a higher Fe uptake compared with Cu and Zn, and an excess of Mn uptake compared with Zn, resulted in increased leaf DM.

Macro- and micronutrient concentrations were more sensitive to fertilization rate than to the media components, as decreasing the ψ_s was associated with an increase in N and K concentration and a decrease in P, Ca, Mg, and most micronutrients (despite the concentration of B, Mn, Cu, and Zn in the nutrient solutions were maintained constant in all the nutrient solutions). The lower shoot concentration of most of the nutrients may be a consequence of the lower demand of *Laelia* because plants exhibited less growth when irrigated with solutions of low ψ_s .

Conclusions

The maximum *Laelia* leaf growth was observed in plants irrigated with nutrient solutions at -0.076 MPa in growing media formulated with charcoal and peat, at varying proportions, as a result of the favorable combination of their physical and chemical properties. However, pseudobulb and root growth was greater when plants were irrigated with solutions of a lower fertilizer rate, -0.051 MPa, and the media were formulated with low proportions of volcanic rock for maximum root growth. For maximum pseudobulb growth, the media should contain either a high charcoal or low volcanic rock content combined with peat. Fertilizer concentration and the growing media composition affected growth of *Laelia* by resulting in an imbalanced shoot nutrient concentration. These findings may serve as a basis for the

commercial production of this threatened species.

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