Research, Society and Development, v. 9, n. 10, e7449109096, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.9096

Nutrient concentrations in trifoliate orange as affected by lime and gypsum

Concentrações de nutrientes em citros trifoliata sob aplicação de calcário e gesso

Concentraciones de nutrientes en *Poncirus trifoliata* como respuesta a la aplicación de caliza y yeso

Received: 10/06/2020 | Reviewed: 10/13/2020 | Accept: 10/14/2020 | Published: 10/17/2020

Maciel Korzune
ORCID: https://orcid.org/0000-0001-8944-0317
State University of Mid-West, Brazil
E-mail: korzune12@hotmail.com
Fabricio William de Ávila
ORCID: https://orcid.org/0000-0003-0301-2720
State University of Mid-West, Brazil
E-mail: fwavila@unicentro.br
Renato Vasconcelos Botelho
ORCID: https://orcid.org/0000-0001-9580-2572
State University of Mid-West, Brazil
E-mail: rbotelho@unicentro.br
Poliana Horst Petranski
ORCID: https://orcid.org/0000-0002-4203-1951
State University of Mid-West, Brazil
E-mail: polianahorstp@hotmail.com
Karin Kamila Brick Lopes de Matos
ORCID: https://orcid.org/0000-0002-7795-2814
State University of Mid-West, Brazil
E-mail: Karin_kamila@hotmail.com
Leandro Rampim
ORCID: https://orcid.org/0000-0001-8300-7424
State University of Mid-West, Brazil
E-mail: lrampim@unicentro.br
Marcelo Marques Lopes Muller
ORCID: https://orcid.org/0000-0002-5466-2398
State University of Mid-West, Brazil

E-mail: mmuller@unicentro.br

Abstract

Use of trifoliate orange [*Poncirus trifoliata* (L.) Raf.] as a rootstock has intensified in recent years in Brazil. Objectives of this study were to evaluate the effects of lime and agricultural gypsum on concentration of nutrients in trifoliate orange. Seedlings of trifoliate orange were grown in PVC pipe columns, presenting 15 cm in diameter and 35 cm in length. The columns were sectioned in two rings: the upper ring, 15 cm high, and the lower ring, 20 cm high. The factorial scheme (2×4)+1 was used, being two liming treatments and four agricultural gypsum doses (carried out only in the soil of the upper ring), and an additional treatment (with liming carried out in the soil of both upper and lower ring). Liming increased Mg and S concentrations in roots of the superficial soil layer (0-15 cm). Ca concentration was higher in roots of both superficial (0-15 cm) and subsuperficial (15-35 cm) layers. Gypsum without liming resulted in higher N, K, and Mn concentrations and lower Mg concentration in roots of the soil subsurface layer. Ca and S concentrations in root of the soil superficial layer were higher with gypsum. In plant shoot, the concentrations of K, Ca, S and Cu were higher with liming, and concentrations of Ca and P were higher and lower, respectively, with gypsum application.

Keywords: *Citrus* sp.; Acid soil; Soil fertility; Plant nutrition; Citriculture.

Resumo

O uso de citros trifoliata [*Poncirus trifoliata* (L.) Raf.] como porta-enxerto tem-se intensificado nos últimos anos no Brasil. Objetivou-se avaliar os efeitos de calagem e da gessagem sobre as concentrações de nutrientes em mudas de citros trifoliata, cultivadas sob condições de casa de vegetação. As colunas de tubo de PVC (vasos), com 15 cm de diâmetro e 35 cm de comprimento, foram seccionadas em dois anéis: o anel superior (0-15 cm de profundidade), e o anel inferior (15-35 cm de profundidade). Os tratamentos foram dispostos em um esquema fatorial (2×4)+1, sendo dois tratamentos de calagem e quatro doses de gesso agrícola (aplicadas apenas no solo do anel superior), e um tratamento adicional (com realização de calagem no solo de ambos os anéis, superior e inferior). A calagem aumentou os teores de Mg e S nas raízes da camada superficial do solo (0-15 cm), havendo também maior teor de Ca nas raízes de ambas as camadas de solo (0-15 e 15-35 cm). Para o Ca e S, em geral, houve também aumentos de teores das raízes da camada superficial com as doses de gesso.

Na parte aérea das mudas de citros trifoliata, a calagem elevou os teores de K, Ca, S e Cu, enquanto a gessagem elevou o teor de Ca e reduziu o de P.

Palavras-chave: *Citrus* sp.; Solo ácido; Fertilidade do solo; Nutrição de plantas; Citricultura.

Resumen

El uso de *Poncirus trifoliata* (L.) Raf. como portainjerto se ha intensificado en los últimos años en Brasil. Los objetivos de este estudio fueron evaluar los efectos de las dosis de encalado y yeso sobre las concentraciones de nutrientes en *Poncirus trifoliata*. Las columnas de tubo de PVC (jarrones), de 15 cm de diámetro y 35 cm de largo, se seccionaron en dos anillos: el anillo superior (0-15 cm de profundidad) y el anillo inferior (15-35 cm de profundidad). Los tratamientos se dispusieron en un esquema factorial (2×4)+1, con dos tratamientos de encalado y cuatro dosis de yeso (aplicado solo al suelo del anillo superior), y un tratamiento adicional (con encalado en el suelo de ambos anillos, superior e inferior). El encalado aumentó el contenido de Mg y S en las raíces de la capa superior del suelo (0-15 cm), y también hubo un mayor contenido de Ca en las raíces de ambas capas del suelo (0-15 y 15-35 cm). Para Ca y S, en general, también hubo incrementos en el contenido de las raíces de la capa superficial con las dosis de yeso. En la parte aérea de *Poncirus trifoliata*, el encalado aumentó los contenidos de K, Ca, S y Cu, mientras que el enyesado aumentó el contenido de Ca y redujo P.

Palabras clave: *Citrus* sp.; Suelo ácido; Fertilidad del suelo; Nutrición vegetal; Citricultura.

1. Introduction

O Brazil is one of the largest producers of citrus fruits and derivatives as well as largest exporter of concentrated orange juice in the world (FAO 2017). Rootstock directly influences precocity, tolerance to biotic and abiotic stresses, water and nutrient uptake, yield and quality of the fruits harvested from citrus orchard.

Trifoliate orange [*Poncirus trifoliata* (L.) Raf.], also known as trifoliate, bitter lemon or ‘Flying Dragon’, has been used as a rootstock since the beginning of the first millennium in Asia. Currently it is one of the most used rootstocks in the world, for example in the USA (State of California), Argentina, Australia and Japan. In the latter country the species is widely used as a rootstock for the 'Satsuma' tangerine (*Citrus unshiu* Marc.).

In Brazil, use of trifoliate orange as a rootstock has intensified in recent years. As advantages it is tolerant to gummosis, nematodes and sadness virus and exhibits good quality fruits. In addition, trifoliate orange presenting dormancy after continuous periods of low temperatures, followed by loss of leaves, which provides greater tolerance to cold. Thus, trifoliate orange has provided good yield in citrus orchards located in the Southern region of Brazil (Bastos *et al.*, 2014; Nesom, 2014; Passos, Peixouto, Santos, Caldas, & Soares Filho, 2006). However, as disadvantages, there is lower yield and low tolerance to water deficit (Passos *et al.*, 2006).

Yield increase in citrus orchards driven by programs to improve varieties and use suitable rootstocks resulted in higher nutrient export from citrus areas, such as calcium (Ca) and sulfur (S). One of the alternatives for provide these nutrients is use of agricultural gypsum, which has about 23% Ca and 15% S as sulfate anion (SO_4^{2-}) (Caires, Joris, & Churka, 2011). When supplied through gypsum application, both Ca^{2+} and SO_4^{2-} percolate into soil profile, forming ionic pairs between Al^{3+} and SO_4^{2-} in soil subsurface layer. Consequently, gypsum reduces Al^{3+} activity and provides Ca^{2+} and SO_4^{2-} in deepest layers of the soil, stimulating growth of roots in depth, which results in greater tolerance of plants to water deficit stress. However, high-dose agricultural gypsum changes availability of nutrients in the soil and may influence the nutrient uptake by plant (Michalovicz *et al.*, 2019; Rampim, Lana, Frandoloso, & Fontaniva, 2011; Ritchey & Sousa, 1997; Sousa, Lobato, & Rein, 2005; Vicensi *et al.*, 2016). Thus, gypsum may affect accumulation of macronutrients and micronutrients in root and shoot of trifoliate orange plants.

The management of soil acidity may be enhanced with association of liming and gypsum, instead of using only the first practice in isolation. Liming has function of correcting acidity and providing bases (Ca^{2+} and Mg^{2+}) in soil superficial layer, while gypsum, although not a soil acidity corrective, reduces the harmful effects of subsurface acidity and provides Ca and S to plant nutrition (Raij, 2011; Silva, Van Raij, Carvalho, De, Bataglia, & Kondo, 1997; Sousa, Miranda, & Oliveira, 2007; Vicensi *et al.*, 2020).

The aim of this study was to evaluate the effects of liming and doses of agricultural gypsum on concentration of nutrients in root and shoot of the trifoliate orange rootstock.

2. Materials and Methods

In this study, a qualitative and quantitative experimental research was carried out (Pereira, Shitsuka, Parreira, & Shitsuka, 2018). Trifoliate orange (*Poncirus trifoliata* (L.) Raf.) seedlings were obtained from germination of seeds and kept in pots with intermittent irrigation, under greenhouse conditions of the CEDETEG *Campus* of the State University of Mid-West (UNICENTRO), Guarapuava, Paraná State, Brazil.

In November 2017, trifoliate orange seedlings were transplanted into PVC pipe columns filled with subsurface soil, a clayey dystrophic Oxisol (Typic Hapludox), collected from an area under native forest, located on CEDETEG *Campus* of the UNICENTRO. Soil samples were dried, ground, sieved in a 2 mm mesh and analyzed chemically (EMBRAPA, 2009; Pavan, Bloch, Zempulski, Miyazawa, & Zocoler, 1992), with results as follows: pH ($CaCl_2$) = 3.83; P-Mehlich I = 2.09 mg dm^{-3}; K^+ = 0.23 $cmol_c$ dm^{-3}; Ca^{2+} = 1.42 $cmol_c$ dm^{-3}; Mg^{2+} = 0.55 $cmol_c$ dm^{-3}; Sum of Bases = 2.20 $cmol_c$ dm^{-3}; Al^{3+} = 3.30 $cmol_c$ dm^{-3}; H+Al = 11.69 $cmol_c$ dm^{-3}; Cation Exchange Capacity = 13.89 $cmol_c$ dm^{-3}; Saturation of Bases = 15.84%; Saturation of Al = 43,02%; and Organic Matter = 43.02 g dm^{-3}.

The PVC pipe columns, 15 cm in diameter and 35 cm in length, were composed of two rings: upper ring, 15 cm high (surface layer, 0-15 cm deep), and lower ring, 20 cm high (subsurface layer, 15-35 cm deep). To avoid contact between roots and inner wall of the PVC pipes, a coating of kaolin on the inner face of the PVC pipes was used before filling them with the soil. The upper and lower rings of each soil column were joined with “silver tape” adhesive. Both planting and topdressing fertilizations were carried out only in the soil of the upper ring, in order to provide macronutrients (except Ca and S, which were provided only with liming and gypsum dose treatments) and micronutrients recommended (Malavolta, Vitti, & Oliveira, 1997), followed by the planting seedlings.

The experimental design was of randomized blocks (DBC), with four replicates, in a factorial scheme (2×4)+1, with two liming treatments (without and with applications of calcium and magnesium carbonates, carried out only in soil of upper ring), combined with four gypsum dose treatments (0.0, 2.5, 5.0 and 10.0 g kg^{-1} of agricultural gypsum, also applied only in soil of upper ring). An additional treatment (with applications of calcium and magnesium carbonates, carried out in soil of both rings, upper and lower) was carried out to evaluate the potential of agricultural gypsum, applied on soil surface, in improving the conditions of soil subsurface, comparing with the soil subsurface corrected with liming. Each experimental plot consisted of a PVC pipe column with two plants of trifoliate orange.

Based on the results of the chemical analysis of the soil, in treatments that received liming, the application of calcium ($MgCO_3$) and magnesium ($MgCO_3$) carbonates was made aiming to raise the saturation of bases to 70% and to adjust Ca/Mg ratio to 4/1, with a dose of 3.84 and 1.87 g kg^{-1} of $CaCO_3$ and $MgCO_3$, respectively (both reagent p.a.). In treatments with agricultural gypsum, the doses were 0.0, 2.5, 5.0 and 10.0 g kg^{-1} (dry weight basis),

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.9096

simulating the approximate doses of 0, 5, 10 and 20 Mg ha^{-1} gypsum in the field. The agricultural gypsum was obtained from local commerce of Guarapuava, Paraná State, Brasil.

Plant shoot and roots in upper and lower rings were harvested in each separately, on April 10, 2018, at 153 DAT. Roots were previously washed in running water and deionized water to separate the soil particles, dried on paper towels and, subsequently, in an oven with forced air circulation at 58 °C for four days.

Dry shoot and root were ground in a Willey mill. Nutrient analyzes in root (0-15 and 15-35 cm depths) and shoot samples were carried out at Soil and Plant Nutrition Laboratory of the CEDETEG *Campus* of the UNICENTRO, according to EMBRAPA (2009).

The data obtained were submitted to analysis of variance (ANOVA, $P \leq 0.05$), considering the design in DBC in a scheme (2×4)+1, using software R version 3.5.1. (R Development Core Team 2016). In ANOVA, degrees of freedom were adjusted according to treatments of liming, gypsum doses and interaction between liming × gypsum doses, and a contrast between additional treatment and factorial (2×4) experiment average. When there was a significant effect of gypsum dose treatments, linear and quadratic regression analysis was performed ($P \leq 0.05$). The normality of data was tested by Shapiro-Wilk test ($P \leq 0.05$).

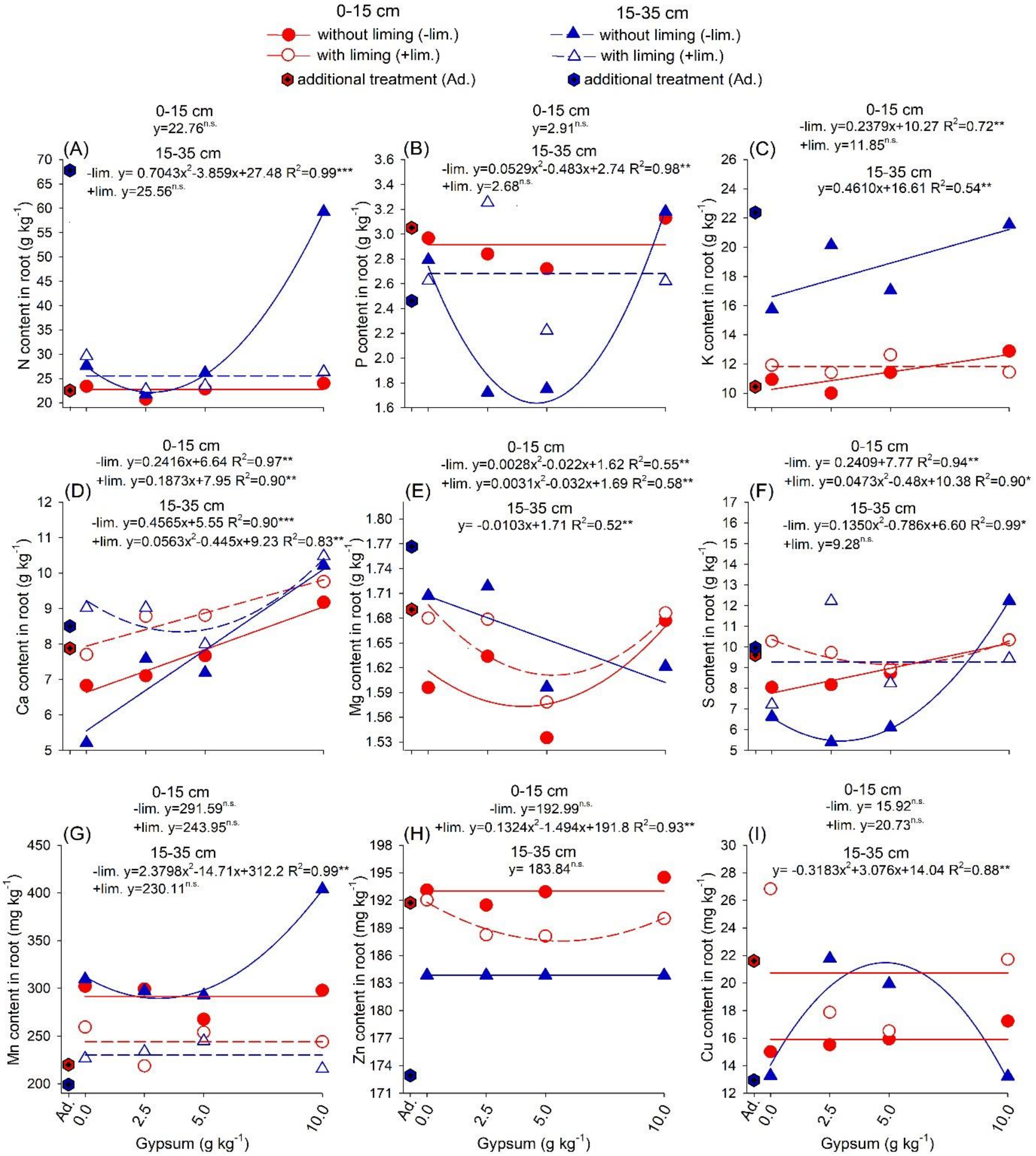
3. Results and Discussion

Figure 1 shows nutrient concentrations in roots collected from both 0-15 and 15-35 cm depths.

It may be seen in Figure 1 that nitrogen (N) (Figure 1A) and phosphorus (P) (Figure 1B) concentrations of the root had similar behaviors. In roots collected from 0-15 cm depth, N and P concentrations were not significantly influenced by the liming and gypsum dose treatments, presenting an average of 22.76 and 2.91 g kg^{-1} of N and P, respectively. In this soil layer there was also no significant difference between additional treatment and factorial experiment average.

However, in roots collected from 15-35 depth, N ($P \leq 0.001$) and P ($P \leq 0.05$) concentrations were affected by the interaction between liming × gypsum doses. Without liming, N and P concentrations responded to gypsum doses in a quadratic form, decreasing at the lowest gypsum doses (0 and 5 g kg^{-1}) but with a strong increase (126 and 82%, respectively) of values between the two highest gypsum doses (5 and 10 g kg^{-1}). However, with liming, there was no significant variation in N and P concentrations of the root with gypsum doses.

Figure 1. Nutrient concentrations in trifoliate orange roots collected from both soil layers (0-15 and 15-35 cm depths) at 153 DAT, as a function of liming and gypsum dose treatments applied in soil of upper ring (0-15 cm deep). Additional treatment (ad.) received liming in both upper (0-15 cm deep) and lower (15-35 cm deep) rings. Figures 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H and 1I corresponding to root concentrations of N, P, K, Ca, Mg, S, Mn, Zn and Cu, respectively. ***, **, * and ^{ns} = P ≤ 0.001, P ≤ 0.01, P ≤ 0.05 e P > 0.05, respectively, for linear and quadratic regression equations for gypsum dose response.



Source: Authors (Guarapuava-PR, Brazil, 2020).

Figure 1 shows that agricultural gypsum contains calcium and its application may decrease the P solubility in the soil due to precipitation. Lower P availability in soils treated with gypsum were reported (Anderson, Tuovinen, Faber, & Ostrokowski, 1995; Boruvka &

Rechcigl, 2003; Callahan, Kleinman, Sharpley, & Stout, 2002; Stout, Sharpley, & Weaver, 2003). On the other hand, under acidic soil, agricultural gypsum in high dose may improve the uptake efficiency of some nutrients by root system (Backes *et al.*, 2018; Vicensi *et al.*, 2020), as it was reported that gypsum increased both N and P uptake in pawpaw (*Asimina triloba*) (Picchioni, Graham, & Ulery, 2004). Agricultural gypsum is a by-product of phosphate fertilizer manufacture and may contain small concentrations of P (about 0,3%) (Caires *et al.*, 2011). Thus, high gypsum doses in the soil provide considerable amounts of P.

In this soil layer (15-35 depth), N and P concentrations of the root in additional treatment was 128% higher ($P \leq 0.001$) and without significant variation ($P > 0.05$), respectively, compared to the average N and P concentrations of the factorial experiment. Thus, liming application also in soil subsurface layer improved the availability of N for trifoliate orange plants. As is known, liming improves the soil N availability for plants by correcting soil acidity (Raij, 2011; Troeh & Thompson, 2005).

Potassium (K) concentration of the roots (Figure 1C) collected from 0-15 cm depth was affected by the interaction between liming × gypsum doses ($P \leq 0.05$). Without liming, K concentration increased linearly with gypsum doses, showing values of 10.93 to 12.88 g kg^{-1} (18% increase). However, with liming, K concentration did not vary significantly. In this soil layer (0-15 cm depth), additional treatment caused a significant decrease ($P \leq 0.05$) of 10% in K concentration of the root when compared to factorial experiment average. Thus, without liming, agricultural gypsum improved K uptake of the soil surface layer by trifoliate orange plants, possibly by decreasing acidity and increasing cation exchange capacity (CEC) of the soil (Kumar *et al.*, 2014; Troeh & Thompson, 2005).

For the roots of the 15-35 cm depth, liming did not significantly affect K concentration, however, there was a linear increase in K concentration by increasing gypsum doses ($P \leq 0.01$), ranging from 15.75 to 21.56 g kg^{-1} (increase of 37%). However, K concentration in additional treatment did not differ statistically of the factorial experiment average in this soil layer.

Picchioni *et al.* (2004) also found a considerable increase in K accumulation in the root of pawpaw (*Asimina triloba*) with gypsum application, showing that gypsum may increase K uptake by root system. Moreover, sulfur as sulfate (SO_4^{2-}) added with agricultural gypsum provides greater mobility of nutrients, such as Mg^{2+} and K^+, from the surface layers, together with water, transferring them to soil deeper layers (Caires *et al.*, 2011; Vicensi *et al.*, 2016). This experiment was carried out in a greenhouse with intermittent irrigation

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.9096

throughout the experimental period, which may have contributed even more to redistribution of some nutrients into the soil.

Calcium (Ca) concentration in the roots (Figure 1D), in general, increased with liming and gypsum, with significant interaction between liming × gypsum doses ($P \leq 0.01$). The roots of the 0-15 cm depth had about 13% more Ca concentration with liming, verified in treatments without gypsum (0.0 g kg^{-1} treatment). However, increased variation in Ca concentration was found with gypsum, responding in a linear way to gypsum doses. On average, Ca concentration of the root varied from 7.27 to 9.47 g kg^{-1} (30% increase) between doses 0 and 10 g kg^{-1} of agricultural gypsum, respectively.

In roots of the 15-35 cm depth, there was also a significant effect of the interaction between liming × gypsum doses ($P \leq 0.01$) for Ca concentration. Unlike the superficial layer, in treatments without gypsum, liming considerably increased Ca concentration (73% increase). Without liming, there was also a strong increase in Ca concentration with gypsum doses, ranging from 5.21 to 10.22 g kg^{-1} (96% increase) between doses 0 and 10 g kg^{-1} of gypsum, respectively. With liming, variations in Ca concentration with gypsum doses were of lesser magnitude, probably due to effect of liming in also supplying Ca to soil. In this case, there was a quadratic effect of gypsum doses on Ca concentration of the root, showing an increase between the two highest gypsum doses. For the roots of both soil layers (0-15 cm and 15-35 cm depths), additional treatment did not show significant variation in Ca concentration when compared to factorial experiment average.

In field conditions, limestone applied on soil surface, without incorporation, has effects only on soil superficial layer, due to low solubility and mobility of limestone into soil. However, in this study, correctives ($CaCO_3$ and $MgCO_3$) were incorporated into soil of the upper ring (0-15 cm depth), associated with intermittent irrigation throughout the experimental period, potentiating the effect of liming also on soil subsurface layer (15-35 cm depth), which is a favorable factor for production of rootstocks under greenhouse conditions. On the other hand, gypsum has good effect on soil subsurface layer, even when applied on soil surface, without incorporation (Michalovicz *et al.*, 2019; Raij, 2011; Vicensi *et al.*, 2016). In general, liming together with gypsum application may enhance the benefits on plant development, in relation to the management of only one of these (Raij, 2011; Silva *et al.*, 1997; Sousa *et al.*, 2007).

Without or with liming, the gypsum doses did not provide a defined behavior of the magnesium (Mg) concentration in roots (Figure 1E) of the 0-15 cm depth, which were significant the quadratic equations, but with a low determination coefficient ($R^2 \leq 0.58$). In this case, without liming, it was found that Mg concentration was high with highest gypsum dose.

In roots of the 15-35 cm depth, there was no significant effect of liming on Mg concentration of the root. Gypsum doses resulted in a decrease ($P \leq 0.01$) of Mg concentration, especially between 5 and 10 g kg^{-1} of gypsum (the linear equation was statistically significant, but with a low determination coefficient). In additional treatment, there was a significant increase in Mg concentration of the root of both soil depths, compared to factorial group average.

In general, the effect of liming on Mg concentration of the roots was small. A more pronounced effect was expected, due to addition of $MgCO_3$ to soil of the upper ring with liming. In a study by Fochesato, Souza, Schäfer and Maciel (2006), it was found that trifoliate orange naturally has a lower potential for Mg^{2+} accumulation in roots, in comparison with other rootstocks, such as lemon (*Citrus limonia* Osbeck) and citrange C13 [*Citrus sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.].

Sulfur (S) concentration in roots (Figure 1F) collected from 0-15 cm depth varied with liming and gypsum treatments ($P \leq 0.01$). Without gypsum (0 g kg^{-1} treatment), liming increased S concentration by 28%. The gypsum effect on S concentration in this soil layer was increased without liming application, increasing the S concentration of the root by also 28%.

In roots of the 15-35 cm depth there was also interaction ($P \leq 0.01$) of the treatments on S concentration. As in soil surface layer, gypsum was more efficient in raising the S concentration when liming was not carried out, with an increase of 84% between doses 0 and 10 g kg^{-1} of gypsum. With liming, there was no defined behavior of gypsum doses on S concentration of the root. For both soil layers, there was no significant variation in S concentration between additional treatment and factorial experiment average.

Agricultural gypsum has about 15% S as SO_4^{2-} and its application in the soil increases the uptake of this nutrient by plants (Vicensi *et al.*, 2020). However, the availability of SO_4^{2-} in soil depends on both density and type of predominant electrical charges. On weathered soils from tropical and subtropical regions, SO_4^{2-} is more adsorbed in more acidic soil layers, where there is a higher density of positive charges. Associated with this, in agricultural soil surface layer, there is a high concentration of phosphate anion ($H_2PO_4^-$ and HPO_4^{2-}), which competes with SO_4^{2-} for adsorption on soil colloid surfaces (Novais & Smyth, 1999; Raij, 2011).

As for the micronutrient concentrations of the root, there was no significant effect ($P > 0.05$) of liming and gypsum treatments on iron (Fe) concentration, with roots exhibiting 934 mg kg^{-1} of Fe.

Manganese (Mn) concentration of the root (Figure 1G) in both soil depth decreased with liming ($P \leq 0.01$). In 15-35 cm depth, this liming effect was reinforced by reducing Mn concentration in the additional treatment when compared to factorial experiment average. Due to liming effect in decreasing Mn concentration of the root, gypsum effect was significant only in 15-35 cm depth and without liming. In this treatment, Mn concentration increased by 38% between 5 and 10 g kg^{-1} of gypsum.

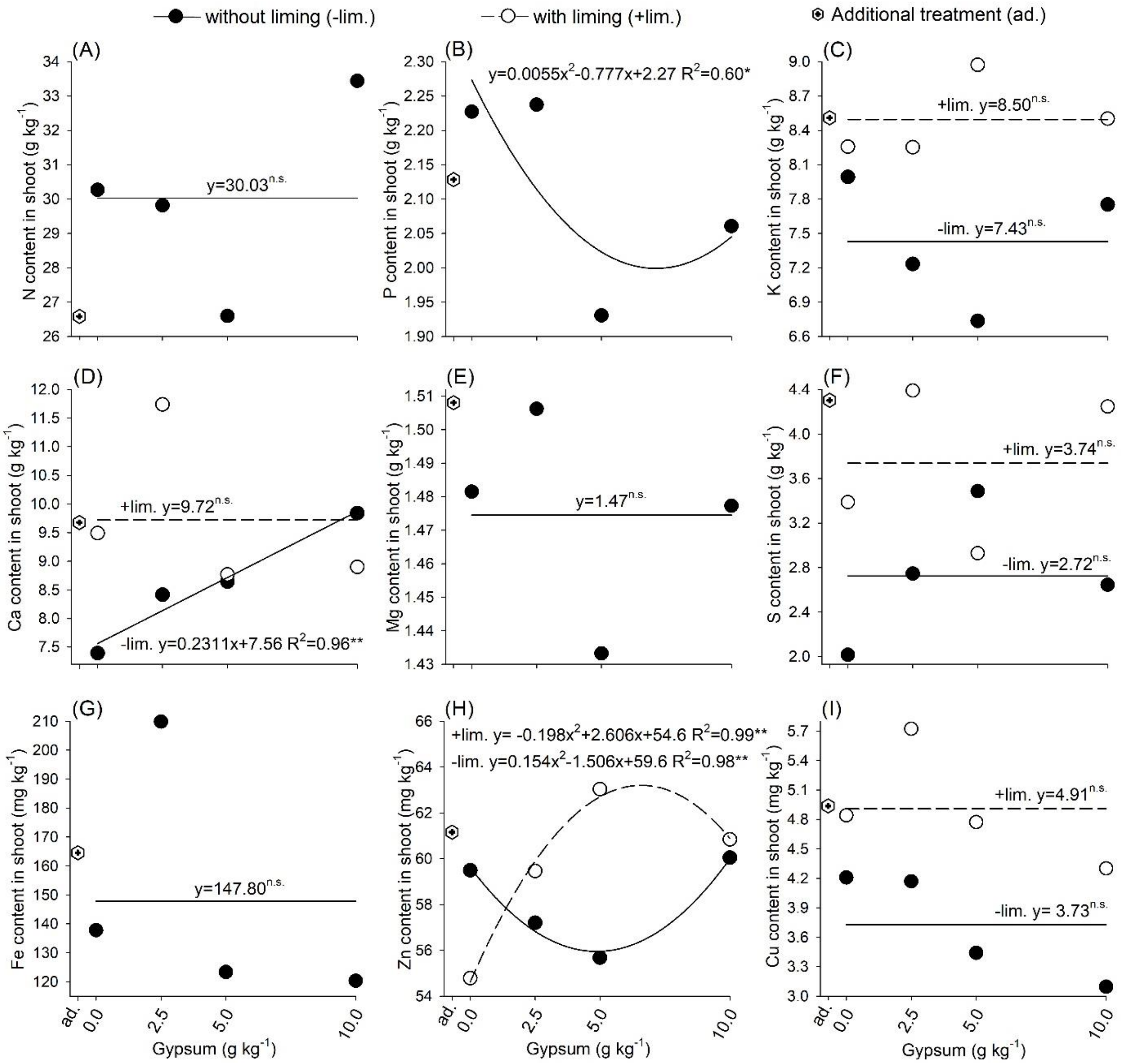
Zinc (Zn) concentration of the root (Figure 1H), considering both soil depth, had little variation, although there was a significant effect of the interaction between liming × gypsum doses in 0-15 cm layer. Higher variation range was observed in the additional treatment, which in 15-35 cm layer showed a 6% reduction ($P \leq 0.01$) of Zn concentration of the root when compared to factorial experiment average.

Copper (Cu) concentration of the root (Figure 1I) in 0-15 cm depth was 30% higher ($P \leq 0.01$) with liming and did not vary significantly with gypsum doses. In 15-35 cm depth there was only gypsum effect ($P \leq 0.05$), with intermediate doses (2.5 and 5.0 g kg^{-1} of gypsum) showing the highest values of Cu concentration. In both soil layers, there was no significant difference in Cu concentration between additional treatment and factorial experiment average.

The liming effect in raising soil pH value decreases the micronutrient availability for uptake by plants (Raij, 2011), as found in common bean (*Phaseolus vulgaris* L.) by Ndakidemi, Bambara and Makoi (2011). Corroborating this study, higher Mn availability for plants with gypsum application was found in other researches (Caires, Blum, Barth, Garbuio, & Kusman, 2003; Habte & Soedarjo, 1995; Moraes, Brito, Fioretto, & Moreira, 2016; Walker, 1961).

Figure 2 shows nutrient concentrations in trifoliate orange shoot.

Figure 2. Nutrient concentrations in trifoliate orange shoot at 153 DAT, as a function of liming and gypsum dose treatments applied in soil of upper ring (0-15 cm deep). Additional treatment (ad.) received liming in both upper (0-15 cm deep) and lower (15-35 cm deep) rings. Fig. 3A, 3B, 3C, 3D, 3E, 3F, 3G, 3H and 3I corresponding to root concentrations of N, P, K, Ca, Mg, S, Fe, Zn and Cu, respectively. **, * and ^{ns} = P ≤ 0.01, P ≤ 0.05 e P > 0.05, respectively, for linear and quadratic regression equations for gypsum dose response.



Source: Authors (Guarapuava-PR, Brazil, 2020).

It may be seen in Figure 2 that nitrogen (N) concentration of the shoot (Figure 2A) was not significantly affected by the liming and gypsum dose treatments, exhibiting about 30.03 g kg^{-1} of N in shoot. For phosphorus (P) concentration of the shoot (Figure 2B) there was no significant effect of liming, but the two highest gypsum doses (5 and 10 g kg^{-1}) resulted in 11% decreased in P concentration (P ≤ 0.05), with values varying from 2.24 to 2.0 g kg^{-1}. For both N and P concentrations, there was no significant difference between additional treatment and factorial experiment average, reinforcing that liming had no influence on the concentrations of these two macronutrients in trifoliate orange shoot.

Nitrogen is present in all proteins and many other components of plant cells, acting on plant growth and development (Marschner, 2012). Critical level for leaf N concentration in citrus is about 25 g kg^{-1} (Silva, Silva, & Simão, 2016). Low gypsum dose (from 0 to 5 g kg^{-1}) without liming also decreased P concentration of the root collected from 15-35 cm depth (Figure 1B). Phosphorus has great importance for energy transfer and physiological processes in plants (Marschner, 2012; Tofanelli, Santos, & Kogeratski, 2018).

Potassium (K) concentration of the shoot (Figure 2C) was not significantly affected by the gypsum doses, but increased on average by 14% ($P < 0.01$) with liming application. In the plant, K plays a role in osmotic, ionic balance and stomatal regulation, in addition to activating many enzymes. The supply of this nutrient in adequate doses for plants promotes positive relationships in leaf growth and photosynthesis (Silva *et al.*, 2016; Tofanelli *et al.*, 2018).

Calcium (Ca) concentration of the shoot (Figure 2D) was affected by the interaction between liming × gypsum doses ($P < 0.01$). Without liming, Ca concentration increased linearly with gypsum doses, ranging from 7.39 to 9.84 g kg^{-1} (33% increase). However, with liming, there was no significant gypsum effect, and average Ca concentration was 9.72 g kg^{-1} (that is, a value close to the treatment without liming and with 10 g kg^{-1} of gypsum). Corroborating these results, in roots (especially in the 15-35 cm depth) (Figure 1D) it was shown that Ca concentration was strongly high when liming was not done, however, with liming, the effect was less, reinforcing that gypsum doses were more effective in providing Ca^{2+} to plants when liming was not carried out. Agricultural gypsum contains about 24% of Ca (Caires *et al.*, 2011), which explains the strong increase of Ca concentration in trifoliate orange root and shoot by increasing gypsum doses.

Magnesium (Mg) concentration of the shoot (Figure 2E) there was no significant effect of the liming and gypsum dose treatments, and average Mg concentration was 1.47 g kg^{-1}, also confirming the results observed in the roots (Figure 1E) whose values varied in small magnitude.

For the three cationic macronutrients (K, Ca and Mg), there was no significant difference in shoot concentrations between additional treatment and factorial experiment average. In general, this behavior was also verified for the roots (Figure 1C, 1D and 1E), showing that liming in soil subsurface layer did not contribute significantly to increase of K, Ca and Mg concentrations in the trifoliate orange plant.

Sulfur (S) concentration of the shoot (Figure 2F) increased ($P < 0.05$) by an average of 37% with liming, however, there was no significant effect of gypsum doses. This result is contrary to those observed in the roots (Figure 1F), which was found a considerable increase in S concentration with gypsum doses in treatments without liming. In this case, it seems that the S uptake by the plants without gypsum application was sufficient for the development of the shoot, and it is assumed that the surplus of S added to the soil with the gypsum doses was uptake by the plant and accumulated in the roots.

Additional treatment had no significant difference in S concentration of the shoot when compared to factorial experiment average, the same behavior also was evident in the root (Figure 1F).

It is still rare to find in the literature information about research that addressed the effects of agricultural gypsum associated with liming on citrus rootstock growth under greenhouse conditions. In fieldwork, Minato, Esper Neto, Sakurada, Inoue and Batista (2017) observed that gypsum doses did not influence the K, Ca and Mg concentrations in corn leaves, collected at phenological stage R1 (beginning of the reproductive period). Santos *et al.* (2012) found that gypsum did not affect Mg, K, P and S concentrations in the shoot biomass of elephant grass varieties. However, in annual crops under field conditions, agricultural gypsum has been responsible for raising leaf concentrations of Ca and S in soybeans, corn, beans, wheat and barley (Michalovicz *et al.*, 2019; Vicensi *et al.*, 2016).

As for micronutrients, manganese (Mn) concentration of the shoot was not significantly altered by the liming and gypsum dose treatments, presenting an average value of 29.6 mg kg^{-1}.

Iron (Fe) concentration of the shoot (Figure 2G) was also not significant with liming and gypsum dose treatments (presenting an average value of 147.8 mg kg^{-1}), although there was an increase in Fe concentration at the dose 2.5 g kg^{-1} of gypsum.

Zinc (Zn) concentration of the shoot (Figure 2H) was affected by the interaction between liming × gypsum doses. Without liming, gypsum reduced Zn concentration between doses 0 and 5 g kg^{-1}. However, with liming, the effect was opposite, which was observed an increase in Zn concentration of the shoot.

Copper (Cu) concentration of the shoot (Figure 2I) was higher with liming (32% increase) and there was no significant effect of gypsum doses. This behavior was similar to that seen in the roots of the soil superficial layer (Figure 1I).

For the four micronutrients (Fe, Mn, Zn and Fe), the concentrations of the shoot did not vary significantly between additional treatment and factorial experiment average, differently from the Mn and Zn concentrations of the roots (Figure 1G and 1H, respectively), which showed a significant difference between additional treatment and factorial experiment average. The Zn and Mn are among the most important micronutrients for citrus growth and yield. Orchards in formation may show a certain Cu deficiency. The application of certain fungicides has been a form of Cu supplementation for citrus orchards (Mattos Junior, Cantarella, Quaggio, & Boaretto, 2009).

4. Final Considerations

Liming increased Ca content and reduced Mn content of the root in both soil layers, and increased Mg and S contents of the root in soil surface layer. Gypsum provided without liming resulted in increased contents of N, K, and Mn and decreased Mg content of the root in soil subsurface layer. There were also increased Ca and S contents of the roots in soil surface layer with the gypsum doses.

Contents of K, Ca, S and Cu in the trifoliate orange shoot were increased by liming, while gypsum increased Ca content and decreased P content.

Future studies with different genotypes are necessary to better understand the effect of the interaction between liming and agricultural gypsum on nutrition and development of trifoliate orange [*Poncirus trifoliata* (L.) Raf.] seedlings.

Acknowledgment

F.W.A. thanks the Brazilian National Research Council (CNPq) for the research fellowship (Grant numbers 461553/2014-7). The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível (CAPES) – Finance Code 001.

References

Anderson, D. L., Tuovinen, O. H., Faber, A., & Ostrokowski, I. (1995). Use of soil amendments to reduce soluble phosphorus in dairy soils. *Ecological Engineering*, *5*, 229–246. DOI: https://doi.org/10.1016/0925-8574(95)00025-9

Backes, C. C., Santos, A. M., de Bessa, S. V., Ribon, A. A., Teodoro, A. G., Rodrigues, L. M., Tomazelo, D. A., Leite, L. L. F. (2018). Taxa de cobertura verde e exportação de macronutrientes pelo capim Marandu em função da aplicação de gesso. *Archivos de Zootecnia*, *67*, 234–242. DOI: https://doi.org/10.21071/az.v67i258.3659

Bastos, D. C., Ferreira, E. A., Passos, O. S., Sá, J, F. de, Ataíde, E. M., & Calgaro, M. (2014). Cultivares copa e porta-enxertos para a citricultura brasileira. *Informe Agropecuário*, *35*(281), 36–45. Available: https://www.alice.cnptia.embrapa.br/handle/doc/1007492

Boruvka, L., & Rechcigl, J. E. (2003). Phosphorus retention by the Ap horizon of a Spodosol as influenced by calcium amendments. *Soil Science*, *168*, 699–706. DOI: https://doi.org/10.1097/01.ss.0000095143.68539.55

Caires, E. F., Blum, J., Barth, G., Garbuio, F. J., & Kusman, M. T. (2003). Alterações químicas do solo e resposta da soja ao calcário e gesso aplicados na implantação do sistema plantio direto. *Revista Brasileira de Ciência do Solo*, *27*, 275–286. DOI: https://doi.org/10.1590/S0100-06832003000200008

Caires, E. F., Joris, H. A. W., & Churka, S. (2011). Long-term effects of lime and gypsum additions on no-till corn and soybean yield and soil chemical properties in southern Brazil. *Soil Use and Management*, *27*(1), 45–53. DOI: https://doi.org/10.1111/j.1475-2743.2010.00310.x

Callahan, M. P., Kleinman, P. J., Sharpley, A. N., & Stout, W. L. (2002). Assessing the efficacy of alternative phosphorus sorbing soil amendments. *Soil Science*, *167*, 539–547. Retrieved from https://journals.lww.com/soilsci/Abstract/2002/08000/ASSESSING_THE_EFFICACY_OF_ALTERNATIVE_PHOSPHORUS.5.aspx

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária (2009). *Manual de análises químicas de solos, plantas e fertilizantes*. Brasília: Embrapa informação tecnológica.

FAO (2017). *Citrus fruit fresh and processed statistical bulletin*. Food and Agriculture Organization of the United Nations, Rome.

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.9096
Fochesato, M. L., Souza, P. V. D. D., Schäfer, G., & Maciel, H. S. (2006). Produção de mudas cítricas em diferentes porta-enxertos e substratos comerciais. *Ciência Rural*, *36*(5), 1397–1403. DOI: https://doi.org/10.1590/S0103-84782006000500008

Habte, M., & Soedarjo, M. (1995). Limitation of vesicular-arbuscular mycorrhizal activity in *Leucaena leucocephala* by Ca insufficiency in an acid Mn-rich Oxisol. *Mycorrhiza*, *5*(6), 387–394. Available: https://link.springer.com/content/pdf/10.1007/BF00213437.pdf

Kumar, R., Chatterjee, D., Kumawat, N., Pandey, A., Roy, A., & Kumar, M. (2014). Productivity, quality and soil health as influenced by lime in ricebean cultivars in foothills of northeastern India. *The Crop Journal*, *2*(5), 338–344. DOI: https://doi.org/10.1016/j.cj.2014.06.001

Malavolta, E., Vitti, G.C.; & Oliveira, S. A. (1997). *Avaliação do estado nutricional das plantas: Princípios e aplicações. (*3a ed.), Piracicaba: Potafos.

Marschner, H. (2012). *Mineral nutrition of higher plants*. (3a ed.) London: Academic Press.

Mattos Junior, D., Cantarella, H., Quaggio, J. A., & Boaretto, R. M. (2009). *Citros: manejo da fertilidade do solo para alta produtividade*. Piracicaba: International Plant Nutrition Institute (IPNI), Informações Agronômicas. 128. Available: http://www.ipni.net/publication/ia-brasil.nsf/0/0431445A9BC1C48F83257A90001 2439B/$FILE/Page5-12-128.pdf

Michalovicz, L., Müller, M. M. L., Tormena, C. A., Dick, W. A., Vicensi, M., & Meert, L. (2019). Soil chemical attributes, nutrient uptake and yield of no-till crops as affected by phosphogypsum doses and parceling in southern Brazil. *Archives of Agronomy and Soil Science*, *65*(3), 385–399. DOI: https://doi.org/10.1080/03650340.2018.1505041

Minato, E. A., Esper Neto, M., Sakurada, R. L., Inoue, T. T., & Batista, M. A. (2017). Foliar macronutrient contents and maize (*Zea mays* L.) production in response to gypsum application in an Oxisol. *Scientia Agraria Paranaensis*, *16*(2), 219–224. Available: http://saber.unioeste.br/index.php/scientiaagraria/article/view/14208

Moraes, V. R., Brito, O. R., Fioretto, R. A., & Moreira, A. (2016). Changes in chemical properties of an Oxisol due to gypsum application. *Communications in Soil Science and Plant Analysis*, *47*(5), 571–580. DOI: https://doi.org/10.1080/00103624.2016.1141929

Ndakidemi, P. A., Bambara, S., & Makoi, J. H. (2011). Micronutrient uptake in common bean ('Phaseolus vulgaris' L.) as affected by Rhizobium inoculation, and the supply of molybdenum and lime. *Plant Omics*, *4*(1), 40–52. Retrieved from https://search.informit.com.au/documentSummary;dn=729351375108082;res=IELHSS

Nesom, G. L. (2014). *Citrus trifoliata* (Rutaceae): Review of biology and distribution in the USA. *Phitoneurum*, *46*, 1–14.

Novais, R. F., & Smyth, T. J. (1999). *Fósforo em solo e planta em condições tropicais*. Viçosa: Universidade Federal de Viçosa (UFV).

Passos, O. S., Peixouto, L. S., Santos, L. C. D., Caldas, R. C., & Soares Filho, W. D. S. (2006). Caracterização de híbridos de *Poncirus trifoliata* e de outros porta-enxertos de citros no estado da Bahia. *Revista Brasileira de Fruticultura*, *28*(3), 410–413. DOI: https://doi.org/10.1590/S0100-29452006000300016

Pavan, M. A., Bloch, M. D. F., Zempulski, H. D. C., Miyazawa, M., & Zocoler, D. C. (1992). Manual de análise química de solo e controle de qualidade. Londrina: Instituto Agronômico do Paraná (IAPAR), Circular 76.

Picchioni, G. A., Graham, C. J., & Ulery, A. L. (2004). Gypsum effects on growth and macroelement uptake of field-grown *Asimina triloba* (pawpaw) irrigated with low-saline, sodic water. *Hortscience*, *39*(5), 1104–1109. DOI: https://doi.org/10.21273/HORTSCI.39.5.1104

R Development Core Team (2016). R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available: URL https://www.r-project.org/

Raij, B. van. (2011). *Fertilidade do solo e manejo de nutrientes*. Piracicaba: International Plant Nutrition Institute (IPNI).

Rampim, L., Lana, M. D. C., Frandoloso, J. F., & Fontaniva, S. (2011). Atributos químicos de solo e resposta do trigo e da soja ao gesso em sistema de semeadura direta. *Revista Brasileira de Ciência do Solo*, *35*, 1687–1698. DOI: https://doi.org/10.1590/S0100-06832011000500023

Ritchey, K. D., & Sousa, D. M. G. (1997). Use of gypsum in management of subsoil acidity in Oxisols. In Moniz, A. C., Furlani, A. M. C., Schaffert, R. E., Fageria, N. K., Rosolem, C. A., & Cantarella, H. (Ed.). *Plant-soil interactions at low pH: sustainable agriculture and forestry production* (pp. 165–178). Campinas: Brazilian Soil Science Society.

Santos, R. L. D., Azevedo, V. M. D., Freire, F. J., Rocha, A. T. D., Tavares, J. A., & Freire, M. B. G. D. S. (2012). Nutrient uptake and use efficiency of elephant grass in the presence of crude gypsum. *Revista Brasileira de Ciência do Solo*, *36*(2), 497–505. DOI: https://doi.org/10.1590/S0100-06832012000200019

Silva, J. T. A. D., Silva, I. P. D., & Simão, F. R. (2016). Produção e nutrição de limoeiro 'Tahiti' em função da adubação com nitrogênio e potássio em cinco safras. *Pesquisa Agropecuária Brasileira*, *51*(4), 357–363. DOI: https://doi.org/10.1590/S0100-204X2016000400008

Silva, N., Van Raij, B., Carvalho, L. H. de, Bataglia, O. C., & Kondo, J. I. (1997). Efeitos do calcário e do gesso nas características químicas do solo e na cultura do algodão. *Bragantia*, *56*(2), 389–401. DOI: https://doi.org/10.1590/S0006-87051997000200018

Sousa, D. M. G., Miranda, L. N., & Oliveira, S. A. (2007). Acidez do solo e sua correção. In: Novais, R. F. et al. (Eds.). *Fertilidade do solo* (pp. 205–274). Viçosa, MG: Sociedade Brasileira de Ciência do Solo.

Sousa, D.M.G., Lobato, E., & Rein, T.A. (2005). *Uso de gesso agrícola nos solos do Cerrado*. Planaltina, DF: Embrapa Cerrados.

Stout, W. L., Sharpley, A. N., & Weaver, S. R. (2003). Effect of amending high phosphorus soils with flue-gas desulfurization gypsum on plant uptake and soil fractions of phosphorus. *Nutrient Cycling in Agroecosystems*, *67*, 21–29. DOI: https://doi.org/10.1023/A:1025163319889

Tofanelli, M. B. D., Santos, R. T. dos, & Kogeratski, J. F. (2018). Complexo hidrossolúvel na formação de mudas do porta-enxerto limoerio "Cravo". *Revista de Ciências Agroveterinárias*, *17*(4), 564–570. DOI: https://doi.org/10.5965/223811711732018564

Troeh, F. R., & Thompson, L. M. (2005). *Soils and Soil Fertility*. New York, USA: Blackwell.

Vicensi, M., Lopes, C., Koszalka, V., Umburanas, R. C., Vidigal, J. C. B., de Ávila, F. W., & Müller, M. M. L. (2020) Soil fertility, root and aboveground growth of black oat under gypsum and urea rates in no till. *Journal of Soil Science and Plant Nutrition*, Published online 20 March 2020. DOI: https://doi.org/10.1007/s42729-020-00211-3

Vicensi, M., Müller, M. M. L., Kawakami, J., Nascimento, R. D., Michalovicz, L., & Lopes, C. (2016). Do rates and splitting of phosphogypsum applications influence the soil and annual crops in a no-tillage system? *Revista Brasileira de Ciência do Solo*, *40*, 1–17. DOI: https://doi.org/10.1590/18069657rbcs20150155

Walker, T. W. (1961). Fertility requirements of pastures and their diagnosis. *Proceedings of the New Zealand Grassland Association*. *23*, 85–91.

Percentage contribution of each author in the manuscript

Maciel Korzune – 25%

Fabricio William de Ávila – 25%

Renato Vasconcelos Botelho – 20%

Poliana Horst Petranski– 5%

Karin Kamila Brick Lopes de Matos – 5%

Leandro Rampim – 5%

Marcelo Marques Lopes Muller – 15%