Plant and Soil **147**: 13–23, 1992.

PLSO 9419

The influence of nitrogen concentration and ammonium/nitrate ratio on N-uptake, mineral composition and yield of citrus

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Received 26 November 1991. Revised July 1992

Key words: ammonium, citrus, nitrate, NO_3^-/NH_4^+ ratio, nitrogen, nutrient solution

Abstract

In short-term water culture experiments with different ^{15}N labeled ammonium or nitrate concentrations, citrus seedlings absorbed NH_4^+ at a higher rate than NO_3^-. Maximum NO_3^- uptake by the whole plant occurred at 120 mg L^{-1} NO_3^-–N, whereas NH_4^+ absorption was saturated at 240 mg L^{-1} NH_4^+–N. $^{15}NH_4^+$ accumulated in roots and to a lesser degree in both leaves and stems. However, $^{15}NO_3^-$ was mostly partitioned between leaves and roots.

Adding increasing amounts of unlabeled NH_4^+ (15–60 mg L^{-1} N) to nutrient solutions containing 120 mg L^{-1} N as ^{15}N labeled nitrate reduced $^{15}NO_3^-$ uptake. Maximum inhibition of $^{15}NO_3^-$ uptake was about 55% at 2.14 m*M* NH_4^+ (30 mg L^{-1} NH_4^+–N) and it did not increase any further at higher NH_4^+ proportions.

In a long-term experiment, the effects of concentration and source of added N (NO_3^- or NH_4^+) on nutrient concentrations in leaves from plants grown in sand were evaluated. Leaf concentration of N, P, Mg, Fe and Cu were increased by NH_4^+ versus NO_3^- nutrition, whereas the reverse was true for Ca, K, Zn and Mn.

The effects of different NO_3^-–N:NH_4^+–N ratios (100:0, 75:25, 50:50, 25:75 and 0:100) at 120 mg L^{-1} total N on leaf nutrient concentrations, fruit yield and fruit characteristics were investigated in another long-term experiment with plants grown in sand cultures. Nitrogen concentrations in leaves were highest when plants were provided with either NO_3^- or NH_4^+ as a sole source of N. Lowest N concentration in leaves was found with a 75:25 NO_3^-–N/NH_4^+–N ratio. With increasing proportions of NH_4^+ in the N supply, leaf nutrients such as P, Mg, Fe and Cu increased, whereas Ca, K, Mn and Zn decreased. Yield in number of fruits per tree was increased significantly by supplying all N as NH_4^+, although fruit weight was reduced. The number of fruits per tree was lowest with the 75:25 NO_3^-–N:NH_4^+–N ratio, but in this treatment fruits reached their highest weight. Rind thickness, juice acidity, and colour index of fruits decreased with increasing NH_4^+ in the N supply, whereas the % pulp and maturity index increased. Percent of juice in fruits and total soluble solids were only slightly affected by NO_3^-:NH_4^+ ratio.

Introduction

Plants absorb nitrogen either as nitrate or as ammonium. Ammonium-N as compared to nitrate-N, nutrition results in lower plant Ca, Mg, and K concentration and higher anion concentration, especially phosphate (Barker and Maynard, 1972; Blair et al., 1970; Cox and Reisenauer, 1973; de Classen and Wilcox, 1974; Edwards and Horton, 1982; Gashaw and Mugwira, 1981; Hartman et al., 1986; Kirkby and Mengel, 1967; Pill and Lambeth, 1977; Shelp, 1987).

Numerous studies have shown that ammoniacal-N as a sole source of N is deleterious to the growth of many higher plants (Bennett et al., 1964; Goyal et al., 1982; Pill and Lambeth, 1977). The addition of nitrate however, alleviated the inhibitory effects of NH_4^+ on growth (Goyal et al., 1982; Ota and Yamamoto, 1989). In several crops, combinations of NH_4^+ and NO_3^- usually result in greater vegetative growth than when either N form is used alone (Edwards and Horton, 1982; Gashaw and Mugwira, 1981; Hartman et al., 1986; Schrader et al., 1972). However, some species of the genus Vaccinium (blueberry and cranberry) grow better when NH_4^+ is the N source rather than NO_3^- (Peterson et al., 1988; Rosen et al., 1990).

Some plants grown in nutrient solutions under controlled conditions absorb nitrate more readily than ammonium (Blair et al., 1970; Scherer and MacKown, 1987; Viets, 1965), while other plants prefer ammonium (Edwards and Horton, 1982; Fried et al., 1965; Minotti et al., 1969; Peterson et al., 1988). The relative absorption rates of NH_4^+ and NO_3^- are influenced by N concentration, NO_3^-/NH_4^+ ratio, pH and temperature (Criddle et al., 1988; Fried et al., 1965; Frota and Tucker, 1972; Gashaw and Mugwira, 1981; Hartman et al., 1986; Warncke and Barber, 1973). In several species NH_4^+ may inhibit NO_3^- uptake when both NH_4^+ and NO_3^- are present (Breteler and Siegerist, 1984; Fried et al., 1965; Frith and Nichols, 1975; Mackown et al., 1982; Minotti et al., 1969; Pan et al., 1985).

However, little information is available on Citrus response to the different nitrogen forms. This study was undertaken to determine the influences of different NO_3^- and NH_4^+ concentrations and varying NO_3^-/NH_4^+ ratios on N uptake rates, tissue nutrient concentrations, and yield of citrus trees. In addition, we examine the effect of ammonium on nitrate absorption in citrus.

Since drip irrigation is an important management practice for supplying water and nutrients to citrus cultivated in areas with a low rainfall, this research should give information about the form of N most appropriate to apply via this irrigation system. Furthermore, the pollution of ground-water by nitrate fertilizers has an environmental impact. A better understanding of plant response to nitrate and ammonium should allow a more rational use of nitrogen fertilizers.

Material and methods

Experiment I

Seeds of "Hamlin" orange (*C. sinensis* (L.) Osbeck) were germinated and the seedlings were grown in sand under greenhouse conditions for four months. Seedlings were removed from the sand, washed in distilled water and transferred to 0.5 liter polyethylene pots covered with aluminium foil. Each pot contained 0.45 liters of nutrient solution and one seedling. Nutrient solutions were aerated using an air pump. Six uniform plants were used for each treatment. Pots were randomized over the experimental area and the plants analyzed individually. Nutrient concentrations in the basic solution were: 6.6 m*M* $Ca(CH_3–COO)_2$, 2.4 m*M* H_3PO_4, 1.6 m*M* $MgSO_4$, 17.9 μM Fe EDDHA (ethylene diamine dihydroxiphenyl acetic acid), 27.3 μM $MnSO_4$, 1.5 μM $ZnSO_4$, 46.2 μM H_3BO_3, 0.02 μM $CuSO_4$ and 0.5 μM MoO_3. Nitrogen was supplied at concentrations of 0, 30, 60, 120, 180, 240, or 300 mg L^{-1} as ^{15}N-labeled KNO_3 or $(NH_4)_2SO_4$ (9.7 atom $^{15}N\%$ excess in both cases). The K concentration was adjusted to 21.4 m*M* with K_2SO_4. Nutrient solutions were autoclaved for 15 min at 121°C.

Another group of plants was treated with the nutrient solution containing 120 mg L^{-1} N as $K^{15}NO_3$ (9.7 atoms $^{15}N\%$ excess) and 0, 15, 30, 45 or 60 mg L^{-1} of N as unlabeled $(NH_4)_2SO_4$. Nutrient solutions were adjusted to pH 6.5 with 1 *N* NaOH or 1 *N* HCl. Changes in pH were less than 0.5 unit in two days. The nutrient concentrations did not vary more than 10%.

Experiments were conducted in a controlled-environment growth chamber operated at a light/dark cycle of (16 h, 26°C/8 h, 18°C). Relative humidity was approximately 80%. The photosynthetic photon flux density at plant height was 400 $\mu E\ m^{-2}\ s^{-1}$, supplied by incandescent and cool-white fluorescent lamps.

Plants were harvested after two days, washed with distilled water, separated into leaves, stems and roots, freshly weighed and dried in a forced

air-oven (65°C, 48 h). The dried samples were weighed and ground in a hammermill to pass a 40 mesh screen before analysis of concentration of total N and ^{15}N.

Experiment II

Two-year old Valencia orange plants (*C. sinensis* (L.) Osbeck) grafted on Troyer citrange rootstock were grown between January and December under greenhouse conditions with supplementary light (>50 μE m^{-2} s^{-1}, 400–700 nm) to extend the photoperiod to 16 h. During the heating season, temperatures ranged between 16–18°C at night and 24–28°C by day. During summer upper temperature was held at 32°C or below with evaporative coolers. Individual plants were grown in 50 liter tanks filled with coarse sand. Plants were irrigated three times a week with nutrient solution which was renewed after each irrigation. Large volumes of solutions ensured sufficient leaching, thus minimizing concentration and pH changes in the substrate.

Nutrient concentrations in the basic solution were: 3.1 m*M* K_2SO_4, 2.4 m*M* H_3PO_4, 1.6 m*M* $MgSO_4$, and trace elements as in Experiment I. Treatments consisted of 2 N sources, $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ with N increasing from 15 to 240 mg L^{-1} (15, 30, 60, 120, 180 or 240 mg L^{-1}). Equal Ca levels (8.6 m*M*) were maintained in all nutrient solutions with the addition of $Ca(CH_3COO)_2$, and pH was adjusted at 6.5 ± 0.5 by adding either 1 *N* HCl or 1 *N* NaOH.

Six uniform plants were used in each treatment. Tanks were randomized over the experimental area and the plants analyzed individually.

At the end of the experiment, 5–8 month old mature leaves were sampled, washed and freshly weighed. The leaves were dried in a forced air-oven (65°C, 48 h), weighed and ground in a hammermill to pass a 40 mesh screen before analysis of mineral elements.

Experiment III

Five-year old Valencia orange plants (*C. sinensis* (L.) Osbeck) grafted on Troyer citrange rootstock were grown individually outdoors in 500 liter tanks filled with coarse sand. Plants were irrigated three times a week with nutrient solution. Nutrient solutions were renewed every week. Large volumes of solutions were used to minimize concentration and pH changes. Nutrient concentrations changed less than 10% within this period. The pH of the initial solution remained relatively unchanged, varying less than 0.5 pH unit in two days. The pH was monitored and readjusted with 1 *N* HCl or 1 *N* NaOH after each irrigation.

The basic nutrient solution was the same as in Experiment II. Treatments consisted of five NO_3^-–N:NH_4^+–N ratios (100:0, 75:25, 50:50, 25:75 and 0:100) at 120 mg L^{-1} total N. Three sources of nitrogen were used to obtain the desired NO_3^-:NH_4^+ ratios. For the 100:0 and 0:100 treatments $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ were used, respectively; the 50:50 treatment was obtained with NH_4NO_3; the 75:25 and 25:75 treatments were obtained adding a suitable ratio of NH_4NO_3, and either $Ca(NO_3)_2$ or $(NH_4)_2SO_4$. Calcium was adjusted to 6.6 m*M* using $CaCl_2$. Nitrogen treatments were initiated in January and continued until December during the same year.

Six uniform plants were used in each treatment. Tanks were randomized over the experimental area and the plants analyzed individually.

At the end of the experiment, 5–8 month old mature leaves were harvested as in Experiment II. Ripe fruits were also harvested for quality analysis.

Analytical procedures

The K, Mg, Ca, Fe, Cu, Zn and Mn concentrations in leaves were determined by atomic absorption (Chapman and Pratt, 1961), and phosphorus was determined by the ammonium molybdate method as described by Jackson (1965). Total N concentration was determined by the semi-micro Kjeldahl method (Bremner, 1965). The distillates were also used for ^{15}N analysis. For the emission spectrometer determination of ^{15}N, each titrated sample was evaporated to 1 mg nitrogen per 2 mL after the addition of 0.8 mL 0.32 *N* H_2SO_4. An appropriate amount (200 μg nitrogen) of concentrated digest was transferred to Rittenberg tubes. After degas-

sing under high vacuum, the ammonia was oxidized to N_2 gas with excess sodium hypobromite solution. In the preparation of the alkaline hypobromite, KI was used to inhibit oxygen formation. The gas samples were analyzed by emission spectrometry with a JASCO-15 analyzer. ^{15}N enrichment was determined from peaks 28, 29 and 30.

Yield, number and size (weight and diameter) of fruits, juice content, rind thickness, total soluble solids (TSS) and titratable acidity (TA) at the time of harvest, were measured in Experiment III. Sixty fruits per treatment (10 fruits per tree) were randomly picked for quality measurements. The juice was extracted with uniform pressure in a mechanical extractor. Total soluble solids (TSS, °Brix) were determined with a refractometer and titratable acidity (TA) by titration with 0.1 *N* NaOH. Colour index (CI) of fruit rind was calculated by measuring the 'L', 'a' and 'b' parameters with a Hunter lab D-25 P-2 colorimeter (Jimenez Cuesta et al., 1981).

Results

Experiment I

In plants fed with $^{15}NO_3^-$, ^{15}N accumulation increased in leaves and roots with increasing NO_3^- concentration in the solution, until a plateau was reached at 120–180 mg L^{-1} N (Table 1, Fig. 1). However, in plants fed with $^{15}NH_4^+$, ^{15}N accumulation increased almost linearly in roots up to 240–300 mg L^{-1} N in the solution, whereas a much lower ^{15}N accumulation was observed in leaves and stems (Table 1, Fig. 2).

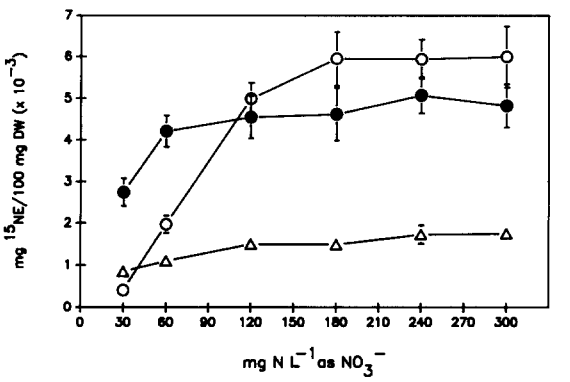

Fig. 1. The effect of $^{15}NO_3^-$ concentration on ^{15}N uptake by leaves, stems and roots during a 2-day period. (Experiment I; ○ leaves; △ stems; ● roots). Values are the means of six replication sand vertical *bars* represent twice the S.D.

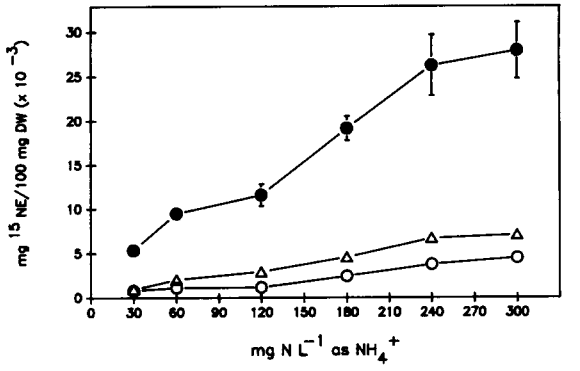

Fig. 2. The effect of $^{15}NH_4^+$ concentration on ^{15}N uptake by leaves, stems and roots during a 2-day period. (Experiment I; ○ leaves; △ stems; ● roots). Values are the means of six replication sand vertical *bars* represent twice the S.D.

Nitrogen uptake by the whole plant was much lower in plants supplied with NO_3^- than in NH_4^+ fed plants (Fig. 3). Maximum ammonium uptake was reached at 240 mg L^{-1} N in the solution,

Table 1. Concentration of ^{15}N (%AE ^{15}N) in organs from plants treated with increasing $^{15}NO_3^-$ or $^{15}NH_4^+$ levels in the nutrient solution (Experiment I; ±S.D., n = 6)

Treatment mg L^{-1} N	Leaves		Stems		Roots	
	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
30	0.014 ± 0.001	0.028 ± 0.002	0.051 ± 0.009	0.073 ± 0.017	0.124 ± 0.018	0.251 ± 0.010
60	0.066 ± 0.003	0.036 ± 0.004	0.066 ± 0.013	0.132 ± 0.023	0.194 ± 0.007	0.415 ± 0.056
120	0.159 ± 0.014	0.043 ± 0.007	0.094 ± 0.015	0.191 ± 0.029	0.210 ± 0.026	0.505 ± 0.085
180	0.192 ± 0.019	0.074 ± 0.030	0.100 ± 0.013	0.278 ± 0.022	0.214 ± 0.023	0.750 ± 0.064
240	0.188 ± 0.008	0.123 ± 0.027	0.114 ± 0.010	0.438 ± 0.017	0.249 ± 0.026	1.066 ± 0.043
300	0.193 ± 0.006	0.137 ± 0.003	0.109 ± 0.008	0.443 ± 0.044	0.225 ± 0.004	1.076 ± 0.049

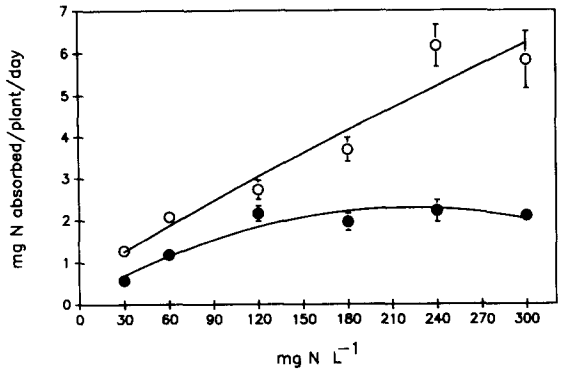

Fig. 3. Uptake rate of nitrogen during a 2-day period by whole plants from solutions containing increasing levels of ^{15}N labeled ammonium or nitrate as the sole nitrogen source. (Experiment I; ○ NH_4^+; ● NO_3^-). Values are the means of six replications and vertical *bars* represent twice the S.D.

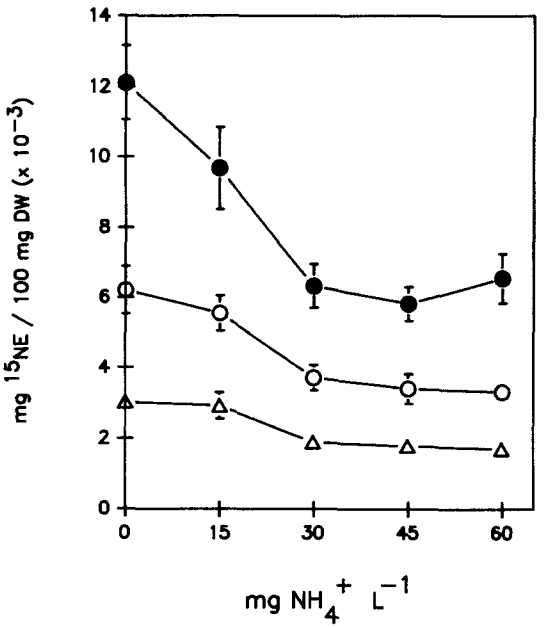

Fig. 4. The effect of increasing NH_4^+ in the nutrient solution on $^{15}NO_3^-$–N uptake by leaves, stems and roots during a 2-day period. (Experiment I; ○ leaves; △ stems; ● roots). Values are the means of six replications and vertical *bars* represent twice the S.D.

whereas nitrate uptake was saturated at 120 mg L^{-1} N. These results indicated that ammonium was absorbed more readily than nitrate by citrus plants. The differences in ^{15}N concentrations between roots and leaves found in both $^{15}NO_3^-$ and $^{15}NH_4^+$ fed plants also indicate that nitrate is transported from roots to shoots more easily than ammonium assimilatory products.

Uptake of ^{15}N by plants exposed to $^{15}NO_3^-$ (120 mg L^{-1} N) was markedly inhibited by addition of NH_4^+ (Table 2). The presence of 30 mg L^{-1} NH_4^+–N reduced the concentration of ^{15}N in leaves (40.2%), in roots (47.6%) and in stems (35.1%) (Fig. 4). Nitrate uptake by the whole plant was depressed by 54.6%, when the concentration of NH_4^+ in the solution was raised to 30 mg L^{-1} N (Fig. 5).

Experiment II

The concentration of N in the leaves was significantly enhanced by increasing the NO_3^-–N level in the nutrient solution from 15 to 120 mg L^{-1} (Fig. 6). However, higher levels of NO_3^- did not further increase leaf-N concentration. In contrast, leaf-N concentration continued to increase with increasing NH_4^+–N levels up to 240 mg L^{-1} (Fig. 6).

With increasing NO_3^- supply, P levels in leaves decreased. In contrast, P concentration increased progressively with increasing levels of NH_4^+, reaching higher values than in NO_3^- supplied plants at N levels in solution higher than 60 mg L^{-1} (Fig. 7). Plants fed with NH_4^+ showed lower leaf-K concentrations than plants fed with NO_3^-.

Table 2. Concentration of ^{15}N (%AE ^{15}N) in organs from plants treated with different NO_3^-/NH_4^+ ratios in the nutrient solution (Experiment I; ±S.D., n = 6)

Treatment (NO_3^-–N/NH_4^+–N)	Leaves	Stems	Roots
120/0	0.234 ± 0.011	0.213 ± 0.003	0.604 ± 0.009
120/15	0.196 ± 0.010	0.186 ± 0.004	0.454 ± 0.008
120/30	0.140 ± 0.009	0.144 ± 0.012	0.323 ± 0.009
120/45	0.123 ± 0.009	0.127 ± 0.010	0.312 ± 0.007
120/60	0.117 ± 0.008	0.122 ± 0.013	0.332 ± 0.007

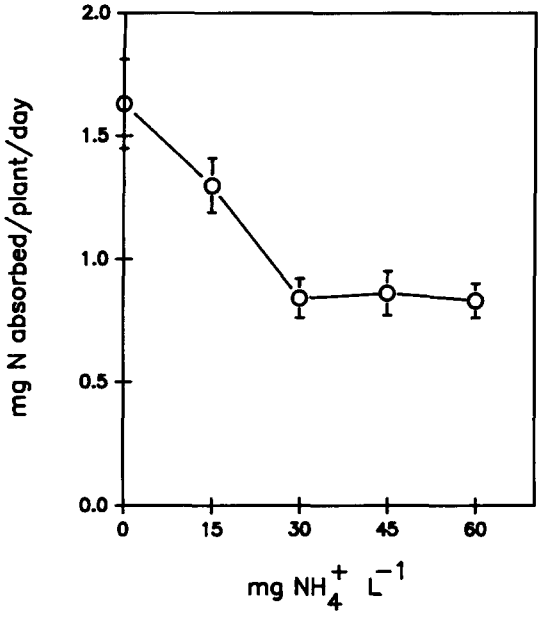

Fig. 5. The effect of increasing NH_4^+ in the nutrient solution on the rate of $^{15}NO_3^-$–N uptake by whole plants (Experiment I). Values are the means of six replications and vertical *bars* represent twice the S.D.

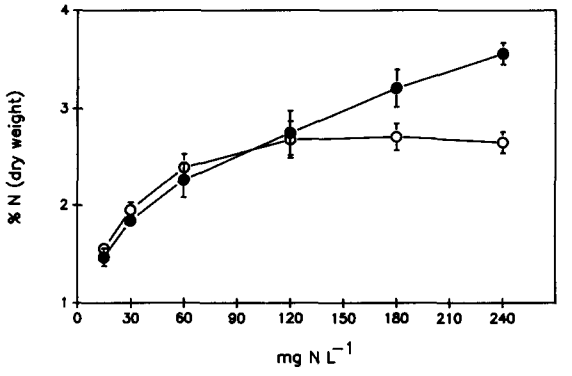

Fig. 6. Concentrations of N in leaves of plants grown in sand fed with different concentrations of NO_3^- or NH_4^+ as the sole N source. (Experiment II; ○ N applied as NO_3^-; ● N applied as NH_4^+). Values are the means of six replications and vertical *bars* represent twice the S.D.

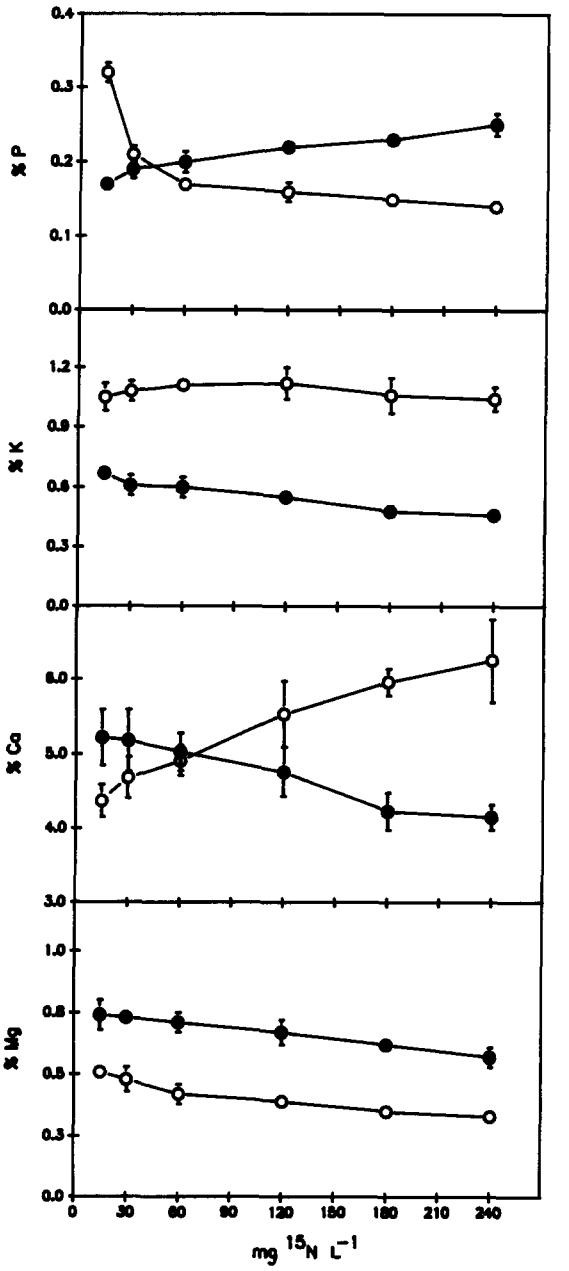

Fig. 7. P, K, Ca and Mg concentrations of leaves as affected by form and concentration of applied N (Experiment II; ○ N applied as NO_3^-; ● N applied as NH_4^+). Values are means of six replications and vertical *bars* represent twice the S.D.

An increase in the level of NH_4^+ in solution reduced K concentration in leaves, whereas NO_3^- levels had little effect (Fig. 7). Increasing NH_4^+ levels in solution, significantly decreased Ca concentration in leaves while nitrate had the opposite effect. Leaf-Mg concentration was greater with N supplied as NH_4^+ (Fig. 7). Nitrate nutrition resulted in higher leaf concentrations of Zn and Mn and lower concentrations of Fe and Cu, when compared to ammonium nutrition (Fig. 8).

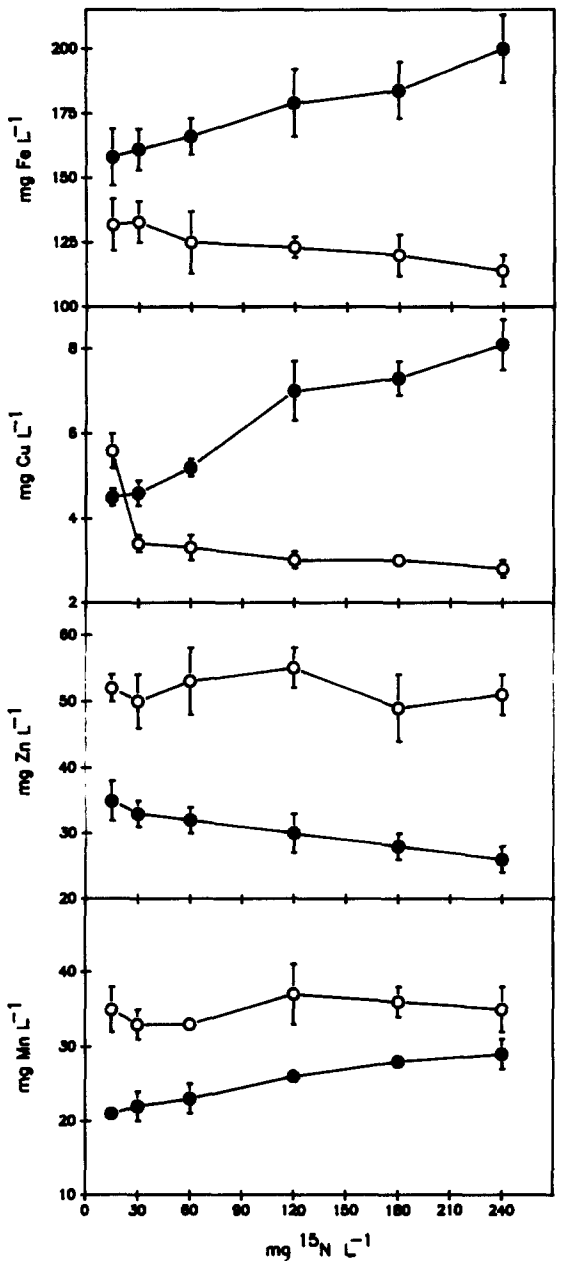

Fig. 8. Fe, Cu, Zn and Mn concentrations of leaves as affected by form and concentration of applied N (Experiment II; ○ N applied as NO_3^-; ● N applied as NH_4^+). Values are the means of six replications and vertical *bars* represent twice the S.D.

Experiment III

Highest N concentrations in leaves were observed at NO_3^-–N:NH_4^+–N ratios of 100:0 and 0:100 in the culture solution (Table 3), being not markedly different for the two treatments. When ammonium and nitrate were supplied together, leaf N concentration was lower than with NO_3^- or NH_4^+ alone. The lowest N concentration in leaves was obtained with the 75:25 NO_3^-:NH_4^+ treatment.

Higher proportions of NH_4^+ in the nutrient solution increased the leaf concentrations of P, Mg, Fe, and Cu, and decreased those of K, Ca, Zn and Mn (Table 3).

Table 4 shows the effect of different NO_3^-/NH_4^+ ratios in the nutrient solution on plant yield and fruit characteristics. With all N supplied in the NH_4^+ form, 20% more fruits per tree was produced than when all N was in the NO_3^- form. Treatments containing 25% and 50% of the N as NH_4^+ produced 36.5% and 19% less fruits per tree, respectively, as compared to 100% NO_3^-. In contrast, the highest fruit weight was obtained with the 25:75 NO_3^-:NH_4^+ treatment and the lowest with 100% NH_4^+. The percentage of peel with respect to total fruit weight, peel thickness and acidity of juice decreased with increasing proportions of NH_4^+, whereas the percentage of pulp with respect to total fruit weight and maturity index (MI = % TSS/% TA in juice) increased. The percentage of juice with respect to total fruit weight, and the content of total soluble solids in the juice were not influenced by the NO_3^-:NH_4^+ ratio in solution. The treatment containing 100% of the N as NH_4^+, slightly enhanced fruit colour.

Discussion

Nitrate and ammonium uptake rates by Citrus plants, as a function of the external concentration of each ion, exhibit two distinct curves. Nitrate uptake rates increase with increasing external nitrate levels until 120 mg L^{-1} NO_3^-–N, when a plateau is reached (Fig. 3). The stabilization in NO_3^- uptake at external NO_3^- concentrations higher than 120 mg L^{-1} N could be due to a saturation of the NO_3^- uptake system or to NO_3^- filling of the root vacuolar compartment as suggested by Glass et al. (1985). This effect might have been produced after the system of NO_3^- transport from roots to leaves was satu-

Table 3. Influence of NO_3^-/NH_4^+ ratio in the nutrient solution on the concentration of nutrients in leaves from plants grown in sand (Experiment III)

	NO_3^-–N/NH_4^+–N ratio (total N = 120 mg L^{-1})				
	100/0	75/25	50/50	25/75	0/100
N (%)	2.94 b	2.19 a	2.29 a	2.69 b	2.97 b
P (%)	0.13 a	0.13 a	0.14 ab	0.16 b	0.18 c
K (%)	0.99 c	0.70 b	0.63 ab	0.52 a	0.47 a
Ca (%)	5.71 b	5.67 b	5.27 ab	4.72 a	4.55 a
Mg (%)	0.33 a	0.38 a	0.38 a	0.47 ab	0.54 b
Fe (mg L^{-1})	130 a	141 a	158 ab	166 b	165 b
Cu (mg L^{-1})	3.4 a	3.4 a	4.4 b	4.9 b	6.1 c
Zn (mg L^{-1})	51 b	56 b	46 ab	35 a	36 a
Mn (mg L^{-1})	27 b	25 b	26 b	20 a	21 a

The values are the means of six replications. Within a row, means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 4. Yield and fruit characteristics of plants grown in sand as affected by NO_3^-/NH_4^+ ratio in the nutrient solution (Experiment III)

	NO_3^-–N/NH_4^+–N ratio (total N = 120 mg L^{-1})				
	100/0	75/25	50/50	25/75	0/100
Fruit number/tree	69.7 b	44.2 a	56.5 ab	69.2 b	84.2 c
Fruit weight (g)	154.3 a	196.4 c	171.7 b	143.9 a	137.5 a
Rind (% by weight)	38.9 b	35.8 b	35.4 b	30.7 a	27.9 a
Rind thickness (mm)	4.3 b	4.6 b	4.2 b	3.7 ab	3.2 a
Juice (% by weight)	53.5 a	53.9 a	57.6 a	51.7 a	52.9 a
Total soluble solids, TSS (%)	12.2 a	11.3 a	11.3 a	11.6 a	11.5 a
Total acidity, TA (%)	2.7 c	2.2 b	2.4 b	1.7 a	1.7 a
Maturity index, MI (=TSS/TA)	4.4 a	5.1 a	4.7 a	6.8 b	6.8 b
Pupl (% by weight)	5.9 a	8.2 b	9.2 b	18.2 c	18.0 c
Colour index, CI	−14.9 a	−13.6 a	−13.3 a	−15.3 ab	−16.3 b

The values are the means of six replications. Within a row, means followed by the same letter are not significantly different at the the 5% level by Duncan's multiple range test.

rated as consequence of an elevated NO_3^- influx in the roots. In contrast, NH_4^+ uptake continued to increase at external NH_4^+ levels up to 240 mg L^{-1} N. Therefore, at high N levels in solution, NH_4^+ is absorbed at higher rates than NO_3^-, whereas intakes of NO_3^- and NH_4^+ were similar below 120 mg L^{-1} N. The results obtained in the short-term experiment with labeled N (Experiment 1) are consistent with the data obtained in the long-term experiment (Experiment II) in which similar response curves were obtained of leaf-N concentrations versus external NO_3^- or NH_4^+ levels (Fig. 6).

Some plant species absorb NH_4^+ at higher rates than NO_3^- (Edwards and Horton, 1982; Fried et al., 1965; Minotti et al., 1969; Peterson et al., 1988). In plants treated with $^{15}NH_4^+$ for 2 days (Experiment I), most of the ^{15}N was accumulated in the roots, indicating a lack of translocation capacity at day 2 of ammonium and/or subsequent assimilatory products. In contrast, the partitioning of ^{15}N between roots and leaves in $^{15}NO_3^-$ treated plants indicates that part of NO_3^- is readily translocated into the xylem and transported to the leaves for enzymatic reduction by nitrate reductase. These results are consistent with the findings of Kato (1981), who observed that in plants fed with NO_3^-, nitrate was the main nitrogen compound found in the xylem sap, while in plants fed with NH_4^+ most nitrogen in the sap was as amino-acids.

The results of Experiment I and II clearly

demonstrated an inhibition of NO_3^- assimilation by NH_4^+ and a reduction in N uptake when both N sources were supplied together. The inhibition of nitrate assimilating processes by ammonium has been observed in ryegrass (Lycklama, 1963), wheat (Minotti et al., 1969), apple (Frith and Nichols, 1975), corn (Mackown et al., 1982; Pan et al., 1985), and dwarf bean (Breteler and Siegerist, 1984). Several mechanisms have been proposed to explain the inhibition of nitrate uptake by ammonium. Ammonium or the products of its assimilation may: (a) decrease the synthesis and activity of nitrate reductase (Lycklama, 1963), (b) inhibit the uptake system (Frith and Nichols, 1975; Mackown et al., 1982; Minotti et al., 1969), (c) reduce the availability of carbon substrates and ATP due to active ammonium assimilation (Ohmori and Hattori, 1978), and (d) increase the efflux of NO_3^-. More work needs to be done to demonstrate which modes of regulation of the nitrate uptake system by ammonia are operating in Citrus plants.

Intake of cations and anions was markedly influenced by the form of N supplied. NO_3^--nutrition stimulated accumulation of K, Ca, Zn, and Mn, whereas NH_4^+-nutrition affected the uptake of P, Mg, Fe, and Cu positively (Experiment II; Figs. 6 and 7). These results are consistent with those of Experiment III, where lower NO_3^-:NH_4^+ ratios in the nutrient solution decreased leaf concentrations of K, Ca, Zn and Mn and increased those of P, Mg, Fe and Cu (Table 3).

A decrease of K, Ca and Mg concentrations and an increase in P concentration of plant tissues with NH_4^+ nutrition, have been observed in other plants (Edwards and Horton, 1982; Hartman et al., 1986). This NH_4^+ stimulated uptake of anions and reduced uptake of cations is generally considered to be the result of the cation:anion balance adjustment in response to an increase in cation (NH_4^+) uptake. Also, loss of membrane integrity, possibly as a result of Ca-ions deficiency under NH_4^+ nutrition, may explain reduced uptake and/or increased loss of ions.

Except for Mg, the results in the literature are in general agreement with those obtained in the present experiments. Probably Mg-ion uptake is more conditioned by the antagonism from other cations such as K and Ca, than by the presence of NH_4^+. Ammonium would act indirectly on Mg-ion uptake by reducing the influx of K and Ca-ions.

One of the problems in comparing ion uptake from a solution containing NO_3^- or NH_4^+, arises from the fact that NH_4^+ uptake reduces the pH of the solution while NO_3^- uptake increases it. It is necessary, therefore, to separate possible indirect pH effects from the direct effects on ion uptake due to N source.

Although a relatively large volume of solution was used per plant, the pH of the solution did not remain stable for more than 24 h. Plants grown in solutions for two days (Experiment I) caused pH changes by 0.5 to 1.0 pH unit. The irrigation procedure and the renewal of nutrient solutions used for the plants grown in sand (Experiments II and III) ensured that the pH of the solutions in the different experiments were kept between 5.5 and 7.5 for the entire experimental period. We checked in an independent culture solution experiment (results not presented), that ion uptake by Citrus plants was not significantly affected in this pH range (5.5–7.5).

Yield of citrus trees (Experiment III) was affected by NO_3^-/NH_4^+ ratio in the nutrient solution. The number of fruits produced per tree was highest when NH_4^+–N was the sole form of N, and lowest for the NO_3^-–N:NH_4^+–N ratio of 75:25. The results indicate that Citrus plants utilized ammonium more efficiently than nitrate for fruiting and that nitrate utilization was improved in the absence of ammonium. Also, a negative interaction was observed between the total number of fruit per tree and unit fruit weight.

Other effects of NH_4^+ and NO_3^- in fruit characteristics might possibly be mediated by the effects of either nitrogen form on the uptake of other ions. Embleton et al. (1973) indicated that reducing K and increasing P levels in Citrus leaves, reduced fruit peel thickness and juice acidity and increased the maturity index (MI) and colour index (CI). Fruits from plants treated with increasing proportions of NH_4^+ in the N

supply showed similar effects in fruit characteristics while P levels in leaves increased and K levels decreased (Tables 3 and 4).

Acknowledgements

We are grateful to the Generalidad Valenciana and the Instituto Nacional de Investigaciones Agrarias for financial support.

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