

# Effect of ammonium/nitrate ratio on growth and development of avocado plants under hydroponic conditions

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Lobit, P., López-Pérez, L., Cárdenas-Navarro, R., Castellanos-Morales, V. C. and Ruiz-Corro, R. 2007. **Effect of ammonium/nitrate ratio on growth and development of avocado plants under hydroponic conditions**. *Can. J. Plant Sci.* **87**: 99–103. An experiment was carried out to study the effects of the proportion of ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) in the nutrient solution on avocado plants in a hydroponic system. Five treatments were applied, consisting of the following proportions of ammonium to nitrate ions in the solution: T0 = 0:4, T1 = 1:3, T2 = 2:2, T3 = 3:1 and T4 = 4:0 at a constant nitrogen concentration of 4 mM. After 4 months, the plants were harvested and fresh and dry matter accumulation, leaf area, and leaf nitrogen and carbon content were measured. An architectural description of the shoot (length, number of nodes and branching) was made. Dry matter of shoots and leaves all decreased with increasing  $\text{NH}_4^+$  concentrations. In contrast, water content in aerial organs increased with  $\text{NH}_4^+$  concentration. Nitrogen and carbon content and the relationship between dry matter production and leaf nitrogen were not significantly affected by the treatments. The architectural analysis showed that the shoots developed over time in one or two distinct growth units. While nitrogen form affected both growth units, the second one was more severely affected, showing decreased length, number of nodes, and branching when  $\text{NH}_4^+$  concentration increased.

**Key words:** Avocado, nitrogen, nitrate, ammonium, development

Lobit, P., López-Pérez, L., Cárdenas-Navarro, R., Castellanos-Morales, V. C. et Ruiz-Corro, R. 2007. **Incidence du ratio ammonium/nitrate sur la croissance et le développement des plants d'avocat cultivés en hydroponique**. *Can. J. Plant Sci.* **87**: 99–103. Les auteurs ont entrepris une expérience afin de préciser les effets de la proportion d'ions ammonium ( $\text{NH}_4^+$ ) et nitrate ( $\text{NO}_3^-$ ) dans la solution nutritive sur les plants d'avocat cultivés en hydroponique. Ils ont appliqué cinq traitements qui correspondaient aux proportions d'ions ammonium et nitrate que voici, dans la solution : T0 = 0:4, T1 = 1:3, T2 = 2:2, T3 = 3:1 et T4 = 4:0, la concentration d'azote demeurant constante à 4 mM. Quatre mois plus tard, ils ont récolté les avocats et déterminé l'accumulation de matière humide et sèche, la surface foliaire ainsi que la teneur des feuilles en azote et en carbone. Les auteurs ont également décrit la structure de la pousse (longueur, nombre de nœuds et ramifications). La quantité de matière sèche dans les pousses et les feuilles diminue quand la concentration de  $\text{NH}_4^+$  augmente. La concentration d'eau dans les organes aériens, en revanche, s'accroît avec celle des ions  $\text{NH}_4^+$ . La teneur en azote et en carbone et les liens entre la production de matière sèche et la teneur des feuilles en azote ne sont pas touchés de façon significative par les traitements. L'analyse de la structure du plant révèle que les pousses se développent selon une ou deux unités de croissance distinctes. Si la forme d'azote affecte ces deux unités, la seconde est plus touchée puisqu'on assiste à une diminution de la longueur, du nombre de nœuds et des ramifications avec la hausse de la concentration d'ions  $\text{NH}_4^+$ .

**Mots clés:** Avocat, azote, nitrate, ammonium, développement

The state of Michoacán, México, is one of the main producers of avocado in the world, with more than 80 000 ha cultivated annually (INEGI 2003). The nitrogen sources most commonly used are urea and ammonium sulfate. However, with the ongoing adoption of new irrigation techniques such as drippers or micro-sprinklers, nitrogen is increasingly applied through the irrigation water in the form of potassium or calcium nitrate. In spite of the variety of nitrogen forms being used, few studies have been carried out to evaluate the influence of the ionic form of nitrogen ( $\text{NH}_4^+$  –  $\text{NO}_3^-$ ) on growth and development of avocado.

The avocado can metabolize both  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , apparently with a preference for  $\text{NO}_3^-$  uptake (Zilkah et al. 2000). In principle, the assimilation cost of ammonium is smaller than that of nitrate (Hopkins 1999). At low concentrations, ammonium often enhances nitrate uptake and plant growth

(Cox and Reisenauer 1973; Haynes and Goh 1978; Thibaud and Grignon 1981; Smart and Bloom 1988, 1993, 1998). However, at higher concentrations, it usually reduces growth (Salsac et al. 1987; Raab and Terry 1994; Gerendás et al. 1997; Cárdenas-Navarro et al. 2006).

The effects of the ionic form of nitrogen ( $\text{NH}_4^+$  or  $\text{NO}_3^-$ ) on uptake, assimilation, photosynthesis and growth have been extensively studied in many species, but to our knowledge not in avocado. The purpose of this work was to study the effect of the ammonium:nitrate ratio in the nutrient solution on the growth and architecture of avocado plants (*Persea americana* Mill 'Hass').

## MATERIALS AND METHODS

The experiment was carried out in a greenhouse (shade type, equipped with a shade net and rain shelter but no climate

control) belonging to the Instituto de Investigaciones Agropecuarias y Forestales of the Universidad Michoacana de San Nicolás de Hidalgo, in Morelia, Michoacán, México (lat. 19°45'95"N, long. 101°09'16", alt. 1900 m).

Prior to the experiment, indigenous avocado plants (*Persea americana* Mill.) were planted in black plastic bags (25 cm diameter and 50 cm depth) filled with soil (60% sand, 30% silt and 10% clay) fertilized with 17-17-17 NPK (50 g bag<sup>-1</sup>). The plants were then cut at the base and the rootstocks were grafted with cultivar Hass and allowed to grow for 7 mo. One month before the experiment, the plants were dug out and transplanted into a hydroponics system made of 20 containers filled with 20 L of volcanic gravel ("tezontle", particle size 2 cm) and fertirrigated with a complete nutrient solution [KH<sub>2</sub>PO<sub>4</sub> 1.0 mM, K<sub>2</sub>SO<sub>4</sub> 1.0 mM, Ca(NO<sub>3</sub>)<sub>2</sub> 1.5 mM, CaSO<sub>4</sub> 2.0 mM, MgSO<sub>4</sub> 1.5 mM, H<sub>3</sub>BO<sub>3</sub> 20 µM, CuSO<sub>4</sub>·5H<sub>2</sub>O 0.5 µM, Fe-EDTA 15 µM, MnSO<sub>4</sub>·H<sub>2</sub>O 12 µM, (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O 0.05 µM, ZnSO<sub>4</sub>·7H<sub>2</sub>O 3 µM] prepared using deionized water (Cárdenas et al. 1998). Irrigation was applied three times a day, for 1.5 h each time. The hydroponics system was built so that the leached solution could be collected after each irrigation and returned to the irrigation tank. Water was added daily to compensate for the loss by transpiration and the complete solution of each treatment was replaced every 10 d.

Treatments were applied 1 mo after transfer to the hydroponic system. The nutrient solution used to establish the plants was modified to obtain the five treatment proportions of NH<sub>4</sub><sup>+</sup>:NO<sub>3</sub><sup>-</sup> (T0 = 0:4, T1 = 1:3, T2 = 2:2, T3 = 3:1 and T4 = 4:0) at a total nitrogen concentration of 4 mM. Nitrate was supplied as Ca(NO<sub>3</sub>)<sub>2</sub> and ammonium as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, keeping the balance of cations and anions at 14 Eq m<sup>-3</sup> and producing variable concentrations of SO<sub>4</sub><sup>=</sup> (from 4 mM to 8 mM) and Ca<sup>++</sup> (from 9 mM to 13 mM). Each treatment consisted of four replicates (bags) and each bag was positioned randomly in the greenhouse in a 4 × 5 arrangement. The pH of the solution was adjusted every third day between 5.5 and 6.

The plants were allowed to grow for 4 mo and harvested at the end of the experiment. Before collecting organs for analysis, the architecture of the shoots was described as follows. Each newly formed shoot was cut at the base and the distance between each node and the base was measured, so that the total length of the shoot, the number of nodes, and the length of each internode could be calculated. For each node, the development of the bud was recorded (no development or development of a secondary shoot, in which case the length of the secondary shoot was recorded). All primary shoots appeared to develop either one growth unit (between 10 and 15 internodes), or two growth units separated by a region where the length of the internodes was much smaller. In this case, the position of the smallest internode was taken as the limit between the first and second growth unit. The following parameters were calculated for each growth unit: number of nodes, total length, average length of the internodes, length of the longest internode, number of axillary leaves, number of secondary shoots, and average length of secondary shoots. The average values for all these variables were calculated for each plant.

Leaves and shoots were separated and their total fresh and dry weights (after drying at 105°C for 48 h) per plant were measured on a balance (Mettler Toledo PR8002; Mettler Toledo Inc., Columbus, OH). Total leaf area per plant was measured with a leaf area meter (Li-Cor LI-2001; LI-COR, Lincoln, NE). Water content in leaves and shoots was calculated as the ratio (fresh weight – dry weight)/dry weight for each plant. Specific leaf area for each plant was calculated as the leaf area/leaf dry weight ratio. Total nitrogen and carbon concentration were determined in a 6 mg sample of ground leaves (ball mill Fritsch Pulverisette 5; Fritsch GmbH, Idar-Oberstein, Germany) per plant, using the Dumas method (Elemental Analyzer Carlo Erba Flash EA 1110; Carlo Erba Reagenti SpA, Milan, Italy).

Data were analyzed using R software for linux (R Development core team, 2004), using ANOVA and the pairwise t-test (95% confidence level) with the Benjamini and Hochberg (1995) correction for differences between means.

## RESULTS

### Growth and Composition

There was a general trend towards a reduction of dry matter accumulation in all aerial parts as NH<sub>4</sub><sup>+</sup> proportion in solution increased (Table 1). The biomass accumulated in shoots was most affected, with a 56% reduction of dry matter accumulation between the pure NO<sub>3</sub><sup>-</sup> (T0) and pure NH<sub>4</sub><sup>+</sup> (T4) treatments ( $P \leq 0.05$ ). A similar trend was observed in leaves (47% reduction of dry matter accumulation) and in the whole plant (41% reduction of dry matter accumulation), although with a low level of significance ( $P \leq 0.10$  in both cases).

No significant differences in total leaf area were found among treatments, where treatment means ranged from 4386 to 8633 cm<sup>2</sup> per plant and from 40.48 to 57.75 cm<sup>2</sup> per leaf. However, specific leaf area increased significantly with the proportion of NH<sub>4</sub><sup>+</sup> in solution (Fig. 1). No significant differences in N and C content in leaf dry matter were observed in response to the proportion of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> in the solution, where treatment means for N ranged from 0.026 to 0.028 g N g dry weight<sup>-1</sup> and the C/N ratio ranged from 18.02 to 19.70 g g<sup>-1</sup>. Furthermore, when considering each plant individually, a unique linear relationship was found between the total amount of nitrogen in leaves and the dry matter accumulated in the aerial portion. In other words, the relationship between total plant biomass and total leaf nitrogen was independent of the form of nitrogen supplied (Fig. 1).

Water content in all organs was found to increase with the proportion of NH<sub>4</sub><sup>+</sup> in solution (Table 1). The overall increase was 28% for leaves, 17% for shoots (both significant with  $P \leq 0.05$ ), and 22% for the whole plant (significant at  $P \leq 0.10$ ) between the pure NO<sub>3</sub><sup>-</sup> (T0) and pure NH<sub>4</sub><sup>+</sup> (T4) treatments.

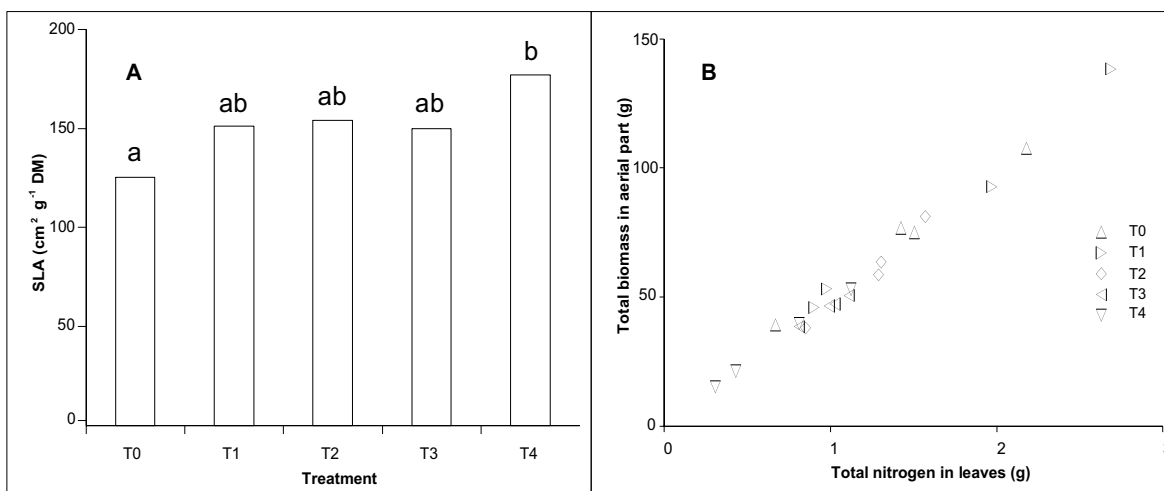
### Architecture

The length of primary shoots decreased with the proportion of NH<sub>4</sub><sup>+</sup> in solution (Table 2). When NH<sub>4</sub><sup>+</sup> increased from nil (T0) to 100% (T4), shoot length presented a 36% decrease (significant at  $P \leq 0.05$ ).

**Table 1.** Effect of the proportion of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  on dry matter accumulation and water content in whole plant, leaves and shoots. The treatments correspond to the following  $\text{NH}_4^+:\text{NO}_3^-$  ratios: T0 = 0:4, T1 = 1:3, T2 = 2:2, T3 = 3:1 and T4 = 4:0. The significance level ( $P$  value) was determined by analysis of variance

	Dry matter (g)			Water content (g fresh wt – g dry wt/g dry wt)		
	Plant	Leaves	Shoots	Plant	Leaves	Shoots
T0	74.66	54.83	19.83a	2.55	2.15a	3.67a
T1	82.58	60.30	22.27ab	2.81	2.47ab	3.76ab
T2	60.34	45.56	14.77ab	2.82	2.46ab	3.94ab
T3	45.72	35.70	10.02ab	2.73	2.38ab	3.98ab
T4	32.26	24.92	7.33b	3.19	2.84b	4.41b
P Value	0.07	0.10	0.04	0.07	0.04	0.05

*a, b* Means within columns with different letters indicate pairwise means comparison tests with statistical difference at  $P \leq 0.05$ .



**Fig. 1.** A. Effect of the proportion of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  on specific leaf area. Treatment means with different letters indicate statistical difference at  $P \leq 0.05$ . B. Relationship between the total amount of N in the leaves of each plant and the biomass accumulated in its aerial part (shoots + leaves). Each symbol represents one plant, different symbols mark different treatments. The treatments correspond to the following  $\text{NH}_4^+:\text{NO}_3^-$  ratios: T0 = 0:4, T1 = 1:3, T2 = 2:2, T3 = 3:1 and T4 = 4:0.

When the first and second growth units of the shoots were analyzed separately, differences in shoot length could be attributed mostly to differences in the development of the second growth unit (Table 2). There was a 49% reduction (significant at  $P \leq 0.05$ ) in the length of the second growth unit compared with a 12% reduction in the first growth unit (significant at  $P \leq 0.01$ ) with increasing  $\text{NH}_4^+$ . Similarly, the number of nodes was reduced by 37% in the second growth unit (significant at  $P \leq 0.05$ ), and was unaffected in the first one.

The number and average length of secondary shoots were also strongly influenced by the proportion of  $\text{NH}_4^+$ . However, the branching patterns of the first and second growth unit were different. While there were no statistically significant differences in the number of secondary shoots on the first growth unit, their length was significantly reduced as the proportion of  $\text{NH}_4^+$  in solution increased (Table 3), with a 48% reduction (significant at  $P \leq 0.05$ ) between the pure  $\text{NO}_3^-$  (T0) and pure  $\text{NH}_4^+$  (T4) treatments. In contrast, the number of secondary shoots on the second growth unit

showed a 94 % reduction (significant at  $P \leq 0.05$ ) between the pure  $\text{NO}_3^-$  (T0) and pure  $\text{NH}_4^+$  (T4) treatments (significant at  $P \leq 0.05$ ) while their size was not significantly affected (Table 3).

### DISCUSSION

The nitrogen source used in the nutrient solution affected the vegetative growth of the avocado plants. In general, increasing the proportion of ammonium in solution decreased vegetative growth and produced plants with lower dry matter content, and shorter primary shoots with fewer secondary shoots.

Among the mechanisms proposed in the literature to account for growth inhibition by  $\text{NH}_4^+$ , are: toxic effects of free ammonium ions (Takács and Técsi 1992; Raab and Terry 1994; Claussen and Lenz 1999), interactions between the absorption of  $\text{NH}_4^+$  and that of other nutrients like  $\text{NO}_3^-$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  (Jungk 1979), excessive demand for assimilates for  $\text{NH}_4^+$  assimilation and/or excretion (Krozucker et al. 2001), perturbation of water regulations (Chaillou et al.

**Table 2. Effect of the proportion of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  on the growth of the primary shoots. The treatments correspond to the following  $\text{NH}_4^+ : \text{NO}_3^-$  ratios: T0 = 0:4, T1 = 1:3, T2 = 2:2, T3 = 3:1 and T4 = 4:0. The significance level (P Value) was determined by analysis of variance**

	Length (cm)			Number of nodes		
	Whole shoots	1st growth unit	2nd growth unit	Whole shoots	1st growth unit	2nd growth unit
T0	42.79a	14.74a	28.04a	27.44	12.75	14.69a
T1	30.68ab	12.19b	18.49ab	22.97	11.90	11.07ab
T2	29.78ab	9.69b	20.09ab	22.64	11.12	11.53ab
T3	25.06ab	9.00b	16.06ab	22.49	11.98	10.51ab
T4	27.30b	12.94b	14.36b	23.09	13.88	9.21b
P value	0.02	0.007	0.02	0.10	0.12	0.03

a, b Means within columns with different letters indicate pairwise means comparison tests with statistical difference at  $P \leq 0.05$ .

**Table 3. Effect of the proportion of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  on the development of secondary shoots on the first and second growth units of primary shoots. The treatments correspond to the following  $\text{NH}_4^+ : \text{NO}_3^-$  ratios: T0 = 0:4, T1 = 1:3, T2 = 2:2, T3 = 3:1 and T4 = 4:0. The significance level (P Value) was determined by analysis of variance**

	Average length of secondary shoots (cm)		Number of secondary shoots	
	1st growth unit	2nd growth unit	1st growth unit	2nd growth unit
T0	21.89a	14.56	1.06	2.69a
T1	15.7ab	21.78	1.36	2.00ab
T2	14.19ab	15.99	0.81	1.80ab
T3	13.44ab	16.26	0.80	1.11ab
T4	11.39b	13.15	3.25	0.16b
P Value	0.03	0.26	0.16	0.05

a, b Means within columns with different letters indicate pairwise means comparison tests with statistical difference at  $P \leq 0.05$ .

1986; Salsac et al. 1987; Raab and Terry 1995), or signalling effects, either through a reduction of  $\text{NO}_3^-$  concentration or through alterations of the hormonal balance (Walsh-Liu et al. 2000).

Direct toxic effects of ammonium seem unlikely in the present experiment. These effects should have appeared soon after  $\text{NH}_4^+$  was added to the nutrient solution. On the contrary, parameters associated with the development of the first growth unit (total length of the growth unit and number of nodes formed) appeared to be affected little by the N form supplied, which is consistent with previous reports that avocados in early stages of their development do not respond to nitrogen form (Lovatt 1996). Also, in spite of differences in leaf morphology, the N form did not seem to affect their functioning: leaf nitrogen concentration remained around 2.69% N in all treatments, which is within the limits considered as optimal by several authors (Lovatt 1996; Rengrudkij 2003; Wolstenholme 2004), no effect on the nitrogen/carbon ratio was observed, and the relationship between the amount of N in leaves and biomass accumulation suggests that the photosynthetic efficiency of leaf N was not affected by the N form supplied.

Indirect effects of  $\text{NH}_4^+$  on the uptake of mineral nutrients cannot be discarded, as  $\text{NH}_4^+$  has been shown to decrease root growth and root hair formation and, therefore, the uptake of elements such as zinc and iron (Crowley 1997). The acidification of the substrate associated with  $\text{NH}_4^+$  uptake may also have affected the absorption of mineral ions. However, this was unlikely to be the case in this experiment since there were no visible symptoms of deficiency, all elements in the solution were maintained at rather

high levels by renewing the solution and the pH was adjusted regularly.

The most likely effect of nitrogen is that of affecting hormonal signals in functions related to morphogenesis. The nitrate ion by itself is known to be involved in signaling in a variety of physiologic processes mainly related with nitrogen reduction and assimilation, but also with the architecture of the root (Takei et al. 2002). Nitrogen nutrition also affects the synthesis and translocation of cytokinins that affect morphogenesis (Kende and Zeevaart 1997).

Plant response to  $\text{NH}_4^+$  changed during development. In the first growth unit, the number of nodes was not affected, and the total length only slightly, with no progressive response to  $\text{NH}_4^+$ . In contrast, during the second growth unit, these variables were significantly reduced by an increase in the proportion of  $\text{NH}_4^+$  in the solution. The branching pattern is another indication of this change in response to  $\text{NH}_4^+$ . As the fate of an axillary bud is determined by the conditions prevalent at the time of its formation (Kervella et al. 1995), the fact that the number of axillary shoots was not affected during the formation of the first growth unit indicates that the plants were insensitive to nitrogen form during the formation of the first growth unit, and became affected during the second one. The length of the axillary shoots confirms this response over time; these shoots were initiated during the first growth unit, but their elongation occurred at the same time as the second growth unit developed. The fact that their average length decreased with the proportion of  $\text{NH}_4^+$  in the solution confirms that the sensitivity to N form appeared at the time the second growth unit started to develop.

The reasons for this change in sensitivity to the form of nitrogen remain unknown. A change in hormonal response during the juvenile stage of the plant is possible. Also, much of the first growth unit probably relied on the reserves of the scion (both nitrogen and carbohydrate reserves), which may have made the plant less dependent on external N sources, while the second growth unit required uptake and assimilation of external nitrogen, and was affected by nitrogen form. The alternating periodicity of shoot and root development is also a well known phenomenon, particularly in tropical trees (Thaler and Pagès 1996). In this experiment, nitrogen during the first growth unit may have affected the following root development, while the effect on aerial development may have appeared only during the second growth unit.

### CONCLUSIONS

This study presents the effects of the proportion of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in nutrient solution on vegetative growth and architecture of avocado plants. In general, increasing the proportion of  $\text{NH}_4^+$  depressed dry matter accumulation, shoot extension, and branching. There were no effects of the proportion of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in solution on leaf nitrogen and carbon content nor on the relationship between leaf N content and biomass production.

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