



Environmentally Friendly and
Safe Technologies for Quality
of Fruits and Vegetables

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The papers contained in this book report some of the peer reviewed Proceedings of the International Conference “Environmentally friendly and safe technologies for quality of fruit and vegetables”, but also other papers related with the subject were included. The manuscripts were reviewed by the Editor and Editorial Board, and only those papers judged suitable for publication were accepted. The Editor wish to thank to all the reviewers and authors for their contribution.

Authors are responsible for content and accuracy of their papers.

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SECTION 2. PRE-HARVEST FACTORS AFFECTING
POSTHARVEST QUALITY AND SAFETY

10. AMMONIUM FERTILISERS WITH NITRIFICATION INHIBITORS IMPROVE THE NUTRITIONAL QUALITY OF HORTICULTURAL CROPS FOR INDUSTRIAL PROCESSING

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Abstract

Nitrogen management in plant nutrition is an agronomic and environmental key factor. Under soil conditions the main form of N available for the plant is nitrate. Nitrification inhibitors reduce the nitrification rate and increase the ammonium absorbed by the plant. This effect reduces the risk of groundwater pollution by nitrates. Nitrate may be dangerous for human health and can increase oxalate concentration and decrease vitamin C (vit C) content in vegetables. This paper presents the results of three field trials on tomato, spinach and broccoli, all of them for industrial processing (spinach and broccoli for frozen industry and tomato for tomato paste). In the three trials a conventional ammonium plus nitrate fertilization was compared with the application of ammonium plus nitrate plus nitrification inhibitor, using similar rates of N. All of the trials were conducted in Badajoz (Spain) during 2006 and 2007. In the spinach trial the ammonium increase (with the nitrification inhibitor) reduced nitrate and oxalate in leaves and increased vit C. Greater yields were obtained with mixed fertilization (nitrate plus ammonium plus nitrification inhibitor). In the Broccoli trial there was no differences in yields but using the nitrification inhibitor, a slightly increasing of vit C content was obtained. The trial performed with fertigated industrial tomato shown that the use of nitrification inhibitor increased yield on 8% and also increased the lycopene concentration (also colour a/b ratio) from 32 mg kg⁻¹ (conventional fertilization) to 45 mg kg⁻¹. These results presented shows that important nutritional parameters, as nitrates, oxalates, vit C or lycopene are influenced by the quantity and characteristics of the N fertilizer applied, and that a mixed nutrition nitrate plus ammonium with nitrification inhibitors, can increase vit C contents and reduce nitrates or oxalates.

Keywords: broccoli, industrial tomato, nitrate, Nitrification inhibitor, oxalate, spinach, vitamin C

Introduction

Nitrogen is an essential plant nutrient but it is involved in environmental problems associated with crops fertilization. Groundwater pollution due to nitrates is increasing in all Europe. Also high nitrate concentration in waters is one of the factors that produce the eutrophication of superficial waters.

For animal and human health nitrates *per se* are not toxic, but its metabolites and reaction products such as nitrite (NO₂-), nitric oxide, or N-nitrous compounds have adverse implications as, methaemoglobinaemia or certain carcinogenesis (Lee 1970; Farré & Oliver 1982; Hartman 1983; Fraser 1985; Forman *et al.* 1988; Bruning-Fann *et al.* 1993; EFSA 2008). In spite recent research shows positive aspects of antimicrobials as nitrites and nitrates within the blood vessels (EFSA 2008), the main scientific opinion is that nitrate must be controlled and reduced in vegetables and water. Several authors (Hall 2002; Palaniswamy *et al.* 2002; Glaxo-SmithKline 2006) refer the relationships between high intake of oxalate and the formation of kidney stones, as an inadequate absorption of calcium (Okutami & Sugiyama 1994; Ahmed & Johnson 2000). The low consumption of oxalate is particularly important in patients with intestinal problems like Bowel Syndrome (SBS) or patients with a high tendency to form kidney stones. Overall estimates of kidney stones due to calcium oxalate represents between 75 and 90% of the total (Hall 2002; Glaxo-SmithKline 2006). As example in the United States more than 12% of the population is affected by this pathology clinic (Glaxo-

SmithKline 2006). In animal nutrition the high concentration of oxalates in pasture crops is harmful because of its ability to precipitate with calcium, which produces hypocalcaemia in animals (Ramos *et al.* 1998).

Nitrogen fertilization influences the accumulation of nitrate and oxalate in foods (Elia *et al.* 1998; Zhang *et al.* 2005). In field trials with spinach, Elia *et al.* (1998) concluded that the increase of N fertilization increase the concentration of nitrate and oxalate in leaves. In the same trial the increase of ammonium fertilizers reduce the concentration of harmful compounds. Baku & Gawish (1997) showed that the use of ammonia fertilisers with a nitrification inhibitor reduce the accumulation of nitrate in lettuce and spinach, lowering the total and free oxalate, and producing a slight increase in vitamin C (vit C). This result is due to one of the possible routes of formation of oxalate and breaking of the link C2/C3 in ascorbic acid (Libert & Franceschi 1987). Vit C is the main soluble antioxidant present in plants and is essential for humans and animals (Tedone 2005).

An interesting technique to improve the efficiency of N fertilisers is the control of N chemical cycle in the soil. When urea or ammoniac fertilisers are applied to the ground they are quickly transformed to nitric forms by the action of the *Nitrosomona* spp. bacteria (part of the process known as nitrification). In the nitrate form, N can be easily absorbed by the plant, because it is very soluble and mobile in the soil solution. Salt-washing irrigations, rainfalls or inadequate irrigation management can produce important losses by leaching.

Nitrification inhibitors are added to any type of fertilizers and delay the bacteria oxidation of NH_4^+ to NO_2^- (Prasad & Power 1995), due to the competition for the active site in the enzyme AMO (McCarty 1999). Nitrification inhibitors increase ammonium-N and decrease nitrate-N in soils, as a result they also decrease N losses (Fettweis *et al.* 2001). Nitrogen use efficiency is improved and the doses and number of applications can be reduced, producing an economical and environmental benefit (Trenkel 1997). In Europe the most spread nitrification inhibitor is 3,4-dimethylpyrazol phosphate (DMPP) developed by BASF during the late 90's. DMPP is a high efficiency molecule, with a soil optimal activity at 1 kg ha^{-1} , and without negative effects in the crops and the environment (Zerulla *et al.* 2001).

The objective of this work was to study the effects of different N treatments (with and without nitrification inhibitors) on the yield and the nutritional quality of tomato, spinach and broccoli for industrial processing.

Material & Methods

The trials were conducted in the farm "El Bercial" (Badajoz, Spain), which belongs to the Agro Technology Center of Extremadura (CTAEX). Soils have pH=7, the texture is sandy-clay-loam, have low organic matter content (0.9%) and low electrical conductivity, low levels of total N, high available P (Olsen method), medium-low calcium and potassium, and medium-high magnesium.

Spinach trial

The trial was developed in a plot of 2400 m^2 with 5 treatments \times 4 replications. The variety used was 'El Paso' (Syngenta) planted on March 20 at $0.25 \times 0.08 \text{ m}$ seeding spacing. The treatments were: a control without N fertilization (T1); a conventional N fertilization (100 kg ha^{-1}) applied in two fractions (T2); a single application of a granular fertilizer (70% of N as ammonium and 30% of N as nitrate) with a nitrification inhibitor (3,4-Dimethylpyrazole phosphate, DMPP) at 100 kg ha^{-1} (T3); a similar treatment to T3 but applied in two fractions (T4); and two applications of a 100% ammonium fertiliser plus the nitrification inhibitor DMPP, at 100 kg N ha^{-1} (T5).

Tomato trial

Plants of industrial tomato 'UG 8168' were transplanted to field at May 16 with a fertigation system for each treatment. The harvesting was conducted in late August when fruits reached the commercial maturity

(85% of fruits with optimal red colour). Five different treatments, with 4 repetitions, were established: (T1 and T2) - conventional fertilization using the recommended dose of N (T1=150 kg N ha⁻¹) and a reduction of 20% (T2=120 kg N ha⁻¹); (T3 and T4) - an initial base fertilization with N was done and then the remaining N was applied by fertigation; (T5) - all the nutrients was applied by fertigation. In T3 and T5 150 kg N ha⁻¹ and in T4 120 kg N ha⁻¹ was applied. Fertigation program was adjusted to nutritional requirements of this crop throughout its growing cycle.

The effects on yield were assessed by measuring the total yield, the commercial yield, and firmness of the fruits, average weight and the percentage of damage fruits. In each repetition, 4 kg of tomatoes was sampled and processed at the pilot plant of CTAEX. Soluble solids, juice pH, viscosity, colour and the concentration of lycopene, vit C and nitrate in the fruit was determined.

Broccoli trial

'Monaco' variety was used, at a density of 35000 plants m⁻². The treatments were: conventional nitrogen fertilization carried out in 2 applications (T1) with 2 formulations of mixed ammonium/nitrate plus nitrification inhibitor (DMPP): the first of them at the same dose of N and the second reducing 25% the N applied, both in a single application at the start of the crop cycle. Plants were transplanted on August 30 and three harvests were done during November (20, 23 and 29).

The marketable yield, number of broccoli heads per ha, and their average weight and diameter were registered. The concentration of nitrates and vit C was done in the broccoli heads. At the beginning and end of the harvest dates the concentration of N-NO₃⁻ and NH₄⁺-N in the soil were measured.

Results & Discussion

Spinach trial

The results were greatly influenced by the fertilization treatments (Fig 1A). The yield of spinach plants grown without N (T1) was 3 times lower than plants of T3 treatment (N plus N fertilizer with DMPP); the other treatments (T2 and T4) presented intermediate values. Nitrate content in leaves had a high variability (coefficients close to 100% in some cases), however the highest values was obtain in plants fertilised with nitrate (and the lowest values with ammonium (T5- 100% ammonium fertilization plus DMPP). The values were below the maximum limit (2 g nitrate kg⁻¹) proposed by the Regulations N° 1822/2005 of the European Union Journal (9/11/2005) for spinach to frozen.

The oxalic acid content in leaves was distributed according to this order (Fig 1B): T2>T3>T1>T4>T5. The inverse order was obtained in the concentration of vit C (Fig 1C). The highest content of vit C was obtained in the treatments with ammonium plus DMPP (T3, T4 and T5). In T1 and T2 treatments, oxalic acid to ascorbic acid ratio was 39 while in T3 and T4 treatments was 22/1, and in T5 treatment was 18/1. These results were in accordance with those obtained in spinach (Zhang *et al.* 2005), in cabbage (Elia *et al.* 1998) and in *Portulaca oleracea* L. (Palaniswamy 2002).

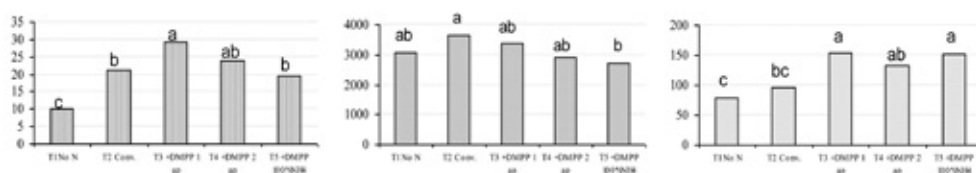


Fig 1. A - total yield (t ha⁻¹) and some nutritional parameters of spinach leaves for each treatment: B - oxalic acid (mg kg⁻¹) and C - vit C (mg kg⁻¹). Different letters in the same column indicate significant differences (P<0.05).

Broccoli trial

Fertilization with nitrification inhibitors slightly increased crop production. The ammonium fertilization had effects on the yield classes and anticipates the harvest date. The nitrate content of broccoli was very low and similar in all treatments (data not shown). However, in previous trials conducted with fertigated broccoli, Egea *et al.* (2003) found levels of nitrates 100 times lower in ammonium+DMPP treatments. Moreover, treatments with nitrification inhibitors presented decreases in the nitrates content of final product. There were no statistical differences in the concentration of ascorbic acid, however in treatment with a lower contribution of N plus nitrification inhibitor (T3) ascorbic acid content (257 ± 62 mg kg⁻¹ fresh weight) was higher compared to T1 (184 ± 35 mg kg⁻¹) and T2 (183 ± 44 mg kg⁻¹) treatments.

Tomato trial

Production in T1 was abnormally low compared to other treatments which had similar values (Table 1). The results shown that the dose of 120 kg ha⁻¹ was sufficient for this crop, considering the high N-mineral in soil at the start of the trial. T3, T4 and T5 treatments (with nitrification inhibitor DMPP) had slightly higher production (Table 1). Colour and lycopene content of tomatoes were affected by N-fertilization treatments (Table 1). Lycopene is one of the main antioxidants in food, along with vit A and C, and is one of the causes of red colouring in fruits (Dumas *et al.* 2003). Antioxidants are important for preventing cardiovascular diseases or cancer (Agarwal & Rao 2000; Dumas *et al.* 2003). In order to improve the colour of the tomato paste it is important to get a high content of lycopene in fruit, which is principally affected by environmental issues (temperature and sunlight) but also by fertigation. The review done by Dumas *et al.* (2003) indicates that the information about the effects of N fertilization on antioxidants contents of tomato is scarce and may provides contradictory and inconclusive results. In the present study the treatments had a clear effect on the content of lycopene (Table 1), but not on the concentration of ascorbic acid. Higher doses of N reduced the content of lycopene (T1 compared with T2 and T3, T4) but if N in ammonium form was used (with nitrification inhibitors; T3 and T4) there was an increase in lycopene content. In any case, these results should be supported in the followings campaigns.

Table 1. Marketable yield and industrial parameters analyzed in tomato trial.

Treatments	Yield t ha ⁻¹	°Brix	pH	Viscosity cm 30s ⁻¹	Firmness g	Colour a/b	Lycopene mg kg ⁻¹	Vit C mg kg ⁻¹	NO ₃ mg kg ⁻¹
T1	58.4 ^b	4.6 ^a	4.4 ^a	26.7 ^b	4723 ^a	1.99 ^b	17.9 ^c	86.7 ^a	<5
T2	79.1 ^a	4.6 ^a	4.4 ^a	33.1 ^{ab}	4356 ^a	1.99 ^b	32.7 ^{bc}	83.2 ^a	<5
T3	84.2 ^a	4.7 ^a	4.4 ^a	33.8 ^{ab}	4546 ^a	2.09 ^a	30.9 ^{bc}	72.7 ^a	<5
T4	87.5 ^a	4.8 ^a	4.4 ^a	30.6 ^{ab}	4134 ^a	2.09 ^a	40.5 ^{ab}	68.6 ^a	<5
T5	82.6 ^a	4.8 ^a	4.4 ^a	34.9 ^a	4496 ^a	2.03 ^{ab}	45.8 ^a	73.7 ^a	<5

(T1 and T2) - Conventional fertilization (T1=150 kg N ha⁻¹) and (T2=120 kg N ha⁻¹); (T3=150 kg N ha⁻¹ and T4=120 kg N ha⁻¹) - an initial base fertilization with N was done and then the remaining N was applied by fertigation; (T5=150 kg N ha⁻¹) - all the nutrients was applied by fertigation. Different letters in the same column indicate significant differences P<0.05.

Conclusions

The three trials presented shown that the quantity and characteristics of the N fertilizer applied influenced in the concentration of important nutritional substances as ascorbic acid (vit C) or lycopene and over anti-nutrients as nitrate or oxalate. Mixed nutrition nitrate plus ammonium with nitrification inhibitors increase the ammonium presence in the soil and its absorption by the plant. This effect induces modifications in N metabolism. These trials shown that in field conditions ammonium fertilisers plus DMPP increased vit C in spinach and broccoli plants, and lycopene in tomato plants, compared to conventional fertilization. Besides, the increase of lycopene let to reddish tomatoes which are more profitability for the growers.

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