

**Interactions between nitrogen fertilization with the growth and leaf macronutrients of citrus rootstocks**

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Abstract - Rootstocks of ‘Rangpur’ Lime and ‘Swingle’ Citrumelo were grown in containers with substrate in a greenhouse, aiming to evaluate the effects of N (urea) fertilization on the vegetative growth and macronutrient content of the plant tissue. The experimental design was a factorial randomized block design with four repetitions, and each experimental plot was composed of five plants. Four doses of N (0, 2.0, 4.0 and 8.0 g.plant⁻¹) were evaluated and applied every week (15 applications) to both of the rootstocks. After 200 days of transplanting, the following parameters were evaluated: vegetative growth and total content of macronutrients on the dry weight of the leaves, stems, and roots. ‘Rangpur’ Lime was more vigorous than ‘Swingle’ Citrumelo. ‘Rangpur’ Lime showed the greatest accumulation of plant dry weight with 3.38 g.plant⁻¹ of N and a greater root dry weight with 2.03 g.plant⁻¹. For ‘Swingle’ Citrumelo, 2.03 g.plant⁻¹ of N provided a greater plant dry weight, however, nitrogen fertilization reduced the root:canopy ratio of the rootstocks. The leaf content of N and P were favored by high doses of N in the tested range. Intermediate doses favored the Ca and Mg leaf contents. The leaf K content was decreased by nitrogen fertilization.

Key words: *Citrus* spp. Propagation. Greenhouse. Mineral nutrition.

Interação entre adubação nitrogenada e crescimento e conteúdo foliar de micronutrientes em porta-enxertos de citros

Resumo - Porta-enxertos limoeiro ‘Cravo’ e citrumeleiro ‘Swingle’ foram cultivados em recipientes contendo substrato comercial em condições de ambiente protegido, visando avaliar os efeitos da aplicação de doses de nitrogênio (ureia) sobre o desenvolvimento vegetativo e o conteúdo foliar de macronutrientes nos mesmos. O delineamento experimental foi em blocos casualizados com quatro repetições e cinco plantas por parcela. Foram avaliados dois fatores: cultivar (dois) e doses de nitrogênio (0,0; 2,0; 4,0 e 8,0 g.planta⁻¹). As doses foram aplicadas semanalmente. Após 200 dias do início do experimento avaliou-se o desenvolvimento vegetativo e o conteúdo total de macronutrientes nas folhas, hastes e raízes. Limoeiro ‘Cravo’ foi mais vigoroso que citrumeleiro ‘Swingle’, bem como apresentou maior acúmulo de “N” na matéria seca de parte aérea (dose estimada de N de 3,38 g. planta⁻¹) e nas raízes (2,03 g.planta⁻¹). Para o citrumeleiro ‘Swingle’ a dose estimada de N de 2,03 g.planta⁻¹ promoveu o maior incremento em matéria seca. A adubação nitrogenada provocou uma redução na relação raiz/parte aérea das plantas em ambos porta-enxertos. Os conteúdos foliares em N e P foram favorecidos nas maiores doses de N aplicadas, enquanto que doses intermediárias de N favoreceram os conteúdos foliares de Ca e Mg. Os conteúdos foliares de K decresceram com o aumento das doses de N.

Palavras-chave: Ambiente protegido. Citricultura. Propagação. Nutrição mineral.

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Introduction

For the successful production of an orchard the first fundamental step is the use of good quality seedlings. In this way, the nurseryman needs to use appropriate management practices so that he has a healthy and well nourished seedling for the producer. Among these practices, the proper management of fertilization (FOCHESATO et al. 2006).

The substrates that are used for seedling production are basically constituted of vermiculite, perlite, peat, pine-bark, sawdust, coconut fiber, carbonized or burned rice husk, and other organic materials, commonly requiring the addition of mineral fertilizers. These substrates are frequently applied through fertigation, adjusting the frequency of application accordingly to the dose, crop, and cultivation system (SCHÄFER et al., 2008).

In growing systems in which containers are used, the dynamics of the nutrients in the substrates are different compared with those in the soil, intensifying synergisms and antagonisms between nutrients due to the greater temperature and humidity in the system. Factors such as container type, substrate, species, variety, age, vegetal tissue (leaves, stems, and roots), and interactions between nutrients can interfere with the vegetative growth and nutritional content of citrus plants (SCIVITTARO et al., 2004; SERRANO et al., 2004; FOCHESATO et al., 2006; BACK et al., 2017).

Nitrogen is one of the nutrients that are required in large amounts by plants and consequently by citric rootstocks, participating in the main metabolic processes of the plant. This element is poorly utilized in the composition of substrates, and there are studies demonstrating the response of citrus plants to nitrogen fertilization during the nursery phase, with diverse responses due to the application form and dose (ESPOSTI; SIQUEIRA, 2004; SCIVITTARO et al., 2004; PRADO et al., 2008; GIRARDI et al., 2010; REZENDE et al., 2010). Most of these studies were performed using seedlings after the grafting process, with few examples of fertilization during the growing phase of the rootstock (before grafting), which can reduce the time of seedling production due to the anticipation of grafting (RUSCHEL et al., 2004; SORGONÁ et al., 2006; MATTOS JÚNIOR et al., 2010).

In addition, the source of nitrogen also influences these responses. Carvalho et al. (2000) reported that the increased the dose and frequency of nitrogen application as KNO_3 increased the N tissue content, probably related to greater plant growth. However, there was a reduction in the P, K, Ca, Mg, and S levels and in the plant total dry weight when the dose and application frequency of KNO_3 increased, indicating that better responses would be obtained with a better monitoring of fertilization when using other sources of N and supplementation with the other macronutrients, avoiding nutritional imbalances and reducing the time between the transplant and grafting of the rootstocks.



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In southern Brazil, citrus seedling production in greenhouses is recent, and the interactions between the several factors affecting this production require more detailed studies due to the region being classified as humid subtropical with a hot summer (Cfa), where the hottest months are January and February, with an average temperature of approximately 25 °C, and the coldest months are June and July, with an average temperature of 9 °C (BERGAMASCHI et al., 2003).

This research aimed to study the action of nitrogen doses applied as urea through fertigation on the vegetative growth and foliar contents of nitrogen, phosphorus, potassium, calcium, and magnesium on the rootstocks of ‘Rangpur’ Lime (*Citrus limonia* (L.) Osbeck) and ‘Swingle’ Citrumelo (*Poncirus trifoliata* [L.] Raf × *Citrus paradisi* Macf.) when grown in a greenhouse.

Material and Methods

This experiment was performed in a greenhouse located at 30° 6' 87"S; 51°39' 58"W. The rootstocks were grown initially in conical tubes (volume of 120 cm³) with the commercial substrate Plantmax Hortaliças HT (Eucatex®), irrigated by capillarity (twice per day, 10 minutes each, one in the morning and another in the afternoon) in greenhouses. When the rootstocks reached 15 cm in height and/or complete root development in April they were transplanted to special 4-L containers for citrus with Rendmax Citrus (Eucatex®) substrate. According to the substrate company, as printed on the product label, the substrate presented pH 5.8 (±0.5) (water); CE 1.7 mS cm⁻¹ (±0.3); density of 200 kg m⁻³ (dry) and 550 kg m⁻³ (humid); and the following chemical characteristics: P, K, S, Zn, Cu, B, and Mn extractable (mg Kg⁻¹) and Al, Ca, and Mg exchangeable (cmol_c Kg⁻¹) amounts of 385, 2763, 7.3, 7.7, 0.5, 0.8, 19, 0.5, 28, and 19, respectively.

Two citrus rootstocks were evaluated, ‘Rangpur’ Lime (*Citrus limonia* (L.) Osbeck) and ‘Swingle’ Citrumelo (*Poncirus trifoliata* [L.] Raf × *Citrus paradisi* Macf.), and four doses of N (0, 2.0, 4.0, and 8.0 g.plant⁻¹) were tested, with an experimental design of randomized blocks with four repetitions, totaling eight treatments, and each experimental unit constituted five plants.

The total dose of nitrogen corresponding to each treatment was divided into 15 top-dressing applications, with urea (44%), occurring every seven days. The first application occurred forty days after the rootstock transplanting. During the first three applications, only 50% of the scheduled dose of nitrogen was applied. The fertilizer was applied through a fertigation system, with urea diluted in water, and every container received 50 mL application⁻¹. The micronutrients were applied every other week via foliar application uniformly for all treatments (commercial fertilizer composition: 11% S, 3.5% B, 0.1% Cu, 0.2% Fe, 1% Mn, 0.1% Mo and 6% Zn).



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Throughout the experimental period, the local temperature was monitored in a shelter that was close to the greenhouse in which the experiment was conducted. The maximum and minimum temperatures of the period were divided into three periods of ten days per month.

The irrigation was performed through drip irrigation two to three times per day for five to ten minutes each. The emitter had a flow of 1.65 L hour⁻¹.

Throughout the experiment, the plant height and the trunk diameter were evaluated at 40, 70, 140, 170, and 200 days after transplanting (DAR). At 200 DAR in November 2007, the vegetative growth of the rootstocks was evaluated through the determination of the plant height (cm), trunk diameter (mm), plant leaf area (cm² plant⁻¹) as obtained by a leaf area measurer (LI-Cor® LI – 3100), root dry weight (MSR), stem dry weight (MSH), leaf dry weight (MSF), and the plant dry weight (g plant⁻¹), which were obtained after drying the tissues in a dryer oven at a temperature of 65 °C until constant tissue weight. Using the equations that were generated by regression on ANOVA, the root:canopy (MSF+MSR) ratio was obtained for each treatment. The foliar nutrient content of N (total), P, K, Ca, and Mg was determined following the protocol that is described by Tedesco et al. (1995).

The means were submitted to a variance analysis (ANOVA), and the curves were determined, analyzing the regressions on ANOVA, with a 5% error probability.

Results and Discussion

The tested citrus rootstocks were different in vegetative growth, where ‘Rangpur’ Lime showed superior values for all of the evaluated characteristics when compared with the ‘Swingle’ Citrumelo (Figures 1 and 2), indicating a greater vigor of the first during this phase.

The regression analysis indicated a quadratic response to the accumulation of the dry weight of the leaves, stem, and roots and the total dry weight of the rootstocks when relating the different doses of nitrogen (Figure 1), with the exception of the accumulation of the root dry weight of ‘Swingle’ Citrumelo, which showed a decreasing linear response to nitrogen fertilization (Figure 1c).

Most of the responses of the dry matter accumulation of the ‘Rangpur’ Lime occurring within the interval of [2.03; 4.17] g of N per plant (Figure 1). For ‘Swingle’ Citrumelo, the interval with a greater response was [0.0; 3.61] g of N per plant. The greater accumulation of the total dry matter for ‘Rangpur’ Lime was at a dose of 3.38 g of N per plant, providing an accumulation, as estimated by the equation, of 20.0 g.plant⁻¹. A greater accumulation of the roots dry matter occurred with 2.03 g of N per plant, with 8.15 g.plant⁻¹ MSR. For ‘Swingle’ Citrumelo, the greatest accumulation of the total dry weight was with the dose of 2.74 g of N per plant and



provided an accumulation, as estimated by the equation, of 9.58 g of N per plant. Nitrogen fertilization provided a reduction in the root dry weight accumulation.

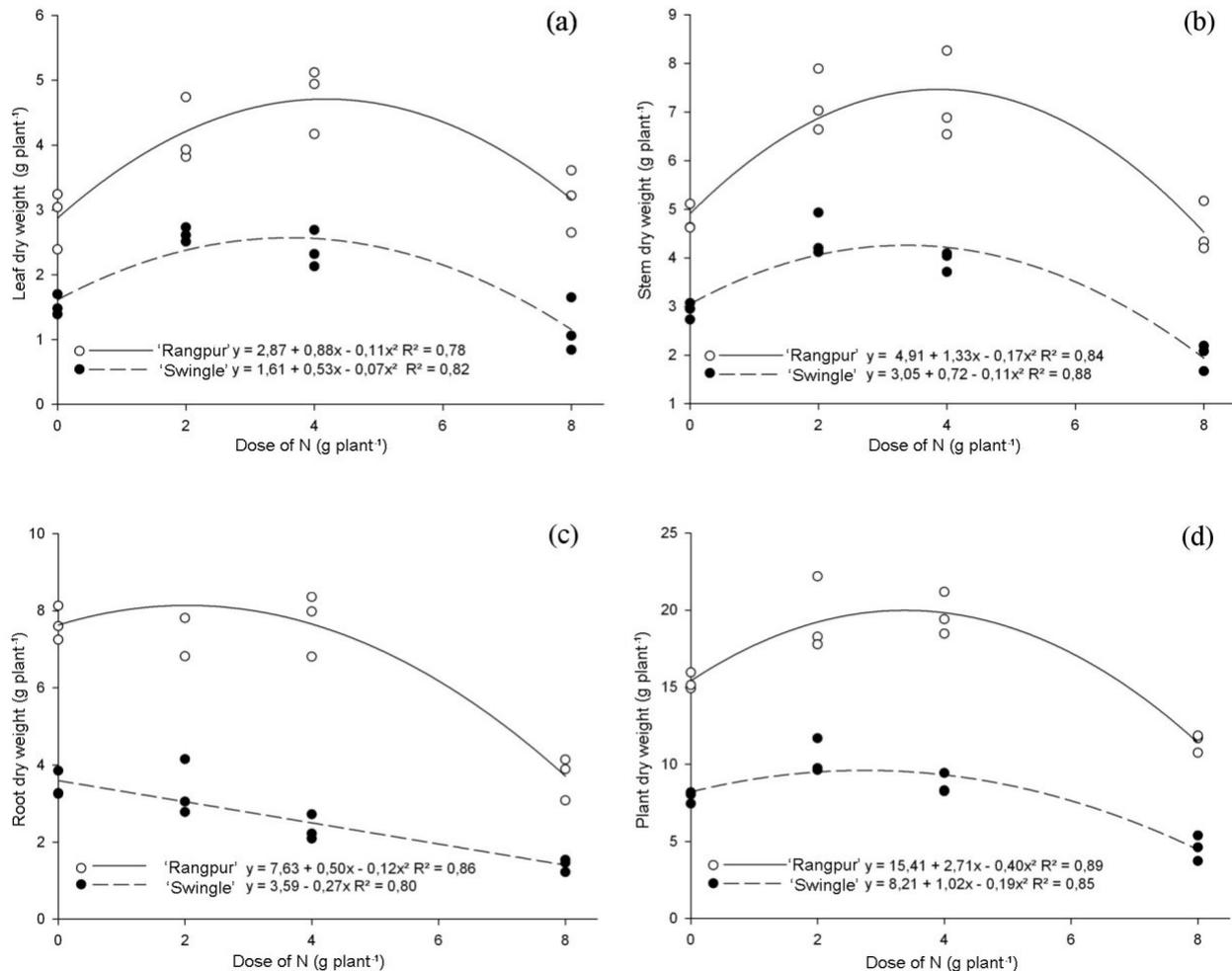


Figure 1. Leaf (a), stem (b), root (c) and total plant dry weight (d) of ‘Rangpur’ Lime and ‘Swingle’ Citrumelo rootstocks that were cultivated in pots under different doses of N fertigation 200 days after transplanting.

The trunk diameter showed the greatest growth, at 0.17 and 0.88 g of N per plant (Figure 2a), as estimated by the equations, of 7.84 mm in ‘Rangpur’ Lime and 6.09 mm in ‘Swingle’ Citrumelo, respectively. For the growth in height, doses of 3.88 g of N per plant and 3.54 g of N per plant stimulated a greater growth in ‘Rangpur’ Lime (62.95 cm) and ‘Swingle’ Citrumelo (61.09 cm), respectively (Figure 2b). Doses of 3.92 and 3.20 g of N per plant provided a greater leaf area per plant in the rootstocks ‘Rangpur’ Lime (492.09 cm²) and ‘Swingle’ Citrumelo (166.86 cm²), respectively (Figure 2c).



There was a change in the balance of the root:canopy ratio with the different N doses that were tested, in which there was a reduction in the proportion of roots compared with the canopy of the plant for both of the tested rootstocks (Figure 3). For the tested greatest dose of N, the reduction of the accumulation of roots as related to the canopy volume for ‘Swingle’ Citrumelo was approximately 12%. For ‘Rangpur’ Lime, this reduction was of approximately 15%.

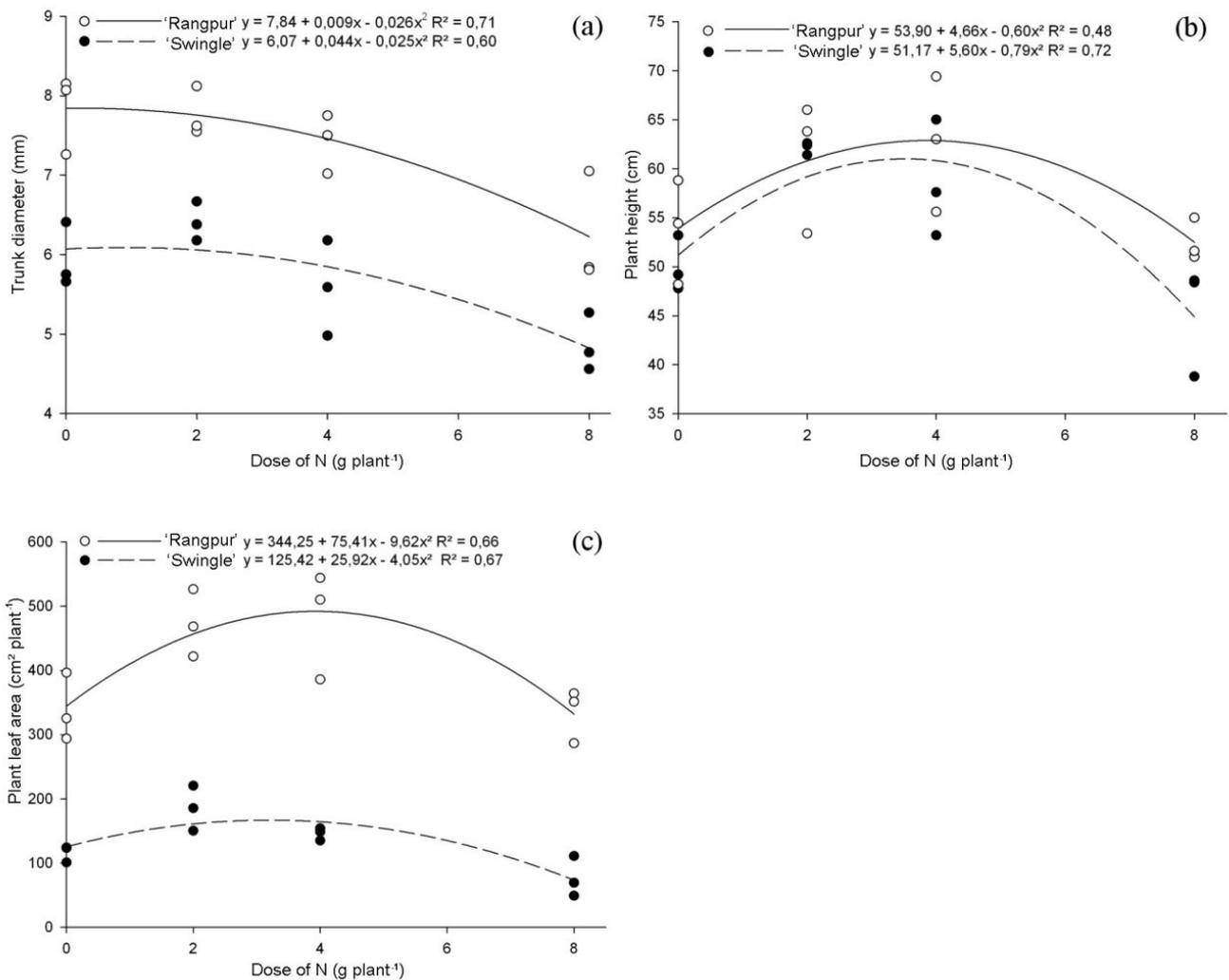


Figure 2. Trunk diameter (a), plant height (b) and plant leaf area (c) of ‘Rangpur’ Lime and ‘Swingle’ Citrumelo rootstocks that were cultivated in pots under different doses of N fertigation 200 days after transplanting.

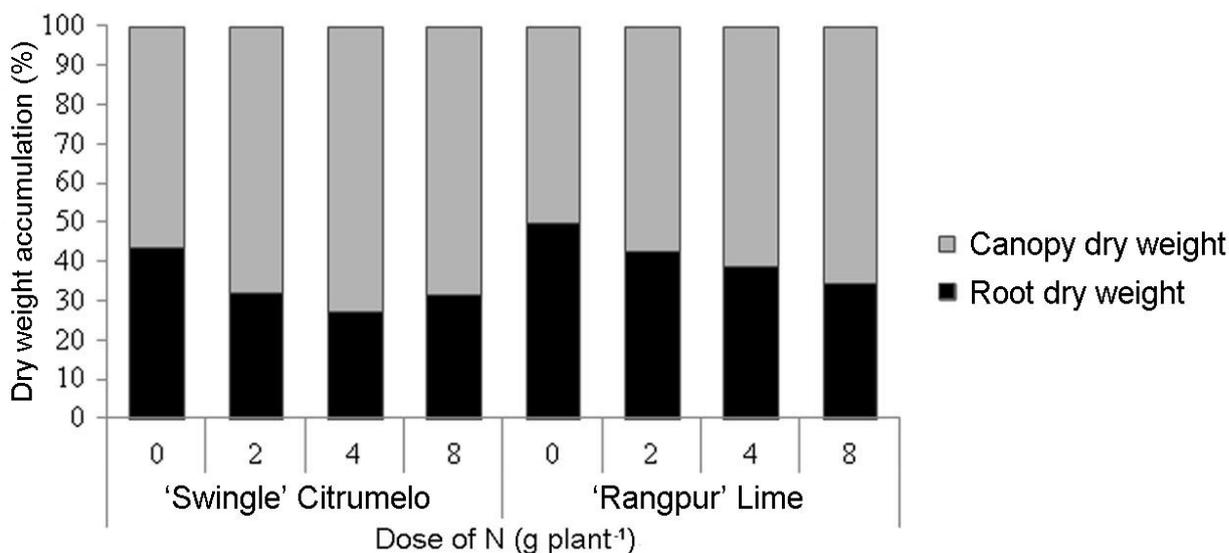


Figure 3. Canopy and root dry weight accumulation of ‘Rangpur’ Lime and ‘Swingle’ Citrumelo rootstocks that were cultivated in pots under different doses of N fertigation 200 days after transplanting.

Figure 4 presents the height and trunk diameter of the rootstocks throughout the 200 days after transplanting. The growth in the height of the ‘Rangpur’ Lime exhibited a sigmoidal behavior, indicating that it is earlier/premature compared with ‘Swingle’, which exhibited an exponential behavior. The diameter of the plants of both of the rootstocks for the four doses of N that were tested exhibited an exponential growth behavior. The dose of 2 g of N per plant provided an anticipation of the growth in diameter and height of the ‘Swingle’ Citrumelo, whereas for ‘Rangpur’ Lime, the response to this dose was not different from that of the control treatment.

Generally, neither of the studied rootstocks, independently of the applied N dose, showed any increasing in the diameter for up to 100 days after transplanting due to the low temperatures in May and July, with minimum and maximum temperatures that were lower than 12 °C between days 20 and 60 after transplanting, even though the experiment was conducted inside a greenhouse. There was an interaction between the tested rootstocks and the different doses of N applied for the nutritional content of K, Ca, and Mg. The nutritional content of N and P did not present an interaction, having significant effects only in response to nitrogen fertilization (Figure 5).

The dose of 7.59 g of N plant⁻¹ induced a greater accumulation of foliar N, presenting a maximum content of 5.29% as estimated by the second-degree quadratic equation (Figure 5a). The maximum foliar P content as estimated by this equation was 0.139% under the dose of 11 g of N per plant (Figure 5b).

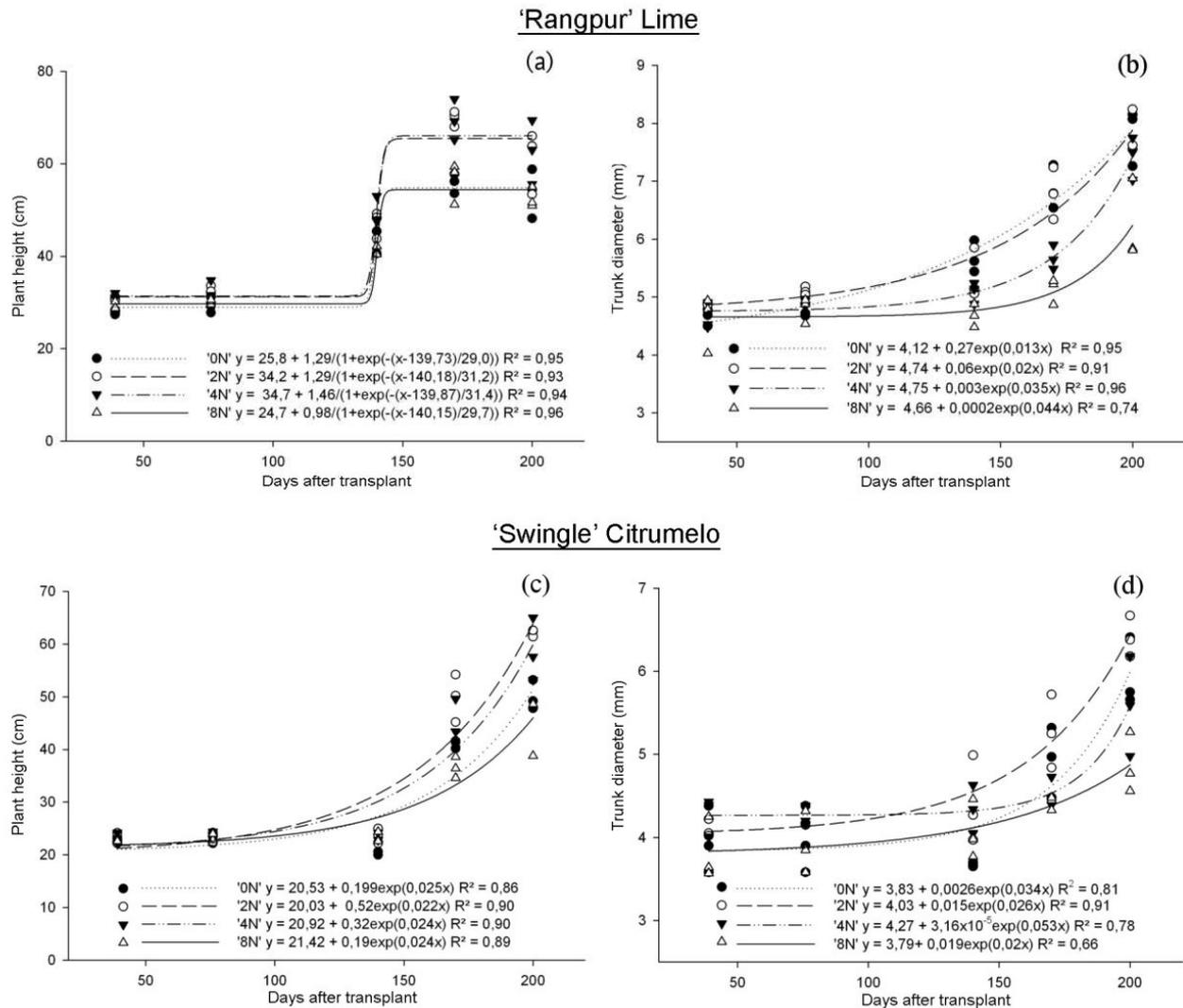


Figure 4. Trunk diameter and plant height of 'Rangpur' Lime (a; b) and 'Swingle' Citrumelo (c; d) rootstocks that were cultivated in pots under different doses of N fertigation 200 days after transplanting.

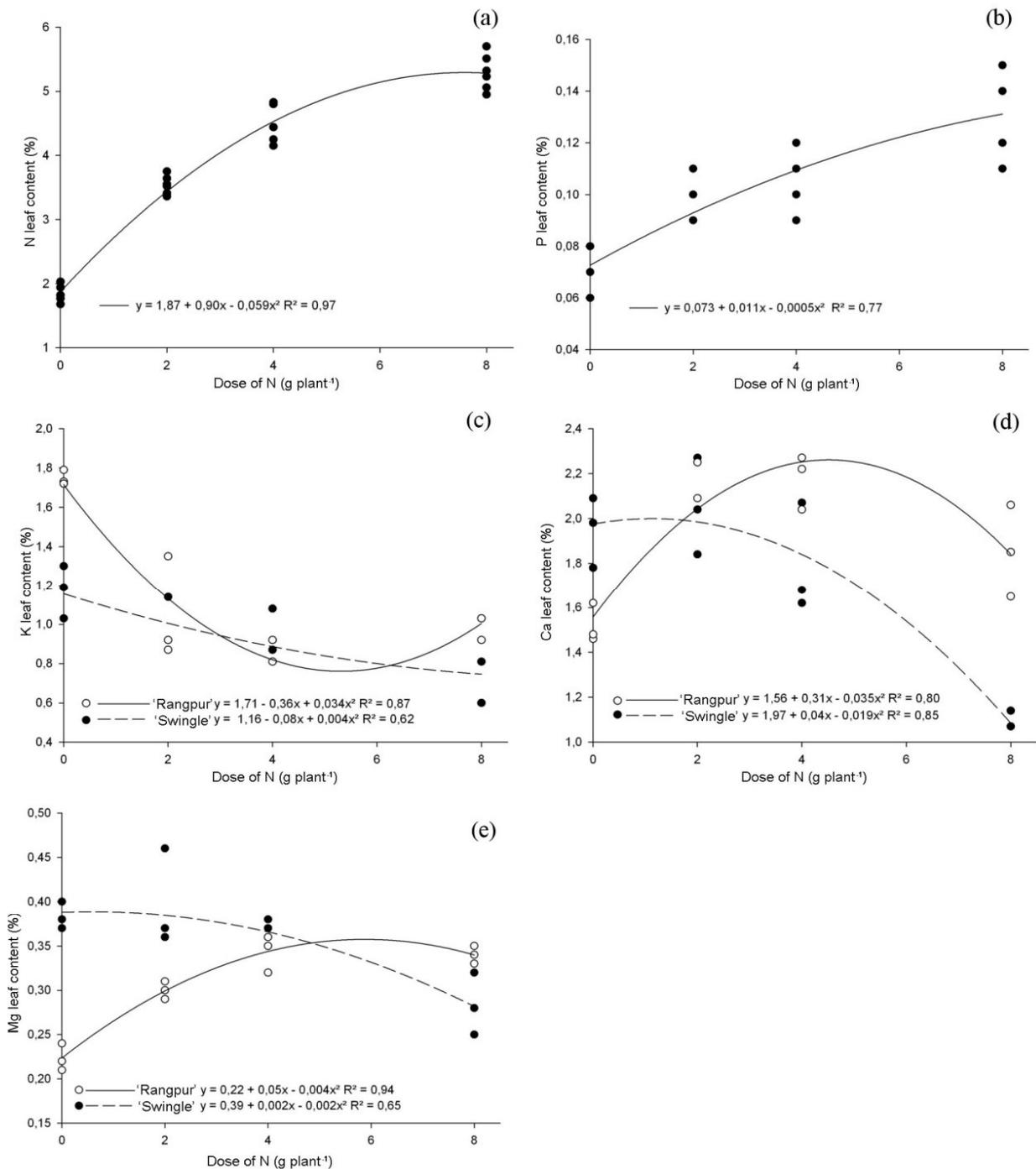


Figure 5. Nitrogen (a), phosphorus (b), potassium (c), calcium (d), and magnesium (e) leaf contents of 'Rangpur' Lime and 'Swingle' Citrumelo rootstocks that were cultivated in pots under different doses of N fertigation 200 days after transplanting. *Nitrogen (a) and phosphorus (b) do not show difference between rootstocks.



The foliar content of K of 'Swingle' Citrumelo and 'Rangpur' Lime showed a negative effect in relation to the N doses that were applied via top-dressing (Figure 5c). The maximum contents that were observed were in plants that did not receive nitrogen fertilization, showing contents of 1.71 and 1.16% for 'Rangpur' and 'Swingle', respectively. The maximum Ca foliar content was of 2.26% under the dose of 4.52 g of N per plant for 'Rangpur' Lime and was 1.99% for 'Swingle' Citrumelo under the dose of 1.13 g of N per plant. The Mg on the leaves presented a maximum content of 0.36 and 0.39% with doses of 5.85 and 0.58 g of N per plant for 'Rangpur' Lime and 'Swingle', respectively (Figure 5e).

The high doses of nitrogen fertilizer negatively influenced the absorption of K, Ca, and Mg. For Ca and Mg, the nitrogen fertilizer in intermediate doses benefited the accumulation of leaves, especially for 'Rangpur' Lime, due to the increase in root (Figure 1c) and canopy (Figures 2a and 2b) development.

These results agree with those of other studies of citric rootstock production in greenhouses, where it is common to find differences between rootstocks for the evaluated variables (BACK et al., 2017; ESPOSTI; SIQUEIRA 2004; FOCESATO et al., 2006; SORGONÁ et al., 2006). The different responses of the rootstocks can be attributed to the different genetic characteristics that influence the ability to use light and CO₂, affecting the absorption, transport, and interaction of the nutrients inside the plant and consequently the vigor (SCIVITTARO et al., 2004).

The greatest accumulation of the dry matter of 'Rangpur' Lime occurred within the interval [2.03; 4.17] g of N per plant (Figure 1). A similar result was found by Ruschel et al. (2004) after five months of cultivation of 'Rangpur' Lime, where the greater dry matter production (23.15 g plant⁻¹) was obtained under the dose of 3.41 g of N plant⁻¹.

Nitrogen can interfere with the root:canopy growth ratio (Figure 3). This balance is useful to study the balance between the organs due to modifications in the environment because this ratio indicates the existence of an interdependency between organs on the balance for water, nutrients, and carbon (ALVA et al., 2006). This change in the root:canopy balance occurred under the different tested doses, where there was a reduction in the proportion of roots as related to the plant canopy for both of the tested rootstocks.

Rozane et al. (2007) evaluate the growth components and the nutritional status of Swingle citrumelo, as a function of N, P and K doses and the partitioned application of N and K by fertigation. The use of N standard doses (920 mg dm⁻³), P (100 mg dm⁻³), K (790 mg dm⁻³) and N and K partition through weekly fertigation produced a higher quality seedling.

Machado et al. (2011) evaluated the effect of different rates of the organic fertilizer Humato Macota® compared with the slow-release fertilizer Osmocote® on the growth and nitrogen content in the dry matter of Rangpur lime. Means of all growth characteristics (plant height, total dry matter, root/shoot ratio and leaf area)



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and the potential quantum yield of photosystem II (Fv/Fm) were higher when plants were fertilized with the slow-release fertilizer.

Maust and Williamson (1994) also reported that the increasing nitrogen fertilization reduced the root:canopy ratio after 30 days of cultivation of ‘Hamlin’ oranges (*C. sinensis* (L.) Osb.) that were grafted onto ‘Cleópatra’ tangerine (*C. reticulata* Blanco) and onto ‘Carrizo’ citrange (*C. sinensis* × *P. trifoliata*) due to the greater growth rate of the canopy compared with that of the root system.

In seedling production, one of the objectives is to make the rootstock reach the diameter-to-graft as fast as possible (the minimum diameter should be 7 mm at a 10-cm height) because it allows an anticipation of the grafting, thereby generating a new plant (BACK et al., 2018; RIETH et al., 2012). However, the plant should be produced with care to develop a well-developed root system. In general, neither of the studied rootstocks, regardless of the nitrogen dose that was, showed an increase in diameter up to 100 days after transplanting due to the low temperatures that occurred during May and July because the minimum and average temperatures between 20 and 60 days after transplanting were lower than 12 °C, even though the experiment was conducted inside a greenhouse. Citric plants do not open buds or grow at temperatures lower than 12 °C. Near this temperature, the photosynthesis rate is too small or even zero if below 10 °C, whereas the range of temperatures at which the photosynthesis is more intense is between 20 to 30 °C (KOLLER, 2006). This behavior was also found by Back et al. (2018), where they tested the vegetative development of plants of the citrange Fepagro C37 rootstock under the inoculation of arbuscular mycorrhizal fungi, obtained absence of primary growth due to the low temperatures that occurred in June to September. In southern Brazil, the minimum temperatures inside the greenhouse are similar to those of the outside environment, and there is a strong influence of the temperature on the time necessary to grow citric seedlings in a greenhouse (FOCHESATO et al., 2006).

Some authors cite an inverse interaction between N and P, with a predominance of N on P. An excessive supply of N limits the accumulation of P in the tissues, possibly due to the smaller production of the dry matter of the canopy or due to the occurrence of antagonist interactions of N and P absorption (CARVALHO et al., 2000; ROZANE et al., 2007). This fact can be explained due to the use of potassium nitrate in other studies because urea permits the greater accumulation of P in the tissues due to the liberation of nitrogen in the fertigation (NH₄), in which the competition between phosphate anions and nitrate on the absorption sites is reduced (CARVALHO et al., 2000). Scivittaro et al. (2004), comparing the source of used N, calcium nitrate and urea, verified that urea provides a greater accumulation of P in the tissues of ‘Rangpur’ Lime and that the behavior of the accumulation of P in the leaves was quadratic, as in this study (Figure 5b).

High doses of nitrogen fertilizer negatively influenced the K, Ca, and Mg absorption, possibly due to the urea nitrogen fertilizer because ammonium (generated from the urea solubilization) competes by the same adsorption sites for K⁺, Ca²⁺ and Mg²⁺ (SCIVITTARO et al., 2004). The competition for ammonium by the



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absorption et al. sites on the roots was greater with K^+ , where the foliar contents were greater where nitrogen fertilization did not occur. For Ca^{2+} and Mg^{2+} , the nitrogen fertilization at intermediate doses benefited leaf accumulation, mainly for 'Rangpur' Lime, due to the increased root development (Figure 1c) and canopy (Figures 1a and 1b) because the absorption of these nutrients occurs mainly by radicular interception and mass flow (MARSCHNER; RENGEL, 2012).

Previous studies have demonstrated differences in the nutritional content according to the cultivar and rootstock. Fochesato et al. (2006) demonstrated the greater absorption of nitrogen by the 'Trifoliata' rootstocks in relation to 'Rangpur' Lime and to 'C13' citrange. However, 'Rangpur' Lime absorbs more potassium in the leaves than doe 'C13' citrange and 'Trifoliata', but the absorption of phosphorus (normal levels), calcium (insufficient levels), and magnesium (normal levels) is similar in the three rootstocks. Fochesato (2006) also demonstrated that despite high levels of calcium in the substrates, the foliar content in all of the treatments was insufficient, and the N, P, K, and Mg levels were normal or excessive. The same behavior was observed in this study, with exception of P.

Corroborating the data from this experiment, Carvalho et al. (2000) found in the rootstocks of 'Rangpur' Lime and 'Cleopatra' Tangerine that were subjected to applications of different doses and frequencies of application of potassium nitrate (KNO_3) a trend of reduced K levels under high doses and frequencies of fertilization application, coinciding with the results of this study (Figure 5c).

In other studies, increased fertilization resulted in a crescent linear response of the leaf nutrient contents of N in 'Rangpur' Lime and a quadratic response of the leaf nutrient contents of Ca in different production systems and doses of slow-release fertilizer NPK (14-14-14). The higher content of K in the substrate and NH_4 of the fertilizer may have influenced the low content of Mg in the tissue, in addition the Ca:Mg ratio in the substrate, which is very high, and may have prejudiced the absorption of Mg. Plants that demand high levels of K, such as citric species, may present a lower absolute content of Mg (SERRANO et al., 2004).

With the results of this research, the importance of the determination of an adequate level of nitrogen becomes evident because at high doses, the growth in height is accelerated, but the trunk diameter and root volume are reduced, which are the most important variables in rootstock formation before grafting to prepare a seedling to be grafted sooner without prejudicing the development of roots, as obtaining seedlings with a large volume of roots is fundamental, favoring implantation in the field.

Conclusions

Under greenhouse conditions in southern Brazil, it can be concluded that 'Rangpur' Lime is more vigorous and has a greater response to nitrogen fertilization in vegetative growth than 'Swingle' Citrumelo; 'Rangpur' Lime shows a greater accumulation of dry matter of the plant under a dose of 3.38 g of N per plant.



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For 'Swingle' Citrumelo, a dose of 2.03 g of N per plant stimulates a greater accumulation of dry matter of the plant; however, nitrogen fertilization at the tested doses prejudices root accumulation. Nitrogen fertilization reduces the root:canopy ratio of the rootstocks. The leaf contents of N and P are favored by high doses of N within the tested range. Intermediate doses favored the leaf contents of Ca and Mg. The leaf content of K decreased due to nitrogen fertilization.

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