**Crop response of greenhouse soil-grown cucumber to total available N in a Nitrate Vulnerable Zone**

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**ABSTRACT**

The effects of increasing total available N (TAN, i.e. the sum of soil mineral N at planting, N mineralized from organic matter, and mineral fertilizer N applied by fertigation) on soil-grown cucumber were evaluated. Parameters assessed were: dry matter production (DMP), yield, crop N uptake, nitrogen use efficiency (NUE) and the potential $NO_3^-$ leaching loss. The study was conducted in three growing seasons, in autumn, spring and late spring. Additionally, three commercial cultivars were examined to assess possible cultivar differences. Five N treatments were applied, in the Autumn and Spring crops, as different N concentrations in nutrient solution that were applied in all irrigations throughout the crops. The applied N concentrations were N1: 0.7-1.0 mmol $L^{-1}$, N2: 4.7-5.7 mmol $L^{-1}$, N3: 12.1-13.8 mmol $L^{-1}$, N4: 16.3-17.6 mmol $L^{-1}$ and N5:

19.7-21.1 mmol $L^{-1}$. The cultivar ´Strategos´ was used in both crops. Three N treatments (N1: 2.4 mmol $L^{-1}$; N2: 8.5 mmol $L^{-1}$and N3: 14.8 mmol $L^{-1}$) were continuously applied throughout the Late Spring crop to three different cultivars (´Strategos´, ´Padrera´, and ´Miltre´). DMP, total and marketable yield, relative to maximum value, were strongly related to TAN in linear-plateau relationships for the three growing seasons and three cultivars. Using relationships that include data from the three cropping seasons and cultivars, TAN values for maximum DMP, total yield, and marketable yield were 222±15 kg $ha^{-1}$, 228±15 kg $ha^{-1}$ and of 221±14 kg $ha^{-1}$, respectively, for the Autumn, Spring and Late Spring crops. The relationships of crop N uptake to TAN, and DMP to crop N uptake, were asymptotic and described by a logarithmic equation. The relationship of N uptake efficiency to TAN (e.g. N uptake/TAN) was described by an exponential decay equation. Considering all crops and cultivars, these relationships were described by individual equations with a $R^2$ value of 0.75-0.96. The consistency of these relationships and those for yield and DMP indicate that there is general response of greenhouse-grown cucumber to N, which is not affected by growing season or cultivar. Measured $NO_3^-$ leaching losses were low because of good irrigation management. Residual mineral N was considered equivalent to potential leaching loss; residual soil mineral N increased exponentially with TAN, being 196 and 330 kg N $ha^{-1}$ for the highest N treatments in the Autumn and Spring crops, respectively.

Keywords: N fertilizer requirements, N management, *cucumis sativus*, nitrogen, yield, fertigation, NUE, apparent recovery, nitrate leaching

## 1. Introduction

Intensive vegetable production systems are generally characterized by large applications of mineral nitrogen (N) fertilizer (Neeteson, 1994; Ramos et al., 2002; Thompson et al., 2007). Commonly, the N supply considerably exceeds crop N requirements (Ju et al., 2006; Soto et al., 2015) resulting in nitrate ($NO_3^-$) leaching loss (Padilla et al., 2018). The greenhouse-based intensive horticultural system of SE Spain, located close to the coast of southeastern (SE) Spain, is associated with substantial nitrate ($NO_3^-$) contamination of aquifers, and eutrophication of natural water bodies. In the province of Almeria, where most of greenhouses are concentrated, the aquifers underlying these areas have high and increasing $NO_3^-$ concentrations (Pulido-Bosh, 2005). Because of the $NO_3^-$ contamination and eutrophication of surface water bodies, most of the areas in SE Spain with appreciable areas of greenhouses have been declared “Nitrate Vulnerable Zones” in accordance with the European Union (EU) Nitrate Directive (Anonymous, 1991), which requires the adoption of improved management practices. Additionally, production certification schemes are increasingly requiring the adoption of improved nutrient management practices.

In Almeria, approximately 90% of the greenhouse vegetable crops are grown in soil, and the rest in soilless systems. Cucumber is grown on approximately 5,000 ha in greenhouses in province of Almeria (MAPAMA, 2017), in two different seasonal growing cycles, in autumn or spring. In each of these two growing seasons, there is appreciable variation in planting dates (Reche Mármol, 2011).

Common practice for N management of commercial soil-grown cucumber in Mediterranean greenhouses, using fertigation, is the use of standard recipes in which N concentrations of 12–16 mmol $L^{-1}$ (Fernández and Camacho Ferre, 2007; Reche Mármol, 2011) are applied throughout the crop. Additionally, manure applications supplying approximately 1,000 kg N $ha^{-1}$ are commonly made once every several years (Thompson et al., 2007). N balance calculations for greenhouse-grown vegetable crops in SE Spain indicated that the supply of N was more than two times crop N uptake, and that N mineralized from the manure applications are an appreciable part of the excess (Jadoski et al., 2013). Additionally, considerable amounts of soil mineral N can be present at planting (Granados et al., 2013). The amounts of soil mineral N present at planting and of N mineralized from manure can be appreciable; however, they are normally not considered when planning mineral N fertilizer applications (Thompson et al., 2007).

Most published studies examining the effect of different N rates on greenhouse cucumber production and/or N losses have been conducted in soilless systems (Jasso-Chaverria et al., 2005; Kotsiras et al., 2005; Ruiz and Romero, 1998). The few studies conducted with greenhouse cucumber in soil, focused on applied mineral N fertilizer without consideration of soil N sources (Güler et al., 2006; Zhang et al., 2011). Effective N management of soil-grown vegetable crops, such as cucumber, requires that all sources of N be considered (Thompson et al., 2017a; 2017b). Soto et al. (2015) demonstrated, in greenhouse tomato, that by considering mineral N fertilizer as a supplement to other N sources (soil mineral N at planting, mineralized N), maximum production was achieved with a relatively small application of mineral N, a high recovery of total available N, and with a reduced risk of N loss to the environment.

Nitrogen use efficiency (NUE) is an indicator of the production of dry matter or yield per unit of supplied N, for example of TAN (Moll et al., 1982). It can be partitioned into two components, crop N uptake efficiency ($N_{upt}E$), i.e. N uptake per unit of supplied N, and N utilization efficiency ($N_{ut}E$), i.e. dry matter or yield per unit of crop N uptake ($N_{ut}E_{DMP}$ and $N_{ut}E_{Y}$, respectively). It has been suggested that $N_{upt}E$ is the more influential component under high N availability, whereas $N_{ut}E$ is more influential under low N availability (Moll et al., 1982). NUE and its components are well established indicators that have been used in many agronomic studies to characterize the response of crops to N supply (Sadras and Lemaire, 2014).

Most studies that have been conducted, examining the response of greenhouse cucumber to applied N, have used individual cultivars; very few studies have explored possible differences between cultivars in their response to N. Additionally, the response of cucumber to N could vary with growing season, as observed by Kotsiras et al. (2005).

Understanding the agronomic response of greenhouse cucumber to increasing total available N (TAN) will assist in determining optimal N fertilizer application, thereby reducing the large $NO_3^-$ leaching losses that are associated with these production systems. Using this approach, the optimal TAN is determined and mineral N fertilizer is considered as a supplement to soil N sources.

The objectives of this study were to evaluate the effects of increasing amounts of TAN on soil-grown cucumber on: (1) dry matter production, crop N uptake, and the distribution of dry matter and N between organs, (2) yield and yield components, (3) NUE and its constituent components, and (4) possible $NO_3^-$ leaching loss. An additional major aim was to determine optimal N requirements using segmented linear regression analysis. The study was conducted in three contrasting growing seasons in autumn-winter, spring, and in late spring, and with three different cultivars in the Late Spring crop to determine possible effects of growing season and cultivar on the response of cucumber to N.

## 2. Material and Methods

### *2.1. Experimental site*

Three cucumber (*Cucumis sativus* L.) crops were grown, in soil, in a greenhouse under similar conditions to those of commercial intensive vegetable production in southeastern (SE) Spain. The experimental work was conducted in a plastic greenhouse at the Experimental Station of the University of Almeria, located in Retamar, Almeria, SE Spain, (36°51 N, 2°16 W and 92 m elevation). The greenhouse had a multi-span structure with polycarbonate walls and a roof of low density polyethylene cladding with transmittance to photosynthetically active radiation (PAR) of approximately 60%. It had no heating, passive ventilation (two lateral side panels and flap roof windows), an east-west orientation, and crop rows aligned north-south. The cropping area was 1,300 $m^2$.

The soil of the greenhouse was an artificial layered “enarenado” typical of the region (Thompson et al., 2007), consisting of a 30 cm layer of imported silty loam textured soil placed over the original loam soil and a 10 cm layer of fine gravel (mostly 2–5 mm diameter) placed on the imported soil as a mulch. The main properties of the soil were described by Soto et al. (2014). At greenhouse construction in July 2007, before adding the final gravel layer, 200 $m^3$ $ha^{-1}$ of mature sheep manure (63% dry matter, 1.7% N content and 0.7 Mg $m^{-3}$ density) was mixed into the top layer of the imported soil following local practices (Céspedes et al., 2009; Thompson et al., 2007).

Above-ground drip irrigation was used which combined irrigation and mineral fertilizer application (fertigation). Nutrients were applied by fertigation every 1–2 days depending on crop demand. Each plant was immediately adjacent to an emitter, which had a discharge rate of 3 L $h^{-1}$. The uniformity coefficient of the drip system was >95% at the beginning of the crops.

For the Autumn and Spring cucumber crops, the greenhouse was organized into a total of 24 plots, measuring 6 m by 6 m; 20 plots were used in the current study. Each plot contained three paired lines of drip tape with 12 drip emitters in each line. In each line, the distance between plants was 0.5 m. Separation between lines within a paired line was 0.8 m, and the distance between adjacent paired lines was 1.2 m, giving a plant density of 2 plants $m^{-2}$ and 72 plants in each replicate plot. For the third cucumber crop, the greenhouse was divided into 12 plots of 12 x 6 m each. Each plot contained 6 paired lines of plants, with 24 plants per line and 144 plants per replicate plot, with the same configuration and planting density as in the previous crops. Sheets of polyethylene film (250 $\mu$m thickness) buried to 30 cm depth acted as a hydraulic barrier between plots. The greenhouse was divided longitudinally into northern and southern plots by a 2 m path along its east-west axis, with two plots of each N treatment in the northern and southern sides. There were border areas along the edges of the greenhouse.

*2.2. Cucumber crops and experimental N treatments*

Three cucumber crops were grown, each in different growing seasons, all with different N treatments; one crop was grown with different cultivars. All cultivars used in the three crops were long, Dutch-type cucumber. The first crop was grown with an autumn cycle in 2013 (hereafter referred to as the Autumn crop), the second with a spring cycle in 2014 (hereafter referred to as the Spring crop), and the third with a late spring cycle in 2018 (hereafter referred to as the Late Spring crop). In the Autumn and Spring crops, the cultivar ‘Strategos’ (Syngenta International AG, Basel, Switzerland) was used; five different N treatments were applied by fertigation, and there were four replicate plots per treatment arranged in a randomized block design.

In the Late Spring crop, three different cucumber cultivars, ‘Strategos’, ´Pradera’ (Rijk Zwaan Zaadteelt en Zaadhandel B.V., De Lier, The Netherlands) and ‘Mitre’ (Semillas Fitó, Barcelona, Spain) were examined. The three cultivars were planted in each plot, with two paired lines of plants of each cultivar per plot. The three paired lines of the three cultivars were randomly distributed within each plot. There were three different treatments of N applied by fertigation, with four replicate plots per treatment. The distribution of N treatments in the plots followed a randomized block design.

All crops were transplanted as four-week old seedlings upon development of the first true leaf. The Autumn crop was grown from 5 September to 22 November 2013 (78 days from transplant to end of crop), the Spring crop from 4 March to 28 May 2014 (85 days from transplant to end of crop), and the Late Spring crop from 24 April to 3 July 2018 (70 days from transplant to end of crop). In all crops, the plant density was 2 plants $m^{-2}$. In the Autumn and Spring crops, there were five treatments that consisted of five different N concentrations in the nutrient solution applied by fertigation, in every irrigation, from 15 days after transplanting (DAT) (Autumn) and from 4 DAT (Spring) until the end of the crops. The N treatments were very N deficient (N1), N deficient (N2), conventional N management (N3), excessive N (N4) and very excessive N (N5), according to the N concentration in the applied nutrient solution (Table 1). There were some slight differences in the applied N concentration of equivalent treatments between the two crops. In the Late

**Table 1**

General description of the N treatments at each crop including total irrigation volume and drainage, soil mineral N at transplanting, N fertigation treatments defined on the basis of N concentration of the applied nutrient solution, total amount of N applied, total available N (TAN) and $NO_3^-$ leaching. N mineralization, included in the calculation of TAN was 71 and 43 kg N $ha^{-1}$ for the Autumn and Spring crops respectively. NA: data not available

| Crop | N Treatment | Irrigation amount (mm)[a] | Drainage (mm) | Mineral N at planting (kg N $ha^{-1}$) | N concentration in nutrient solution (mmol $L^{-1}$)[b] | Total N applied (kg N $ha^{-1}$)[a] | TAN (kg N $ha^{-1}$) | $NO_3^-$ leaching (kg N $ha^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| Autumn | N1 | 113 | 27 | 47 | 0.7 | 12 | 131 | 8.6 |
| | N2 | 101 | 25 | 53 | 4.7 | 62 | 186 | 7.8 |
| | N3 | 137 | 24 | 49 | 12.1 | 218 | 339 | 6.7 |
| | N4 | 133 | 29 | 57 | 16.3 | 281 | 409 | 8.4 |
| | N5 | 147 | 34 | 86 | 19.7 | 380 | 537 | 8.7 |
| Spring | N1 | 122 | 36 | 16 | 1.0 | 16 | 75 | 1.1 |
| | N2 | 140 | 20 | 19 | 5.7 | 109 | 172 | 1.6 |
| | N3 | 182 | 20 | 30 | 13.8 | 344 | 418 | 2.1 |
| | N4 | 197 | 25 | 28 | 17.6 | 472 | 544 | 9.4 |
| | N5 | 195 | 33 | 47 | 21.1 | 565 | 655 | 10.9 |
| Late-Spring | N1 | 114 | NA | 24 | 2.4 | 38 | 62 | NA |
| | N2 | 253 | NA | 35 | 8.5 | 302 | 337 | NA |
| | N3 | 247 | NA | 63 | 14.8 | 515 | 578 | NA |

[a] Total N and total irrigation applied correspond to the complete cropping cycle
[b] N concentration values are for the period of N treatments, which commenced at 15, 4 and 9 days after transplanting for the Autumn, Spring and Late-Springs crops respectively

Spring crop, the N treatments applied from 9 DAT until the end of the crop, were very deficient (N1), optimal (N2) and excessive (N3) treatments (Table 1). For the first 3 to 5 days depending on the crop, seedlings were irrigated with water only (0.04-0.12 mmol N $L^{-1}$). From then until the beginning of N treatments, all plots received a common nutrient solution of 1.5 and 1.0 mmol N $L^{-1}$ in the Autumn and Late Spring crops, respectively; in the Spring crop, the different N treatments commenced immediately after water application. In all treatments, most N was applied as nitrate ($NO_3^-$) (92% of applied N), the rest as ammonium ($NH_4^+$). The other macro and micronutrients were applied in the nutrient solution to ensure they were not limiting. Before transplanting the crops, a series of large irrigations were applied to leach residual $NO_3^-$ from the soil and to homogenize the soil profile between plots. The soil mineral N contents, in the first 60 cm depth at transplanting, for each crop are shown in Table 1.

The crops were managed following local practices, being periodically pruned and supported by nylon cord guides. Irrigation was scheduled to maintain the soil matric potential in the root zone, at 15 cm depth, within −10 to −30 kPa; one tensiometer (Irrometer, Co., Riverside, CA, USA) per plot was used to measure soil matric potential. Topping (the removal of the main apical shoot to arrest stem elongation) was conducted at 35 DAT in the Autumn crop, at 66 DAT in the Spring crop, and at 46 DAT in the Late Spring crop, following local practice. High temperature within the greenhouse was controlled by white-washing the plastic cladding with a $CaCO_3$ suspension, which was applied nine days before transplanting for the Autumn crop (0.30 kg $L^{-1}$), at 34 DAT for the Spring crop (0.18 kg $L^{-1}$), and 4 days before transplanting (0.20 kg $L^{-1}$) for the Late Spring crop.

*2.3. Measurements*

Air temperature and relative humidity were measured inside the greenhouse with a relative humidity/temperature probe (model 41382V, R.M. Young Company, MI, USA ) encased in an aspirated protective radiation shield (model 43502, R.M. Young Company, MI, USA) and solar radiation with a pyranometer (model SKS 1110, Skye Instruments, Llandrindod Wells, Wales, UK). All data were recorded and stored using a data logger (model CR10X, Campbell Scientific Inc., Utah, USA).

In all treatments, all measurements of plant and soil parameters were the mean of four values, each from an individual replicate plot. In the Late Spring crop, plant measurements were the mean of four replicate measurements from each N treatment and cultivar. In the Autumn and Spring crops, soil was sampled and analyzed for mineral N ($NO_3^-$ and $NH_4^+$) immediately before and at the end of each crop. In the Late Spring crop, soil mineral N was only measured before planting. Soil was sampled to 60 cm in each replicate plot of each treatment, in 15 cm depth increments in the Autumn and Spring crops, and 20 cm increments for the Late Spring crop. To deal with the heterogeneity associated with combined drip irrigation and fertigation, each soil sampling in each plot was conducted in three sampling positions in relation to a representative emitter and plant, being (1) 5 cm from the drip emitter, (2) mid-way between lines within paired lines, and (3) mid-way between two paired lines. In the Late Spring crop, only positions 1 and 3 were sampled because no notable differences in mineral N between positions 1 and 2 were observed in previous work. Each depth increment from each sampling position within each plot was treated as a separate sample. Soil mineral N content was determined following extraction with potassium chloride (KCl) (40 g moist soil: 200 mL 2 mol $L^{-1}$ KCl). $NO_3^-$ and $NH_4^+$ concentrations in the extracts were determined with an automatic continuous segmented flow analyzer (model SAN++, Skalar Analytical B.V., Breda, The Netherlands). Soil mineral N ($NO_3^-$–N plus $NH_4^+$–N) was calculated as: (0.50 x position 1) + (0.15 x position 2) + (0.35 x position 3), and for the Late Spring crop as (0.65 x position 1) + (0.35 x position 3).

The volume of each irrigation volume applied to each treatment was measured with volume meters. Three times per week, two replicate samples of applied nutrient solution of each treatment were collected from separate emitters, to determine the concentration of $NO_3^-$

and $NH_4^+$. In the Autumn and Spring crops, drainage was collected from each treatment using two replicate free-draining, re-packed lysimeters (4 m long x 2 m wide x 0.7 m deep) located in the southern side of the greenhouse. The bottom and walls of the lysimeters were lined with butyl rubber. The soil profile in the lysimeter reproduced that of the outside area described above, to a depth of 0.7 m, with a layer of gravel placed between the butyl rubber sheet on bottom of the lysimeter and the layered soil. Lysimeter drainage volumes were measured three times per week. Representative sub-samples of drainage from each lysimeter were analyzed for concentration of $NO_3^-$; the concentration of $NH_4^+$ was negligible. The concentration of $NO_3^-$ in drainage was analyzed using the automatic segmented flow analyzer described previously.

Dry matter production of pruned material, fruit production and final above-ground biomass was determined in each crop. Pruned dry matter was determined from eight marked plants in each replicate plot in the Autumn and Spring crops, and from each cultivar in each replicate plot in the Late Spring crop. In the Autumn, Spring and the Late Spring crops, pruning started at 20, 22 and 24 DAT respectively; there were a total of six prunings in the Autumn and Late Spring crops, and eight in the Spring crop. The dry matter content of removed material, from each pruning was determined by oven-drying at 65°C until constant weight. Fruit was collected from the same eight marked plants every three or four days depending on fruit maturity, during the fruit harvest period. There were six fruit harvests in the Autumn crop, and eight in both the Spring and Late Spring crops. At each fruit harvest, fresh and dry weight were determined. Fresh production was separated into marketable and non-marketable fruit, and the fruit number and mean fresh fruit weight were determined.

The total fresh weight of four of the eight marked plants, previously used for pruning and fruit production, was determined after the final harvest, in the Autumn and Spring crops. The sampled plants were cut at ground level, and were then separated into leaves, stems and un-harvested fruit. Representative sub-samples of each component (approximately 20% of fresh weight) were oven-dried at 65°C until constant weight. In the Late Spring crop, at the end of the crop, two complete plants per cultivar and replicate plot were sampled from the eight marked plants, and total fresh weight of each component (i.e. leaves, stems and fruit) was measured, and the dry matter content determined as described previously. Total dry matter production (DMP) at the end of each crop was determined as the sum of dry matter mass of leaves, stems and fruits of the final biomass, plus all previously sampled pruned material and harvested fruit.

Representative individual samples of leaves, stems, and fruit from the final biomass sampling, and of pruned material and harvested fruits, from each replicate plot and cultivar, were ground sequentially in knife and ball mills. Total N content of each sample was determined using a Dumas-type elemental analyzer system (model Rapid N, Elementar, Analysensysteme GmbH, Hanau, Germany). The mass of N in each component was calculated from the N content of the sample and the corresponding dry matter mass of the sample. Total crop N uptake (kg N $ha^{-1}$) in each replicate plot and variety, was the sum of the amounts of N in all relevant components, including previously pruned material and harvested fruit, as was done for the calculation of total DMP.

In the Autumn and Spring crops, total available N (TAN) was calculated as the sum of soil mineral N at planting, mineral N applied in fertigation, and N mineralized from soil organic matter.  The amount of N mineralized from soil organic matter was calculated for each crop using the N balance approach of Feller and Fink, (2002) based on the linear relationship between N supply (initial soil mineral N plus N applied by fertigation) and N recovery (crop N uptake plus N leached plus soil mineral N at the end of the crop). In this approach, N mineralization is the independent term of the linear regression equation relating N supply to N recovery (Feller and Fink, 2002). All the component of the N balance were measured as described above; it was assumed that gaseous N losses were negligible. Using this N balance calculation, mineralized N was determined to be 71 and 43 kg N $ha^{-1}$ for the Autumn and

Spring crops, respectively. In the Late Spring crop, TAN was calculated as the sum of soil mineral N at planting and mineral N applied in fertigation. For this crop, mineralization of N was considered to be negligible on account of (i) the period of eleven years since manure application at greenhouse construction, (ii) the short growing season, and (iii) the negligible value of mineralized N determined, using a N balance calculation, in a preceding pepper crop. There was a three year period between the Spring crop and the Late Spring crop. In this work, the treatments were evaluated in the context of the amounts of N available to the crop (TAN) rather than the amounts or the concentration of mineral N fertilizer applied by fertigation.

*2.4. Data analysis*

The experimental data were evaluated using analysis of variance (ANOVA), after verifying assumptions of normality and equal variance. If the main effects or interactions were significant at $P<0.05$, the least significant difference (LSD) test was conducted for multiple comparison of means. The results of the analysis of variance are presented as: no significant difference (ns), significant at $P \leq 0.05$ (*), very significant at $P \leq 0.01$ (**) and highly significant at $P \leq 0.001$ (***). All statistical procedures were conducted with the STATISTICA V. 10.0 software (StatSoft Inc. Tulsa, OK, USA).

For the relationships of both yield and DMP with TAN, segmented linear regression analysis was conducted following Gianquinto et al., (2011) and Ordoñez et al., (2015). For this analysis, yield, marketable yield and total dry matter production (DMP) were expressed as relative values in relation to the maximum value obtained in each crop. This analysis was conducted using combined data from the three crops and cultivars. Segmented linear regression consists of two linear regression lines, an inclined segment described by the equation $y = ax + b$ (*if* $x < x_0$), and a horizontal segment described by the equation $y = c$ (*if* $x \geq x_0$), where $a$ and $b$ are, respectively, the slope and intercept values of the inclined segment, and $x_0$ is the $x$ value of the intersection of the two segments. The first segment implies a linear increase in yield ($y$) (or

DMP) with increasing N, in this case with increasing TAN (*x*). The second segment implies that the yield response is flat at higher N addition, in this case with higher TAN values. The value $x_0$ is the amount of TAN at which the maximum response occurs and after which the maximum response is constant. The software GraphPad Prism 6.01 (GraphPad Software, Inc., La Jolla, CA, USA) was used to perform the segmented regression analysis and to obtain values of $R^2$ and SEE and the intersection of the relationship ($x_0$). Additional regression analyses were made with the CurveExpert Professional 2.2.0 software (Daniel G. Hyams) to determine relationships between variables, and the best-fitting model was selected according to the $R^2$ value.

Nitrogen use efficiency (NUE) was calculated, for each treatment in each crop, as the ratio between DMP or total fresh yield and TAN (Moll et al., 1982). The components of the NUE were calculated following Moll et al., (1982). N uptake efficiency ($N_{upt}E$), was calculated as the ratio between crop N uptake and total available N (TAN), N utilization efficiency for biomass ($N_{ut}E_{DMP}$) as the ratio between DMP and N uptake, and N utilization efficiency for total yield ($N_{ut}E_{Y}$) as the ratio between total fruit production and N uptake.

## 3. Results

### *3.1. Climatic conditions*

Daily average values of air temperature and of the daily integral of solar radiation, inside the greenhouse for the growing periods of the three crops, are presented in Fig. 1. The climatic conditions reflected the different growing seasons, with values of temperature and solar radiation decreasing during the autumn-winter period and increasing during the spring or late spring periods. Air temperature ranged from 25 to 12°C in autumn-winter, from 16 to 21°C in spring, and from 20 to 26°C in late spring (Fig. 1a). Solar radiation decreased from 11 to 4 MJ $m^{-2} d^{-1}$ in the autumn-winter period, and was relatively constant, with fluctuations, at approximately 11 MJ $m^{-2} d^{-1}$ during spring, and increased to 10–13 MJ $m^{-2} d^{-1}$ in late spring (Fig.

1b). In general, the values of climatic parameters were within the normal ranges for plastic greenhouses, without active climate control, on the Mediterranean coast.

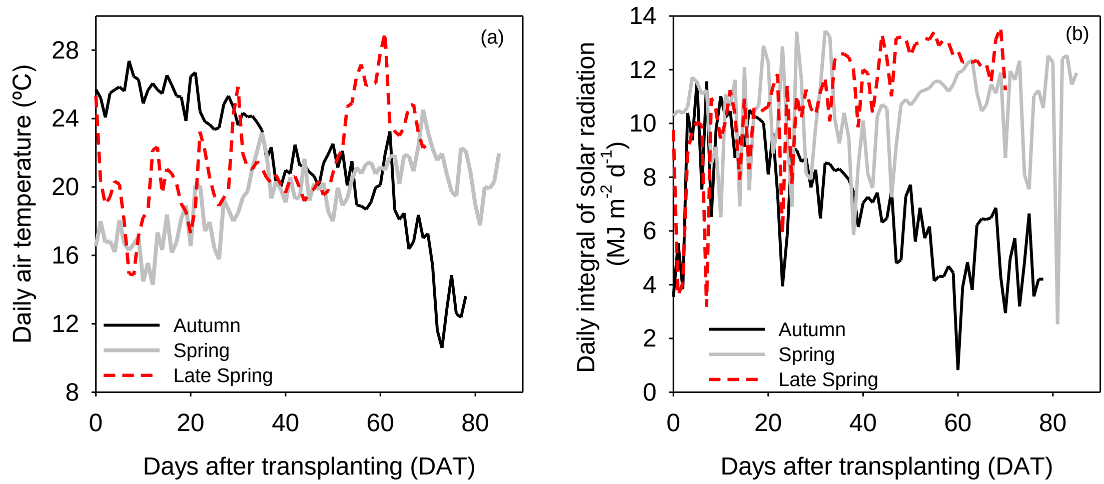


**Fig. 1.** (a) Daily average air temperature and (b) daily integral of solar radiation in the greenhouse during the Autumn, Spring and Late Spring cucumber crops, from transplant to the end of the crop.

*3.2. Effect of N treatments on dry matter production, N uptake and yield*

The effect of the N treatments on dry matter production (DMP), crop N uptake, yield and yield components are presented for the Autumn and Spring crops in Table 2, and for the Late Spring crop in Table 3. The N treatments affected total DMP with highly significant differences between N treatments in the three crops. In the Late Spring crop, the effects of cultivar and of the interaction N x cultivar were not significant indicating similar response of DMP to N for the three cultivars. In the Autumn crop, total DMP was significantly higher (25% more) in treatments N3, N4 and N5 compared to N1 and N2; there were no significant differences between N1 and N2, or between N3, N4 and N5. In the Spring crop, treatment N1 had significantly lower DMP (55% less) than the other four N treatments, which were not significantly different from one another. In the Late Spring crop, DMP was significantly lower in N1 compared to N2 and N3, which were not significantly different from one another (Table 3). In the Autumn crop, there were no significant differences between treatments in the proportion of total DMP as fruit (i.e. harvest index, HI). In the Spring crop, treatment N1 had a significantly lower HI, with no significant differences between treatments N2 to N5 (Table 2). Similar results were obtained in the Late Spring crop where HI in treatment N1 was significantly less than the other two treatments (Table 3). HI was significantly higher in ´Strategos´ than in ´Pradera´ and ´Miltre´ which were similar (Table 3). There were no significant differences in the interaction N x cultivar indicating that N had the same effect on the three cultivars.

There were highly significant differences in crop N uptake between treatments in both the Autumn and Spring crops (Table 2). In the Autumn crop, crop N uptake was significantly higher in treatments N3, N4 and N5 compared to N1 and N2, with no significant differences between N1 and N2 or between N3, N4 and N5. Crop N uptake was almost double in treatments N3–N5 compared to N1 and N2. In the Spring crop, crop N uptake increased progressively from treatments N1 to N3 and then was relatively constant; there were significant differences between N1 and N2 and between N2 and N3 (Table 2). In all cultivars in the Late Spring crop, crop N uptake was significantly lower in treatment N1 than in the others; there were no significant differences in crop N uptake between the three cultivars (Table 3), and the interaction N x cultivar was not significant.  In the Autumn crop, differences between treatments were significant for the proportion of N uptake by the fruits (e.g. NHI) (Table 2). In the spring crop, despite a tendency for NHI to decrease from treatment N2 on as N supply increased, and a lower value in treatment N1, the differences between treatments in NHI were not statistically significant (Table 2). In the Late Spring crop, differences between cultivars and between N treatments in NHI were very significant, with higher NHI in the cultivar ´Strategos´ than ´Miltre´ and lower NHI in treatment N1 than in N2 and N3 (Table 3) as observed in the Spring crop. However, there no significant differences occurred in the interaction N x cultivar.

**Table 2**

Total above-ground dry matter production (DMP), harvest index (HI), total crop N uptake and nitrogen harvest index (NHI), total and marketable yield, total fruit number and mean fruit weigh for each N treatment of the Autumn and Spring crops. Different letters indicate significant differences ($P$<0.05) between means according to the LSD procedure. A summary of the analysis of variance is presented with: no significant (ns), significant at $P$≤0.05 (*), very significant at $P$≤ 0.01 (**) and highly significant at $P$≤0.001 (***).

| Crop | DMP (t ha$^{-1}$) | HI | Crop N uptake (kg N ha$^{-1}$) | NHI | Total yield (t ha$^{-1}$) | Marketable yield (t ha$^{-1}$) | Total fruit number (Fruits m$^{-2}$) | Mean fruit weight (g) |
|---|---|---|---|---|---|---|---|---|
| Autumn | | | | | | | | |
| N1 | 3.9 a | 0.56 | 97 a | 0.64 a | 78 a | 69 a | 16.5 a | 473 |
| N2 | 3.7 a | 0.55 | 98 a | 0.62 a | 80 a | 71 a | 16.5 a | 478 |
| N3 | 4.9 b | 0.58 | 163 b | 0.60 ab | 105 b | 96 b | 23.3 b | 454 |
| N4 | 5.2 b | 0.58 | 189 b | 0.61 ab | 112 b | 106 b | 27.3 c | 419 |
| N5 | 5.3 b | 0.55 | 190 b | 0.57 b | 111 b | 103 b | 26.1 cb | 441 |
| *Significance* | *** | ns | *** | ** | *** | *** | *** | ns |
| Spring | | | | | | | | |
| N1 | 2.6 a | 0.33 a | 37 a | 0.48 | 24 a | 11 a | 7.7 a | 313 a |
| N2 | 5.4 b | 0.46 b | 125 b | 0.56 | 87 b | 69 b | 17.8 b | 484 b |
| N3 | 5.8 b | 0.48 b | 224 c | 0.52 | 105 c | 96 c | 26.0 c | 408 c |
| N4 | 5.5 b | 0.48 b | 230 cd | 0.52 | 100 c | 91 c | 25.8 c | 383 c |
| N5 | 6.0 b | 0.48 b | 247 d | 0.52 | 112 c | 100 c | 27.4 c | 409 c |
| *Significance* | *** | *** | *** | ns | *** | *** | *** | *** |

**Table 3**

Total above-ground dry matter production (DMP), harvest index (HI), total crop N uptake and nitrogen harvest index (NHI), total and marketable yield, total fruit number and mean fruit weigh for each N treatments and cultivar of the Late Spring crop. Different letters indicate significant differences (P<0.05) between means according to the LSD procedure; upper case letter refer to comparison between cultivars and lower case between N treatments. A summary of the analysis of variance is presented with: no significant (ns), significant at $P$≤0.05 (*), very significant at $P$≤0.01 (**) and highly significant at $P$≤0.001 (***).

| Cultivar | DMP ($t\ ha^{-1}$) | HI | Crop N uptake ($kg\ N\ ha^{-1}$) | NHI | Total yield ($t\ ha^{-1}$) | Marketable yield ($t\ ha^{-1}$) | Total fruit number (Fruits $m^{-2}$) | Mean fruit weight (g) |
|---|---|---|---|---|---|---|---|---|
| Strategos | 4.4 | 0.37 A | 151 | 0.45 A | 59 | 31 A | 21.7 A | 269 |
| N1 | 2.4 a | 0.32 a | 51 a | 0.43 a | 20 a | 6 a | 10.9 a | 190 a |
| N2 | 5.6 b | 0.38 b | 193 b | 0.44 b | 79 b | 40 b | 23.8 b | 332 b |
| N3 | 5.3 b | 0.42 b | 208 c | 0.47 b | 77 b | 47 b | 27.3 c | 285 c |
| Pradera | 4.4 | 0.34 B | 149 | 0.41 AB | 60 | 40 B | 23.4 B | 237 |
| N1 | 2.1 a | 0.26 a | 40 a | 0.39 a | 19 a | 9 a | 12.6 a | 151 a |
| N2 | 5.3 b | 0.38 b | 180 b | 0.43 b | 79 b | 51 b | 27.8 b | 283 b |
| N3 | 5.9 b | 0.38 b | 228 c | 0.42 b | 82 b | 60 b | 29.9 c | 276 c |
| Miltre | 4.5 | 0.33 B | 156 | 0.38 B | 60 | 31 A | 23.1B | 244 |
| N1 | 2.1 a | 0.23 a | 45 a | 0.29 a | 18 a | 6 a | 10.5 a | 170 a |
| N2 | 5.7 b | 0.39 b | 202 b | 0.43 b | 86 b | 43 b | 27.3 b | 321 b |
| N3 | 5.7 b | 0.38 b | 221 c | 0.41 b | 75 b | 42 b | 31.3 c | 240 c |
| Analysis of variance | | | | | | | | |
| N | *** | *** | *** | ** | *** | *** | *** | *** |
| Cultivar | ns | * | ns | ** | ns | ** | ** | ns |
| N x Cultivar | ns | ns | ns | ns | ns | ns | ns | ns |

The N treatments had highly significant effects (*P*≤0.001) on total yield and marketable yield in the three crops (Tables 2 and 3). The highest total yield and marketable yield were obtained in treatments N3, N4 and N5 in the Autumn and Spring crops, and in treatments N2 and N3 in the Late Spring crops. There were no statistical differences between these treatments (Tables 2 and 3). In the Autumn crop there were no significant differences in total yield and marketable yield between treatments N1 and N2, whereas the differences between N1 and N2 were significant (*P*≤0.05) in the Spring crop (Table 2). In all crops, the number of fruits increased with N treatment, being highest in N4=N5 in the Autumn crop, in N3=N4=N5 in the Spring crop, and in N3 in the Late Spring crop. In the Spring and Late Spring crops, there were differences between N treatments in the mean fruit weight which was lowest in the N1 and highest in the N2 treatment (Tables 2 and 3).

In the Late Spring crop, there were no significant differences between cultivars in total yield, marketable yield, and mean fruit weight. There were differences between cultivars in the number of fruit, with the highest values for the cultivar ´Strategos´ (Table 3). There were no significant differences in the interaction N x cultivar. Compared to the Autumn and Spring crops, total yield was lower and total yield and marketable yield were considerably lower in the Late Spring crop, due to (i) the shorter cycle, (ii) the less favourable (hotter) climatic conditions, and (iii) the incidence of powdery mildew and thrips in the second part of the growing season. The thrips affected fruit quality of fruits thereby reducing the fraction of marketable fruit.

*3.3. Relationships of dry matter production and yield to crop N uptake*

In the three crops, DMP and total fruit production had an asymptotic response to crop N uptake with an initial rapid response followed by progressively smaller increases in DMP and yield as crop N uptake increased (Fig. 2). The relationship of DMP to crop N uptake for the combined data set of the Autumn and Spring crops, and the three cultivars of the Late Spring crop, was described by a logarithmic equation, which had a $R^2$ of 0.96 (Fig. 2a). The relationship between total yield and crop N uptake of the combined Autumn and Spring crops was described by a logarithmic equation with a $R^2$ of 0.92 (Fig. 2b). The three cultivars in the Late Spring crop were not included in this regression analysis because of the low production and HI values that were commented upon previously (Fig. 2b). The similarity of the values of both DMP and N crop uptake for treatments N3 to N5 in the Autumn and Spring crops (Fig. 2a; Table 2; Table 4), and for N2 and N3 in the Late Spring crop (Fig. 2a; Table 3), indicated that there was very little luxury consumption of N (Lemaire and Gastal, 1997) at the highest N application rates in the three crops

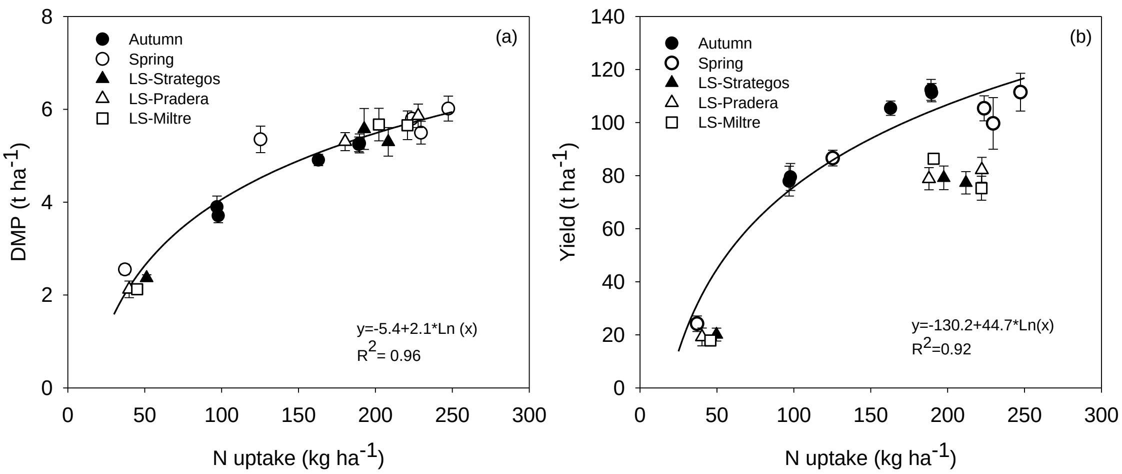


**Fig. 2.** Relationship of N uptake with (a) dry matter production and (b) total yield, derived from the three crops (Autumn, Spring and Late Spring) under five N treatments (Autumn and Spring crops) and under three N treatments and three cultivars (Late Spring crop). Values are means of four replications. The best fit equations and the corresponding lines for each relationship are presented. In Figure 2b only data from the Autumn and Spring crops were used to fit the equation.

Nitrogen utilization efficiency (ratio of yield or DMP to crop N uptake) values related to total yield ($N_{ut}E_Y$) and to DMP ($N_{ut}E_{DMP}$) are presented in Tables 4 and 5. In the three crops, $N_{ut}E_Y$ and $N_{ut}E_{DMP}$ decreased appreciably as N increased. $N_{ut}E_Y$ values ranged from 830 to 590 kg $kg^{-1}$ in the Autumn crop, from 695 to 432 kg $kg^{-1}$ in the Spring crop, and from 325 to 120 kg $kg^{-1}$ in the Late Spring crop (Tables 4 and 5) which had much lower yield. $N_{ut}E_{DMP}$ values ranged from 42 to 28, 69 to 24, and 38 to 9 kg $kg^{-1}$ in the Autumn, Spring and Late Spring crops, respectively (Tables 4 and 5). In both the Autumn and Spring crops, there were no significant differences in $N_{ut}E_{Y}$ and $N_{ut}E_{DMP}$ values between the three highest N treatments (Table 4). In the Late Spring crop, there were no significant differences between cultivars in $N_{ut}E_{Y}$ and $N_{ut}E_{DMP}$ values (Table 5).

**Table 4**

For the Autumn and Spring crops, nitrogen use efficiency related to total yield ($NUE_{y}$ , total yield/TAN) and to total dry matter ($NUE_{DMP}$; DMP/TAN), N uptake efficiency ($N_{upt}E$; N uptake/TAN) and N utilization efficiency in relation to yield ($N_{ut}E_{y}$ ; Yield/N uptake) or to total dry matter ($N_{ut}E_{DMP}$; DMP/N uptake). Different letters indicate significant differences (*P*<0.05) between means according to the LSD procedure. A summary of the analysis of variance is presented with: no significant (ns), significant at *P*≤0.05 (*), very significant at *P*≤0.01 (**) and highly significant at *P*≤0.001 (***).

| Crop | $NUE_{y}$ (kg $kg^{-1}$) | $NUE_{DMP}$ (kg $kg^{-1}$) | $N_{upt}E$ (kg $kg^{-1}$) | $N_{ut}E_{y}$ (kg $kg^{-1}$) | $N_{ut}E_{DMP}$ (kg $kg^{-1}$) |
|---|---|---|---|---|---|
| Autumn | | | | | |
| A-N1 | 597 a | 29.9 a | 0.74 a | 830 a | 41.7 a |
| A-N2 | 427 b | 19.9 b | 0.53 b | 822 a | 38.7 a |
| A-N3 | 311 c | 14.5 c | 0.48 b | 648 b | 30.1 b |
| A-N4 | 275 cd | 12.8 cd | 0.46 b | 594 b | 27.7 b |
| A-N5 | 207 d | 9.8 d | 0.35 b | 590 b | 27.8 b |
| *Significance* | *** | *** | ** | *** | ** |
| Spring | | | | | |
| S-N1 | 323 a | 34.1 a | 0.50 ac | 648 a | 69.4 a |
| S-N2 | 505 b | 31.2 a | 0.73 b | 695 a | 42.8 b |
| S-N3 | 252 ac | 13.9 b | 0.54 c | 472 b | 25.9 c |
| S-N4 | 183 cd | 10.1 c | 0.42 cd | 432 b | 23.9 c |
| S-N5 | 170 d | 9.2 c | 0.38 d | 451 b | 24.3 c |
| *Significance* | *** | *** | *** | *** | *** |

*3.4. Relationships between total available N and yield, crop N uptake and soil N*

The relationships between (i) relative total yield and total available N (TAN), (ii) relative marketable yield and TAN, and (iii) relative total dry matter production (DMP) and TAN are presented in Fig. 3, for the Autumn and Spring crops and for the three cultivars of the Late Spring crop. Relative yields and DMP are expressed relative to the maximum value for that crop or cultivar. For each of three parameters (relative total yield, relative marketable yield, relative DMP), all of the crops and cultivars followed very similar linear-plateau relationships (Fig. 3). The three parameters were strongly related to TAN in linear-plateau relationships with $R^2$ values of 0.96–0.97 (Fig. 3). These relationships indicated that both yield and DMP increased with TAN to a maximum value after which there was a flat response.

The linear-plateau analysis determined that the intersection of the inclined and horizontal lines corresponded to 96% of the maximum yield obtained. This was equivalent to a total yield of 108 t $ha^{-1}$ for the Autumn and Spring crops, and of 80, 82 and 86 t $ha^{-1}$ for the cultivars ´Strategos´, ´Pradera´ and ´Miltre´ in the Late Spring crop. For marketable yield, it was equivalent to 106 and 100 t $ha^{-1}$ for the Autumn and Spring crops, to 40, 60 and 43 t $ha^{-1}$ for the cultivars ´Strategos´, ´Pradera´ and ´Miltre´ in the Late Spring crop. The intersection of the inclined and horizontal lines for DMP corresponded to 97% of the maximum DMP obtained. This was equivalent to 5.3 and 6.0 t $ha^{-1}$ for the Autumn and Spring crops, and to 5.7 t $ha^{-1}$ for the three cultivars in the Late Spring crop.

The TAN values that corresponded to maximum yield and DMP values as identified by the intersection of the inclined and horizontal lines are the minimum amounts of TAN required for maximum yield or DMP production. The relevant TAN values were 221 ± 14.0 kg N $ha^{-1}$ for maximum total yield (Fig. 3a), 228 ± 14.7 kg N $ha^{-1}$ for maximum marketable yield (Fig. 3b), and 222 ± 15.4 kg N $ha^{-1}$ for maximum DMP (Fig. 3c). These TAN values for maximum yield and DMP corresponded to a theoretical N treatment that was between the N2 and N3 treatments in the Autumn and Spring crops, and between the N1 and N2 treatments in the Late Spring crop.

**Table 5**
For the Late Spring crop, nitrogen use efficiency related to yield ($NUE_y$; total yield/TAN) and to total dry matter ($NUE_{DMP}$; DMP/TAN), N uptake efficiency ($N_{upt}E$; N uptake/TAN) and N utilization efficiency in relation to yield ($N_{ut}E_y$; yield/N uptake) or to total dry matter ($N_{ut}E_{DMP}$; DMP/N uptake). Different letters indicate significant differences ($P$<0.05) between means according to the LSD procedure; upper case letters refer to comparison between cultivars and lower case letters between N treatments. A summary of the analysis of variance is presented with: no significant (ns), significant at $P$≤0.05 (*), very significant at $P$≤ 0.01 (**) and highly significant at $P$≤0.001 (***).

| Cultivar | $NUE_y$ (kg kg$^{-1}$) | $NUE_{DMP}$ (kg kg$^{-1}$) | $N_{upt}E$ (kg kg$^{-1}$) | $N_{ut}E_y$ (kg kg$^{-1}$) | $N_{ut}E_{DMP}$, (kg kg$^{-1}$) |
|---|---|---|---|---|---|
| Strategos | 231 | 21.4 | 0.59 | 396 | 33.8 |
| N1 | 325 a | 38.4 a | 0.83 a | 418 a | 47.0 a |
| N2 | 235 b | 16.5 b | 0.57 b | 405 a | 29.0 b |
| N3 | 134 c | 9.2 c | 0.36 c | 366 b | 25.4 c |
| Pradera | 229 | 20.1 | 0.53 | 423 | 34.3 |
| N1 | 311 a | 34.4 a | 0.70 a | 496 a | 52.2 a |
| N2 | 234 b | 15.7 b | 0.54 b | 420 a | 29.5 b |
| N3 | 142 c | 10.1 c | 0.39 c | 370 b | 25.7 c |
| Miltre | 225 | 20.3 | 0.57 | 395 | 33.8 |
| N1 | 289 a | 34.4 a | 0.73 a | 391 a | 47.6 a |
| N2 | 256 b | 16.8 b | 0.60 b | 456 a | 28.1 b |
| N3 | 130 c | 9.8 c | 0.38 c | 339 b | 25.6 c |
| Analysis of variance | | | | | |
| N | *** | *** | *** | ** | *** |
| Cultivar | ns | ns | ns | ns | ns |
| N x Cultivar | ns | ns | ns | ns | ns |

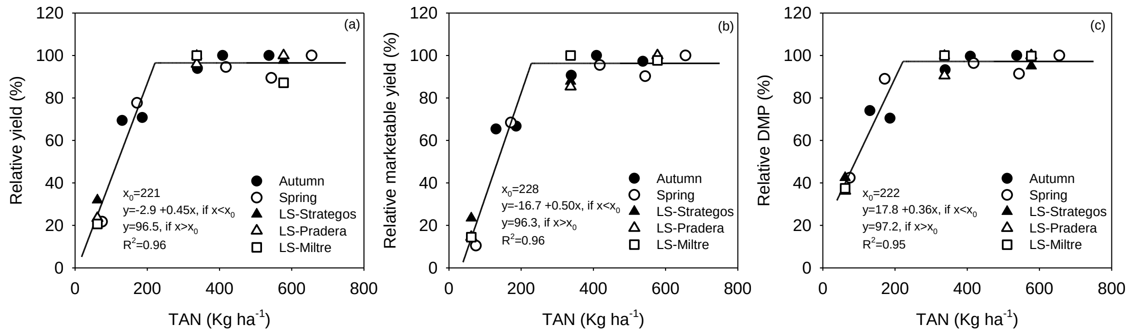


**Fig. 3.** Relationships of (a) relative total yield, (b) relative marketable yield and (c) relative dry matter production with total available N (TAN) for combined datasets of the Autumn and Spring cucumber crops and of the three cultivars of the Late Spring cucumber crop, using segmented linear regression analysis. Relative values were calculated separately for each crop in relation to the maximum value of each parameter. Values are means of four replications. The xo is the abscissa of the breakpoint of the relationship (i.e. the minimum value of TAN for maximum yield or DMP). The two equations of the segmented regression are presented.

Crop N uptake increased asymptotically with increasing TAN; at the highest TAN values the slope was much reduced but it never became horizontal (Fig. 4a). This relationship, for all crops and cultivars considered together, was strongly curvilinear being described by a logarithmic equation with a $R^2$ of 0.95 (Fig. 4a). N uptake efficiency ($N_{upt}E$) decreased rapidly with increasing TAN; the relationship for the three crops and three cultivars was described by an exponential decay equation with a $R^2$ of 0.75 (Fig. 4b). At the lower amounts of TAN, corresponding to N1 treatments, $N_{upt}E$ was approximately 0.8; at the highest amounts of TAN corresponding to the N5 treatments, $N_{upt}E$ had reduced to 0.4 (Tables 6 and 7).

The potential N loss was assessed by determining the amount of N leached as $NO_3^-$ (Table 1) and the residual soil mineral at the end of each crop (Fig. 4c). Nitrate leaching was very low (between 1 and 11 kg N ha$^{-1}$) for all treatments and did not increase with increasing TAN because optimal irrigation management resulted in very small total drainage volumes of 20–34 mm during an entire crop (Table 1). Considering the Autumn and Spring crops together, residual soil mineral N at the end of the crop increased exponentially with TAN (Fig. 4c); the exponential equation describing this relationship had a $R^2$ of 0.92. In the highest N treatments, residual mineral N was 196 and 330 kg N $ha^{-1}$ for the Autumn and Spring crops, respectively.

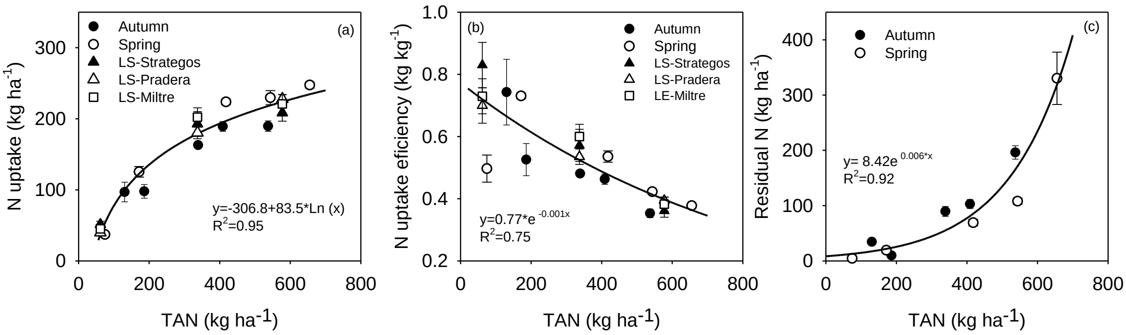


**Fig. 4.** Relationships (a) crop N uptake, (b) N uptake efficiency (e.g. N uptake/TAN) and (c) mineral N at the end of each crop in the top 60 cm of soil, with total available N (TAN). For figures 4a and 4b, data used were from the three crops (Autumn, Spring and Late Spring) for five N treatments from the Autumn and Spring crops, and from three N treatments and three cultivars (Late Spring crop). In Figure 4c, data are from the Autumn and Spring crops. Values are means of four replications. The best fit equations and the corresponding regression lines for each relationship are presented. In Figure 4b, data from treatment N1 of the Spring crop was excluded from the relationship because they were anomalous.

*3.7. Nitrogen use efficiency*

In the three crops, NUE related to total yield ($NUE_Y$) and to biomass ($NUE_{DMP}$) decreased with increasing TAN (Tables 4 and 5). The range of values for the Autumn and Spring crops was 170–600 kg $kg^{-1}$ for $NUE_Y$ and 9–34 kg $kg^{-1}$ for $NUE_{DMP}$ (Table 4). The following significant differences between N treatments for both $NUE_Y$ and $NUE_{DMP}$ were observed; for the Autumn crop N5<N3<N2<N1, and for the Spring crop N5<N3<N2 and N1<N2. The responses of $NUE_Y$ and $NUE_{DMP}$ to increasing TAN were similar in the Late Spring crop; both $NUE_Y$ and $NUE_{DMP}$ N decreased significantly with increasing TAN (Table 5). There were no significant differences between cultivars in $NUE_Y$ and $NUE_{DMP}$ in the Late Spring crop (Table 5).

Unlike $NUE_Y$ and $NUE_{DMP}$ which continually decreased with increasing TAN, the N utilization efficiency values related to both yield ($N_{ut}E_y$) and dry matter production ($N_{ut}E_{DMP}$) initially declined with increasing TAN; however, values associated for the three highest N treatments were similar for both parameters for the Autumn and Spring crops (Table 4).

## 4. Discussion

### *4.1. Response of cucumber yield and DMP to N*

The response of crop production (i.e. total yield, marketable yield and DMP) to TAN, was described by the segmented linear regression model, which is a commonly-used and effective model to describe yield responses to N addition (Gianquinto et al., 2011; Ordoñez et al., 2015). This model allows calculation of an optimal TAN value above which no yield increase is obtained. In this work, the TAN values that maximized total and marketable yield were 221± 14.0 and 228 ± 14.7 Kg $ha^{-1}$, respectively, which corresponded to a theoretical N fertigation treatment between treatments N2 and N3 for the Autumn and Spring crops, and between treatments N1 and N2 for the Late Spring crop. These values agree with the value of 200 kg N $ha^{-1}$ for optimization of yield and fruit quality of greenhouse cucumber grown in soil in Almeria reported by Ruiz and Romero (1998). However, they are much lower than the recommended value of 450 kg N $ha^{-1}$ for cucumber in solar greenhouses in China (Zhang et al., 2011).

The optimum values of N in the present work are comparable to the recommendations by the Spanish Ministry of Agriculture of 187–238 kg N $ha^{-1}$ for production of 75 and 85 t $ha^{-1}$ of greenhouse cucumber (García-Serrano et al., 2010) which correspond to 2.5 to 2.8 kg N for each tonne of fresh production (i.e. 2.5–2.8 kg N $t^{-1}$). In the present work, a value of 2.0 kg N $t^{-1}$ was obtained for total yield, and 2.3 kg N $t^{-1}$ for marketable yield which is similar to the lower values in the range recommended by García-Serrano et al., (2010), and to the value of 2.5 kg N $t^{-1}$ recommended for greenhouse cucumber by Reche Mármol, (2011). In the Late Spring crop, due to the low total yield and the poor fruit quality, the corresponding values were 2.8 kg N $t^{-1}$ (total yield) to 5.9 Kg N $t^{-1}$ (marketable yield). The legislation for N management within the nitrate vulnerable zones (NVZ) of the Andalusia region (BOJA, 2015), in which this study was conducted, established a maximum value for N applications to cucumber of 4 kg N $t^{-1}$. The threshold value determined in the present work is well below the maximum value in the local legislation.

To calculate a recommended N concentration in the nutrient solution for greenhouse-grown cucumber, from the present results, the following observations were considered: (i) the irrigation volumes applied to treatments N3 in the Autumn and Spring crops, and to treatment N2 in the Late Spring crop, were representative of a well-irrigated crop, (ii) the optimal TAN for commercial production was 228 kg N $ha^{-1}$, (iii) the amounts of soil mineral N soil at planting and mineralized N were insignificant, and (iv) all N was supplied by fertigation. The subsequent recommended N concentrations are 11.9 ± 0.8 mmol $L^{-1}$ for an autumn crop, 9.0 ± 0.6 mmol $L^{-1}$ for a spring crop, and of 6.5 ± 0.4 mmol $L^{-1}$ for a late spring crop. The recommended N concentration for an autumn crop is similar to the value of 11.5 mmol $L^{-1}$ proposed by Cadahia, (2005) for cucumber grown in SE Spain. It is lower than the value of 13.0  mmol $L^{-1}$ proposed by Fernández and Camacho Ferre, (2007) for cucumber. The results of this work suggest that the applied N concentration should consider the cropping season to take into account both N and irrigation requirements. Generally, growers apply fixed concentrations of N regardless of crop water requirements and the cropping season (Thompson et al., 2007). When taking into account soil mineral N and mineralized N, the required concentrations will be lower than those calculated above.

Most of the published studies analyzing the response of greenhouse cucumber to applied N have been conducted in soilless systems with the N concentration of the applied solution expressed in mg $L^{-1}$.  Integrating several studies conducted in different regions and climates, and converting the units to mmol $L^{-1}$, the values of N concentration that maximize production ranged from 7.2 to 16 mmol $L^{-1}$ (e.g. Altunlu et al., 1999 in Turkey; Kotsiras et al., 2005 in Greece;  Dai et al., 2011 in China and Jasso-Chaverria et al., 2005 in Florida). In cucumber grown in greenhouses in soil, the maximum yield corresponded to concentrations of N of 200 mg $L^{-1}$ (14.3 mmol $L^{-1}$) (Güler et al., 2006). However, the studies examining the effect of N rates on soil-grown cucumber have focused on mineral fertilizer N rather than on TAN (e.g. Güler et al., 2006; Ruiz and Romero, 1998; Zhang et al., 2011). Considering soil N sources will likely result in a lower N concentration for optimal production.

The yield decrease with reduced TAN was associated to a smaller number of fruits in the three crops. In all crops, the mean fruit weight was highest in the N2 treatments and decreased as N application increased. These results indicated that with more N, more fruits were produced per plant but they were smaller.  Similar results were reported by Güler et al., (2006).  However, in the study of Zhang et al., (2011) with cucumber in a Chinese solar greenhouse, the N supply did not significantly affect fruit number while the effect on fruit weight was significant.

The TAN value for maximum DMP obtained in this work of 222 kg N $ha^{-1}$, was very similar to that obtained for maximum total yield and maximum marketable yield. This suggest that the same amount of N is required to optimize both DMP and fruit production. This is consistent with the similarity of harvest index (HI) values between N treatments.  Taken together, these results suggest that for greenhouse cucumber, vegetative and reproductive growth respond very similarly to increasing N supply. This contrasts observations with tomato where excess N favoured vegetative growth and decreased HI (Elia and Conversa, 2012; Soto et al., 2015).

The lack of significant differences in DMP and crop N uptake between the three highest N treatments (N3, N4 and N5) in the Autumn and Spring crops and between the two highest treatments in the Late-Spring crop is indicative of negligible or very small luxury N uptake by cucumber. We are unaware of other studies that have assessed whether luxury N uptake occurred in greenhouse cucumber.

*4.2. Effect of N treatments on N pollution processes*

In this work, $NO_3^-$ leaching was negligible which contrasts with values of 100 –240 kg N $ha^{-1}$ reported in comparable studies with greenhouse-grown vegetable crops in soil in the same region (Granados et al., 2013; Thompson et al., 2013). In the current study, the use of tensiometers to manage irrigation resulted in only 20–36 mm of total drainage which substantially restricted $NO_3^-$ leaching loss. This is consistent with the meta-analysis of Quemada et al., (2013) which reported that irrigation management is the most influential management factor for $NO_3^-$ leaching. However, the results of the present study demonstrate that unless the total N supply is also managed optimally, a substantial accumulation of soil mineral N can occur which is likely to result in a delayed large $NO_3^-$ leaching loss when subsequent drainage occurs from salt leaching irrigations or from rainfall in open field crops.

The optimal TAN value determined in the present study of approximately 222 kg N $ha^{-1}$ for a cucumber crop of 70 –84 day duration, was associated with a very small $NO_3^-$ leaching loss, attributable to good irrigation management, a very small accumulation of soil mineral N of 28 kg N $ha^{-1}$, and a N uptake efficiency of 0.60. Similar results were obtained by Soto et al., (2015) in greenhouse tomato for N management based on optimal TAN.

*4.3. Interaction between N responses, climate and cultivars*

Despite that the three crops were grown under very different climatic conditions in autumn, spring and late spring cropping cycles, the same relationships described the three crops for (i) yield with TAN, (ii) both crop N uptake and N uptake efficiency with TAN, and (iii) DMP with crop N uptake, suggesting that (a) the response of cucumber to N is not affected by climate, and (b) general recommendations can be made for N requirements for cucumber. However, the N recommendations expressed as N concentration should be modified depending on the cropping season, as discussed earlier. In contrast, Kotsiras et al., (2005) reported that for cucumber grown in rockwool in southern Greece, that there were different responses to increasing N in a spring-grown crop compared to a winter-grown crop.

The current work examined possible differences between cultivars in the response to N. We found similar responses to increasing N applications for the three cultivars in terms of DMP and yield. Similar results were reported by Jasso-Chaverria et al., (2005) in Florida, with the same response of fruit production to N applications in two different greenhouse cucumber cultivars that differed in fruit type.

*4.4. Nitrogen Use efficiency*

NUE decreased appreciably with the increasing N supply, as observed in other studies with cucumber (Zhang et al., 2011) and pepper (Yasour et al., 2013) grown in greenhouses. The lower NUE at higher N supply is mainly associated with the lower capacity of the crop to absorb N from soil. In contrast when the crop is N limited, N is absorbed more efficiently (Moll et al., 1982). The values of $NUE_Y$ obtained in this work of 200 –600 kg kg$^{-1}$ are much higher than the range of 40 –140 kg kg$^{-1}$ reported by Zhang et al., (2011) for cucumber in Chinese solar greenhouses. The difference with the values of Zhang et al., (2011) can be explained by the appreciably higher yields and lower N applications in our study.

In the present work, NUE which indicates the amount of dry matter or yield produced per unit of N supply (in this case TAN), was partitioned into its two components according to Moll et al., (1982) and to Sadras and Lemaire (2014), being (1) N uptake efficiency ($N_{upt}E$), i.e. N uptake per unit of TAN, and (2) N utilization efficiency ($N_{ut}E$), i.e. dry matter or yield per unit of N uptake ($N_{ut}E_{DMP}$ and $N_{ut}E_Y$, respectively). The NUE related to yield ($NUE_Y$) and to DMP ($NUE_{DMP}$) decreased as TAN increased. This decrease was mostly explained by a decrease in the N uptake efficiency rather than in N utilization efficiency which was constant for three highest N treatments in the Autumn and Spring crops. These results are in agreement with studies in cereals where the low efficiency of N was due to a low efficiency in N uptake by the crop (e.g. Arregui and Quemada, 2008). The three cultivars compared in the Late Spring crop, all had similar $NUE_Y$ and $NUE_{DMP}$ values for equivalent N treatments which suggests that a higher efficiency in nitrogen use may not be a trait to consider in breeding programs for vegetables to be grown in intensive greenhouse production.

## 5. Conclusions

For cucumber grown in soil in a greenhouse, the minimum amount of total available N (TAN) that produced the highest yield was 228±14.7 Kg $ha^{-1}$ for a 70–84 day crop. This TAN supply was associated with negligible $NO_3^-$ leaching, attributable to good irrigation management, very small N accumulation in soil of 28 kg N $ha^{-1}$, and an N uptake efficiency of 0.60. The same relationships between production and TAN was found for three crops grown with different seasons and climatic conditions, and for the three cultivars suggesting that general recommendations for cucumber in terms of N requirements can be made.


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## References


Altunlu, H., Gül, A., Tunc, A., 1999. Effect of nitrogen and potassium nutrition on plant growth, yield and fruit quality of cucumbers grown in perlite. Acta Hortic. 486, 377–381.

Anonymous, 1991. Council directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources, Official Journal of the European Communities.

Arregui, L.M., Quemada, M., 2008. Strategies to improve nitrogen use efficiency in winter cereal crops under rainfed conditions. Agron. J. 100, 277–284. doi:10.2134/agronj2007.0187.

BOJA, 2015. Orden de 1 de junio de 2015, por la que se aprueba el programa de actuación aplicable en las zonas vulnerables a la contaminación por nitratos de fuentes agrarias designadas en Andalucia, Boletín Oficial de la Junta de Andalucía nº 111, 8-18 (In Spanish).

Cadahia, C., 2005. Fertirrigación: cultivos hortícolas, frutales y ornamentales, 3ª Edición. ed. Mundi-Prensa, Madrid, Spain (In Spanish).

Céspedes, A.J., García, M.C., Pérez-Parra, J.J., Cuadrado Gómez, I.M., 2009. Caracterización de la explotación hortícola protegida de Almería. FIAPA, Fundación Cajamar, Almeria, Spain.

Dai, J., Liu, S., Zhang, W., Xu, R., Luo, W., Zhang, S., Yin, X., Han, L., Chen, W., 2011. Quantifying the effects of nitrogen on fruit growth and yield of cucumber crop in greenhouses. Sci. Hortic. 130, 551–561. doi:10.1016/j.scienta.2011.08.004.

Elia, A., Conversa, G., 2012. Agronomic and physiological responses of a tomato crop to nitrogen input. Eur. J. Agron. 40, 64–74. doi:10.1016/j.eja.2012.02.001.

Feller, C., Fink, N., 2002. Nmin target values for field vegetables. Acta Hortic. 571, 195–201.

Fernández, E.J., Camacho Ferre, F., 2007. Manual práctico de Fertirrigación en riego por goteo. Ediciones Agrotécnicas S.L., Madrid, Spain (In Spanish).

García-Serrano, P., Juan, J., Lucena, J., Sebastián, M., Criado, R., García, M.N., López Bellido, L., Betrán, J., Álvaro, A., Monreal, R., López, H., Prudencio, C., Fuster, L., Luis, J., Corrales, B., Urbano, P., Juan, T., Andión, P., Castro, J., Ricardo, I., Rodríguez, B., Ramos, C., Fernando, M., García, P., Quiñones, A., Belén, O., Alcántara, M., Primo-Millo, E., Paredes, F.L., Carbó, E., García-Escudero Domínguez, E., García, C., Jésica, G., Rodríguez, P., 2010. Guía práctica de la fertilización racional de los cultivos en España. Parte I, Parte II. Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid, Spain.

Gianquinto, G., Orsini, F., Sambo, P., Paino D'Urzo, M., 2011. The use of diagnostic optical tools to assess nitrogen status and to guide fertilization of vegetables. Horttechnology 21, 287–292. doi: 10.21273/HORTTECH.21.3.287.

Granados, M.R., Thompson, R.B., Fernández, M.D., Martínez-Gaitán, C., Gallardo, M., 2013. Prescriptive-corrective nitrogen and irrigation management of fertigated and drip-irrigated vegetable crops using modeling and monitoring approaches. Agric. Water Manag. 119, 121-134. doi:10.1016/j.agwat.2012.12.014.

Güler, S., Ibrikci, H., Büyük, G., 2006. Effects of different nitrogen rates on yield and leaf nutrient contents of drip-fertigated and greenhouse-grown cucumber. Asian J. Plant Sci. 5, 657–662. doi:10.3923/ajps.2006.657.662.

Jadoski, S., Thompson, R.B., Peña-Fleitas, M.T., Gallardo, M., 2013. Regional N balance for an intensive vegetable production system in South-Eastern Spain, in: Fontana, E., Grignani, C., Nicola, S. (Eds.), Book of Abstracts of NEV 2013 International Workshop on Nitrogen, Environment and Vegetables. Turin, Italy, pp. 15–17.

Jasso-Chaverria, C., Hochmuth, G.J., Hochmuth, R.C., Sargent, S. A., 2005. Fruit yield, size, and color responses of two greenhouse cucumber types to nitrogen fertilization in perlite soilless culture. Horttechnology 15, 565–571.

Ju, X.T., Kou, C.L., Zhang, F.S., Christie, P., 2006. Nitrogen balance and groundwater nitrate contamination: comparison among three intensive cropping systems on the North China Plain. Environ. Pollut. 143 (1), 117-125. doi:10.1016/j.envpol.2005.11.005.

Kotsiras, A., Olympios, C.M., Passam, H.C., 2005. Effects of nitrogen form and concentration on yield and quality of cucumbers grown on rockwool during spring and winter in southern Greece. J. Plant Nutr. 28, 2027–2035. doi:10.1080/01904160500311102.

Lemaire, G., Gastal, F., 1997. Nitrogen uptake and distribution in plant canopies, in: Diagnosis of the Nitrogen Status in Crop. Heidelberg: Springer-Verlag, pp. 3–43.

MAPAMA, 2017. Superficies y producciones anuales de cultivo. Ministerio de Agricultura Pesca y Alimentación [WWW Document]. URL https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/superficies-producciones-anuales-cultivos/

Moll, R.H., Kamprath, E.J., Jackson, W.A., 1982. Analysis and Interpretation of Factors Which Contribute to Efficiency of Nitrogen Utilization1. Agron. J. 74, 562-564. doi:10.2134/agronj1982.00021962007400030037x.

Neeteson, J.J., 1994. Nitrogen management for intensively grown arable crops and field vegetables. In: Bacon, P. (Ed.), Nitrogen Fertilization and the Environment. Marcel Dekker, New York, USA, pp. 295-325.

Ordoñez, R.A., Savin, R., Slafer, G.A., 2015. Genetic variation in the critical specific leaf nitrogen maximising yield among modern maize hybrids. F. Crop. Res. 172, 99–105. doi:10.1016/j.fcr.2014.12.002.

Padilla, F.M., Gallardo, M., Manzano-Agugliaro, F. 2018. Global trends in nitrate leaching research in the 1960-2017 period. SCI TOTAL ENVIRON 643, 400-413. doi.org/10.1016/j.scitotenv.2018.06.215.

Pulido-Bosh, A., 2005. Recarga en la Sierra de Gádor e Hidrogeoquímica de los acuiferos del Campo de Dalías. Estación Experimental Cajamar, El Ejido, Almería, (In Spanish).

Quemada, M., Baranski, M., Nobel-de Lange, M.N.J., Vallejo, A., Cooper, J.M., 2013. Meta-analysis of strategies to control nitrate leaching in irrigated agricultural systems and their effects on crop yield. Agric. Ecosyst. Environ. 174, 1–10.

Ramos, C., Agut, A., Lidón, A.L., 2002. Nitrate leaching in important horticultural crops of the Valencian community region (Spain). Environ. Pollut. 118, 215-223. doi: 10.1016/S02697491(01)00314-1.

Reche Mármol, J., 2011. Cultivo del pepino en invernadero. Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid, (In Spanish).

Ruiz, J.M., Romero, L., 1998. Commercial yield and quality of fruits of cucumber plants cultivated under greenhouse conditions: response to increases in nitrogen fertilization. J. Agric. Food Chem. 46, 4171–4173. doi:10.1021/jf980503c.

Sadras, V.O., Lemaire, G., 2014. Quantifying crop nitrogen status for comparisons of agronomic practices and genotypes. F. Crop. Res. 164, 54-64. doi:10.1016/j.fcr.2014.05.006.

Soto, F., Gallardo, M., Giménez, C., Peña-Fleitas, T., Thompson, R., 2014. Simulation of tomato growth, water and N dynamics using the EU-Rotate_N model in Mediterranean greenhouses with drip irrigation and fertigation. Agric. Water Manag. 132, 46–59.

Soto, F., Gallardo, M., Thompson, R.B., Peña-Fleitas, M.T., Padilla, F.M., 2015. Consideration of total available N supply reduces N fertilizer requirement and potential for nitrate leaching loss in tomato production. Agric. Ecosyst. Environ. 200, 62-70. doi:10.1016/j.agee.2014.10.022.

Thompson, R.B., Martínez-Gaitán, C., Giménez, C., Gallardo, M., Fernández, M.D., 2007. Identification of irrigation and N management practices that contribute to nitrate leaching loss from an intensive vegetable production system by use of a comprehensive survey. Agric. Water Manag. 89, 261–274. doi:10.1016/j.agwat.2007.01.013.

Thompson, R.B., Gallardo, M., Rodríguez, J.S., Sánchez, J.A., Magán, J.J., 2013. Effect of N uptake concentration on nitrate leaching from tomato grown in free-draining soilless culture under Mediterranean conditions. Sci. Hortic. 150, 387-398. doi:10.1016/j.scienta.2012.11.018.

Thompson, R.B., Tremblay, N., Fink, M., Gallardo, M., Padilla, F.M., 2017a. Tools and strategies for sustainable nitrogen fertilisation of vegetable crops, in: Tei,F.,  Nicola, S., Benincasa, P. (Eds.), Advances in research on fertilization management of vegetable crops. Advances in Olericulture. Springer, Cham, Switzerland, pp. 11–63.

Thompson, R.B., Incrocci, L., Voogt, W., Pardossi, A. and Magán, J.J., 2017b. Sustainable irrigation and nitrogen management of fertigated vegetable crops. Acta Hortic. 115, 363– doi: 10.17660/ActaHortic.2017.1150.52.

Yasuor, H., Ben-gal, A., Yermiyahu, U., Beit-yannai, E., Cohen-Shabtai, 2013. Nitrogen Management of Greenhouse Pepper Production : Agronomic, Nutritional , and Environmental Implications. HortScience 48, 1241–1249.

Zhang, H.X., Chi, D.C., Wang, Q., Fang, J., Fang, X.Y., 2011. Yield and quality response of cucumber to irrigation and nitrogen fertilization under subsurface drip irrigation in solar greenhouse. Agric. Sci. China 10, 921–930. doi:10.1016/S1671-2927(11)60077-1.