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Artigo/Article

Nitrogen source and nitrification inhibitors affect soil nutrient status and Star Ruby grapefruit performance

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SUMMARY

Nitrogen (N) source is known to affect ion composition and organic acid production in plants. We determined the effects of N source and nitrification inhibitors (NI) on soil solution ionic composition and nutrient availability, grapefruit yield and quality, and accumulation of carboxylic acids in fruits as expressed by fruit acidity. The individual and combined effects of N source (fertilizers or effluent) and NI on mature grapefruit trees were studied in a commercial orchard planted on a sandy loam soil and drip-fertigated. Fertilization with ammonium sulfate (AS) resulted in soil acidification during the irrigation season from pH 7.4 to 6.1, and increased P, Mn and Ca concentrations in the soil solution relative to the ammonium nitrate (AN) treatment. Consequently, under AS treatment, P and Mn uptake, chlorophyll content in leaves and fruits, total soluble solids (TSS) in fruits, were higher than in the other treatments. Excess ammonium concentration in the soil solution inhibited Ca and K uptake without significantly reducing fruit acidity. The positive correlation found between Ca in leaf and yield was unexpected since the soils are calcareous. The presence of dissolved organic N in the effluent treatment diminished the impact of ammonium on soil pH. Application of NI had a negligible effect on fruit yield and quality but enhanced the capacity of AS to raise N uptake above that achieved with AN or effluent. Consequently, it reduced the potential for N leaching from the soil.

Index terms: ammonium, nitrate, dicyandiamide, 3,4-dimethylpyrazole phosphate, chlorophyll, effluent, nitrate pollution.

Fontes de nitrogênio e inibidores de nitrificação alteram o estado nutricional do solo e a produção de pomelo Star Ruby

RESUMO

As fontes de nitrogênio (N) afetam a composição iónica e a produção de ácido orgânico pelas plantas. Neste trabalho foram determinados os efeitos de fontes de N e inibidores de nitrificação (NI) sobre a composição iónica da solução do solo e disponibilidade de nutrientes, rendimento e qualidade de pomelo e acumulação de ácidos carboxílicos em frutos, conforme expressado pela acidez da fruta. Os efeitos individuais e combinados da fonte de N (fertilizantes ou efluentes) e NI em árvores adultas de pomelo foram avaliados em um pomar comercial, plantado em um solo franco-arenoso e fertirrigado por gotejamento. A fertilização com sulfato de amônio (AS) resultou

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em acidificação do solo durante ao período de irrigação de pH 7,4 para 6,1 e aumentou as concentrações de P, Mn e Ca na solução do solo em relação ao tratamento com nitrato de amônio (AN). Consequentemente, no tratamento de AS, a absorção de P e Mn, teor de clorofila em folhas e frutos, sólidos solúveis totais (TSS) em frutas, foram maiores do que nos outros tratamentos. O excesso de amônio na solução do solo inibiu a absorção de Ca e K, porém sem reduzir significativamente a acidez da fruta. A correlação positiva encontrada entre o Ca na folha e a produtividade foi inesperada uma vez que os solos são calcários. A presença de N orgânico dissolvido no tratamento de efluentes diminuiu o impacto do amônio no pH do solo. A aplicação da NI teve um efeito insignificante sobre a produtividade e qualidade dos frutos, mas aumentou a capacidade de AS para aumentar a absorção de N acima do alcançado com AN ou efluente. Consequentemente, reduziu o potencial de lixiviação de N a no perfil do solo.

Termos de indexação: amônio, nitrato, dicianodiamida, fosfato 3,4-dimetilpirazol, clorofila, efluente, poluição por nitrato.

INTRODUCTION

During the last decade, the internal quality of citrus fruit, especially the total sugar and acid contents and the acid-to-sugar ratio, has become a critical value-determining parameter. To achieve high fruit quality, the fruit should contain low acid (0.8-1.2%) and sufficiently high sugar (12-15%) (Davis & Albrigo, 1994). Climate is a dominant factor in controlling sugar and acid contents in fruit (Reuther, 1973) and under certain climatic conditions, some varieties, e.g. grapefruit and Minneola tangelo, contain excess acid and do not meet the acid concentration requirements (Wilson & Ubrezaa, 1988).

The role of plant nutrition in citrus fruit acidity has long been known. For example, K has been shown to strongly stimulate carboxylic acid accumulation in fruits (Embleton et al., 1973; Erner et al., 1993, 2004a). This is because the high rate of K uptake increases the cation-to-anion ratio in the plant tissue and carboxylates are synthesized to maintain electroneutrality (Marschner, 1995). Nitrate uptake also stimulates carboxylation in plant tissue, but in this case it is to remove hydroxyls produced in the nitrate-reduction process (Marschner, 1995).

The ammonium-to-nitrate ratio is an important N-management tool, particularly under fertigation (Bar-Yosef, 1999). For a given N dose and irrigation regime, the NH_4:NO_3 ratio affects nitrate leaching (Feigin et al., 1981, 1982a, 1982b), N-uptake efficiency (Marschner, 1995), Ca, Mg, and K uptake (Bar-Tal et al., 2001a, 2001b; Kirkby & Mengel, 1967; Neilsen et al., 1995), carboxylic acid biosynthesis (Kirkby, 1968; Kirkby & Mengel, 1967; Mengel & Kirkby, 2001), and soil pH (Imas et al., 1997a, 1997b; Neilsen et al., 1995). Soil pH is also affected by nitrification, which acidifies the entire soil volume, and by H^+ excretion from roots, which decreases the pH in the rhizosphere only. For alkaline soils, acidification is beneficial as it dissolves P, Ca, Fe, Zn, and Mn (Lindsay, 1979) thus enhancing their availability to plants; however, excess NH_4, particularly at root temperatures above 28 °C, impairs root development (Ganmore-Neumann & Kafkafi, 1980a, 1980b, 1983). Low NH_4:NO_3 ratios in the soil increase nitrate uptake and thus carboxylic acid biosynthesis, whereas high ratios increase NH_4 absorption at the expense of NO_3absorption, thus reducing organic acid synthesis in the plant and stimulating H^+ excretion by the roots (Imas et al., 1997a, 1997b; Kirkby & Mengel, 1967).

Nitrification inhibitors (NI) are intended to prolong the beneficial effects of a high NH_4:NO_3 ratio in the soil. Dicyandiamide (DCD) is not adsorbed by soil and it is expected to operate throughout the wetted soil volume (Bock et al., 1981), whereas 3,4-dimethylpyrazole phosphate (DMPP) has been reported to disperse in the soil in a manner similar to NH_4 (Azam et al., 2001; Zerulla et al., 2001). As a result of the narrow margin between beneficial and deleterious effects of increasing NH_4:NO_3 ratio, NI should be used carefully, with due attention to the specific crop, soil, and fertigation conditions.

Reclaimed municipal wastewater (effluent) is sometimes the only viable source of water for agriculture in arid zones. Typical secondary effluents contain N-NH_4 at about 20 to 30 mg L^{-1}, and dissolved organic N (DON) at about 5 to 15 mg L^{-1} (Feigin et al., 1991). The mineralization rate of DON and how it affects soil pH are not well known. The working hypothesis for this study was that fruit quality and yield would improve if N were supplied as NH_4^+ instead of as NH_4 + NO_3 or NH_4 + DON in alkaline soils. This hypothesis was based on two premises: (i) increasing the NH_4:NO_3 ratio decreases soil pH (Marschner, 1995; Mengel & Kirkby, 2001), thus increasing the plant's Fe and Mn contents, and leaf and peel chlorophyll concentrations; (ii) enhanced NH_4^+ uptake reduces carboxylic acid contents in leaves and possibly in fruit (Serna et al., 1996), thereby decreasing fruit acidity.

The objectives of this study were to determine the effects of N source, fertilizers or effluent, and NI on soil solution ionic composition and nutrient availability, fruit yield and quality, and accumulation of carboxylic acids in fruits as expressed by fruit acidity

MATERIALS AND METHODS

Experimental layout

A field experiment was conducted for 3 successive years in the center of Israel on Star Ruby grapefruit (*Citrus paradisi* Macfad.) grafted on Sour orange (*C. aurantium* L.) rootstock planted on sandy loam soil. The trees were 20 years old, spaced at 6 × 4 m and irrigated with drip irrigation, one lateral per tree row, 0.5 m between adjacent emitters and a discharge rate of 3.8 L h^{-1}. Four trees in a row served as the plot boundary and the two at the center of the plot were sampled. The treatments are summarized in Table 1. Fertigation with ammonium nitrate (AN), ammonium sulfate (AS) and effluent water (Eff) (Table 2) was computer-controlled and applied according to a weekly irrigation schedule: three times per day on Sunday, Monday, Wednesday, Thursday and Friday, and 2 days—Tuesday and Saturday—without irrigation. The total application from mid-April to mid-November was

Table 1. Summary of treatments during 3 years

Treatment[z]	Source			Total N (mg L^{-1})
	Water	N	NI[z]	
AN[y]	Fresh	60% NO_3: 40% NH_4	-	30
Eff[x]	Effluents	77% NH_4-N: 19% DON[u]	-	46
Eff+NI[w]	Effluents	77% NH_4-N: 19% DON	+	46
AS[v]	Fresh	100% $(NH_4)_2SO_4$	-	55
AS+NI	Fresh	100% $(NH_4)_2SO_4$	+	55

[z]Treatment initiated in 2nd year (all others in Mar. 1st year); [y]Ammonium nitrate; [x]Effluent water; [w]Nitrification inhibitors (DCD in first 2 years; DMPP in the 3rd year); [v]Ammonium sulfate; [u]Dissolved Organic Nitrogen.

Table 2. Ionic composition of the irrigation solutions for three years

Water source	AN[z]	Eff[y]	AS[x]
pH	7.5	8.0	5.8
		dS m^{-1}	
EC	1.2	1.7	1.2
		mg L^{-1}	
TOC[w]	2.5	70.0	2.5
NO_3-N	20.0[v]	1.5	5.0
NH_4-N	15.0	30.0[u]	55.0
P	7.0	7.0	12.0
K	20.0	32.0	50.0
Mg	25.0	25.0	25.0
Na	60.0	160.0	60.0
Ca	60.0	81.0	60.0
SO_4	33.6	58.0	202.0
Cl	100.0	225.0	145.0
HCO_3	234.0	482.0	210.0

[z]Ammonium nitrate; [y]Effluent water; [x]Ammonium sulfate; [w]Total organic carbon; [v]Including NO_3-N from fresh water at 5 mg L^{-1}; [u]Including organic nitrogen at 16 mg L^{-1}.

about 580 mm. The N inhibitors (NI) were DCD at 10% of the N rate during the first 2 years, and DMPP at 1% of the N rate during 3rd year. Each treatment was replicated five times in a complete randomized block design.

Soil data

Soil samples were taken from the center of each treatment, along a line between two trees perpendicular to the irrigation line, at distances of 15, 30 and 45 cm from the emitter, at depths of 20, 40, and 60 cm. The samples were transferred in a cool box to a forced-air oven at 40 °C and dried for 48 h to minimize NH_4 nitrification. Soil water extracts were obtained by shaking 40 g of soil with 20 mL of double-distilled water for 2 h in a plastic centrifuge tube, centrifuging at 12,500*g* for 20 min, and vacuum-filtering to obtain the maximum solution volume. Solution electrical conductivity (EC) and pH were determined immediately, and then two drops of concentrated HCl were added to each vial to prevent precipitation. The solution concentrations of nitrate, ammonium, and sulfate were determined with a Lachate Autoanalyzer (quickchm 8000). Chloride was measured with a PCL M3 chloride meter (Jenway, Essex, England) and other elements, except K and Na, were analyzed by ICP (Inductively Coupled Plasma Spectrometer, Spectro, ICP-AES, Kleve Germany). Potassium and Na were determined by flame photometry. The P analysis by ICP included inorganic P and dissolved organic P.

Leaf analysis

Forty leaves were sampled from fruiting branches, transferred to the laboratory in a cool box, washed, and dried in a forced-ventilation oven at 68 ± 1.5 °C (Bar-Akiva & Gotfried, 1972). The dried leaves were ground to pass a 40-mesh sieve, and were stored at room temperature. Cations, P and S were analyzed by ICP after digestion with HNO_3. For N analysis, the tissue was digested with H_2SO_4 plus a few drops of H_2O_2 and analyzed with Nessler reagent (Dittmer & Michael, 1969). Chlorophyll was measured with N, N-dimethylformamide (DMF) (Moran, 1982).

Fruit analysis

Fruits were sampled for yield and fruit quality. All of the fruits from two trees were weighed, and their size distribution was calculated on the basis of 100 fruits per tree. Fruit juice acidity was determined by titration with 0.1 N NaOH; total soluble sugar (TSS) content was measured with a model PR-1 refractometer (Atago, Japan), and the flavedo chlorophyll content with DMF (Moran, 1982).

Statistical analysis

ANOVA, Tukey's highest significant difference (HSD) was applied by means of the appropriate JMP 8.1 software (SAS Inst., Cary, NC).

RESULTS

pH in the irrigated soil volume

The mean pH of the soil volume at the end of the irrigation season (3rd year) was significantly lower in the AS vs. AN treatment (Table 3), but still higher than the pH in irrigation solution (Table 2). The pH in the Eff and Eff + NI treatments was similar to that in the AN treatment throughout the experiment (Figure 1). These pH values are probably the result of the higher NH_4 concentration in the AS vs. Eff treatment solution (N at 55 and 30 mg L^{-1}, respectively) and of the pH buffering capacity of the Eff composition. The differences in 1st and 2nd years were even larger (Figure 1). In all years, the soil pH increased during the winter months, especially in treatments AS and AS + NI. The increase commenced early in September, when fertigation was reduced in response to reduced evapotranspiration, and continued until March (Figure 1).

From March to September, the soil pH decreased due to the ammonium supply. The spatial distribution of pH in the sampled soil volume was quite uniform but in treatment AN, the pH near the emitters was lower than that further away (data not presented), possibly because of the slower movement of NH_4 than NO_3 in soil. The time-dependence of soil pH (Figure 1) revealed differences between the impacts of DCD and DMPP on soil acidification. During the first 2 years, DCD increased the mean soil pH in treatment AS by 0.5 to 1.0, whereas in 3rd year, under DMPP, the pH difference ranged from 0 to 0.4. In the Eff treatment, DCD had a negligible effect on pH over time whereas DMPP reduced it by 0.5 (Figure 1).

Table 3. Concentration of minerals in soil water extract[z] at the end of the experiment, as affected by treatments (Oct. 3rd year)

Treatment	Element (mg L^{-1})							
	pH	N-NO	$N-NH_4$	P	Ca	Mn	Na	SO_4-S
AN[y]	7.10a	17.5c	8.6a	1.67c	65.5b	0.09b	96.5b	66.8ab
Eff[x]	7.26ab	67.7a	2.5c	5.14b	128.9ab	0.25b	155.8a	27.9b
Eff+NI[w]	7.37a	45.8b	3.8c	7.17a	110.1b	0.38b	130.8ab	24.2b
AS[v]	6.12d	38.4b	6.7b	6.39ab	186.9a	2.90a	75.7b	115.7a
AS+NI	6.47c	39.7b	9.1a	5.23b	176.6a	1.56ab	64.9b	99.2a
F	1.01	7.6	22	8.9	5.1	5.5	13.2	7.4
Sign.	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

[z]Each result is the mean of nine soil samples (3 depths x 3 distances); [y]Ammonium nitrate; [x]Effluent water; [w]Nitrification inhibitors (DCD in first 2 years; DMPP in the 3rd year); [v]Ammonium sulfate. Within columns, means followed by different letters differ significantly at P=0.05.

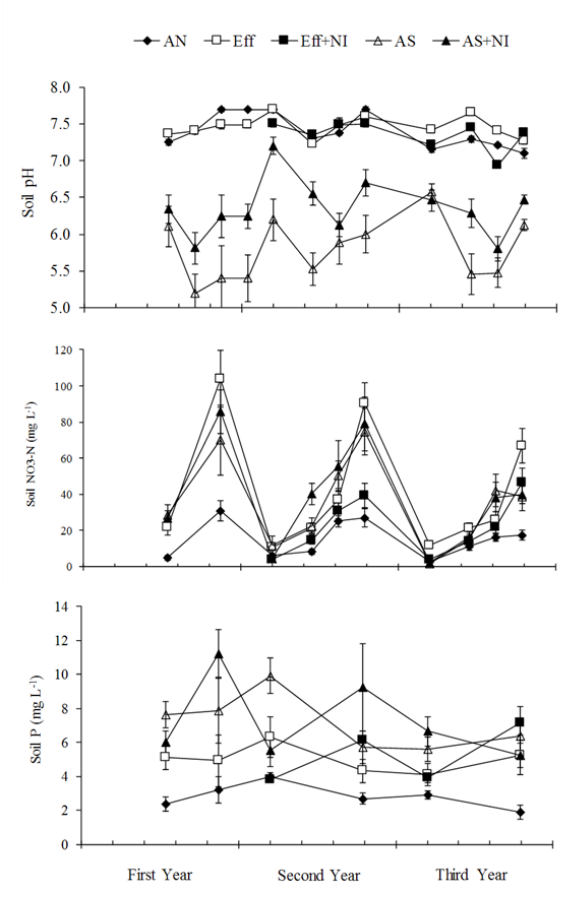

Figure 1. Mean pH, NO_3-N and P of soil water extracts in the irrigated soil volume as a function of treatment and time during 3 years of experiment. Mean values were based on six sampled points in the wetted soil volume (lateral distances of 15, 30, and 45 cm, and depths of 20, 40, and 60 cm). Bars indicate SE of each treatment on a given sampling date.

Nutrients concentrations in the irrigated soil volume

NI increased the soil NH_4 concentration, although, non-significantly in treatment Eff (Table 3). In the fall, when the N supply decreased, the nitrate concentration in each of the nine sampled soil sub-volumes was higher in treatment Eff than in treatment AS (data not shown). This result highlights the contribution of accumulated DON in the soil to the late-season nitrate concentration. The mean temporal nitrate concentration in the soil volume was significantly lower at any given time point in treatment AN than in treatments AS and Eff (Figure 1), stemming from the lower N application rate in the former treatment. NI treatment with AS had a negligible effect on the mean nitrate concentration in the soil volume over time (Figure 1), but in treatment Eff, it significantly reduced the mean nitrate concentration in Oct. of both 2 first years.

The soil volume's mean P concentration in the soil-water extract in Oct. of 3rd year, increased from 1.7 to ~6.4 mg L^{-1} as the mean soil pH decreased from 7.1 (AN) to 6.1 (AS) (Table 3). This difference persisted or even increased throughout the entire growth period (Figure 1), however in the last year the concentration in treatment Eff was similar to that in treatment AS, despite the higher pH in the former (Table 3). This stemmed from the fact that the water in treatment Eff contained dissolved organic P (Table 1) and the solution analysis by ICP included both organic and inorganic P. The mean Ca, Mn, and K concentrations in the soil volume with time were greatest in treatment AS and least in treatment AN (Figure 2).

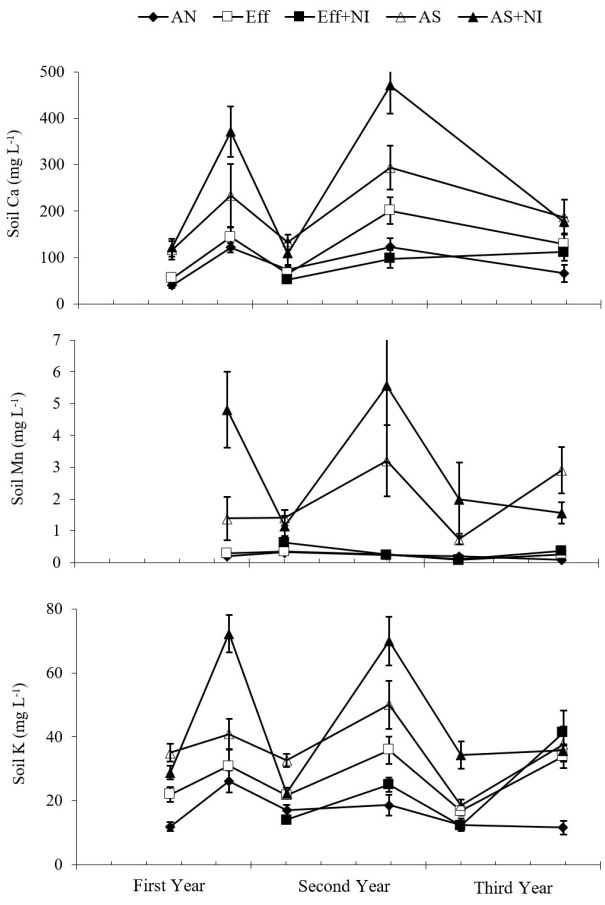

Figure 2. Mean Ca, Mn, and K contents of soil water extracts in the irrigated soil volume as a function of treatment and time during 3 years of experiment. Mean values were based on six sampled points in the wetted soil volume (lateral distances of 15, 30, and 45 cm, and depths of 20, 40, and 60 cm). Bars indicate SE of each treatment on a given sampling date.

The Ca and Mn levels were controlled by the soil pH (Table 3) and their values fluctuated with time like the soil pH (Figures 1 and 2).

The NH_4-K exchange reaction explains the fact that the water-extractable K concentration was higher in the presence of NI than in its absence (Figure 2). The concentrations of Ca in soil-water extracts were greatest at a lateral distance of 30 cm from the emitter in all treatments while that of P at 15 cm and decreasing at 30 and 45 cm (data not shown), whereas the K concentration was uniform laterally but decreased with increasing soil depth (data not shown).

In treatment AS, the impacts of NI on Ca, Mn (Figure 2) and P (Figure 1) concentrations in soil-water extracts exceeded their impact on the apparent soil pH. Most affected was Mn: in the summers of the first 2 years (Figure 2), it increased from 1.5 and 3.0 mg L^{-1}, respectively, in the absence of NI, to 4.9 and 5.5 mg L^{-1}, respectively, even though the pH in the presence of NI was higher (~7.5 vs. ~6.5, Figure 1).

The mean soil-volume Na concentration in the Eff treatments (with and without NI) was significantly higher than in the other treatments, whereas the highest SO_4 concentration was obtained in the AS treatments (with and without NI) (Table 3), due to the differences in Na and SO_4 concentrations in the sources (Table 2).

Element status in leaves

The elevated P and Mn concentrations in the soil solution in treatment AS (Figures 1 and 2) induced an average increase of 25% in diagnostic leaf P and Mn concentrations compared with those in treatments Eff and AN, but the elevated Ca and K concentrations in the solution had no clear effect on their concentration in leaves (Figures 3 and 4). The elevated soil NH_4 concentration increased the leaf N concentration, similar to the behavior of P (Figure 4).

The temporal Mg and Zn concentrations in diagnostic leaves were unaffected by treatment and they showed only a negative trend as the soil NH_4 concentration increased (data not shown). For example, in the summer of 3rd year, the leaf concentration of Mg ranged from 2.0 to 3.2 mg Kg^{-1} in treatment AS, compared with 3.5 to 5.5 mg Kg^{-1} in the other treatments. During most of the growth period, leaf Fe concentration was significantly higher in treatment AS than in the other treatments, but the absolute differences were too small to be of practical importance (Mg, Zn, and Fe data are not shown).

From mid-June through August of each year, the elemental concentrations in the leaves fluctuated considerably with time in all treatments (Figures 3 and 4). The fluctuations occurred in phase with the temporal variations in soil pH (Figure 1), and we therefore tested the correlations between leaf N, P, and Mn concentrations, on the one hand, and their concentrations and pH in the soil solution, on the other, with date as a variable (Table 4). The leaf concentrations of all three tested nutrients were significantly affected by solution pH and date. The highest correlation was found between leaf P concentration and soil-solution P concentration and pH. Although high correlations

Table 4. Correlations between leaf mineral contents and mineral concentrations and pH in the soil water extract[y]

Treatment	Leaf minerals					
	N		P		Mn	
R^2	0.715		0.893		0.713	
	F Ratio	Significance	F Ratio	Significance	F Ratio	Significance
Soil element[z]	1.83	0.1897	3.5643	0.0736	3.45	0.0804
Soil pH	4.83	0.0387	17.0621	0.0005	7.00	0.0170
Date	6.79	0.0006	15.7124	<.0001	4.13	0.0162
Model	7.88	<.0001	23.93	<.0001	7.05	0.0007

[z]Soil elements include total soluble inorganic N, water-extractable soil P, and Mn; [y]concentrations are means of nine soil samples, 3 depths x 3 distances.

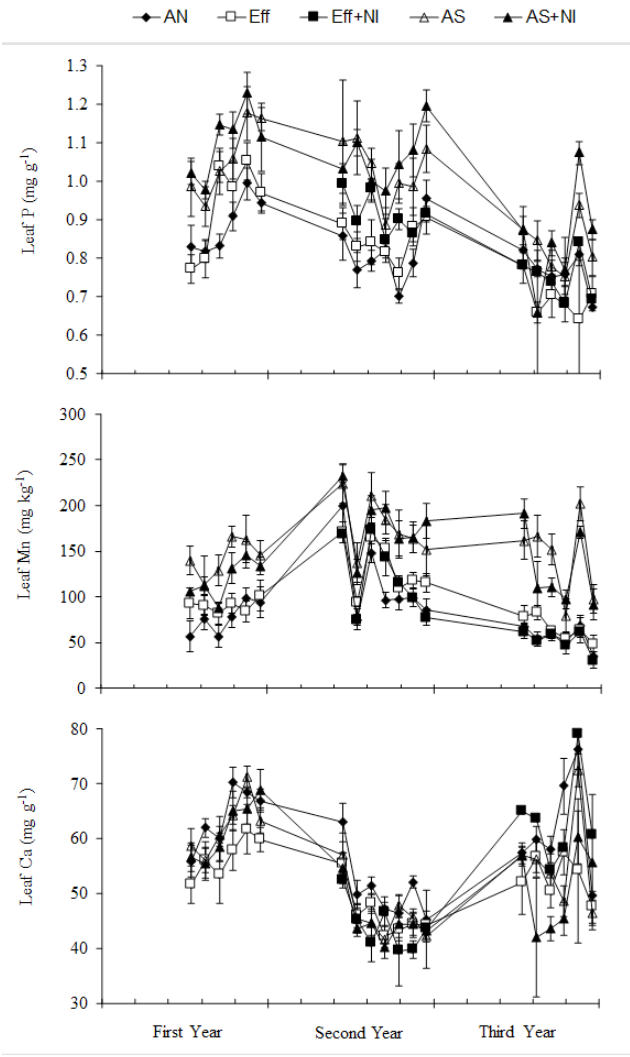

Figure 3. P, Mn, and Ca concentrations in diagnostic grapefruit leaves as a function of time and treatment during 3 years of experiment. The Duncan's mean LSD values ($\alpha = 0.05$) for P, Mn, and Ca are 0.16 ± 0.03, 37.3 ± 3.8 and 11.0 ± 0.6, respectively (mean ± SE of the mean LSD of all presented monthly samplings).

were also obtained between leaf N concentration and soil-solution N concentration and pH, the effect of the soil-solution N concentration was below the significance level of $P = 0.15$.

Chlorophyll and fruit quality

The chlorophyll concentrations in diagnostic leaves from the various treatments decreased in the order AS >AN = Eff (mean of three sampling dates, Table 5). The average leaf chlorophyll concentration over treatments increased with time. In treatment AS, the concentration increased slightly with time whereas in treatment AN, it declined (data not shown). The presence of NI elevated leaf chlorophyll concentrations in July, but in September and December the difference diminished or even reversed (data not shown). The treatment effects on flavedo chlorophyll concentrations were similar to their impacts on the leaves, with the concentrations being 10 times lower at maturity (December) than at the earlier measurements (Table 5). Color break was delayed in the flavedo by ammonium treatments of AS and AS + NI treatments, at the beginning of November, when they contained 56% more chlorophyll than the AN treatment (data not shown). The leaf chlorophyll concentrations in the first 2 years are not presented as they were very similar to those reported above, except that treatment effects were less significant.

TSS was found to be significantly lower in treatment AN than Eff (Table 5). Nitrification inhibition had no effect on fruit TSS. The treatment effects on TSS in the first 2 years were exactly the same as in the 3rd year, and are therefore not presented.

Fruit acidity in the 3rd year was unaffected by treatments (Table 5). However, in Dec. of the 2nd year it was lowest in treatment AN and highest in treatment Eff + NI; treatments AS + NI and AS had no consistent effect on fruit acidity (data not shown).

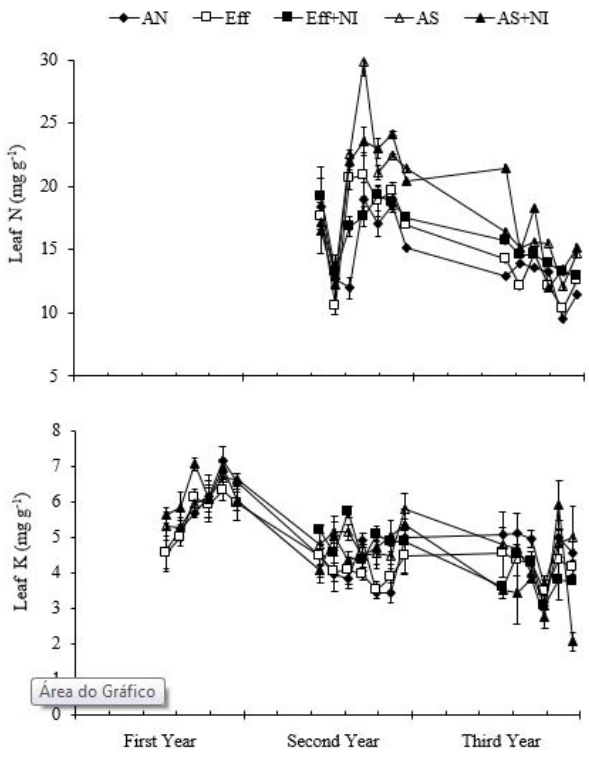

Figure 4. N and K concentrations in diagnostic grapefruit leaves as a function of time and treatment during 3 years of experiment. The Duncan's mean LSD values ($\alpha = 0.05$) for N and K are 2.5 ± 0.6 and 1.3 ± 0.3, respectively (mean ± SE of the mean LSD of all presented monthly samplings).

Fruit size and yield

The individual fruit weight (FW) in the 2nd year was not significantly affected by N or water source, whereas in the 3rd year it was highest in the Eff treatment and lowest in AS and AS + NI treatments, while AN and Eff + NI treatments were not significantly different from the other treatments (Table 6).

In the 2nd year, the highest and lowest fruit yields were obtained in AN and Eff + NI treatments, respectively (Table 6), whereas NI tended to reduce yield. In the 3rd year, the yield response to treatments was similar to that in the second but treatments differed insignificantly (Table 6). A stepwise regression (1) between the yield (Y), in kilograms per tree and leaf element percentages (Q) or leaf chlorophyll concentration at the various sampling dates showed that in the 2nd year the only significant correlation ($P \leq 0.15$) was that with leaf Ca concentration in November (data not shown):

$$Y = 47.94\, Q_{Ca\,Nov} - 45.15 \quad R^2 = 0.95 \qquad (1)$$

The positive correlation found between Ca in leaf and yield was unexpected since the soils are calcareous.

Table 5. Chlorophyll contents in leaves and flavedo, total soluble solids (TSS) and acidity in juice during the 3rd year

Variable	Leaf chlorophyll	Flavedo chlorophyll	Juice acidity	Juice TSS
Treatment	(mg g^{-1} F wt)		%	
AN[z]	1.81 b	0.087 b	1.99	10.63 b
Eff[y]	1.76 b	0.119ab	2.10	12.32a
Eff+IN[x]	1.92ab	0.119ab	2.06	11.62ab
AS[w]	2.12a	0.128a	2.07	11.65ab
AS+NI	2.23a	0.143a	2.06	11.12ab
Date				
July	1.79b	0.224a	2.27a	9.12b
Sept.	1.96b	0.113b	2.05b	12.52a
Dec.	2.16a	0.021c	1.86c	12.77a
Mean	1.97	0.119	2.06	11.49
F sig.				
Model	<0.0001	<0.0001	<0.0001	<0.0001
Treatment	0.0002	0.0042	0.2768	0.0112
Date	0.0003	<0.0001	<0.0001	<0.0001

[z]Ammonium nitrate; [y]Effluent water; [x]Nitrification inhibitors (DCD in first 2 years; DMPP in the 3rd year); [w]Ammonium sulfate. Within columns, means followed by different letters differ significantly at P=0.05.

Table 6. Fruit yield and size in 2nd and 3rd years

Treatment	2nd Year		3rd Year	
	kg tree^{-1}	g fruit^{-1}	kg tree^{-1}	g fruit^{-1}
AN[z]	201.4a	291.2	259.4	306.2ab
Eff[y]	175.4ab	302.6	209.0	337.4a
Eff+NI[x]	140.0 b	335.9	210.5	275.7ab
AS[w]	173.9ab	329.0	232.6	274.7 b
AS+NI	167.5ab	271.7	221.3	262.3 b
ANOVA				
Mean	171.6	306.1	226.5	291.3
F	5.77	0.40	2.52	4.35
Pr>F	0.0029	0.81	0.073	0.0108
LSD	32.8	130.2	44.5	45.3

[z]Ammonium nitrate; [y]Effluent water; [x]Nitrification inhibitors (DCD in first 2 years; DMPP in the 3rd year); [w]Ammonium sulfate. Within columns, means followed by different letters differ significantly at P=0.05.

DISCUSSION

The hypothesis that increasing the NH_4:NO_3 ratio would decrease soil pH (Marschner, 1995; Mengel & Kirkby, 2001), thus increasing the plant's Fe and Mn contents was fully operative in the field experiment, except that leaf Fe content was less affected by the treatments than expected (see Figure 1 for pH, Figure 3 for Mn and Table 5 for chlorophyll and fruit TSS; (Fe data not presented). The impact on Fe content was underestimated because microelements were added via the water as EDTA chelates, and the stability constant of Fe-EDTA is sufficiently high to prevent Fe precipitation even at the highest soil pH. The Mn-EDTA stability constant is several orders of magnitude lower than that of Fe-EDTA (Lindsay, 1979), and therefore the soil-solution Mn concentration depended on soil pH. Reduction of the soil pH led to enhanced leaf P content (Figure 3), but this enhancement did not affect yield. When part of the NH_4 fertilizer was replaced with DON (treatment Eff), the soil pH increased (Figure 1): this is because DON mineralization consumes soil protons. Additional possible factor is the high carbonate concentration in Eff relative to fresh water that buffer pH change (Bernstein et al., 2006). Several studies have reported that irrigation with waste water results in a slight increase in soil pH (Qian & Mecham, 2005; Schipper et al., 1996). Consequently, leaf P and Mn contents decreased (Figure 3).

Addition of NI to the AS treatment raised the soil NH_4 concentration (Table 3) and increased the soil-solution (Figure 1) and leaf P and Mn (Figure 3) concentrations. Notably, this happened despite the fact that the soil pH increased or did not change in the presence of NI (Figure 1). The unexpected response to pH is explained as follows. In the presence of NI, pH can only decrease at the root surface due to NH_4-uptake-mediated proton release by the roots. In the absence of NI, the pH can decrease both in the bulk soil due to nitrification and at the root surface. When soil cores were sampled and extracted with water, the different locations of H^+ accumulation were indistinguishable, although it is plausible to assume that the pH at the root surface in the presence of NH_4 + NI was lower than that in the extract solution and this caused Mn and P dissolution at the soil-root interface.

The time-varying soil NO_3 concentration was always significantly lower in treatment AN than in treatments AS, AS + NI and Eff (Figure 1), although one would expect to find less nitrate in the soil after fertigation with AS + NI than after fertigation with AN. This result could stem from a greater N uptake-to-supply ratio in treatment AN, which received a lower daily dose of N than the other treatments (Table 1). Moreover, we assume that the inhibitors of ammonium nitrification did not fully block the transformation to nitrate. Citrus absorbs ammonium faster than nitrate (Kato, 1986) and will do so as long as there is ammonium in solution; in the present study, in treatment AN, the ammonium supply did not satisfy the crop N demand, and therefore nitrate was absorbed by the roots, whereas in treatment AS, the continuous application of ammonium alone sufficed for growth, and nitrate uptake was minimal. Therefore, nitrate accumulation under treatment AS + NI exceeded that under treatment AN.

Chlorophyll concentrations in the leaves and flavedo are known to be closely correlated with N, Mn and Mg concentrations (Marschner, 1995). The leaves can maintain their chlorophyll concentration over a relatively long period of growth, whereas the fruits turn from green to yellow, or even to red. The changes in color occur when the temperature drops and root activity declines. The higher flavedo chlorophyll concentration in treatment AS (Table 5) required a longer period of chlorophyll breakdown; therefore, early-harvesting varieties should be fertilized with AN to enhance color break and to eliminate the need to apply N toward harvest.

Another consistent finding in the present study was that in all treatments, soil pH increased during the winter months when there was no fertigation (Figure 1). It is possible that the source of OH^- was winter uptake by the trees of nitrate generated by mineralization of soil organic N (Marschner, 1995; Mengel & Kirkby, 2001). Soil leaching by winter rain also increases the preferential adsorption of H^+ at the expense of mono- and divalent cations, which also elevates soil pH (Bolt, 1982). It is also possible that during the rainy season, $CaCO_3$ continues to dissolve, thereby raising the pH of the soil solution.

The hypothesis that NH_4^+ uptake would reduce carboxylic acid contents in leaves and possibly in fruit (Serna et al., 1996), thereby decreasing fruit acidity, could not be confirmed under the present experimental conditions. There is a direct correlation between leaf K concentration and fruit acidity (Embleton et al., 1973; Erner et al., 2004a). In the 3rd year, the supply of NH_4 alone led to lower temporal leaf K content (in agreement with Neilsen et al., 1995) than was obtained with supplies of NH_4 + NO_3 (AN) or NH_4 + DON, but in the first 2 years, this effect was non-significant. This was a result of the higher soil NH_4 concentration in treatment AS than in treatment Eff (N at 6 to 13 mg L^{-1} and 3 to 8 mg L^{-1}, respectively) and of the competition between NH_4 and K uptake (Marschner, 1995; Mengel & Kirkby, 2001). Furthermore, in the third year, the resulting differences in leaf K status were too small to induce the expected decrease in fruit acidity. Another explanation is that carboxylic acid was synthesized in the fruit itself (Sadka et al., 2000), and K and NO_3 concentrations in the fruits (not measured) were not directly related to those in the leaves.

The reduction in leaf K concentration by NH_4 was correlated with the production of smaller fruits (Erner et al., 1993, 2004a), which could be one of the deleterious effects of high NH_4 concentration on yield. In addition, there is antagonism between K and Mg (Embleton et al., 1973; Erner et al., 2004b), so the small reduction in leaf Mg concentration (data not shown) could have resulted from the combined effect of NH_4 and K. The linear correlated between yield and leaf Ca concentration, particularly in the fall was unexpected (Quaggio et al., 2014). The supply of NH_4 alone resulted in a ~0.5 to 0.7% lower leaf Ca concentration than occurred with the supply of NH_4 + NO_3 (treatment AN, Figure 3), and this too could have reduced the yield. Apparently, the inhibition of K and Ca uptake by NH_4 outweighed the beneficial effects of NH_4 in decreasing the soil pH and increasing the soil-solution Ca, Mn and P concentrations. In all treatments the concentrations of elements in the leaves of fluctuated considerably with time (Figures 3, 4), and these fluctuations occurred in phase with time variations in soil pH (Figure 1). They probably resulted from periodic variations in root activity, elicited by competition for photosynthates, between canopy, fruits and roots (Bevington & Castle, 1985).

There is usually a good correlation between leaf N concentration and yield (Embleton et al., 1973). Despite the higher N, P and Mn concentrations in leaves of treatments AS and AS + NI relative to the other treatments (Figures 3 and 4), which might contribute to higher fruit yields, we could not find any significant effects of these treatments on fruit production. This could stem from the relative short period of the differential treatments.

NI have the potential to increase N uptake by trees, thereby reducing nitrate leaching to underground water, since the residual nitrate in the soil at the end of the fertigation season (beginning of the wet season) was significantly lower in Eff with NI than in Eff without NI. Similar results were obtained by Serna et al. (2000) who reported that in the treatment of citrus with Ammonium sulfate + Ammonium nitrate application of DMPP reduced the leached N below 0.60 m after 120 days, from 68.5% to 53.1%.

One can estimate the effectiveness of NI by comparing the soil nitrate concentrations and pH values over time in their presence and in their absence. Comparison of the data from the 3rd year, when DMPP was used, and from both the 1st and the 2nd, when DCD was used, showed that DMPP was more effective than DCD in reducing soil pH and nitrate concentration [If less NH_4 is nitrified, soil pH cannot decline. But NH_4 uptake by roots also decreases soil pH] (Figure 1). This indirectly indicates that under the studied experimental conditions, DMPP was more efficient at inhibiting nitrification.

CONCLUSIONS

Fertilization with AS resulted in soil acidification during the irrigation season from pH 7.4 to 6.1, and increased P, Mn and Ca concentrations in the soil solution. Consequently, under AS treatment, the P and Mn uptake, chlorophyll content in the leaves and fruits, and TSS in fruits, were higher than in the other treatments. Excess ammonium concentration in the soil solution inhibited Ca and K uptake without significantly reducing fruit acidity. The presence of DON in treatment Eff diminished the impact of ammonium on soil pH. Application of NI had negligible effects on fruit yield and quality but enhanced the capacity of AS to raise N uptake above that achieve with AN or Eff and consequently reduced the potential for N leaching from the soil. The correlation found between Ca in leaf and yield was unexpected since the soils are calcareous and we did not expect to get such data. The competition with NH_4 might reduce the uptake of Ca to the leaves and thereafter affect the yield.

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