Nitrogen-fertilizer forms affect the nitrogen-use efficiency in fertigated citrus groves

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Abstract

The fertigated area of the Brazilian citrus industry has grown rapidly during recent years, and an efficient management of nitrogen (N) application at these sites is required for sustainable citrus production. Therefore, a field trial with Valencia orange trees [*Citrus sinensis* (L.) Osbeck] on Swingle citrumelo rootstock (*Citrus paradise* Macfad. x *Poncirus trifoliata* L. Raf.) was conducted for 8 years to evaluate the effects of N rates (80, 160, 240 and 320 kg ha^{-1} y^{-1}) applied by fertigation, either as ammonium nitrate (AN) or calcium nitrate (CN), on soil solution dynamics, fruit yield, nutritional status, and N-use efficiency (NUE) of trees. The maximum fruit yield was reached with 240 kg N ha^{-1} for AN, whereas a linear response and greater fruit yield was observed for N supplied as CN. The NUE was reduced for both N forms with increasing N rates. However, the NUE for CN was 14 to 38% greater than the NUE for AN. The lower fruit yield and NUE for AN compared to CN-treated trees was associated with the increased acidification of the soil solution with increased AN rates (pH $\leq$ 4.0). This limited nitrification resulted in a high ammonium (NH_4^+) concentration in the soil solution and a reduction in the net absorption of cations by the trees, particularly calcium (Ca). Due to the improved ion balance as well as the higher pH of the soil solution (pH $\geq$ 6.3) and diminished NH_4^+ availability, gains in both fruit yield and NUE in fertigated citrus groves in tropical soils can be obtained with the use of CN as a source of N.

Key words: *Citrus sinensis* / fruit yield / soil solution / calcium / ammonium / nitrate

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1 Introduction

Nitrogen (N) and calcium (Ca) are nutrients with high interdependence on citrus nutrition, largely impacting tree growth, fruit yield, and quality. The need for sound management of N fertilizer application and improved N-use efficiency (NUE) is important for the environmental and economic sustainability of citrus production. This is especially important under intensive production systems, such as fertigated groves, since the area grown with citrus has increased in the past years in Brazil and greater amounts of N have been used. Improvements in NUE in fertigated areas have been obtained with the adequate monitoring of the amounts of water applied to the trees (*Alva* et al., 2006; *Morgan* et al., 2009), since this minimizes the losses of water below the root zone and favors N absorption. In addition, *Paramasivam* et al. (2001) and *Quiñones* et al. (2003) showed that the higher frequency of N application is another approach that might contribute to a greater NUE in fertigated groves. The NUE is also affected by the N form when the fertilizer is broadcast on the soil surface in citrus groves (*Cantarella* et al., 2003; *Mattos Jr.* et al., 2003a). However, there is still a lack of detailed information about the influence of N forms on NUE for long-term fertigated areas in tropical soils.

Application of nutrients via fertigation alters the dynamics of N fertilizers applied to the soil compared to the conventional application practice in non-fertigated groves. For instance, the conversion of ammonium (NH_4^+) to nitrate (NO_3^-) in the soil does not occur to the same extent as observed in the traditional production systems, where fertilizers are broadcast to the soil surface (*Souza* et al., 2006). Thus, ammoniacal forms cause soil acidification, which in turn limits nitrification and increases NH_4^+ availability in the soil solution (*Souza* et al., 2012a). As a result, a greater absorption of NH_4^+ by the citrus trees occurs after fertigation with ammoniacal N forms (*Neilsen* et al., 1999).

Although N sources containing NH_4^+, *i.e.*, ammonium nitrate (AN), have been widely used in fertigated groves because of their favorable characteristics, such as low cost, high N concentration, and solubility, there remain doubts regarding the efficiency of AN as an N source for the fertigation of citrus. For instance, citrus trees grown in sand culture with NH_4^+ produced 20% more fruit per tree compared to NO_3^-. However, the highest fruit weight was obtained with a NO_3^- : NH_4^+ ratio of 25 : 75, and the lowest fruit weight was obtained with 100% NH_4^+ (*Serna* et al., 1992). Furthermore, deleterious effects on plant growth and metabolism as a result of excess NH_4^+ availability in the root medium have been observed (*Cramer* and *Lewis*, 1993; *Dou* et al., 1999; *Siddiqi* et al., 2002; *Britto* and *Kronzucker*, 2002), leading to the need of

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evaluating alternative sources of N for fertigated systems. In this context, calcium nitrate (CN) might be an important option because it supplies N exclusively in the form of NO_3^- in association with soluble Ca. These features of CN would contribute to maintain a higher soil pH (*Neilsen* et al., 1993) and also to increase the availability of Ca, which is required in large amounts by citrus (*Mattos Jr.* et al., 2003b).

With the exception of the fruit, which contains high concentrations of potassium (K), N and Ca are the nutrients that are the mostly absorbed and accumulated by the trees (*Mattos Jr.* et al., 2003b). According to this, we based our study on the hypothesis that both fruit yield and NUE of fertigated citrus trees would vary as a function of the regime of N and Ca supply. In order to test this hypothesis and also to add insight into the understanding of responses of fruit yield and NUE of citrus trees to N forms, a long-term field trial was performed to evaluate the effects of N rates applied either as AN or CN on soil solution dynamics and the nutritional status of citrus trees.

2 Material and methods

2.1 Plant material and field conditions

The field trial was set up in 2003 in an Ultisol with 3–4 y-old Valencia sweet orange trees [*Citrus sinensis* (L.) Osbeck] on Swingle citrumelo rootstock (*Citrus paradise* Macfad. x *Poncirus trifoliata* L. Raf.), a combination of scion and rootstock highly responsive to irrigation and fertilization practices. The experimental area was located in a commercial grove (with a population of 444 trees ha^{-1}), in the city of Reginópolis (21°88′ S and 49°14′ W), central-south part of the São Paulo State, Brazil. The local annual average temperature is 20°C, and the rainfall is 1400–1500 mm. The initial chemical characteristics of the soil within the 0–0.20 m top layer determined according to *Raij* et al. (2001) were: pH_{CaCl_2} = 5.4; P-resin = 9 mg dm^{-3}; Ca = 10 $mmol_c$ dm^{-3}, Mg = 10 $mmol_c$ dm^{-3}, and K = 1.9 $mmol_c$ dm^{-3}; and soil base saturation (V,%) of 59. Before the treatments were set up, soil was limed with dolomitic limestone to increase V up to 70%, and re-limed during the conduction of the experiment when V was lower than 50%.

The grove was irrigated with two lines of drippers per row, placed 60 cm away from the tree trunks (one line on each side). The distance between emitters was 85 cm with a flow of 3.5 L h^{-1}. Irrigation management was based on the daily evaporation measured by Class A pan, and the potential evapotranspiration (ETp) and the crop evapotranspiration (ETc) was obtained according to *Allen* et al. (1998). The experiment was also monitored by four batteries of tensiometers, each battery consisting of three tensiometers placed at the depths of 30, 60, and 90 cm.

The N and K were supplied twice a week by fertigation, from early spring (September) to late autumn (May), summing up 70 applications per season. The annual rate of K corresponded to 183 kg K ha^{-1} as potassium chloride. Phosphorus (P) was supplied during the winter by a single application of MAP (26 kg P ha^{-1} y^{-1}). The micronutrients were supplied annually with three foliar applications at optimum rates (*Quaggio* et al., 2005).

The experiment was arranged in a completely randomized block design with four replicates. Each plot consisted of one row with 16 uniform trees, but only the ten central trees were used for sampling and yield evaluation. From 2003 to 2008, the citrus trees received four N rates via fertigation (60, 120, 180 and 240 kg ha^{-1} y^{-1}) applied either as AN or CN. During that period, fruit yield increased to a maximum with AN and linearly with CN rates. Thus, in order to attain maximum fruit yield response to CN application, the N rates for both sources were adjusted to 80, 160, 240 and 320 kg ha^{-1} y^{-1} in the next growth seasons (2009 to 2011). The fruit yield (expressed in t ha^{-1}) was quantified annually in October (during the 2009 to 2011 seasons) by weighting the fruits from the ten central trees of the plot. The NUE (kg of fruit per kg N) was calculated as the ratio between fruit yield (kg fruits ha^{-1}) and rate of applied N (kg ha^{-1}).

2.2 Sap extract analyses

The sap extract was obtained from stems of new shooting branches collected every month during the growth season (September 2010 to August 2011) as described by *Cadahía* and *Lucena* (2005) and adapted by *Souza* et al. (2012b). The samples were composed of 40 stem pieces that were collected from ten trees per treatment at the half height and around four quadrants of the tree canopies during the morning of the day after the fertigation event. After detaching the leaves, stems were placed in paper bags and transported inside coolers with ice bags to the laboratory, where the stems were cleaned with moistured cheesecloths and allowed to dry. Within 24 h after field sampling, the stems were cut into 1 to 2 cm segments and placed into 250 mL plastic flasks, in which ethyl ether (analytical grade) was added until the stems were completely covered by the liquid (the ratio between stems [g] and ethyl ether [mL] was around 1 : 2.4). The flasks containing the stems and ethyl ether solution were frozen at –15°C for 15 d. Samples were then thawed, and the sap extract (formed by a mixture of xylem, phloem and cellular soluble minerals) was separated from the ethyl ether through a separator funnel. The final volume of the sap extract sample corresponded to 2 to 5 mL, depending on the sampling time during the growth season.

The pH was determined directly in sap extracts using a glass electrode. The concentrations of inorganic forms of N (NH_4^+ and NO_3^-) were determined with the Kjeldahl method, while Ca, magnesium (Mg), K and manganese (Mn) were quantified in an argon plasma spectrometer (ICP-AOS). The total N concentration in the sap extract was determined after sulfuric-acid digestion and distillation.

2.3 Leaf sampling

Six-month-old spring flush leaves from fruiting terminals (fruits with 2 to 4 cm in diameter) were annually collected (during the 2009 to 2011 growth seasons) according to the recommendations of *Quaggio* et al. (2005), and analyzed for total nutrient concentrations (*Bataglia* et al., 1983).

2.4 Soil solution analysis

The soil-solution extractor consisted of a PVC tube (12.7 mm diameter), connected at the lower end to a porous ceramic capsule (60 mm length and 19 mm diameter), which was placed at 30 cm soil depth. At the upper end, the extractor was sealed with a rubber stopper. In order to obtain the soil solution, approximately 12 h after fertigation, a suction of 80 kPa was applied to the extractors using a manual vacuum pump. Six hours after this procedure, the solution was collected with a syringe. The soil solution pH and concentrations of NH_4^+, NO_3^-, Ca, Mg, K and Mn were determined directly without digestion or filtering according to *Raij* et al. (2001).

2.5 Statistical analysis

The fruit yield, NUE, and leaf nutrient concentration data were analyzed using a factorial analysis of variance (ANOVA) for the three-way interaction among growth season (2009 to 2011) *vs* N source *vs* N rate. Because no significant ($P > 5\%$) three-way interaction was observed, a statistical analysis for the two-way interaction between N source *vs* N rate was performed using the averaged values across the three growth seasons. For the soil-solution and sap-extract data, the averaged values across the 2010–2011 growing season were obtained from monthly samplings and used to investigate the N source *vs* N rate interaction. The effect of N source was compared using the F test at $P < 5\%$, and regression analysis was used to determine the effect of the N rates. A linear correlation was also used to describe the relationships between selected variables.

3 Results

3.1 Soil solution dynamics

The results of the soil solution revealed a significant interaction between N rate *vs* N source for the pH and concentrations of NH_4^+, Ca and Mn (Table 1). The values of soil-solution pH after CN application were 58 to 130% higher than the pH determined for AN. Furthermore, the application of AN decreased the soil solution pH, whereas CN increased the pH values (Table 1). The concentration of NH_4^+ after application of 240 and 320 kg N ha^{-1} as AN was 9.6 and 5.3-fold higher, respectively, than after the application of CN. In addition, the concentration of NH_4^+ in the soil solution did not vary with the application of CN, but the use of AN linearly increased the concentration of NH_4^+ (Table 1). Regardless of the N source, the concentration of NO_3^- in the soil solution increased with the N rates (Table 1). The Ca concentrations in the soil solution were 10.2 to 23.9-fold higher with CN compared to AN, and the concentration of Ca was proportional to the rate of N when CN was applied (Table 1). Inde-

Table 1: Soil-solution parameters according to nitrogen rate and source. Averaged values across one growth season (2010–2011). ns: non-significant; *$P < 5\%$; **$P < 1\%$; L: linear regression model; Q: quadratic regression model; N source comparison: means (n = 4 or 16) followed by different lowercase letters for the same N rate or averaged values of N rates are significantly different according to the F test ($P < 5\%$).

N rates	pH	NH_4	NO_3	Ca	Mg	K	Mn
/ kg ha^{-1}		/ $mmol_c$ dm^{-3}					
	Ammonium nitrate (AN)						
80	4.0 b	0.06 a	0.8	0.15 a	0.31	1.4	0.017 a
160	3.8 b	0.07 a	2.0	0.32 b	0.36	1.9	0.023 a
240	3.0 b	0.96 a	3.0	0.35 b	0.57	2.6	0.015 a
320	3.0 b	0.95 a	3.2	0.30 b	0.30	1.7	0.009 a
Average	3.5 b	0.51 a	2.3	0.28 b	0.39 a	1.9	0.016 a
	Calcium nitrate (CN)						
80	6.3 a	0.05 a	1.2	1.53 a	0.19	1.6	0.002 b
160	6.7 a	0.10 a	1.5	3.29 a	0.28	2.1	0.002 b
240	6.9 a	0.10 b	2.4	5.43 a	0.33	1.1	0.001 b
320	6.5 a	0.18 b	2.6	7.17 a	0.29	1.3	0.005 a
Average	6.6 a	0.11 b	1.9	4.35 a	0.27 b	1.5	0.002 b
Source (S)	**	**	ns	**	*	ns	*
Rate (R)	**	**	**	**	*	ns	ns
S x R	**	**	ns	**	ns	ns	*
Model of AN	L**	L**	L**	ns	Q*	ns	L*
Model of CN	Q**	ns		L**			ns

Table 2: Values of pH and nutrient concentrations in the sap extract of citrus trees according to nitrogen rate and form. Averaged values across one growth season (2010–2011). ns: non-significant; $^{*}P < 5\%$; $^{**}P < 1\%$; L: linear regression model; Q: quadratic regression model; N source comparison: means (n = 4 or 16) followed by different lowercase letters for the same N rate or averaged values of N rates are significantly different according to the F test ($P < 5\%$).

N rates	pH	NH_4	NO_3	Total N	Ca	Mg	K	Mn
/ kg ha^{-1}		/ mmol$_c$ L^{-1}						
	Ammonium nitrate (AN)							
80	5.5	1.7	2.2	139.8	77.7	89.5 a	111.4	0.11 a
160	5.5	2.1	5.4	186.8	84.2	104.7 a	109.7	0.18 a
240	5.5	2.7	8.9	175.1	87.3	133.7 a	103.0	0.31 a
320	5.5	3.4	9.4	179.4	87.8	151.6 a	105.4	0.46 a
Average	5.5	2.5 a	6.5 b	170.3	84.3 b	119.9 a	107.3	0.26 a
	Calcium nitrate (CN)							
80	5.5	1.2	3.0	163.7	103.1	87.2 a	110.0	0.07 b
160	5.5	1.4	5.1	158.8	105.1	72.6 b	101.0	0.07 b
240	5.5	1.6	10.0	186.7	127.6	63.9 b	98.3	0.06 b
320	5.5	1.7	12.5	188.6	125.6	55.5 b	97.9	0.06 b
Average	5.5	1.4 b	7.6 a	174.5	115.3 a	69.8 b	101.8	0.07 b
Source (S)	ns	**	*	ns	**	**	ns	**
Rate (R)	ns	**	**	**	ns	ns	ns	**
S x R	ns	ns	ns	ns	ns	**	ns	**
Model of AN	ns	L**	L**	L**	L*	L**	L*	L**
Model of CN						L**		ns

pendent of the N source, the concentration of Mg in the soil solution responded positively to N fertilization and, averaged over N rates, the concentration of Mg was 31% greater for AN compared to CN (Table 1). Except for the highest N rate, the Mn concentration in the nutrient solution was 8.5 to 15-fold greater with AN compared to CN. Moreover, the concentration of Mn linearly increased with N supplied as AN, whereas no effect was observed when CN was applied (Table 1).

3.2 Nutrient concentration in the sap extract

The application of AN resulted in greater concentrations of NH_4^+ in the sap extract compared to CN, whereas opposite results were observed for the concentrations of NO_3^- and Ca (Table 2). Regardless of the N form, the concentrations of NH_4^+, NO_3^-, and total N in the sap extract were proportional to the N rate (Table 2). According to the difference between the concentrations of inorganic N (NH_4^+ plus NO_3^-) and total N, the organic N represented, on the average of N rates and N sources, 95% of the total N in the sap extract. Except for the lowest N rate, a greater Mg concentration in the sap extract was found in the AN-treated trees (Table 2). Furthermore, AN application resulted in a linear increase of Mg concentration in the sap extract, whereas the opposite was observed for CN. A linear response and higher concentrations of Mn in the sap extract were obtained when AN was used compared to CN (Table 2).

3.3 Nutrient concentrations in citrus leaves

The leaf N concentration increased with N-fertilizer rate, but except for the application of 160 kg N ha^{-1}, there was no difference between N forms (Table 3). The leaf Ca concentration was only affected by the N form, and for the average N rates, the Ca concentration was 35% higher with CN (Table 3). The N application significantly decreased the leaf K concentration, and this effect was greater with AN than CN (Table 3). Except for the application of 80 kg N ha^{-1}, the leaf Mg concentration was 43 to 127% higher with AN compared to CN (Table 3). Furthermore, the leaf Mg concentration increased with AN application, but decreased with the use of CN (Table 3). The Mn concentration in the leaves of the trees receiving AN was higher than the CN-treated trees. In addition, the application of AN linearly enhanced the leaf Mn concentration, whereas no effect was observed with CN (Table 3).

3.4 Fruit yield and nitrogen-use efficiency

Based on the pooled values of fruit yield during the growth seasons from 2009 to 2011, a significant interaction was observed between the N rate *vs* N source, suggesting that the response to N application depended on the N source (Fig. 1). The maximum fruit yield of 58.2 t ha^{-1} for AN was achieved at approximately 240 kg N ha^{-1}, whereas a linear response was observed for CN, with a maximum estimated fruit yield of 76.2 t ha^{-1}. The NUE showed also a significant interaction for

Table 3: Nutrient concentrations in the leaves of citrus trees according to nitrogen rate and form. Averaged values across three growth seasons (2009–2011). ns: non-significant; $^*P < 5\%$; $^{**}P < 1\%$; L: linear regression model; Q: quadratic regression model; N source comparison: means (n = 4 or 16) followed by different lower-case letters for the same N rate or averaged values of N rates are significantly different according to the F test ($P < 5\%$).

N rates	N	Ca	Mg	K	Mn
/ kg ha^{-1}	/ g kg^{-1}				/ mg kg^{-1}
	Ammonium nitrate (AN)				
80	24.8 a	30.5	3.5 a	18.4 a	71.3 a
160	27.0 a	31.0	4.0 a	18.0 a	141.5 a
240	28.0 a	32.3	4.7 a	15.0 a	238.5 a
320	27.7 a	33.0	5.0 a	13.8 b	330.5 a
Average	26.9	31.7 b	4.3	16.3	195.5 a
	Calcium nitrate (CN)				
80	24.2 a	41.4	3.4 a	16.9 a	42.0 b
160	25.1 b	42.7	2.8 b	16.4 a	49.4 b
240	27.6 a	43.6	2.3 b	16.1 a	38.2 b
320	28.3 a	44.0	2.2 b	15.3 a	54.1 b
Average	26.3	42.9 a	2.7	16.2	45.9 b
Source (S)	ns	*	ns	ns	*
Rate (R)	*	ns	*	*	*
S x R	*	ns	*	*	*
Model of AN	Q**	ns	L**	L**	L**
Model of CN	L**		L**	L*	ns

N rate and N source (Fig 2). Regardless of the N form, the NUE was reduced with increasing N rates, but the NUE of CN was 14 to 38% higher than AN.

The higher fruit yield was obtained when the NO_3^- : NH_4^+ ratio in the sap extract was of 8.5 : 1 (Fig. 3a). Furthermore, the fruit yield was positively correlated with the NO_3^- : Ca ratio in the sap extract (Fig. 3b), with the Ca concentration in the leaves (Fig. 3c), and with the Ca : N ratio in the leaves (Fig. 3d).

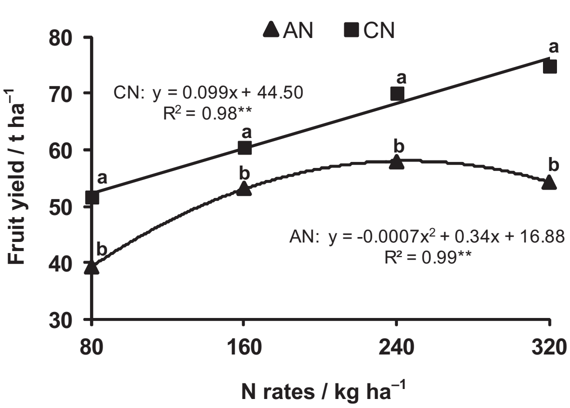

Figure 1: Citrus fruit yield as a function of nitrogen (N) rates applied as ammonium nitrate (AN) or calcium nitrate (CN). Averaged values across three growth seasons (2009–2011). Means followed by the same letter in the same N rate are not significantly different according to the F test at $^*P < 5\%$; $^{**}P < 1\%$.

4 Discussion

The results of our long-term field experiment demonstrated that the fruit-yield response of fertigated citrus trees to N application varies with N form. For instance, the maximum fruit yield was achieved with 240 kg N ha^{-1} applied as AN, whereas for CN, according to a linear model, between the fruit yield and N rate the maximum yield might be achieved with rates greater than 320 kg ha^{-1} N (Fig. 1). Moreover, for the same amount of N applied, higher yields were obtained with CN

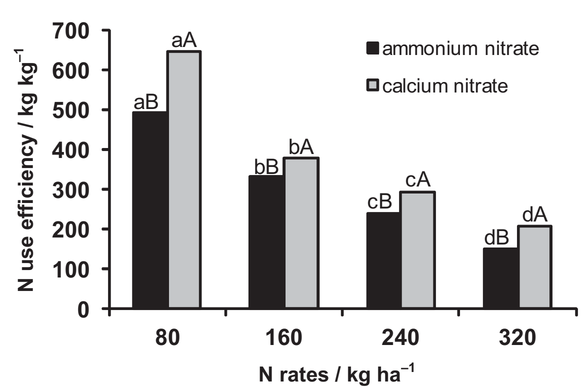

Figure 2: Nitrogen (N)-use efficiency for fruit yield as a function of N rate and source. Averaged values across three growth seasons (2009–2011). Means followed by different uppercase letter in the same N rate and means followed by different lowercase letter for the same N source are not significantly different at $P < 5\%$ according to F and Duncan test, respectively.

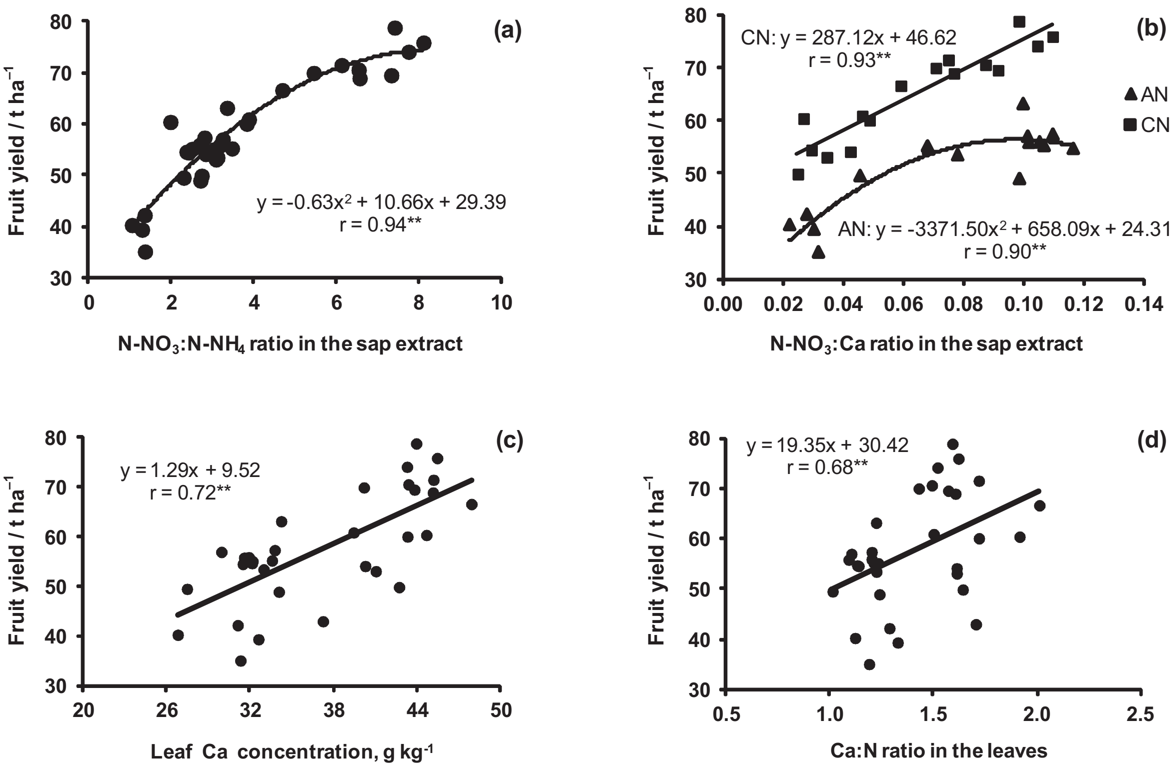

Figure 3: Relationship between fruit yield with NO_3^- : N-NH_4^+ ratio (a), NO_3^- : Ca ratio in the sap extract (b), Ca concentration in the leaves (c) and Ca : N ratio in the leaves (d). $^{**}P < 1\%$.

compared to AN. This greater fruit yield of the CN-treated trees resulted in improved NUE (Fig. 2), suggesting the opportunity of having higher yields and NUE in fertigated citrus groves with the same amount of N applied as CN instead of AN. This is an important finding and provides practical information that might contribute to the definition of novel management practices to improve nutrient use efficiencies and sustainability of fertigated areas.

The NH_4^+ concentration in the soil solution of the trees that received 240 and 320 kg N ha^{-1} as AN was higher than that found for CN-treated trees, whereas no difference was found for the NO_3^- concentration (Table 1). Based on this, the absolute NH_4^+ concentration in the soil solution was the principal factor determining the influence of N form on the responses of citrus to available N in the root medium. According to *Britto* and *Kronzucker* (2002), NH_4^+ toxicity may occur with external concentrations above 0.1 to 0.5 mmol$_c$ dm^{-3} of NH_4^+, probably due to the fact that the input of NH_4^+ may exceed the assimilation capacity of plants (*Kato*, 1986). This constraint can arise in groves that received continuous applications of NH_4^+ and that have poor conditions for nitrification, *i.e.*, in reducing environment with low pH, where the activity of nitrifying microorganisms may be impaired (*Stark* and *Hart*, 1997).

The AN-treated trees increased the extrusion of protons (H^+) into the rooting medium (*Marschner*, 1995), which resulted in pronounced acidification of the soil solution with increased AN rates (pH ≤ 4.0; Table 1). Therefore, based on these very low pH values, direct negative effects of high acidity, *i.e.*, disruption of membrane integrity because of high H^+ concentration (*Marschner*, 1995), likely occurred in roots of the AN-treated trees. Furthermore, the maintenance of very low pH values in the soil solution with the continuous use of AN in fertigated trees led to higher Mn concentrations in the soil solution due to enhanced solubility of Mn (Table 1). As a result, according to the greater Mn concentration in the sap extract and leaf tissue (Tabs. 2 and 3), there was an increased Mn absorption by the AN relative to CN-treated trees. Therefore, it is possible that the greater Mn concentration in the leaves of the AN-treated trees (Table 3) also contributed to the lower fruit yield and NUE compared to those that received the application of CN. This is based on the observation that the Mn concentration in the leaves of the CN-treated trees was in the sufficient range, whereas for the AN-treated trees the values were excessive (> 100 mg kg^{-1}; *Quaggio* et al., 2005). Similar results were observed by *Souza* et al. (2012b), who performed a two-year study with four AN rates in citrus trees with a Mn concentration in the leaves of 238 mg kg^{-1}. The very high concentration of Mn (906 mg kg^{-1}) in the leaves of citrus seedlings was related to reduced photosynthesis, leaf protein concentration and total plant growth (*Li* et al., 2010).

The variation in fruit yield and NUE with the use of AN and CN could not be explained in terms of differences in the N status of the citrus trees, because similar and sufficient N concentrations (*Quaggio* et al., 1998) were found in leaves of trees receiving either AN or CN (Table 3). Additionally, the concentration of total N in the sap extract was not different between AN and CN-treated trees (Table 2), suggesting that similar amounts of total N were available to meet the nutrient demand of the trees. However, the composition of the sap extract rather than the absolute concentration of total N appeared to be more important in affecting the fruit yield. For instance, the fruit yield was negatively correlated with the NH_4^+ concentration in the sap extract (r = –0.36; P = 4%; n = 32), whereas it was positively correlated with the NO_3^- concentration in the sap extract (r = 0.75; P < 0.01%; n = 32). In addition, the fruit yield showed a positive correlation with the NO_3^- : NH_4^+ ratio in the sap extract (Fig. 3a), with the highest fruit yield (74.5 t ha^{-1}) being obtained with an NO_3^- : NH_4^+ ratio of 8.5 : 1. Therefore, the lower NH_4^+ and higher NO_3^- concentrations in the sap extract of the CN-treated compared to the AN-treated trees (Table 2) might have contributed to the greater fruit yield and NUE of the former, supporting the idea that a high N-NH_4^+ concentration in the sap extract was harmful to citrus. In fact, the NH_4^+ concentration in the sap extract of the trees receiving 240 and 320 kg N ha^{-1} as AN were 2.7 and 3.2 $mmol_c$ dm^{-3}, respectively (Table 2), which is close to the value defined as toxic for citrus trees (2.9 $mmol_c$ dm^{-3} of NH_4^+) (*Souza* et al., 2012b).

The positive correlation between fruit yield and Ca concentration in the leaves (Fig. 3c) suggested that the fruit yield responded to improved Ca nutrition of the trees. The Ca concentration in the leaves of the AN-treated trees (Table 3) was in the deficient range (*Quaggio* et al., 2005), which may have contributed to the lower fruit yield and NUE obtained with AN (Fig. 1, 2). The Ca-deficient citrus leaves (21.0 g kg^{-1} Ca) showed lower concentrations of NO_3^-, chlorophyll, and soluble protein, as well as reduced activity of nitrate reductase and RUBPcase compared to the control treatment (52.0 g kg^{-1} Ca; *Lavon* et al., 1999), indicating compromised metabolism in Ca-deficient citrus trees. The ability of CN to increase the availability of Ca for the citrus trees was confirmed by the greater concentration of Ca in the soil solution (Table 1) and higher Ca concentration in the sap extract of CN- compared to AN-treated trees (Table 2). Therefore, the results indicate that despite adequate levels of Ca in the soil (≈ 16 $mmol_c$ dm^{-3} for both fertilizer forms), the exchangeable Ca was not able to replace and to maintain a sufficient concentration of Ca in the soil solution to meet the nutrient demand of the AN-treated trees. However, besides the lower Ca concentration in the soil solution of the AN-treated trees, there was probably a direct effect of the elevated N-NH_4^+ concentration on the diminished absorption of Ca due cation antagonism (*Serna* et al., 1992). Additionally, as indicated by the positive correlation of fruit yield with NO_3^- : Ca ratio in the sap extract (Fig. 3b) and Ca : N ratio in the leaves (Fig. 3d), the use of CN favored fruit yield through improvements in the balance between Ca and N in the citrus trees.

The K concentration was decreased with N rates for both sources, but the values of K in the leaves remained either within or above the sufficient range for citrus (*Quaggio* et al., 2005). Thus, even with high amounts of N applied in this study, the current rate of K recommended for high-yielding groves under fertigation (*Quaggio* et al., 2005) is sufficient to meet the requirement citrus. It is worthy to note that although the use of CN was beneficial for both fruit yield and NUE (except for the application of 80 kg ha^{-1}) the leaf Mg concentration was below the sufficient range (Table 3; *Quaggio* et al., 2005). On the other hand, the citrus trees that were supplied with AN had sufficient Mg concentrations in the leaves (Table 3). Magnesium was exclusively applied as dolomitic limestone. This practice might be insufficient to maintain adequate levels of Mg availability to the trees when CN is continuously used. Therefore, further studies should investigate the impact of this lower Mg concentration in the leaves of CN-treated trees on fruit yield and NUE in order to develop strategies of management for Mg fertilization that allows the maintenance of high levels of fruit yield and NUE with intensive application of CN in fertigated areas.

5 Conclusions

The results demonstrate the differences between N forms for fruit yield and NUE in fertigated citrus production. The reduced fruit yield and NUE with the use of AN as N form compared to CN cannot be associated with the N status of the citrus trees but to an imbalance of the NO_3^- : NH_4^+ ratio in the plant resulting from excessive NH_4^+ supply, which decreases the Ca absorption by citrus trees. In addition, direct toxic effects of H^+ and Mn ions might have also occurred to the AN-treated citrus trees due to the elevated acidity in the soil solution with continuous use of AN. Based on these findings, CN appeared to be a more suitable N source for the fertigation of citrus groves in tropical soils, specially due to the fact that even with the continued application of lime and the maintenance of adequate levels of exchangeable Ca in the soil, the AN-treated trees were not able to maintain leaf Ca concentration in the sufficient range. However, due to the higher cost of CN fertilizer compared to AN, and to the CN-induced Mg deficiency, further studies are required in order to add information about the economic sustainability of supplying N in fertigated citrus groves using a mix of both AN and CN.

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