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Nitrogen fertilization in *Oncidium baueri* seedling growth

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This study was conducted with the objective of evaluating the nitrogen fertilization in orchid seedling formation. The experiment was conducted in a greenhouse environment with a coated polypropylene mesh with retention capacity of 60% of the solar radiation flux at the greenhouse of the Department of Agronomy of State University of Londrina – PR, Brazil. One year old seedlings of the orchid species *Oncidium baueri* has been used (Lindl.) from *in vitro* propagation, with an average height of 8.0 ± 1.0 cm. The experimental design was completely randomized with four replications and the treatments resulted from a 2×7 factorial design in which the factors were two sources (urea and ammonium sulfate) and seven doses of nitrogen applied fortnightly (0.00, 0.75, 1.50, 2.25, 3.00, 3.75, and 4.50 mg/pot). The experiment was conducted for a period of one year and the following variables were evaluated: largest pseudobulb length, plant height, highest root length, leaf area, root number, dry matter, number of leaves, and Dickson quality index. The use of ammonium sulphate resulted in the highest average values to the length of the longest pseudobulb, plant height, leaf area, length of the longest root, and plant dry matter, except for the number of leaves and Dickson quality index. The application of nitrogen between 3.20 and 4.33 mg/pot resulted in the highest values for the length of the longest pseudobulb, number of roots, length of the longest root, number of leaves, plant height and Dickson quality index for *O. baueri* (Lindl.) orchid. The plant height and leaf area variables increased significantly with increasing doses of N regardless of the source used, but the length of the longest pseudobulb, number of roots and dry matter production of plant only increased when urea was used as a nitrogen source.

Key words: Orchidaceae, nitrogen, fertilization, vegetative development.

INTRODUCTION

The Brazilian market of flowers and ornamental plants has been following the world-wide tendency of expansion, especially in exports of flowers and ornamental plants which had an average growth of 11.92% per year in the period of 2000 to 2010. Despite having small reductions in the last years of this period, in 2010 the equivalent of 28.68 million BRL has been sold (Junqueira et al., 2011).

In floriculture, usually species are sold as potted plants

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and, currently, due to an increased demand it is becoming promising to grow species with cut flowers characteristics (Mattiuz et al., 2006). Orchids are grown commercially worldwide as cut flowers or potted and represent about 8% of the commerce of ornamental plants (Chugh et al., 2009).

Orchidaceae is considered the oldest and largest family between monocots and ornamental plants with 600 to 800 types and 25.000 to 35.000 species (Garay, 1960; Sheehan, 1983). Moreover, it represents 7% of sold ornamental plants in the world (Van Der Pijl and Dodson, 1966). It also presents a great diversity of species and hybrids including terrestrial, epiphytes and rock plants. Orchids are among the most prized ornamental plants due to the beauty of its flowers, which vary widely in size, shape, fragrance and color combination that attracts consumers and make the plants reach higher commercial values (Mattiuz et al., 2006). The orchid cultivation has evolved in recent years becoming an economically important activity, especially some genres like *Oncidium, Cymbidium, Dendrobium, Phalaenopsis, Laelia* and *Cattleya* which stand out both for the internal market and for the export market of cut flowers (Hew and Yong, 1997; Mattiuz et al., 2006).

The genus *Oncidium* includes 315 epiphytic species, which 30% (nearly 94 species) native of Brazil (Ferrarezi, 2002). The Brazilian species *Oncidium baueri* (Lindl). is regarded of great ornamental potential for use in landscaping projects and primarily as a cut flower (Lorenzi and Souza, 2001).

The ornamental plants fertilization is primordial because the plant quality is a strategic factor in this activity (Tuzzi, 2011). It is also a key factor for the production of quality flowers, being used as a complement or alternative method in the control of diseases (Locarno et al., 2011). Among the essential nutrients provided by chemical fertilizers, nitrogen, phosphorus and potassium must be applied at appropriate levels to the requirements of each crop (Haag et al., 1993).

According to Lone et al. (2010), the application of water soluble macro and micronutrients can be performed by foliaceous or root application, or both simultaneously. However, to proceed fertilization, it is important to know the different stages of orchids' development cycle, so that it is possible to define which manure will be employed in the correct form (Campos, 2002).

In general, low nitrogen availability is a factor which limits the growth and productivity of plants, since it is required in all phases of plant development (Fernandes and Rossielo, 1995; Marschner, 1995).

In Brazil, among the most consumed nitrogenous fertilizers urea, ammonium sulfate and ammonium nitrate stand out (Sangoi et al., 2003), which have high water solubility and ready availability to plants (Contin, 2007).

Of all nitrogen fertilizers, urea stands out for its handling ease, lower cost, high solubility and compatibility for use in combination with other fertilizers, which makes it, from the economic point of view, potentially superior to the other sources (Scivittaro et al., 2004; Primavesi et al., 2004). On the other hand, it is considered a likely source of nitrogen loss by volatilization, depending on temperature, soil moisture and the amount and form of application (Ribeiro, 1996).

Research on mineral nutrition of orchids is scarce; however, the species need the same nutrients as other crops for their development (Araujo et al., 2007). According to Bernardi et al. (2004) for the orchids production on a commercial scale is necessary to conduct research in order to optimize forms of fertilization that are more efficient and have lower costs.

Considering the lack of information about fertilization of orchids, this study was conducted to evaluate nitrogen fertilization on *O. baueri* (Lindl.) seedlings formation.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse covered with polypropylene mesh with 60% retention capacity of the solar radiation flux belonging to the Department of Agronomy at the State University of Londrina – PR, Brazil (23 23 'S, 51° 11'W; 566 m). The orchid seedlings used were from the species *O. baueri* (Lindl.) from *in vitro* propagation, with one year old and average height of 8.0 ± 1.0 cm.

For the setup of the experiment, plastic pots with 7.3 cm of height and 10.0 cm in diameter were used. At the bottom of each pot, a layer of brick fragments was added to facilitate the drainage of any excess irrigation water. When drainage occurred, the water was collected and reapplied to the vessel to prevent a loss of nutrients.

To avoid possible nutrient limitation at the initial stage of establishment of plants, 15 days before the transfer of the seedlings, the application of a mixture of salts (dc) calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) was made maintaining the ratio Ca:Mg (3:1) applied 1.2 g/pot. On transplantation day, all pots received the application of 10 ml of a complete nutrient solution containing all essential nutrients except nitrogen, calcium and magnesium, being applied 300, 150, 40, 0.81, 1.33, 0.15, 4.0, 3.66 and 1.55 mg/kg of soil (adapted in this experiment to substrate) of P, K, S, B, Cu, Mo, Zn, Mn and Fe, respectively, according to the recommendations of Novais et al. (1991).

The experimental design was completely randomized with four replications and the treatments resulted from a factorial 2×7 in which the factors were two N sources (urea and ammonium sulfate) and seven biweekly doses (0.00, 0.75, 1.50, 2.25, 3.00, 3.75 and 4.50 mg/pot). Fertilization with nitrogen was carried out by applying 15 ml/pot solutions, to ensure the supply of doses to be tested.

The substrate composed of pine bark mixture, carbonized rice husk and coconut fiber in proportion (1:1:1 v/v/v), in each pot was added 70 g of substrate mixture. During the twelve months of the trial period, irrigations were performed daily with the goal of maintaining the humidity of the substrate at 70% of its maximum capacity of water retention, by the weighing and reposition of transpired water.

At the end of a year of installation, the experiment was finished by collecting shoot and root materials which were later sent to the Soil Laboratory of the Department of Agronomy at the State University of Londrina, where the following evaluations were performed: length of the largest pseudobulb, width of the largest pseudobulb, plant height, number of pseudobulbs, number of leaves, leaf area, number of roots, number of shoots, and the

Table 1. The largest pseudobulb length (LPL) (cm), plant height (PH) (cm), the highest root length (HRL) (cm) *Oncidium baueri* according to sources and doses of nitrogen applied every two weeks during the twelve months of cultivation.

N (mg/pot)	SA	LPL	UR	SA	PH	UR	SA	HRL	UR
	cm								
0.00	2.73^{A}*	-	2.85^{A}	15.40^{A}	-	14.03^{A}	12.65^{A}	-	11.45^{A}
0.75	2.83^{A}	-	2.96^{A}	17.00^{A}	-	14.88^{B}	15.75^{A}	-	11.48^{B}
1.50	3.02^{A}	-	3.03^{A}	18.63^{A}	-	19.50^{A}	17.78^{A}	-	16.43^{B}
2.25	3.16^{A}	-	3.20^{A}	19.20^{A}	-	19.98^{A}	19.53^{A}	-	17.45^{B}
3.00	3.60^{A}	-	3.33^{B}	26.33^{A}	-	20.60^{B}	19.60^{A}	-	17.78^{B}
3.75	4.79^{A}	-	3.87^{B}	29.80^{A}	-	25.08^{B}	25.00^{A}	-	19.53^{B}
4.50	2.96^{B}	-	4.03^{A}	24.83^{B}	-	27.08^{A}	17.35^{A}	-	16.45^{A}
CV (%)	-	4.98	-	-	5.05	-	-	5.14	-
DMS	-	0.24	-	-	1.51	-	-	1.25	-

SA: Ammonium sulfate; UR: Urea. Averages followed by the same letter in the lines do not differ between themselves, for the test of Tukey to 5%.

longest root length. After these assessments, the materials were washed with tap and distilled water and packed in paper bags and submitted to drying in a forced air oven maintained at a constant temperature of 65°C to constant mass. Next, all materials were weighed obtaining the dry matter of the area and plant roots. The data were used to calculate the Dickson quality index (DQI).

The leaf area was estimated following the methodology proposed by Stickler (1961) and consisted of using the following equation: AF = 0.7458 × C × L, where AF is the leaf area (cm^2); C is the maximum leaf length (cm), measured between the insertion point in the stem to the end of the leaf blade; L is the web width, measured in the larger position; and 0.7458 is a correction factor.

For the final evaluation of the seedlings quality, the Dickson Quality Index (DQI) was calculated following the proposal of Dickson et al. (1960). This variable is used primarily to assess the quality of forest seedlings such as eucalyptus, pine, anjico, etc. The DQI was calculated by employing the following formula: IQD = MST / [(M / DBP) + (MSPA / MSR)], where MST = total dry weight of the plant; H = shoot height; DBP = pseudobulb base diameter (replacing the original diameter of the formula collect); MSPA = shoot dry mass, and MSR = dry weight of the root system

The data were submitted to variance analysis and when necessary average values were compared by Tukey test at 5% or adjusted by polynomial regression equations.

RESULTS AND DISCUSSION

For width of the largest pseudobulb, pseudobulbs number and number of sprouts, no significant effects of nitrogen sources and tested levels were observed, indicating that these characteristics are independent of the nitrogen fertilization management.

The length of the largest pseudobulb of *O. baueri* (Lindl.) was influenced both by the sources as the nitrogen levels. The use of ammonium sulfate at doses of 3.00 and 3.75 mg N/pot resulted in higher values for the length of the pseudobulbs (3.60 and 4.79 cm, respectively), which significantly differ from urea. With the dose of 4.50 mg/pot, the greater length of pseudobulb (4.03 cm) was obtained with the application of urea (Table 1).

The plant height was influenced by nitrogen sources and doses. The use of ammonium sulfate at doses 0.75, 3.00 and 3.75 mg N/pot resulted in the highest plants of *O. baueri* (Lindl.). For the highest dose tested (4.50 mg/pot), the use of urea determined most of the plant height (Table 1). Comparing the effect of the sources of supply of N in the length of roots of the orchid plant species *O. baueri* (Lindl.), it was observed that the highest values were obtained using ammonium sulfate up to the dose of 3.75 mg/pot of N, applied every two weeks (Table 1).

When considering the effect of the doses of nitrogen, significant variations in the length of the largest pseudobulb was observed for both used sources, the use of ammonium sulfate enhanced the length of the largest pseudobulb adjusted to quadratic models with larger length estimated for the dose of 3.70 mg/pots of N, while for the urea the increases were linear, indicating that it would further increase if higher doses of N were applied (Figure 1A).

It was not found in the literature works which would allow making direct comparisons. For the few studies found, the researchers used formulated fertilizers NPK or NPK + micronutrients in the fertilization of orchids, as in the case of Dronk et al. (2012) who did not observe significant difference in the pseudobulb length in a clone of orchid cultivated in different substrates fertilized with NPK 10-10-10, in the corresponding dose of application of 5 mg/pots of N, and phosphorus and potassium.

For the two tested sources, significant effects of nitrogen rates for height of orchid plants were observed, setting the linear models with slightly higher values for ammonium sulfate (Figure 1B). Linear adjustment in this case indicates that two evaluated sources for the growth of plants still occurring of N rates higher than those tested were applied.

Similar results were obtained by Ferreira et al. (2007) when working with seedlings of bromeliad (*Neoregelia*

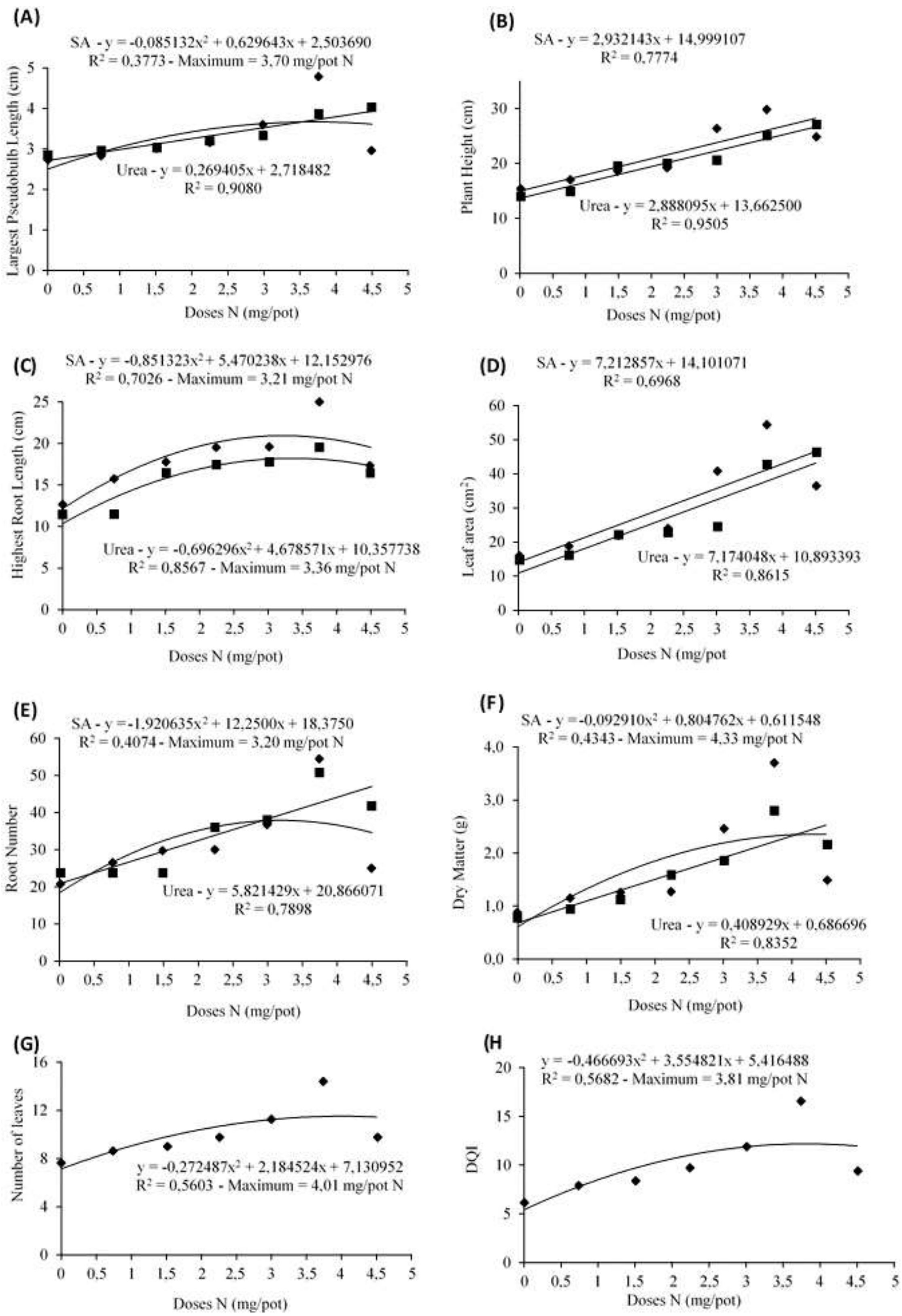

Figure 1. The largest pseudobulb length (A), plant height (B), highest root length (C), leaf area (D), dry matter (E), root number (F), Number of leaves (G) and Dickson Quality Index (DQI) for *Oncidium baueri* seedlings (H) according to sources and doses of nitrogen applied every two weeks during the twelve months of cultivation.

Table 2. Leaf area (LA) (cm²), number of roots/plant (NR), dry plant matter (DPM) of *Oncidium baueri* according to sources and doses of nitrogen applied every two weeks during the twelve months of cultivation.

N (mg/pot)	SA	LA	UR	SA	NR	UR	SA	DPM	UR
		cm^2						g	
0.00	15.96^{A}*	-	14.69^{A}	20.75^{A}	-	23.75^{A}	0.88^{A}	-	0.78^{A}
0.75	18.77^{A}	-	16.12^{A}	26.50^{A}	-	23.75^{A}	1.15^{A}	-	0.94^{A}
1.50	22.00^{A}	-	22.09^{A}	29.75^{A}	-	23.75^{A}	1.26^{A}	-	1.12^{A}
2.25	23.96^{A}	-	22.78^{A}	30.00^{B}	-	36.00^{A}	1.27^{A}	-	1.59^{A}
3.00	40.79^{A}	-	24.48^{B}	36.75^{A}	-	38.00^{A}	2.46^{A}	-	1.86^{A}
3.75	54.40^{A}	-	42.72^{B}	54.50^{A}	-	50.75^{A}	3.70^{A}	-	2.80^{B}
4.50	36.44^{B}	-	46.38^{A}	25.00^{B}	-	41.75^{A}	1.49^{B}	-	2.16^{A}
CV (%)	-	19.14	-	-	7.78	-	-	26.38	-
DMS	-	7.83	-	-	3.66	-	-	0.63	-

SA: Ammonium sulfate; UR: Urea. Averages followed by the same letter in the lines do not differ between themselves, for the test of Tukey to 5%.

cruenta), checked linear increases for height of the plants with the fortnightly application of urea solution of 0.1 and 2% by foliaceous pulverization. However, the results partially agree with those presented by Lone et al. (2010) who checked larger length of the air part of the hybrid *Cattleya intermedia Graham* ex Hooker × *Laelia purpurata* Lindley (Orchidaceae) with the fortnightly application of 200 mg of N/L using the formulated fertilizer 10-10-10. Bernardi et al. (2004) that worked with applications of 5.25, 10.51, 15.76. 21.01, 26.26 and 31.52 mg/pots, of nitrogen in the form of NH4+ and NO3- by solution of Sarruge (Sarruge, 1975) and observed linear growth increases of the *Dendrobium nobile* (Orchidaceae) plants, even working with two plants per pot.

For the purpose of doses, it was observed for the two tested sources, that the length of the largest root increased progressively with increasing N rates, significantly adjusting the quadratic functions. The maximum length of roots was estimated for the application of 3.21 and 3.36 mg/pot of N using ammonium sulfate and urea, respectively (Figure 1C). Similar results were observed by Wen and Hew (1993); Pan and Chen (1994) which found a greater root growth of the *Cymbidium sinense* orchid, with the conjoint application of nitrate and ammonium forms of N as supplemental fertilization. Ferreira et al. (2012) working with Orchid *Cattleya bowringiana,* micropropagated plantlets *in vitro* and observed a greater root length when nitrogen fertilization is used at a rate of 28.88 mg/pot of ammonium nitrate to the crop average.

Regarding the leaf area, significant differences between nitrogen sources only occurred at doses greater than 2.25 mg/pot of N. That is, at doses of 3.00 and 3.75 mg/pot of N, the highest averages were observed when using ammonium sulfate. However, with the dose of 4.50 mg/pot, the largest leaf area was obtained with the application of urea (Table 2). A similar result was observed by Oliveira et al. (2010) who found that the provision of 100 mg/dm^3 of nitrogen using ammonium sulfate provided a larger leaf area in ornamental sunflower plants than for plants fertilized with urea and calcium nitrate.

Concerning the number of plant roots, no differences were observed between the N sources to the dose of 1.50 mg/pot. Above this dose, the behavior was not regular, because sometimes the application of ammonium sulfate and the application of urea had the highest average values for the number of roots per plant (Table 2).

The results partially agree with those presented by Lone et al. (2010) who found an increasing number of hybrid roots *C. intermedia* Graham ex Hooker × *L. purpurata* Lindley (Orchidaceae) when twice a week a solution was applied which provided 10 mg/tray of N using a solution prepared with the fertilizer 10-10-10. In a study evaluating the effects of mineral fertilizer in orchid, Araujo et al. (2007) also had higher numbers of roots/plant in a *Cattleya loddgesii* ("Alba" × *C. loddgesii* "Atibaia") hybrid grown in trays (24 plants/tray) using foliar fertilization twice a week. The best result was obtained when the solution prepared with liquid fertilizer 08-09-09 contained 400 mg/L of N.

For the production of dry matter of the plants differences between sources of N were not observed until the dose of 3.00 mg/pot. At the dose of 3.75 mg/pot of N, the production was higher when using the ammonium sulfate and at the dose of 4.50 mg/pot, the urea use resulted in higher yield (Table 2).

When considering the effect of doses linear increases in leaf area were observed for the two tested sources, indicating that this variable can reach higher values with increases nitrogen rates (Figure 1D). According to Skinner and Nelson (1995) and Garcez Neto et al. (2002), a specific N supply is required because this nutrient is directly related to cell division and the elongation processes and therefore with the size and leaf area of each plant.

The results obtained partly agree with those found by Wang (1996), where by using six different commercial formulations of fertilizers, found linear increases in leaf area from 275 to 355 cm² for orchid plants of the genus *Phalaenopsis,* with increasing N concentration in the irrigation solution from 100 to 200 mg/L of N, regardless of the formula of the used fertilizer. According to Fernandez et al. (1994), nitrogen fertilization, regardless of the source considered, in addition to increasing the size and hence the leaf area also increases the leaf biomass accumulation rate.

When considering the dose effect to the number of plant roots, significant variations were observed for both sources of nitrogen. With the use of ammonium sulfate, it was possible to estimate the maximum number of roots with the application of 3.20 mg/pot. However, when the N source was urea, the root number increased linearly with tested doses, not allowing to estimate the dose which determines the maximum point (Figure 1E).

When considering the unique effects of doses of N in dry matter production of orchid plants, significant differences were observed for the two tested sources. When using ammonium sulfate, dry matter production of plant was set to the quadratic model allowing to estimate the maximum point for the dose of 4.33 mg/pot of N, while for urea, the increases were linear, not being possible to estimate the maximum point (Figure 1F).

The results corroborate those obtained by Oliveira et al. (2010), who worked with ornamental sunflower fertilized with 100 mg dm^{-3} of N and had higher dry matter production of plants with ammonium sulfate than with urea or calcium nitrate as nitrogen sources. The authors attributed the improved performance of ammonium sulfate to the likely contribution of the sulfur contained in the fertilizer to the improved nutritional balance of plants. The results also agree with those presented by Bernardi et al. (2004) who obtained an increase in the accumulation of reserves in the shoots of *Dendrobium nobile* (Orchidaceae) by increasing N rates of 21.01 and 26.26 mg/pot grown with two plants using Sarruge solution with 100 and 125% of the original concentration.

The number of leaves per plant differed significantly only with the tested doses of nitrogen. Thus, it was possible to determine the maximum number of leaves per plant with the dose of 4.01 mg/pot of N (Figure 1G).

Similar results were obtained by Wang and Gregg (1994) who also found increases in the number of leaves in the second year of cultivation of *Phalaenopsis* (Orchidaceae), with the increase of fertilization, using a formulated NPK fertilizer. Wang (1996) working with *Phalaenopsis* found an increase in the number of leaves when the N concentration was changed from 100 to 200 mg/L of irrigation solution with a commercial fertilizer composed of nitrogen, phosphorus and potassium. It can be said also that the results are similar to those presented by Amaral et al. (2009) who obtained more bromélia leaves (*Orthophytum gurkenii*) with an application of 250 mg N/plant using ammonium sulfate.

The Dickson quality index (DQI) is a variable commonly used for overall evaluation of quality seedlings of forest species, fruit, medicinal and perennial crops such as *Eucalyptus grandis* (Gomes et al., 2002), *Mikania glomerate* (Vidal et al., 2006), *Pinus taea (*Rossi et al., 2008), *Coffea arábica* (Marana et al., 2008) and *Erythrina velutina* (Melo and Cunha, 2008). This index is associated with the fixation success and survival of seedlings in the field. For Silva et al. (2002), good quality seedlings will ensure lower mortality reducing spending on replanting.

Adapting these situations to orchid seedlings, it can be said that good quality seedlings (larger DQI) will ensure lower losses for lack of fixation after transplantation and production of best quality flowers to meet the requirements of consumers and the commercial product enhancement. In this study, the DQI for the seedlings orchids *O. baueri* (Lindl.) was influenced only by nitrogen doses regardless of tested sources. In this case, it was possible to estimate that the maximum values for the DQI were obtained with application of dose 3.81 mg/pot of N (Figure 1H).

Conclusions

The use of ammonium sulfate resulted in higher average values for all tested variables except for number of leaves and Dickson quality index. Except for the largest root length, the use of urea in the highest dose tested always resulted in higher average values for other variables evaluated. For the variable largest pseudobulb length, number of root, highest root length and dry matter, maximum values were obtained with doses of 3.70, 3.20, 3.21 and 4.33 mg/pot of N, respectively, for the ammonium sulfate and 3.36 mg/pot of N, the largest root length when using urea. The plant height and leaf area of *O. baueri* (Lindl) increased significantly with increasing nitrogen rates regardless of the source used, but the largest pseudobulb length, number of roots and the production of dry matter only increased when urea was used as a nitrogen source. The number of leaves and the DQI were significantly influenced only by doses of nitrogen, being possible to estimate the maximum points for the doses of 4.01 and 3.81 mg/pot, respectively.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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