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Improvement of N fertilization by using the nitrification inhibitor DMPP in drip-irrigated citrus trees

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Abstract

Nitrogen management in orchards should tend to improve the fertilizer N use efficiency for a sustainable agriculture where productivity, fruit quality and environment are reconciled. The use of specific nitrification inhibitors could increase the N fertilizer uptake and decrease the potential groundwater pollution by nitrate leaching. The aim of this experiment was to assess the effect of application frequency of the ammonium sulphate (AS) and the nitrification inhibitor (NI), 3,4-dimethylpirazole phosphate (DMPP) supply on: nitrate-N and ammonium-N seasonal changes in soil; N and Fe concentrations in the spring-flush leaves; and yield and fruit quality. The experiment was carried out with clementine cv. Nules (*Citrus clementine* Hort. Tanaka x *Citrus reticulate* Blanco) mandarins grafted on Troyer citrange (*Citrus sinensis* x *Poncirus trifoliata*) rootstock under field conditions during three consecutive years. The trees were fertilized with 324 Kg N ha^{-1} from which 192 Kg N ha^{-1} were applied as AS (21% NH_4^+-N) either with or without NI, and the remainder N came from irrigation water. The AS and AS+NI were split into 1, 2 or 4 applications per month by drip irrigation. The NH_4^+-N concentration in the 0-20 and 20-40 cm soil layers was significantly higher in the AS+NI treatment. By contrast, the NO_3^--N concentration was significantly higher in the soil treated only with AS. Moreover, the addition of NI to AS originated a significantly higher N and Fe concentrations in the spring-flush leaves. The yield was higher and some fruit quality parameters improved in trees fertilized with AS+NI compared to those fertilized only with AS.

Additional key words: ammonium sulphate, fruit quality, fruit yield, N frequency, N soil.

Resumen

Optimización de la fertilización nitrogenada en cítricos en riego localizado mediante el inhibidor de la nitrificación DMPP

Las prácticas agrícolas de fertilización nitrogenada deben mejorar la eficiencia de uso del nitrógeno (N) en una agricultura sostenible en que una mayor producción y calidad sea compatible con el medioambiente. El uso de inhibidores de la nitrificación (NI) podría incrementar la eficiencia de absorción de N disminuyendo la contaminación de las aguas subterráneas por lixiviación de nitratos. Este trabajo analiza el efecto de la frecuencia de aplicación del sulfato amónico (AS) y del inhibidor de la nitrificación 4-dimethylpirazol fosfato (DMPP) sobre los cambios estacionales del N-nítrico y N-amónico del suelo, la concentración de N y hierro en hojas de la brotación de primavera y la producción y calidad del fruto. El ensayo se llevó a cabo en una parcela comercial de clementina cv. Nules (*Citrus clementine* Hort. Tanaka x *Citrus reticulata* Blanco) injertadas sobre citrange Troyer (*Citrus sinensis* x *Poncirus trifoliata*) durante tres años consecutivos. Los árboles se fertilizaron con 324 Kg N ha^{-1}; 192 Kg N ha^{-1} se aplicaron como AS (21% NH_4^+-N) sin y con NI, fraccionados en 1, 2 y 4 aplicaciones por mes, el resto procedió del agua de riego. La concentración de NH_4^+-N en los 40 cm superficiales de suelo fue significativamente mayor con AS+NI, mientras que la concentración de NO_3^--N fue significativamente superior en los árboles abonados con AS. El NI dio lugar a una mayor concentración de N y Fe en las hojas de primavera. La producción y algunos parámetros de calidad del fruto fueron mayores en los árboles fertilizados con AS+NI.

Palabras clave adicionales: frecuencia de aplicación, N en suelo, producción y calidad del fruto y sulfato amónico.

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Abbreviations used: AS (ammonium sulphate), ASN (ammonium sulphate nitrate), CI (colour index), DCD (dicyandiamide), DMPP (3,4 dimethylpirazole phosphate), ET_c (crop evapotranspiration), ET_o (reference crop evapotranspiration), K_c (crop coefficient), NI (nitrification inhibitor), NS (not significant), TA (total acidity), TSS (total soluble solids), WHO (World Health Organization).

Introduction

Citrus is an intensively managed crop and the most important economically on the Mediterranean coast of Spain with almost of 3x10^5 ha, where the cultivation of citrus fruits predominates. In these areas, a severe increase in contamination by lixiviation of the nitrate-ion (NO_3^-) has been observed in subterranean waters (Sanchis, 1991; Fernández *et al*., 1998), above the limit of the World Health Organization (WHO) guideline (WHO, 2004), of 50 mg L^{-1} as NO_3^-. There is a growing awareness about the ecological impact of the N fertilizers used in the intensive agriculture zones and it has become a first order environmental problem in Spain since the early 1990s. Most of the fertilizer nitrogen applied is in the form of 100% ammonium (NH_4^+) as ammonium sulphate (AS) and 50% NH_4^+ + 50% NO_3^- as ammonium nitrate (AN). NH_4^+ is usually oxidized quite rapidly to NO_3^- by the nitrifying microorganisms in soils (McCarty and Bremner, 1989). The nitrification process can be performed between 30-40 days after N application, but in summer time, when the majority of N fertilizers are applied in citrus orchards, it can be more rapid (Serna *et al*., 1996a, 2000). Part of the NO_3^- produced is then subject to losses, mainly through leaching or denitrification, which account for the low N efficiency of the N fertilizers used in this crop (Mansell *et al*., 1986; Feigenbaum *et al*., 1987; Martínez *et al*., 2002). The improvement of the fertilizer N use efficiency is necessary for a sustainable agriculture. In this line, the use of specific nitrification inhibitors may increase the N fertilizer uptake and decrease nitrate leaching. Previous studies demonstrated that the nitrification inhibitor (NI) dicyandiamide (DCD) added to ammonium sulphate nitrate (ASN) improved the N-fertilizer efficiency and reduced NO_3^- leaching in young and mature citrus trees (Serna *et al*., 1994, 1996a). More recently, a new NI, 3,4 dimethylpirazole phosphate (DMPP), has been shown to have several distinct advantages compared to the currently used NIs (Zerulla *et al*., 2001). Serna *et al*. (2000) and Bañuls *et al*. (2001) suggested that DMPP could be a more efficient NI than DCD when DMPP is added to AS or ASN in citrus trees grown in containers under glasshouse and outside conditions. Nevertheless, there is no information about the behavior of the DMPP in *Citrus* cultivated under field conditions. For these reasons, the aim of this research was to evaluate the effect of the DMPP and AS application frequency on the seasonal distribution of NO_3^- and NH_4^+ concentration in the upper soil layers, leaf N concentration, yield and fruit quality in a drip-irrigated *Citrus* orchard.

Material and methods

Experimental conditions

The field trial was carried out during three successive years in a drip irrigated orchard of 12-yr-old clementine cv. Nules (*Citrus clementine* Hort. Tanaka x *Citrus reticulata* Blanco) mandarins grafted on Troyer citrange (*Citrus sinensis* x *Poncirus trifoliata*) grown at a spacing of 4 x 5 m (480 trees ha^{-1}). The adult trees were cultivated on a Cambic Arenosol soil (62.5% sand, 19.2% silt, 18.3% clay; pH 8.2; organic matter content 0.95% and a bulk density of 1.6 kg m^{-3}) with low water holding capacity (16%, FAO-Unesco, 1988) and plants were randomized planned across the experimental area.

Fertilization and irrigation scheduling

The trees were fertilized with 675 g N $tree^{-1}$ yr^{-1}, of which 400 g were supplied as AS (21% NH_4^+-N) by fertigation, either without or with 0.5% DMPP, this means 5 mg g^{-1} N applied. The N-remainder was provided by the irrigation water from a well containing 224 mg NO_3^- L^{-1}. The quantity of N contributed by the irrigation water was calculated using the formula described by Martínez *et al*. (2002).

The amount of water applied to each tree was equivalent to the total seasonal crop evapotranspiration (ETc) calculated following the expression $ETc = ET_o \times K_c$ (Doorenbos and Pruitt 1977): where ET_o is reference crop evapotranspiration under standard conditions and K_c is crop coefficient. This coefficient (K_c) accounts for crop-specific effects on overall crop water requirements and is a function of canopy size and leaf properties. The ET_o values were determined using the Penman-Monteith approach (Allen *et al*., 1998). The K_c values were based on guidelines provided by Castel and Buj (1994). Irrigation water requirements were met by the effective rainfall (≥3 mm and ≤ 45 mm which resulted in soil water saturation) of the entire year plus irrigation water (1900+3814, 2020+3626 and 2280+3407 m^3 ha^{-1} for the three years of the assay, respectively). The average amount of irrigation water applied was 3616±204 m^3 ha^{-1}, providing 381±22 g N $tree^{-1}$. According to Legaz and Primo-Millo (2000), between 70-80% of nitrate in irrigation water is available for tree uptake, hence around 275±15 g N tree^{-1} were supplied by irrigation water. Trees were irrigated from 0 to 3 times per week, according to evapotranspiration demand and the effective rainfall, using eight commercial emitters per tree (4 L h^{-1}) resulting in a 33% wetting area (Keller and Karmelli, 1974). In all trees, AS-N was applied in March (5%), April (10%), May (15%), June (20%), July (20%), August (15%), September (10%) and October (5%) according to Legaz and Primo-Millo (2000). These authors analysed the relationship between N application to citrus trees and subsequent N uptake in order to develop nutrient recommendations based on actual crop N demand. The basic dressing of P, K and Fe per tree was also applied according to Legaz and Primo-Millo (2000) and distributed along the growth cycle in similar way as previously indicated for N. Foliar spray treatments of Zn and Mn were applied to correct deficiencies.

Ammonium sulphate (AS) and AS with nitrification inhibitor (AS+NI) was applied in each month at the following frequencies: 1 split (AS_1 and $AS+NI_1$), 2 splits (AS_2 and $AS-NI_2$) and 4 splits (AS_4 and $AS+NI_4$). Thus, the assay consisted of 6 treatments with 4 replications (four trees each) per treatment.

Soil and vegetal sampling

During the first year of assay, soil from the 0-20 and 20-40 cm soil layers was sampled using a 4 cm diameter soil auger. Soil samples were taken monthly in the wetting zone about mid-way between the emitters and the periphery of the wetting front. Each soil sample consisted of 12 subsamples (3 subsamples per tree). All soil samples were air-dried, sieved through a 2 mm screen and stored at room temperature (22ºC) until subsequent analysis, according to Breimer and Slangen (1981) procedure.

Spring flush leaves (10 leaves per tree) from non-fruiting shoots were sampled monthly all around the canopy, and washed in non ionic detergent solution followed by several rinses in distilled water and then frozen in liquid-N_2 and freeze-dried (liophilized). Vegetal samples were ground to pass trough a 0.3 mm mesh sieve using a water-refrigerated mill and stored at –4ºC before further analysis. In November of each year, forty fruits per replication (10 fruits per tree) were collected, weighted and fruit quality parameters were measured immediately.

Analytical procedure

The analysis of the mineral nitrogen of the soil (N as NO_3^- and NH_4^+) was measured by steam distillation with MgO and Devarda's alloy, respectively, using KCl as extracting agent (Bremner, 1965a). Total nitrogen content of plant material was determined using the Semi Micro-Kjeldahl method described by Bremner (1965b). Fruit quality parameters (fruit weight, fruit number per tree, peel thickness, peel weight, juice plus pulp weight, total soluble solids content, total acidity and colour index) were measured following the methods described by Serna *et al*. (1992).

Statistical treatment of the data

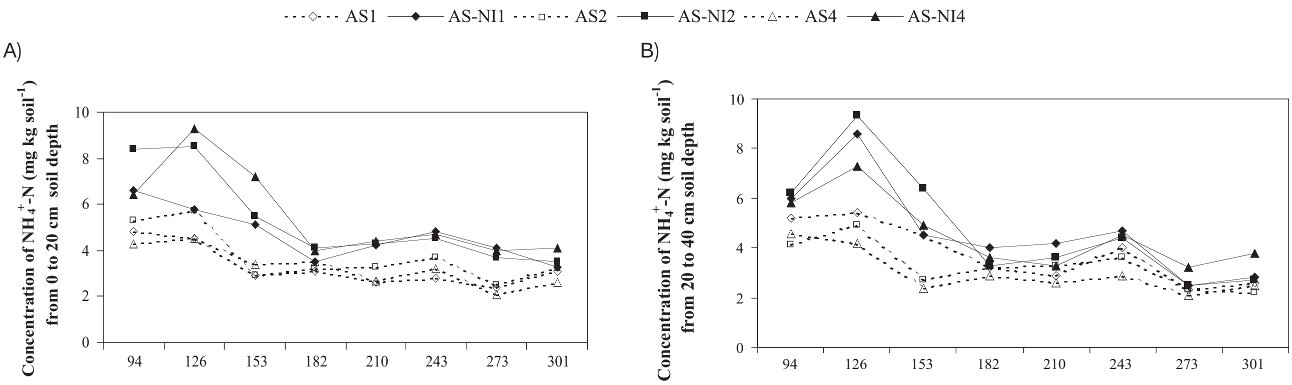
Results were analyzed using standard analysis of variance techniques (ANOVA). Treatment means separation was determined using the LSD-Fisher test with a 95% confidence level.

Results

Seasonal variations of N concentrations in soil

The amounts of NH_4^+–N and NO_3^-–N in the soil were measured in order to estimate the residual concentration of these anions in the upper soil layers. Figs. 1 A and B show the NH_4^+-N and NO_3^--N concentrations, in the first year of the assay, in the upper two layers (0-20 and 20-40 cm), which account for the major of the N available for root uptake. The concentration of NH_4^+-N was significantly higher in the soil treated with DMPP than in soil that only received AS with independence of the frequency of application (Fig. 1 A and B). The most pronounced differences between treatments without or with DMPP were found in spring, from April 7 (day 97) to June 2 (day 153) when average air temperature (17ºC) was milder than in later period (23ºC, average of June and July). The NH_4^+ concentrations were lower when decreasing the application frequency, being statistically significant in the upper 20 cm of soil in May. These differences were higher in soil of DMPP treatments.

Soils treated with AS only (absence of NI) showed higher NO_3^- contents in the upper soil layers, with independence of the application frequency. Differences were statistically significant in the period between April 7 and June 6, as previously observed for NH_4^+ concentra-

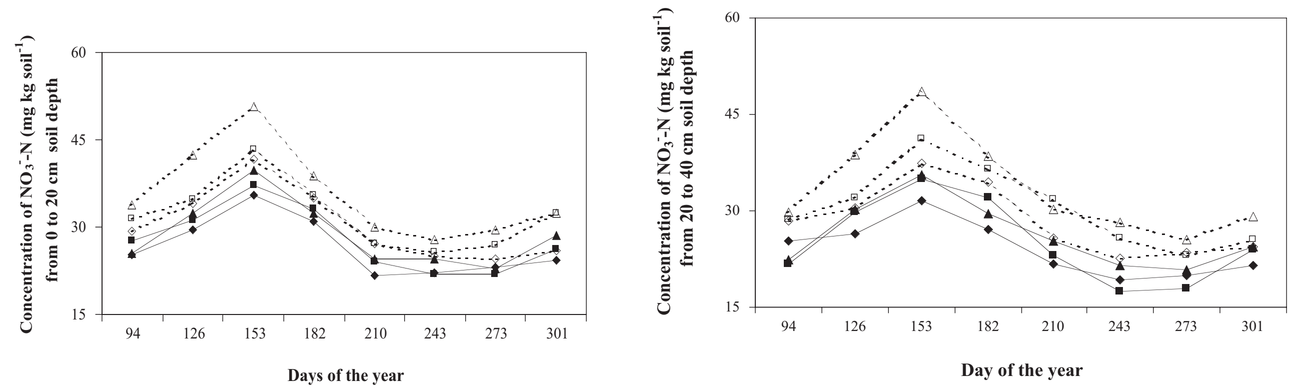


A)

	94	126	153	182	210	243	273	301
F[1]	NS	*	NS	NS	NS	NS	NS	NS
NI[2]	***	***	***	*	**	**	*	NS
FxNI[3]	NS	NS	NS	NS	NS	NS	NS	NS

B)

	94	126	153	182	210	243	273	301
F[1]	NS	NS	NS	NS	NS	NS	NS	NS
NI[2]	***	***	***	*	**	**	*	NS
FxNI[3]	NS	NS	NS	NS	NS	NS	NS	NS



A)

	94	126	153	182	210	243	273	301
F[1]	NS	*	NS	NS	NS	NS	NS	NS
NI[2]	*	***	*	NS	NS	NS	NS	NS
FxNI[3]	NS	NS	NS	NS	NS	NS	NS	NS

B)

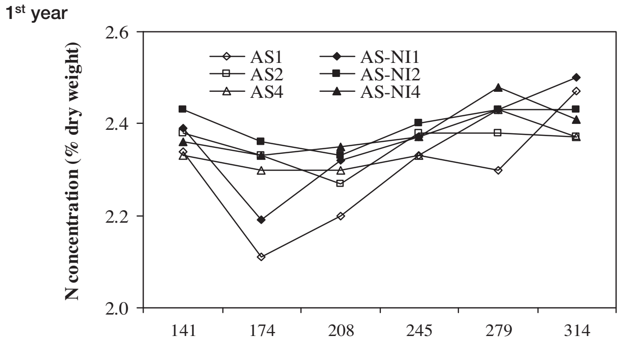
	94	126	153	182	210	243	273	301
F[1]	NS	*	NS	NS	NS	NS	NS	NS
NI[2]	*	***	*	NS	NS	NS	NS	NS
FxNI[3]	NS	NS	NS	NS	NS	NS	NS	NS

Figure 1. Dynamic of the NH_4^+ -N and NO_3^- -N concentrations in A) 0-20 cm and B) 20-40 cm soil layers during the complete growth cycle. Means of four replications per treatment. AS1 and AS+NI1, AS2 and AS+NI2, AS4 and AS+NI4: ammonium sulphate without and with nitrification inhibitor applied in 1, 2 and 4 split each month, respectively. [1] Significant differences between treatments due to frequency of application (F). [2] Significant differences between treatments due to presence of nitrification inhibitor (NI). [3] Significant differences between treatments due to interaction between frequency of applications and presence of nitrification inhibitor (F x NI).

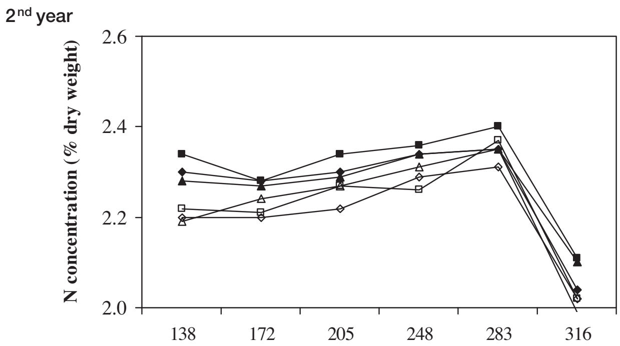
tions. NO_3^- concentrations increased similarly in all treatments in both soil layers from September onwards, possibly due to the remineralization process or a decrease in N uptake.

Nitrogen and iron concentration in the spring flush-leaves

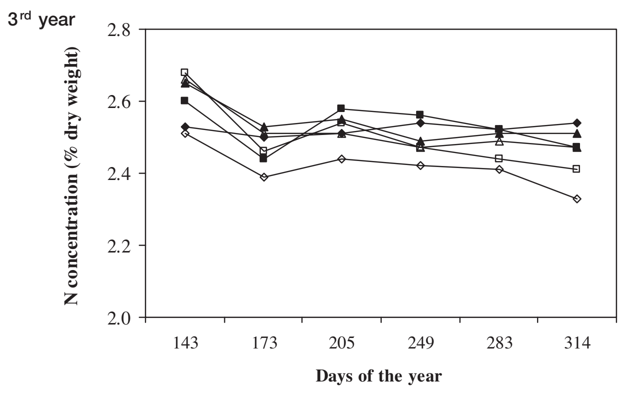
The lowest application frequency (one application each month) originated significantly lower N concentration (Fig. 2) during fruit set in the first and second year of the assay. Foliar N concentration was significantly higher in trees treated with DMPP during the growth cycle and remained within the normal N concentration range of 2.4 and 2.7% (Legaz and Primo-Millo, 2000). The N concentration in the spring flush-leaves decreased from May to June, increasing progressively thereafter until November in the three years of the assay. On 2001, a strong decrease in the foliar N value was found from October onwards (Fig. 2), due to an intensive rainfall event in this month (430 mm). In consequence the 5% of the total N rate corresponding to October was not supplied and no N was added by the irrigation water, since trees were not irrigated. In addition, the development of a massive third flush originated a great remobilization of N accumulated in the spring flush leaves at this time.



F [1]	*	***	*	*	***	**
NI [2]	**	*	**	*	***	*
FxNI [3]	NS	NS	NS	NS	NS	NS



F	NS	NS	NS	NS	NS	NS
I	***	*	*	*	NS	*
F x I	NS	NS	NS	NS	NS	NS



F [1]	**	***	**	NS	NS	NS
NI [2]	*	NS	**	***	**	***
FxNI [3]	NS	NS	NS	NS	NS	NS

Figure 2. Evolution of the N concentration in the spring-flush leaves from non fruiting shoots sampled along the growth cycle. Means of four replications per treatment in the three years of the assay: AS1 and AS+NI1, AS2 and AS+NI2, AS4 and AS+NI4 ammonium sulphate without and with nitrification inhibitor applied in 1, 2 and 4 split each month, respectively. [1, 2, 3]See Fig. 1.

Table 1. Effect of different treatments on foliar Fe (ppm) at harvesting time[1]

Treatment	1st year	2nd year	3rd year
AS_1[2]	83.8b[8]	94.2b	81.6b
$AS+NI_1$[3]	92.3ab	97.0ab	86.2ab
AS_2[4]	86.1b	94.8b	82.4b
$AS+NI_2$[5]	89.2b	100.4a	89.2ab
AS_4[6]	95.4ab	96.7b	84.8ab
$AS+NI_4$[7]	103.8a	100.4a	92.3a
F[9]	NS[12]	NS	NS
NI[10]	*	**	*
FxNI[11]	NS	NS	NS

[1] Means of four replicates, 4 trees each replicate. [2, 3, 4, 5, 6, 7] AS_1 and $AS+NI_1$, AS_2 and $AS+NI_2$, AS_4 and $AS+NI_4$: ammonium sulphate without and with nitrification inhibitor applied in 1, 2 and 4 split each month, respectively. [8] Significant differences between treatments. [9] Significant differences between treatments due to frequency of applications (F). [10] Significant differences between treatments due to presence of nitrification inhibitor (NI). [11] Significant differences between treatments due to interaction between frequency of applications and presence of nitrification inhibitor (FxNI). [12] Significant effects of factors are given at $P>0.05$ (NS), $P\leq0.05$ (*), $P\leq0.01$ (**).

Leaves of the DMPP treated trees showed a higher foliar Fe concentration in comparison to trees with AS without NI (Table 1).

Yield and fruit quality

The addition of the NI resulted in an increase in the final number of fruits per tree (Tables 2 to 4) for all the application frequencies, being significant in the first and second year of the assay. NI also increased total yield only being statistically significant in the second year of the study, while during the first year, the decrease in fruit weight of DMPP treated trees, resulted in a minor increment of total yield. These parameters remained unaffected by application frequency in the three years studied. Split application of DMPP induced similar increments in total yield, 12, 10, and 10% in the $AS+NI_1$, $AS+NI_2$, $AS+NI_4$ treatments, in comparison to AS_1, AS_2, AS_4, respectively, for all years.

A significant effect of the application frequency on peel thickness and peel and juice weight percentages was found. While the highest values of peel thickness and its percentage on the fruit weight were found with four split applications per month, the opposite tendency occurred for the juice relative percentages (Tables 2 to

Table 2. Effect of different treatments on yield and fruit quality parameters of Clementine fruits at harvesting time (1st year of the assay)[1]

Parameters	**AS_1[2]**	**$AS+NI_1$[3]**	**AS_2[4]**	**$AS+NI_2$[5]**	**AS_4[6]**	**$AS+NI_4$[7]**	**F[9]**	**NI[10]**	**FxNI[11]**
Yield (Mg ha^{-1})	62.4a[8]	71.5a	72.2a	78.2a	64.7a	71.8a	NS[12]	NS	NS
Fruit number per tree	622b	764ab	749ab	820a	611b	747ab	NS	*	NS
Fruit weight (g)	100.3ab	93.6c	96.4bc	95.4bc	105.9a	96.1bc	NS	**	NS
Peel thickness (mm)	2.9ab	2.9ab	2.7b	2.7b	3.1a	2.9ab	*	NS	NS
Peel (g kg^{-1})	445b	478ab	460ab	443b	495a	477ab	*	NS	NS
Juice (g kg^{-1})	555a	522ab	540ab	557a	505b	523ab	*	NS	NS
Total soluble solids, TSS (g kg^{-1})	157c	172a	158bc	161abc	161abc	170ab	NS	*	NS
Total acidity, TA (g L^{-1})	8.3a	8.5a	8.2a	8.5a	8.5a	8.4a	NS	NS	NS
Maturity index, TSS/TA	18.9a	20.2a	19.3a	18.9a	19.0a	20.2a	NS	NS	NS
Colour index, CI	10.7a	7.6b	10.4a	8.2b	7.2b	6.8b	***	***	NS

[1] Means of four replicates, 4 trees per replicate. [2, 3, 4, 5, 6, 7] AS_1 and $AS+NI_1$, AS_2 and $AS+NI_2$, AS_4 and $AS+NI_4$ ammonium sulphate without and with nitrification inhibitor applied in 1, 2 and 4 split each month, respectively. [8] Significant differences between treatments. [9] Significant differences between treatments due to frequency of applications (F). [10] Significant differences between treatments due to presence of nitrification inhibitor (NI). [11] Significant differences between treatments due to interaction between frequency of applications and presence of nitrification inhibitor (FxNI). [12] Significant effects of factors are given at $P>0.05$ (NS), $P\leq0.05$ (*), $P\leq0.01$ (**) and $P\leq0.001$ (***).

4). These differences were more remarkable during the second year of the study. The DMPP had not consistent effect on these parameters.

Total soluble solids (TSS) showed significantly higher values when DMPP was added to the fertilizer with independence of the application frequency (Tables 2 to 4). The different treatments did not affect consistently the mature index. The DMPP-treated fruits showed a significantly lower colour index in all the years. The highest application frequency originated a significantly lower colour index.

Discussion

Seasonal variations of N concentrations in soil

The citrus tree root system is comprised of a relative shallow, well-branched framework of woody laterals and fine fibrous roots (Castle, 1980a). The fibrous roots are usually most densely concentrated near the soil surface while few roots are found below 90 cm (Castle, 1980b; Zhang *et al*., 1996; Mattos *et al*., 2003). Serna *et al.* (1994 and 2000) showed that 90% of fine root was located in the upper 45 cm of soil profile and nitrate found beyond this depth could be leached. Hence, NH_4^+ and NO_3^- concentration in the upper 40 cm of the soil profile in this study, thus represents most of the N available for root uptake. These findings suggest that dynamics of soil NH_4^+ and NO_3^- concentration in this trial could be used as an indicator of potential leaching of NO_3-N below the root zone of the trees.

The inhibitor effect of DMPP on the nitrification process resulted in higher NH_4^+ accumulation during the experimental period in comparison to the AS treated soil. The highest differences in the NH_4^+ concentration in the soil found from March to May were due to the fact that DMPP effectiveness decreases with increasing soil temperature (Slangen and Kerkhoff, 1984; Zerulla *et al*., 2001; Irigoyen *et al*., 2003). On the contrary, higher NO_3^- concentrations were found in AS treated soils in comparison to AS+DMPP treatments, as previously reported (Carrasco and Villar, 2001; Wissemeier *et al*., 2001; Zerrulla *et al*., 2001; Muñoz-Carpena *et al*., 2002) in several crops. This could increase the amount of NO_3^- accumulated in deeper soil layers, which can not be explored by the root system, and hence lead to potential risk of NO_3^- losses during intensive rainfalls and/or excessive irrigation events. In previous glasshouse and outside container experiments with *Citrus*, the soil showed higher NH_4^+ and lower NO_3^- concentration in different soil layers (0-45 cm) when a NI, DCD or DMPP, was added (Serna *et al*., 1994, 1996a). The absence of the NI also originated a higher NO_3^- content in the drainage water (Serna *et al*., 2000; Bañuls *et al*., 2001; Martínez-Alcántara *et al*., 2006). Similar response was found by Zerulla *et al*. (2001) in horticultural crops.

Table 3. Effect of different treatments on yield and fruit quality parameters of Clementine fruits at harvesting time (2nd year of the assay)[1]

Parameters	AS_1[2]	AS+NI_1[3]	AS_2[4]	AS+NI_2[5]	AS_4[6]	AS+NI_4[7]	F[9]	NI[10]	FxNI[11]
Yield (Mg ha^{-1})	60.1bc[8]	69.0ab	64.8abc	72.6a	57.6c	66.2abc	NS[12]	*	NS
Fruit number per tree	658ab	726ab	712ab	787a	611b	699ab	NS	*	NS
Fruit weight (g)	91.3a	95.0a	91.0a	91.5a	94.3a	94.7a	NS	NS	NS
Peel thickness (mm)	2.8b	2.7b	2.8b	2.7b	2.8b	2.9a	*	NS	NS
Peel (g kg^{-1})	584a	565ab	535b	534b	588a	587a	***	NS	NS
Juice (g kg^{-1})	416b	435ab	465a	466a	412b	413b	***	NS	NS
Total soluble solids, TSS (g kg^{-1})	141ab	143a	138b	144a	143a	145a	NS	*	NS
Total acidity, TA (g L^{-1})	11.9a	12.1a	10.8a	11.6a	12.7a	12.5a	NS	NS	NS
Maturity index, TSS/TA	11.8a	11.9a	13.2a	12.4a	11.3a	11.6a	NS	NS	NS
Colour index, CI	12.2a	8.3b	12.8a	8.9b	7.7a	7.6b	***	**	NS

[1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12] See Table 2.

Nitrogen and iron concentration in the spring flush-leaves

The foliar N concentration was significantly higher in the trees treated with DMPP during the growth cycle in the years studied. Serna *et al*. (2000) also found a higher N foliar concentration with the addition of the DMPP to ASN in adult trees and the same response was obtained by Bañuls *et al*. (2001) in young citrus trees fed with AS plus DMPP. Pasda *et al*. (2001) found the highest values in crude protein concentration in cereal grain (winter wheat and grain maize) when the DMPP was added to ASN. In other assays, the addition of DCD to ASN resulted in a significant increase in leaf N concentration (Serna *et al*., 1994, 1996a). This could be due to the fact the maintenance of high NH_4^+ concentration in the soil treated with DMPP incorporated to NH_4^+ fertilizers, enhanced N uptake by plants (Serna *et al*., 2000; Bañuls *et al*., 2001; Martínez-Alcántara *et al*., 2006) as a result of a more continuous NO_3^- release in soil and a reduction of N losses by NO_3^- leaching and denitrification. It is well known that the N applied form influences the pH of the soil (Street and Sheat, 1958; Marschner, 1995; Tagliavini *et al*., 1995; Mengel and Kirkby, 2001). These authors indicate that a high NH_4^+/NO_3^- ratio fertilization can induce a decrease in the rhizosphere pH by the nitrification process. The rhizosphere acidification associated with a predominant NH_4^+ induced by NI (Trolldenier, 1981; Thomson *et al*., 1993) improves nutrition by changes on the availability of nutrients such as P, Fe, Mn, Zn, Cu and Al (Gahoonia, 1993; Pasda *et al*., 2001). This is in line with the results by Serna *et al*. (1992) who found a higher foliar Fe concentration in *Citrus* fed with ammonium in comparison with nitrate that it would also explain the higher foliar Fe content found in leaves of AS+NI treated trees.

Yield and fruit quality

The increase in the final number of fruits per tree found in this study, as a result of the NI addition, suggests that the AS +NI inhibitor was more effective than AS alone during the fruit set period. Similar increase to that found in present work, in fruit number of DMPP treated trees, was reported by Serna *et al.* (1992) when the NO_3^-/NH_4^+ ratio applied changed in the range from 75/25 to 0/100 in citrus grown in sand culture. Marschner (1995) and Goos *et al.* (1999) also found higher yield and growing rates in crops fed with a mixture nutrition of ammonium and nitrate. Serna *et al.* (1996a) obtained an increase of about 15% in the yield due to an increase in the fruit number in *Citrus* trees with DCD. Pasda *et al.* (2001) also observed an increase in crop yield of numerous agricultural and horticultural crops fed with ammonium fertilizes with DMPP.

The higher values of peel thickness and lowest percentages of juice weight found in trees treated with four split applications per month could be due to the increase of N foliar concentration observed in these trees. Embleton *et al.* (1973) indicated a similar pattern in these parameters when N foliar concentration increased from 2.0 to 2.6% and inconsistent effects on TSS, total acidity and maturity index. Serna *et al.* (1992) observed a slight effect of the NO_3^-/NH_4^+ ratio on the total solu-

Table 4. Effect of different treatments on yield and fruit quality parameters of Clementine fruits at harvesting time (3rd year of the assay)[1]

Parameters	AS_1[2]	$AS+NI_1$[3]	AS_2[4]	$AS+NI_2$[5]	AS_4[6]	$AS+NI_4$[7]	F[8]	NI[9]	FxNI[10]
Yield (Mg ha^{-1})	65.8a[11]	69.7a	69.2a	75.4a	67.2a	70.6a	NS[12]	NS	NS
Fruit number per tree	625a	660a	680a	723a	614a	668a	NS	NS	NS
Fruit weight (g)	105.3a	105.6a	101.8a	104.3a	109.4a	105.7a	NS	NS	NS
Peel thickness (mm)	3.07b	3.21b	3.16b	3.12b	3.20b	3.52a	**	NS	NS
Peel (g kg^{-1})	541bc	530c	541bc	595a	594a	568ab	**	NS	NS
Juice (g kg^{-1})	459a	470a	459a	405b	406b	432ab	*	NS	NS
Total soluble solids, TSS (g kg^{-1})	132ab	135a	132ab	134ab	132ab	133b	NS	*	NS
Total acidity, TA (g L^{-1})	8.5a	8.4a	8.4a	8.4a	8.9a	8.8a	NS	NS	NS
Maturity index, TSS/TA	15.6a	16.1a	15.8a	16.0a	14.8a	15.0a	NS	NS	NS
Colour index, CI	9.24a	6.85bc	7.97ab	7.51abc	7.73abc	5.88c	*	*	NS

[1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12] See Table 2.

ble solids. In the same line, Serna *et al*. (1996a,b) did not find a significant effect on this parameter when DCD was added to ASN. However, the addition of NI to AS caused a significant increase in the TSS at all years. The different treatments did not affect consistently the total acidity and mature index.

Lower fruit colour index values in the DMPP-treated trees were also observed by Serna *et al*. (1992) when decreasing the NO_3^-/NH_4^+ ratio. NH_4^+ nutrition could be responsible for greener fruit colour, since NH_4^+ applied on citrus fruit peel delays chlorophyll degradation (Huff, 1983). The slight delay in the time colour break found in the DMPP-treated fruits could be an economical advantage for mead-season and late varieties.

From the findings of this experiment, it can be concluded that the addition of the nitrification inhibitor (DMPP) to ammonium sulphate in drip irrigated *Citrus* trees increases foliar N and Fe concentrations and improves yield and some fruit quality parameters. Moreover, the lower $N\text{-}NO_3^-$ content in the deepest layers in presence of DMPP may lead to a reduction of the potential risk of pollution by nitrate leaching to underground water.

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