

## Full Length Research Paper

## Foliar diagnosis in *Phalaenopsis* orchid plants subjected to application of nitrogen

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Production of orchids is important in floriculture, and nitrogen is one of the nutrients most required by plants. However, there is little information on nitrogen leaf analysis of these orchids. We evaluated the nitrogen fertilization on leaf nutritional status and on the production of dry mass and develop a diagnostic sheet for the orchid *Phalaenopsis*. Five concentrations of N (0.0, 0.068, 0.136, 0.204 and 0.272 g L<sup>-1</sup>) in the form of ammonium nitrate, was compared for two hybrids (*Phalaenopsis* Taisuco Swan and *Phalaenopsis* Pink Chiffon). The plants were grown in pots in a greenhouse. Foliar fertilization was done fortnightly during the initial 120 days, and thereafter weekly for over 120 days. From 90 days after onset of the foliar applications, leaf green index with monthly measurements up to 240 days of application was evaluated. Dry matter shoot and the N content in the first and second newly expanded leaf was determined after 240 days. Based on polynomial relationship, a higher dry mass was achieved by applying 0.10 to 0.16 g L<sup>-1</sup> nitrogen. The second newly expanded leaf was the most appropriate plant tissue for assessing nutritional status of *Phalaenopsis* and foliar concentration nitrogen was optimal at 23-28 g kg<sup>-1</sup>.

**Key words:** Orchidaceae, nitrogen fertilization, seedlings.

### INTRODUCTION

Orchids need essential elements for normal growth; however, under deficit may take longer than the cultivated plants to display visual symptoms (Hew and Yong, 2004). Overall, orchid growers use little fertilizer applications, justifying the fact that the nutrients in the culture substrate were sufficient to maintain growth and plant development. However, fertilized plants may exhibit better and faster flowering and increased resistance to pests and diseases. However, the application of nutrients

can only bring excellent results when used in appropriate doses, and can cause problems, when nutrients are supplied in excess. Nitrogen is considered to be the most limiting nutrient for the most plant culture as the biological importance and requirement to higher plants.

Due to lack of information on nitrogen fertilization in *Phalaenopsis* orchids, it is necessary to conduct research to elucidate the appropriate use of foliar application of N and provide important technical information for fertilizer

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applications to producers. Based on this, this research aimed to evaluate the effect of nitrogen fertilization on leaf nutritional status, on the production of dry matter and on diagnostic leaves of orchid *Phalaenopsis*.

## MATERIALS AND METHODS

*Phalaenopsis* orchid seedlings with a pair of leaves were cultivated in the greenhouse for 240 days with light intensity ranging between 110 and 196 W m<sup>-2</sup>, grown in 0.9 L polyethylene black pots. The pots were filled with expanded clay at the bottom layer (25% of total volume) and a 2:1 mixture (v/v) charcoal and pine bark. The pots were placed in suspended 0.65 inches tall tables, and every 15 days position were randomly changed to eliminate the possible edge effects. The irrigation was performed twice weekly in the winter and three times a week in the summer, with 100 ml distilled water (pH = 6.8 and EC = 164.9 µS cm<sup>-1</sup>) per pot. Treatments were designated in factorial scheme 5 x 2, based on the solution of Sarruge (1975), ammonium nitrate was the sole N source, with five N concentrations: 0.0; 0.068; 0.136; 0.204; 0.272 g L<sup>-1</sup> in nutrient solution; and two hybrids *Phalaenopsis* Taisuco Swan (white flowers) and *Phalaenopsis* Pink Chiffon (purple flowers), arranged in a complete randomized design with five replications. The experimental unit consisted of three plants, with one plant per pot. Foliar fertilization, at 40 mL per plant was performed fortnightly during the initial 120 days, becoming weekly for the following 120 days.

After 90 days of onset of biweekly application of the nutrient solutions, monthly measurements were made up to 240 days of application with chlorophyll portable device (CCM-200 OptiScience® model) in the central part of the third upper and lower of the adaxial surface of last leaf fully developed for each plant.

Plant tissue analysis was performed at the end of the period of eight months, when the plants reached 2/3 of the cycle and after 30 days of obtaining the second fully expanded leaf. The first and second sheet newly expanded and total shoots were collected separately from each hybrid. The plant tissue was then rinsed, dried and ground for determination of nitrogen content, according to the method described by Malavolta et al. (1997).

Data were analyzed using an ANOVA and F test and polynomial regressions using the statistical program AgroEstat (Barbosa and Maldonado, 2014).

## RESULTS AND DISCUSSION

At four months of cultivation, when the first leaf chlorophyll reading was made, there was no significant difference in the values on the green color index between hybrids. At five months from the date of implementation, the green color index increased with increasing concentrations of N (Figure 1). The response to foliar nitrogen application was quadratic in index of the color green in the first newly expanded leaf of *Phalaenopsis* Taisuco Swan (A) from the sixth to eighth straight month and the fifth month rated form. Already in *Phalaenopsis* Pink Chiffon (B), there was an increase in the index of the quadratic form of the green color of the fifth to seventh month assessment (Figure 1). Nitrogen is required for synthesis of chlorophyll and influence photosynthesis. In the absence of N, the plant degrades chlorophyll molecules, retranslocating N to regions of active growth,

where it performs its structural function (Mengel and Kirkby, 1987). For this reason, it can be stated that the plants with the lowest rate of green color, grown under the same environmental conditions, may indicate deficiency of leaf N.

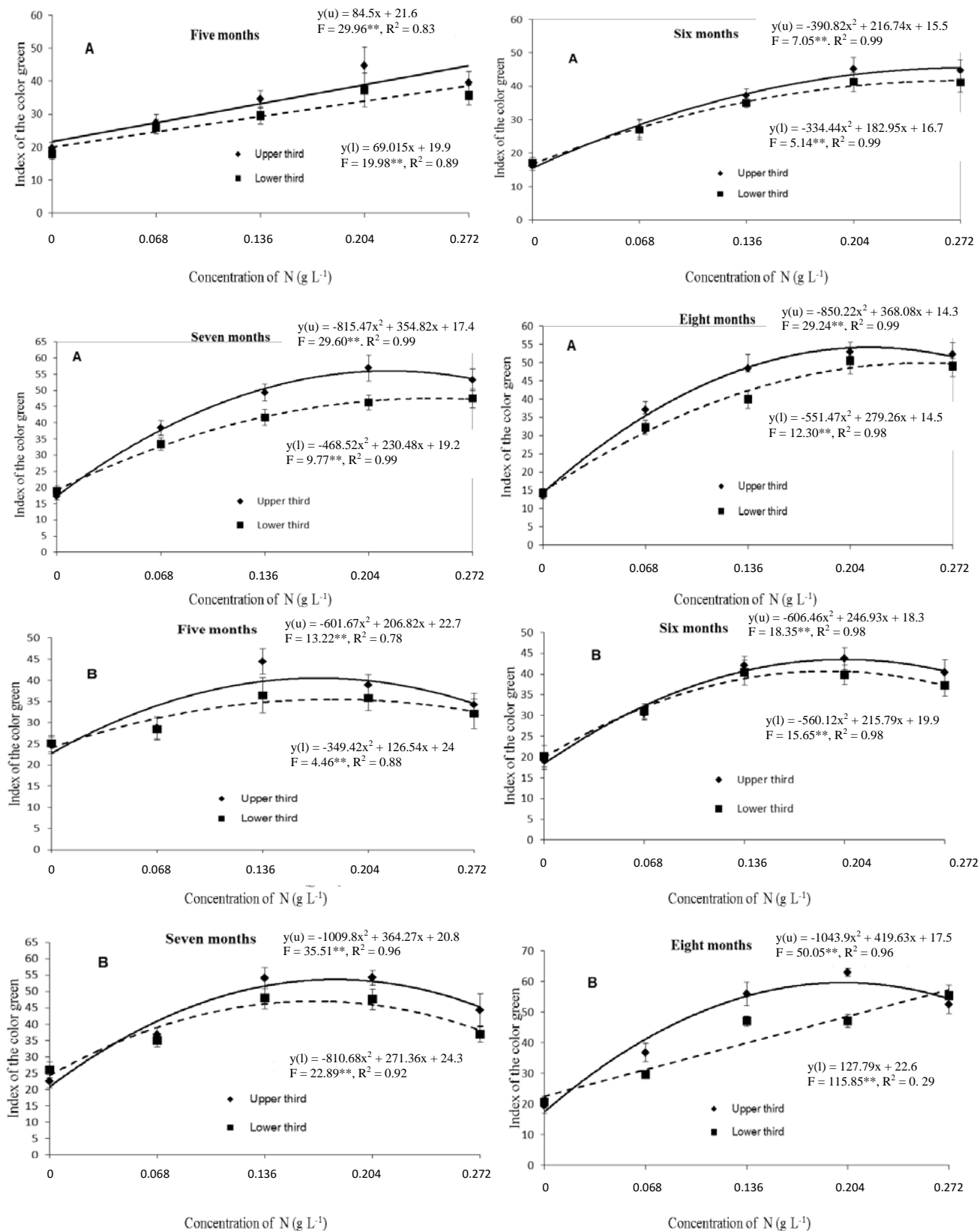
The N concentration in the first and second newly expanded leaf and also in shoots in hybrid *Phalaenopsis* Taisuco Swan (A) increased quadratically and linearly in hybrid *Phalaenopsis* Pink Chiffon (B) (Figure 2). The increase of N content in the plant according the application of foliar N was reflected in the accumulation of this nutrient in the plant. It was found that foliar application of nitrogen promoted quadratic increase in N accumulation in the shoot of hybrids *Phalaenopsis* Taisuco Swan (A) and *Phalaenopsis* Pink Chiffon (B) (Figure 3).

The foliar application of nitrogen promoted a quadratic increase in dry matter, reaching the maximum dry matter at 0.15 and 0.16 g L<sup>-1</sup> nitrogen in hybrids *Phalaenopsis* Taisuco Swan (A) and *Phalaenopsis* Pink Chiffon (B), respectively (Figure 4). This beneficial effect of foliar application of N on dry matter production of orchids occurred because of the improved nutritional status of the plant as seen by increased green color (Figure 1). There was high correlation between dry mass and foliar N content and accumulation of this element (Table 1).

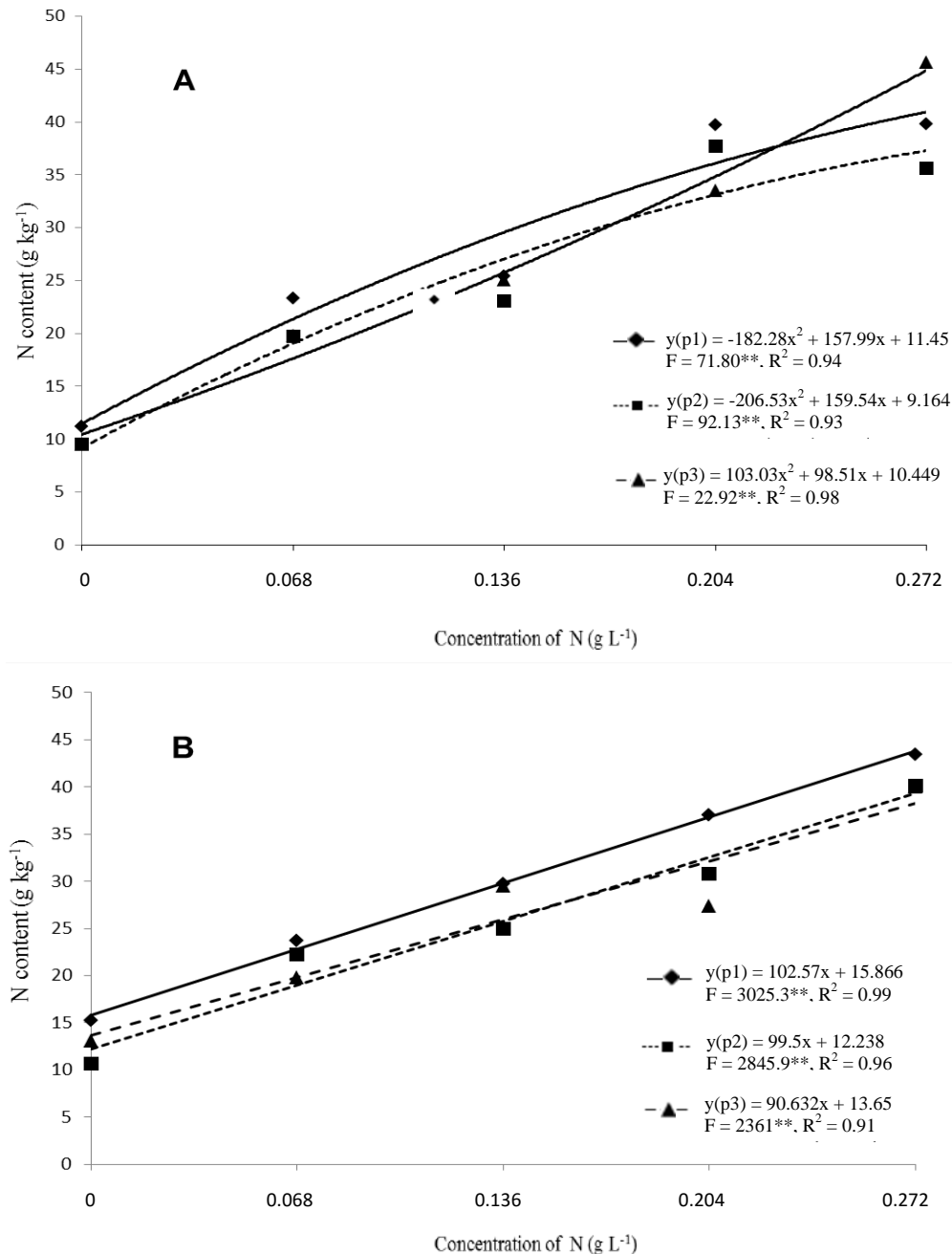
The optimal nitrogen concentration obtained (0.15 to 0.16 g L<sup>-1</sup>) differs from those of other authors such as Amberger-Ochsenbauer (1997) who observed optimal growth of *Phalaenopsis*, with weekly application of 0.4 to 1.5 g L<sup>-1</sup> of 22-09-16 formulated, or 0.064 to 0.24 g L<sup>-1</sup> of N.

The dose of 0.272 g L<sup>-1</sup> N in the current study impaired growth, causing symptoms of excess N in leaf tissue, with 68% reduction in dry mass when compared to the optimal application of N (0.15 to 0.16 g L<sup>-1</sup>). This effect may be related to excessive absorption of ammonia which induces toxicity favoring oxidation of lipids in the plant body structures resulting in necrosis at the edge of leaves (Mengel and Kirkby 1987). The use of higher concentrations resulted in foliar N content in the newly expanded second leaf equal to 37.3 and 39.3 g kg<sup>-1</sup> and it is considered excessive for these orchids.

An appropriate N supply is needed for plants because it is directly related with the processes of cell division and elongation, and consequently with the size and leaf area of the plant (Skinner and Nelson, 1995), and therefore, the supply of this nutrient in either excess or deficiency corresponded to lower values of dry matter per plant (Figure 4). N rates equal to 0.105 and 0.150 g L<sup>-1</sup> provided 90 and 100% of the maximum production of dry matter (Figure 4) respectively, and were associated with foliar (2nd newly expanded leaf) concentrations equal to 23.6 and 28.4 g kg<sup>-1</sup> for *Phalaenopsis* Taisuco Swan and *Phalaenopsis* Pink Chiffon. The respective doses of N equal to 0.104 and 0.162 g L<sup>-1</sup> were associated with foliar (2nd newly expanded leaf) concentration equal to 22.5



**Figure 1.** Index of the color green obtained in the upper third and lower third of the latter recently expanded leaf to 5, 6, 7 and 8 months of cultivation as a response to the foliar application of nitrogen to (A) *Phalaenopsis Taisuco Swan* and (B) *Phalaenopsis Pink Chiffon*. Regressions\*\*, \* Significant at 1% and 5% probability by F test, respectively.



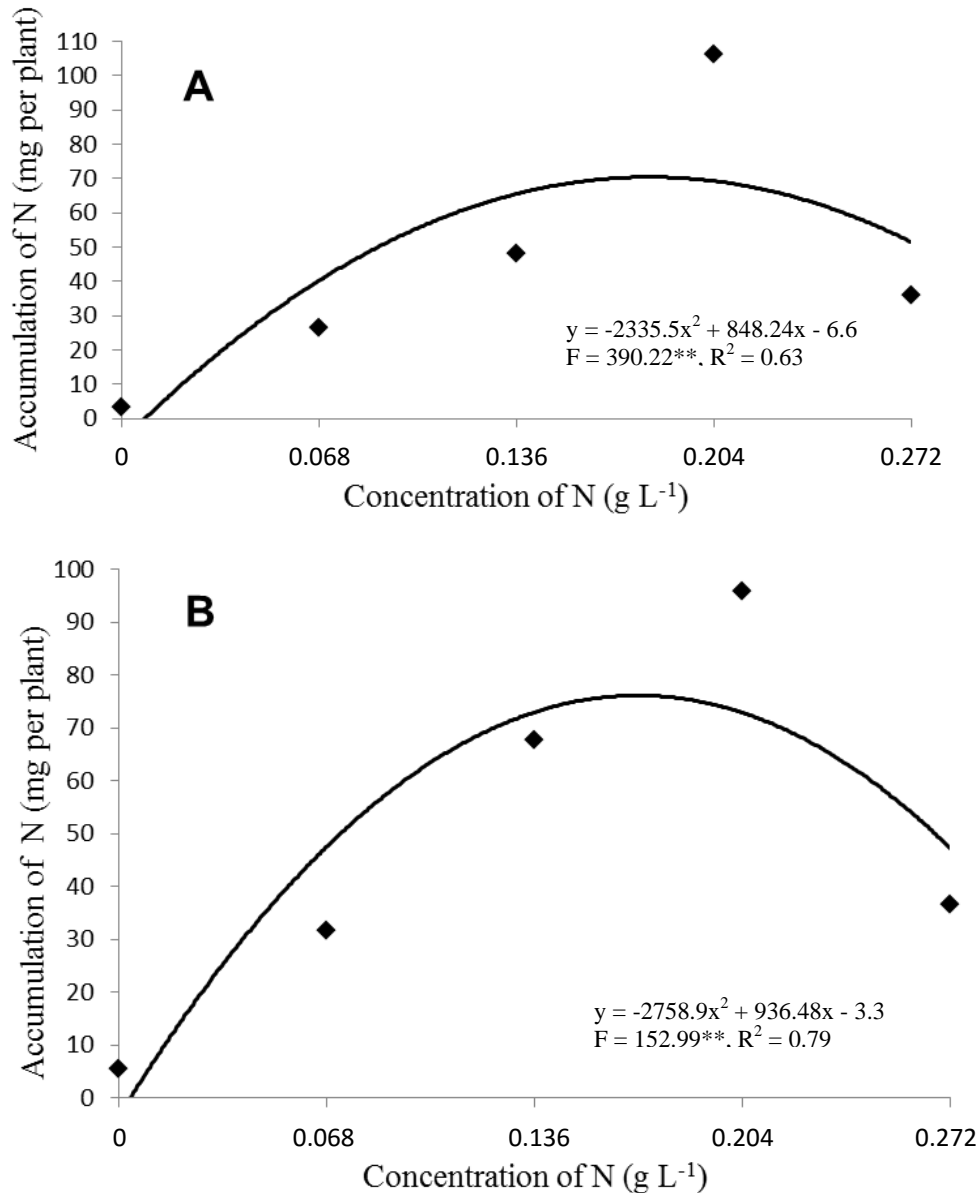
**Figure 2.** Nitrogen concentration for (A) *Phalaenopsis* Taisuco Swan and (B) *Phalaenopsis* Pink Chiffon, in the first newly-expanded leaf (p1) second newly-expanded leaf (p2), and total leaf shoots (p3) as a function of the concentration of nitrogen applied, 240 days after the first fertilization. Regressions\*\* Significant at 1% probability by F test.

and 28.3 g kg<sup>-1</sup>. Therefore, the proper range of values of N for *Phalaenopsis* orchid is 23 to 28 g kg<sup>-1</sup>.

The range of nitrogen sufficiency has not been established for the *Phalaenopsis* orchid. However, some authors indicate suitable ranges for N in orchids of 20 to 35 g kg<sup>-1</sup> in the last fully developed leaf (Jones Jr. et al., 1991), 15 to 25 g kg<sup>-1</sup> of N in the last freshly formed leaf

(Malavolta et al., 1997) but without describing the growing conditions of the plants, hence hindering interpretation of data.

There are reports that the N concentration in leaves of three hybrids of *Phalaenopsis* grown in pine bark varies with age (Poole and Sheehan, 1974). The authors observed that the N content decreases with age/leaf

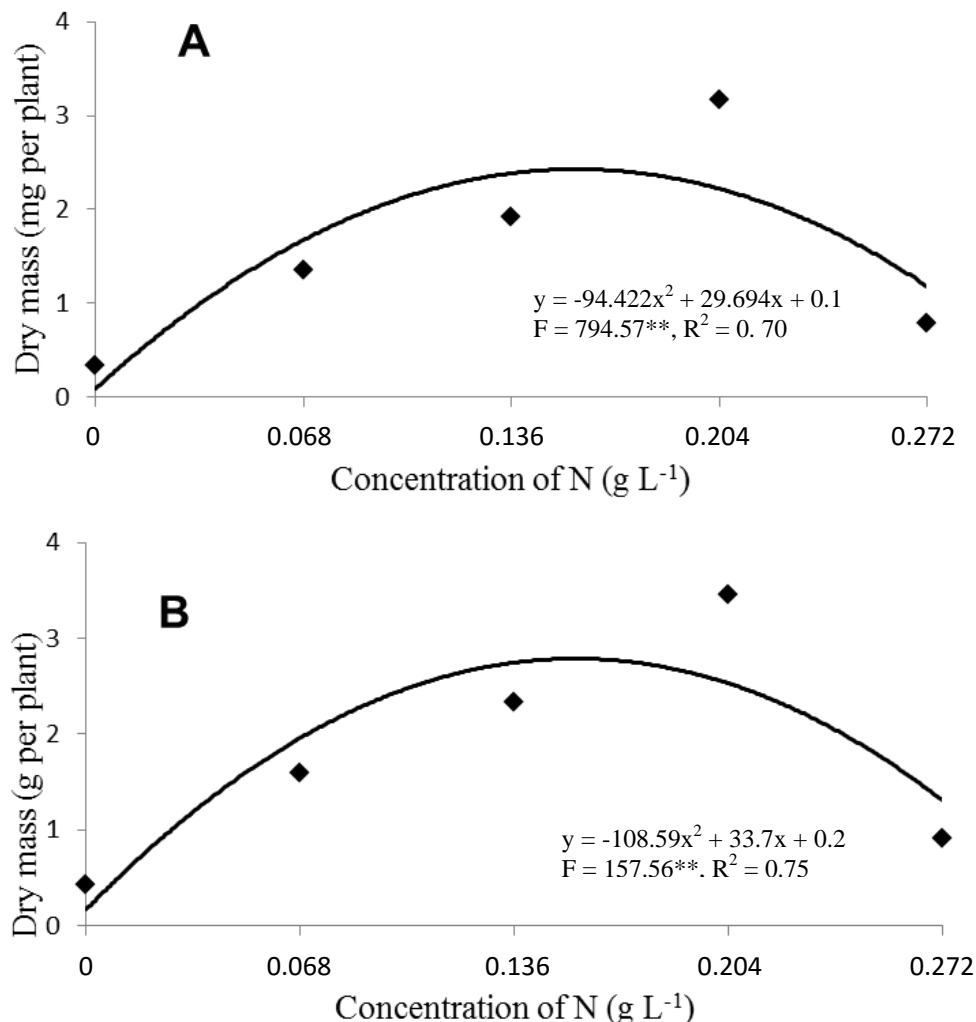


**Figure 3.** Plant nitrogen content of (A) *Phalaenopsis* Taisuco Swan and (B) *Phalaenopsis* Pink Chiffon grown in pots, 240 days after the first fertilization, in response to foliar applied N. Quadratic regressions \*\* Significant at 1% probability by F test.

analysis and in the second (34 g kg<sup>-1</sup>), third (26 g kg<sup>-1</sup>) and fourth leaves (25 g kg<sup>-1</sup>), the content of N is within the optimal range indicated by our study. The variation between the three hybrids was minimal, as was variation between *Phalaenopsis* Taisuco Swan and *Phalaenopsis* Pink Chiffon in our study, indicating that the nutritional management may be similar between orchids of the same genus. In another study, with *Phalaenopsis*, concentration of N depended on the plant part examined (roots, leaves, flowers), however, there was no relationship between higher leaf N content and the number of flowers per plant (Moreno et al., 2000).

A study of *Phalaenopsis* hybrid, grown on different substrates with fertilization of different formulations, revealed that there was no treatment effect on the concentration of nitrogen in the second mature leaf from the base to the apex of the plant, which ranged from 11 to 14 g kg<sup>-1</sup> of N (Wang and Konow, 2002). However, the authors suggest providing 0.2 g L<sup>-1</sup> N in each irrigation for suitable vegetative growth of *Phalaenopsis*, as do Broschat and Klock-Moore (2000), and Wang (1995), which contrasts the maximum dry matter produced in a study with the application from 0.10 to 0.16 g L<sup>-1</sup> N.

A correlation between N content of the second newly



**Figure 4.** Dry mass (A) *Phalaenopsis Taisuco Swan* and (B) *Phalaenopsis Pink Chiffon* as a function of nitrogen application. Quadratic regressions\*\* Significant at 1% probability by F test.

**Table 1.** Correlation between dry mass (g) and N concentration in the first and second newly expanded leaf and shoots of plants and total N per plant (mg per plant) in the shoot of (A) *Phalaenopsis Taisuco Swan* (B) *Phalaenopsis Pink Chiffon*.

Variables	Dry mass (g)	
	A	B
N concentration (first leaf)	0.563 <sup>2</sup>	0.396 <sup>NS</sup>
N concentration (second leaf)	0.993 <sup>1</sup>	0.984 <sup>1</sup>
N concentration (shoot)	0.948 <sup>1</sup>	0.949 <sup>1</sup>
Shoot N content	0.953 <sup>1</sup>	0.966 <sup>1</sup>

<sup>1</sup>significant result ( $p < 0.0001$ ); <sup>2</sup>significant result ( $p = 0.097$ ).

expanded leaf and shoot and the dry mass to the two hybrids of orchid was evident (Table 1). Therefore, this plant tissue is the most suitable for foliar sampling when evaluating the nutritional status of orchids. This indication

of the most appropriate leaf to be collected for orchid may be beneficial in the use of appropriate assessment of the nutritional status of culture and enables the making of adjustments in the fertilization of culture seeking greater

growth and plant development.

## Conclusion

The foliar application from 0.10 to 0.16 g L<sup>-1</sup> of nitrogen fortnightly during the first 120 days of cultivation and weekly during the last 120 days of cultivation promoted the optimal plant growth of *Phalaenopsis* Taisuco Swan and *Phalaenopsis* Pink Chiffon. The second newly expanded leaf was the most appropriate plant tissue for foliar diagnosis for the genus *Phalaenopsis*. The optimal range of leaf nitrogen for *Phalaenopsis* orchids is 23 to 28 g kg<sup>-1</sup>.

## Conflict of Interests

The authors have not declared any conflict of interests.

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