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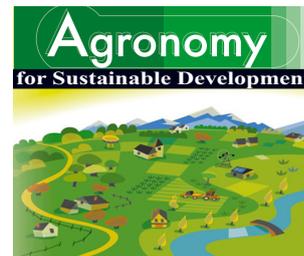
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Research article

Efficient urea-N and KNO₃-N uptake by vegetable plants using fertigation

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Abstract – Vegetable production demands high nitrogen inputs. Fertigation is a means to increase fertilizer-N use by plants. However, the effect of different N sources and doses, and how they relate to the total available N in soils are poorly known. In this study we applied ¹⁵N-labeled fertilizers to green pepper in the field using a drip irrigation system during the dry summer. KNO₃-N and urea-N were applied at a total of 6, 12 and 18 g plant⁻¹. Our results show that urea was as effective as KNO₃ as a N source. The fertilizer-N utilization efficiency was dramatically reduced at higher N doses, from 48% for the 6 g N plant⁻¹ dose to 36% and 26% for the 12 and 18 g N plant⁻¹ doses, respectively. However, the N in plants derived from fertilizer consistently exceeded 60%, indicating high availability of fertilizer-N even at the lowest dose. Negative added nitrogen interactions – the effect of added N on the fate of soil-N – were observed, particularly at high fertilizer-N doses. The fertilizer-N utilization efficiency calculated by the difference method was lower compared with the ¹⁵N enrichment method. This clearly indicates luxury N applications and excess N availability brought about by precise localized placement of fertilizer-N that leads to limited uptake of the available soil-N. N leaching risks in the following rain period should therefore be based on both the residual fertilizer-N and the increased amounts of residual soil mineral-N.

fertigation / ¹⁵N / green pepper / bell pepper / *Capsicum annuum* / nitrate / urea / added nitrogen interaction (ANI)

1. INTRODUCTION

Field production of vegetables is widespread in coastal areas of the Mediterranean basin, typical of the Peloponnese coastal regions in Greece. Although sunlight and temperatures favor vegetable production, problems include shortage of irrigation water during the extended dry summer periods, misuse of groundwater, water quality deterioration and soil salinization (Zalidis et al., 2002; Albiac et al., 2006). Excessive N fertilization appears to be a common practice (Neeteson, 1995; Schenk, 1998) that may lead to NO₃-N leaching during the following rain period, attributed to poor N-use efficiency and large amounts of residual fertilizer-N in the soil (Ramos and Gomez de Barreda, 1991; Ramos et al., 2002; Sanchez Perez et al., 2003; Jago et al., 2008).

Fertigation systems, combining drip irrigation with fertilizer application, allow for precise timing and placement of water and nutrients. This aids farmers to greatly improve both water conservation and the efficiency of fertilizer use (Miller et al., 1981; Papadopoulos, 1988; Waddell et al., 1999; Chawla

and Narda, 2001; Janat and Somi, 2001; Singandhupe et al., 2003), reducing fertilizer costs and protecting soils, groundwater, and surface-water ecosystems.

The “nitrogen utilization efficiency” from a particular N source is the amount of N from this source that is absorbed and utilized by a plant in relation to the size of the source. It may be estimated in various ways and it is also described as “Apparent nitrogen utilization efficiency” (Thompson et al., 2003) or “Nitrogen recovery efficiency” (Rao et al., 1991). Percent Fertilizer Nitrogen Utilization Efficiency (%FNUE) is commonly determined by the “difference method” in which the difference in the amount of N taken up by plants in fertilized and non-fertilized plots is expressed as a percentage of the fertilizer-N applied in the fertilized plots. This approach assumes no change in N immobilization-mineralization processes and no “priming” of soil-N availability by the addition of fertilizer-N (Jenkinson et al., 1985; Rao et al., 1991). Direct measurement of ¹⁵N-labeled fertilizer recovery may be carried out alternatively by applying the isotope dilution technique, but this approach may suffer from pool substitution effects (added labeled N standing proxy for unlabeled soil-N that would otherwise have been removed from the pool). The

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general term “Added Nitrogen Interactions” (ANI) is used to encompass all these interactions between N pools, including priming and pool substitution phenomena (Jenkinson et al., 1985; Hart et al., 1986). ANIs may be of particular importance in the study of fertilizer N-use efficiency in fertigation systems. Fertilizer-N is repeatedly applied in liquid form directly in the “hotspot” of the plant’s root system and could potentially displace native soil-N.

Fast-release nitrate-N fertilizers are immediately available to crops. They are highly soluble nitrate salts that instantly liberate nitrate anions when they come into contact with soil water. Nitrates are prone to leaching following downward water flow and diffusion (Spalding and Exner, 1993; Addiscott, 1996; Di and Cameron, 2002). Plant roots may compete efficiently with microbial immobilization of nitrate-N, since nitrate ions diffuse in bulk soil water and are generally not absorbed in soil colloids. In contrast, ammonium cations may be fixed, or absorbed by cation exchange mechanisms (Barber, 1984). For the majority of soil microorganisms, assimilatory nitrate reductases are suppressed in the presence of even minimal concentrations of $\text{NH}_4\text{-N}$ (Rice and Tiedje, 1989) and microbial nitrate immobilization is partly or completely inhibited (Recous and Mary, 1990).

Hydrolysis and nitrification must occur before the nitrate-N form is released in the soil environment from urea fertilizers and losses of N via ammonia volatilization may potentially arise following surface application of urea in alkaline soils (Ferguson et al., 1984; Bock and Kissel, 1988). Possible slow availability and volatilization losses make farmers reluctant to use urea, despite its lower cost compared with nitrate-N fertilizers. However, urea applied by drip fertigation has been shown to be a good source of N in acidic soils. It does not remain in the soil surface following fertigation and may even lead to increased vegetable production compared with nitrate-N sources, despite inducing significant acidification in the root zone (Haynes and Swift, 1987; Haynes, 1988).

We used a “fast” and a “slow” fertilizer-N source (KNO_3 vs. urea) applied at different doses, to green pepper plants, cultivated under drip fertigation in an alkaline soil in the field to investigate if fertilizer-N uptake and utilization efficiency depend on different N sources and doses. We focused on the effects of fertilizer-N supplied by fertigation to the total available N in the soil; by using different methods for estimating fertilizer-N and soil-N effects we investigated the hypothesis that fertilizer-N may stand proxy for soil-N uptake due to precise fertilizer application timing and placement.

2. MATERIALS AND METHODS

The experiment was carried out in a loam soil (clay: 240, sand: 440, silt: 320 g kg^{-1}) classified as Xerept (Keys to Soil Taxonomy, 2006) in Messinia, Greece (SW Peloponnese) in an experimental field, maintained under fallow for 10 years. The physical and chemical properties of the soil were determined before the start of cultivation (ASA, 1982, 1986) and were as follows: pH 7.8, electrical conductivity 0.9 dS m^{-1} , organic matter 11.7 g kg^{-1} , total N 0.8 g kg^{-1} , CaCO_3 equivalent 3.5%,

CEC 13 $\text{cmol}^{(+)} \text{kg}^{-1}$, Olsen P 27 mg kg^{-1} , exchangeable K 0.59 $\text{cmol}^{(+)} \text{kg}^{-1}$ and exchangeable Na 0.08 $\text{cmol}^{(+)} \text{kg}^{-1}$.

A randomized complete block design was used with two fertilizer sources and three application rates, in a 2×3 factorial arrangement with four replicates per treatment. The treatments were as follows: 6 g $\text{KNO}_3\text{-N}$ per plant ($\text{KNO}_3\text{-6gN}$), 12 g $\text{KNO}_3\text{-N}$ per plant ($\text{KNO}_3\text{-12gN}$), 18 g $\text{KNO}_3\text{-N}$ per plant ($\text{KNO}_3\text{-18gN}$), 6 g urea-N per plant (Urea-6gN), 12 g urea-N per plant (Urea-12gN) and 18 g urea-N per plant (Urea-18gN). A no-fertilizer-N control treatment was also randomized with one replicate within each block to check for the need for N applications and to estimate background N availability.

Green pepper plants (*Capsicum annuum*, “Spartacus” hybrid) were planted at 50-cm distance from each other in parallel rows 1.5 m apart. In each row, seven plants comprised one plot (including two guard plants). Drip irrigation was applied in all treatments using one 16-mm diameter hose per row with emitters (4 L h^{-1} at 1 atm capacity each) 25 cm apart (two trickles per plant). Fertigation with KNO_3 and urea was applied through the irrigation system. The stable isotope labeling of both N sources was 1.5% ^{15}N atom excess (Cambridge Isotope Laboratories, Inc.).

Growing of the crop in the field was carried out during the late spring-summer period from late April to early August (15 weeks). Seeds of green pepper had been pre-sown in a peat-based medium in the greenhouse five weeks earlier and were subsequently transplanted to their final position in the field (9–13 cm height, 7–9 true leaves). Minimum and maximum daily air temperatures increased gradually during the cultivation period, ranging from 12 to 22 °C and from 27 to 39 °C, respectively.

Irrigation was applied daily at rates starting at 0.5 L per plant and reaching 3 L per plant at the end of the cultivation period to compensate for increased evapotranspiration. Fertilizer treatments were applied by 29 fertigations with the irrigation water, distributed evenly during the cultivation period. Fertilizer-N rates were tripled in the later 19 compared with earlier 10 fertigations in order to synchronize applications with plant needs. Potassium was applied in urea-N treatments and to the control in the form of KCl at rates equivalent to the K added with KNO_3 in the respective KNO_3 treatments.

Calculations of N uptake and distribution were performed according to the following formulas (Zapata, 1990; Van Cleemput et al., 2008):

Nitrogen derived from the fertilizer (Ndff) and from the soil (Ndfs):

$$\% \text{Ndff} = \frac{\text{atom } \% \text{ } ^{15}\text{N}_{\text{excess}_{\text{plant}}}}{\text{atom } \% \text{ } ^{15}\text{N}_{\text{excess}_{\text{fertilizer}}}} \times 100 \quad (1)$$

$$\% \text{Ndfs} = 100 - \% \text{Ndff} \quad (2)$$

N yield (g/plant):

$$\text{N yield} = \frac{\text{Dry matter yield (g/plant)} \times \% \text{ total N in plant}}{100} \quad (3)$$

Table I. Shoot weights, fruit weights and N contents of pepper plants treated with KNO₃ and urea-N-labeled fertilizers applied at different N doses. The same letters within columns indicate lack of significant difference between N dose treatments (Tukey's test, $\alpha < 0.05$). Student's t-tests were used to compare the no-N control with N dose treatments.

N dose (g plant ⁻¹)	Dry shoot wt (g plant ⁻¹)	Dry fruit wt (g plant ⁻¹)	Shoot-N (g plant ⁻¹)	Fruit-N (g plant ⁻¹)
<i>no-N</i>	34.9	34.7	0.89	0.85
6	67.0 a	84.4 a	2.19 a	2.24 a
12	76.9 b	99.4 a	2.77 b	2.79 a
18	81.8 b	101.7 a	3.11 b	2.77 a

Fertilizer-N uptake or fertilizer-N yield (g/plant):

$$\text{Fertilizer N yield} = \frac{\text{N yield (g/plant)} \times \% \text{Ndff}}{100} \quad (4)$$

Fertilizer-N utilization efficiency (FNUE):

$$\% \text{FNUE} = \frac{\text{Fertilizer N yield}}{\text{Applied fertilizer N}} \times 100. \quad (5)$$

Main factor and interaction effects were analyzed by a GLM procedure and Tukey's multiple comparison tests were performed. The control was compared with fertilizer-N treatments by t-tests using SPSS v.12 for Windows OS.

3. RESULTS AND DISCUSSION

3.1. Plant growth and productivity

Plants grew well in all treatments and were not affected by pest or disease problems. The control treatments with no N addition resulted in a significant reduction of 48%, 59% and 61% in shoot biomass, fruit biomass and plant-N uptake, respectively, compared with the lowest N dose treatments (6 g N plant⁻¹), clearly indicating the need for N fertilization (Tab. I). Fruit-N content and yields from fertilized treatments were within the range reported in the literature (Guteral, 2000). Haynes et al. (1988) reported about 30% higher fruit yields, reaching a peak at 7.5 g N plant⁻¹, following fertigation with urea-N in a slightly acidic soil. However, they also reported a decline of 15 g N plant⁻¹ attributed to soil acidification and development of Al toxicity, an effect not observed in the alkaline soil used in our experiment.

Analysis of Variance showed no statistically significant effect of fertilizer-N source on fruit production, shoot biomass production or N concentration in plants ($p < 0.05$), indicating similar N availability in soil and uptake efficiency by plants independent of the fertilizer-N source (KNO₃ vs. urea). This is in line with earlier results in acidic soils comparing urea with nitrate fertigation sources (Haynes and Swift, 1987) and denotes the efficiency of urea over a wide soil pH range. Haynes (1990) has shown the efficient conversion of urea-N into nitrate-N in fertigation systems. Partial uptake of N by plants in the form

of NH₄⁺ following urea hydrolysis may also lead to a physiological advantage, since the assimilation of NO₃⁻ requires the energy equivalent of 20 ATP mol⁻¹, whereas NH₄⁺ assimilation requires only 5 ATP mol⁻¹ (Salsac et al., 1987). On the other hand, extreme increase in the NH₄-N-to-NO₃-N ratio in the root environment has been linked to drastic reduction in Ca uptake and the occurrence of Ca deficiencies in pepper plants (Bar-Tal et al., 2001a, b), but no Ca deficiency symptoms were observed even at the high levels of urea application in our experiments. The soils in the area typically show high Ca availability. Moreover, urea molecules may move downwards below the emitters before hydrolysis occurs, in contrast to NH₄ cations derived from ammonium fertilizers which remain directly below the emitters and may locally exchange and displace Ca from the soil colloidal surfaces (Haynes and Swift, 1987).

The low N dose (6 g N plant⁻¹) resulted in significantly reduced shoot growth and shoot-N uptake compared with the 12 and 18 g plant⁻¹ doses (Tab. I). Effects of N dose on fruit production and N content were, however, not significant (Tab. I). The results show that the N doses typically applied in the cultivation region (reaching and often exceeding 18 g plant⁻¹) may be drastically reduced in fertigation systems with no significant effect on fruit production.

3.2. Fertilizer-N utilization and total N availability

Analysis of Variance showed that the fertilizer-N utilization efficiency by plants (%FNUE) did not differ significantly ($P < 0.05$) between the two N sources (urea vs. KNO₃). Direct localized application of urea-N in the rhizosphere and the lack of significant microbial N immobilization under the cultivation conditions probably contributed to the similar %FNUE values observed. Preferential plant uptake of NO₃-N compared with NH₄-N has been repeatedly reported (Powlson et al., 1986), but was mainly related to preferential microbial NH₄-N assimilation, under N immobilization conditions. (Jansson et al., 1955; Wickramasinghe et al., 1985; Ehalotis et al., 1998). During the summer period, however, and in the lack of any organic C inputs to the soil, N immobilization rates are expected to be small. This, combined with localized application and efficient nitrification rates apparently leads to poor antagonism between plant roots and soil microbes for N.

Contrary to the lack of N-source effects, the dose of N had a drastic effect on the %FNUE, which dropped from 48% for 6 g N plant⁻¹ to 36% for 12 g N plant⁻¹ and 26% for 18 g N plant⁻¹ (Fig. 1). Assuming that the small rooting systems, which were not removed from the soil following cultivation, contained minimal amounts of fertilizer-N, this shows that over 70% of nitrogen from either N source remained non-utilized in the high N dose applications and prone to leaching during the following fall and winter period.

The % of N in the plant tissues derived from fertilizer (%Ndff) was consistently high, and exceeded 67% and 61% for KNO₃-N and urea-N, respectively, even at the low N-fertilization dose of 6 g N plant⁻¹ (Fig. 2). This is apparently

Table II. Uptake of fertilizer-N and soil-N by pepper plants and respective estimates of available N pools in soil.

Treatments	Fertilizer-N in plant (g plant ⁻¹) ^b	Soil-N in plant (g plant ⁻¹) ^c	Plant-N attributed to fertilizer-N (g plant ⁻¹) ^d	ANI (g plant ⁻¹) ^a	Ratio of fertilizer-N /Soil-N uptake	Available fertilizer-N (g plant ⁻¹) ^e	Estimated available soil-N ^f
KNO ₃ _6	2.92	1.42	2.6 (-11%)	-0.33	2.05	6	2.91
KNO ₃ _12	4.28	1.26	3.8 (-12%)	-0.49	3.41	12	3.51
KNO ₃ _18	4.60	1.06	3.9 (-15%)	-0.69	4.35	18	4.13
Urea_6	2.78	1.75	2.8 (0%)	0.00	1.58	6	3.78
Urea_12	4.26	1.32	3.8 (-10%)	-0.43	3.23	12	3.70
Urea_18	4.91	1.18	4.3 (-12%)	-0.57	4.16	18	4.31
Control		1.75					

^a Added nitrogen interaction (ANI) due to fertilizer effect on soil-N uptake: calculated as: N derived from soil in fertilized plants minus N derived from soil in control plants or as: fertilizer-N in plant estimated by the “difference method” minus fertilizer-N in plant estimated by ¹⁵N enrichment (the calculations are algebraically equivalent).

^b Directly calculated from ¹⁵N enrichment.

^c Calculated as: fertilizer-N in plant minus total-N in plant.

^d Estimated by the “difference method”. Numbers in parenthesis indicate % underestimation compared with direct, ¹⁵N enrichment-based calculations.

^e The fertilizer-N applied.

^f Available soil-N was estimated as: soil N uptake × (available fertilizer-N/fertilizer-N uptake); all of the applied fertilizer-N is considered potentially available.

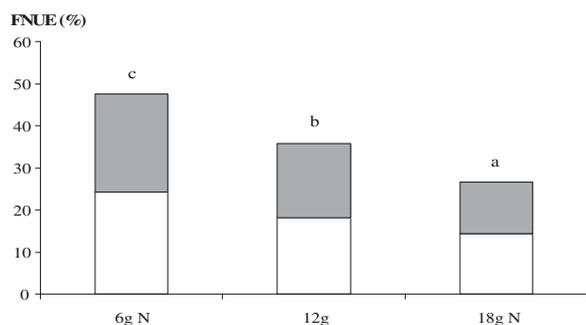


Figure 1. % Fertilizer-N utilization efficiency (%FNUE) in pepper plants treated with KNO₃-N and urea-N fertilizers at 6, 12 and 18 g N plant⁻¹. White bar parts indicate %FNUE in shoots, gray bar parts indicate %FNUE in fruit. The same letters indicate lack of significant difference (Tukey’s test, $\alpha < 0.05$).

attributed to limited availability of soil-N to plants, confirmed by the poor growth of the control plants, and to the high efficiency of the fertilization method. The slight but significant reduction of %Ndff for urea-N compared with KNO₃-N at the low fertilization dose (Fig. 2) was the only indication of potentially reduced availability of urea-N compared with KNO₃-N; this led to a small but significant interaction between N source and N dose. The downward movement of urea deeper in the soil below the emitters (Haynes and Swift, 1987; Haynes, 1990) and the required hydrolysis stage may have reduced urea-derived N accessibility in the low urea dose.

The uptake of a nutrient element by a plant challenged by two or more sources of this nutrient is proportional to the amounts available from each source (Fried and Dean, 1952; IAEA, 2001). The mineralized soil-N and the available fertilizer-N cumulatively contribute to the plant-available N pool. It is generally assumed that the fertilizer-N becomes completely mixed with the soil mineral-N (isotopic

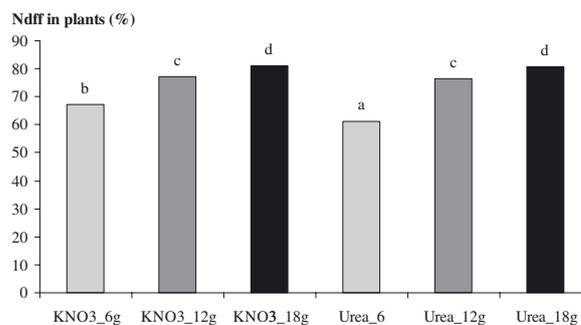


Figure 2. % Nitrogen derived from fertilizer (%Ndff) in pepper plants (shoots+fruits) treated with KNO₃-N and urea-N fertilizers at 6, 12 and 18 g N plant⁻¹. The same letters indicate lack of significant difference (Tukey’s test, $\alpha < 0.05$); comparisons are valid within each fertilizer type (KNO₃ or urea).

equilibrium is achieved) and that plant-N is derived from these N sources, at a ratio proportional to their sizes, according to the assumption of “fractional utilization ratios” (IAEA, 2001). However, when assuming equal accessibility and plant uptake efficiency of fertilizer-N and soil-derived mineral-N, the respective available soil-N in our experiment should have increased considerably as fertilization with N increased (Tab. II).

A real increase in soil-N availability by fertilization (“priming”) is unlikely. “Priming” of soil-N mineralization (Broadbent 1965; Westerman and Kurtz, 1973; Woods et al., 1987) or increased exploitation of soil-N by plant roots (Fried and Broeshart, 1974) may cause a positive “real added nitrogen interaction” (ANI), theoretically described by Jenkinson et al. (1985). However, priming of net soil-N mineralization by higher fertilizer-N applications alone hardly occurs (Jenkinson et al., 1985) when a C substrate is not also applied. Significantly improved root growth leading to better exploitation of

soil-N at higher N doses is also not expected (Burns, 1980); it rarely occurs under fertigation, and was indeed not observed by random destructive sampling in our experiment.

Estimates of N utilization may also be affected by pool substitution phenomena leading to “apparent nitrogen interactions”: accumulation of fertilizer-N may lead to increased immobilization of labeled N, a pool substitution phenomenon during which labeled fertilizer-N stands proxy for soil mineral-N that would have been immobilized in the absence of the fertilizer-N addition. Moreover, accumulation of labeled NH₄-N may lead to displacement reactions with bound NH₄-N in the soil. These positive “apparent ANIs” (Jenkinson et al., 1985) could result in an apparent increase in the available soil-N and may indeed have occurred, especially following urea-N applications. Displacement and pool substitution phenomena, however, lead to overestimations of fertilizer-N uptake by plants when this is calculated as the difference between N in fertilized plants minus N in control plants (Jenkinson et al., 1985; Hart et al., 1986). They do not therefore explain the smaller estimates of fertilizer-N uptake by plants computed by the “difference method” compared with the estimates computed by the ¹⁵N enrichment method and the respective net negative ANIs observed (Tab. II). This underestimation of fertilizer-N uptake by plants by the “difference method” indicates luxury fertilizer-N applications by fertigation, which led to under-exploitation of available soil-N (less soil-N was taken up by the plants compared with control plants). Fertilizer-N may block the uptake of soil-N, once the capacity of the crop to take up N is exceeded (Glendinning et al., 1997), leading to negative ANIs. This is also in line with Pilbeam et al. (2002), who reported underestimation of fertilizer-N uptake by the “difference method” only in a soil poor in organic matter at high fertilization N rates. Indeed, as shown by the ¹⁵N calculations, soil-derived N in plants was progressively reduced with fertilizer-N dose (Tab. II), with the exception of the low urea-N treatment, which showed no reduction in soil-N uptake compared with control (Tab. II). This gave rise to negative ANIs (Tab. II) and led to underestimations of fertilizer-derived N in the fertilized plants when the “difference method” was used (Tab. II, Hamsen, 2003). Negative ANIs may indeed theoretically occur at high fertilizer-N applications that may exceed plant N-uptake capacity; they are favored by lack of N immobilization (Jenkinson et al., 1985) and have been particularly observed at early plant growth stages (Rao et al., 1991).

Further experimentation is needed, to examine the size and consistency of these phenomena in fertigation studies, but it clearly appears from this work that continuous localized fertilizer-N applications may result in efficient N uptake even at low doses. It also appears that urea movement below the emitters, longer retention time in soils and greater potential for N immobilization via pool substitution and displacement reactions with bound soil-N may reduce negative ANIs, at least for small N doses. It is concluded that under-exploitation rather than “priming” of soil-N occurs as a result of N fertigation, leaving more mineral-N prone to leaching than the residual fertilizer-N calculations would assume.

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