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Determination of Supply Rate and Form of Nitrogen on the Growth of Tomato in Greenhouse

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Abstract: *The growth of tomato plants in this experiment was comparable under different supply rate and forms of nitrogen. As high concentrations of ammonium were toxic to tomato growth, a supply rate of 1 mM nitrogen was identified as a low supply rate and 4 mM nitrogen as a high supply rate. The growth of the tomato plants at each concentration were comparable irrespective of N form allowing the effect of N forms and supply rate to be compared directly. Although the growth of the plants was comparable under each condition, there was significant effect on other factor such as foliar P. Tomato plants supplied with 1 mM and 4 mM nitrate, increasing supply rates of N in the form of ammonium led to a decrease in total P content. The growth of tomato and total content of nitrogen in plant tissue were affected by both nitrogen form and supply rate.*
Keywords: *Nitrogen form, supply rate and plant growth.*

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I. INTRODUCTION

Nitrogen is the main component of amino acids, proteins, nucleic acids and other cellular constituents that are necessary for plant development; therefore the availability of nitrogen is often the major nutrient factor limiting the yield of crop plants (Manuel Ruiz and Romero, 1999; Boquet and Breitenbeck, 2000; Nagel et al., 2001; Cechin and de Fatima Fumis, 2004, Lea and Morot-Gaudry, 2001)). Not only is the supply rate of nitrogen important to plant growth but also the form of nitrogen, either as inorganic forms such as nitrate and ammonium (Clarkson and Hanson, 1980; Cramer and Lewis, 1993) or organic forms such as amino acids (Nasholm and Persson, 2001).

The impact of nitrogen form and supply rate on the plant growth has received great attention by plant physiologists, biologists and ecologists; and has increased the current understanding of nitrogen metabolism in plants (Trapani et al., 1999,Walch-Liu et al., 2000). Also it has been reported that abundant nitrogen supply increases the number of meristems produced by plants and their growth, thus encouraging shoot formation and growth in most plants (Lawlor et al., 1988; Lawlor et al., 1989). The impact of nitrogen form and supply rate on plant is not only restricted to plant morphology, but extends to the underlying physiology including photosynthesis (Lawlor et al., 1989) (Claussen and Lenz, 1999; Cechin and de Fatima Fumis, 2004), the activity of a number of plant enzymes and hormones (Lawlor et al., 1989; Claussen and Lenz, 1999; Nagel et al., 2001; Collier et al., 2003), the total content of nitrogen-containing compounds such as amino acids and proteins (Sanchez et al., 2004),carbohydrate content (Paul and Driscoll, 1997) and the interaction with uptake of a number of mineral elements such as Fe, K+, Ca2+, Mg2+ and P (Zou et al., 2001; Zubillaga et al., 2002). In order to accommodate these influences of both nitrogen form and supply rate, plants must be able to adapt their growth, development and metabolism accordingly. For example, plants adjust the balance between shoot growth and root growth in response to changing nitrogen supply. When nitrate (but not ammonium) is detected by the root, a systemic signal is generated that stimulates leaf expansion (Walch-Liu et al., 2000); however the accumulation of nitrate in shoot has a negative effect on root growth (Scheible et al., 1997).

Root-to-shoot signalling mechanisms include the potential role of morphology, physiology and nitrate itself as a long-distance signal communicating nitrogen availability (Wang et al., 2002). It is clear that plant biochemistry are affected by nitrogen form and supply rate;

however nitrogen alters plant composition much more than any other mineral nutrient. (Walch-Liu et al., 2005; Takei et al., 2001; Takei et al., 2002; Sakakibara, 2003; Jang et al., 1997; Moore et al., 2003; Beuve et al., 2004). The aims of those experiments were firstly to characterise plant growth, development and composition under these growth conditions by measuring the plant height, the accumulation and partitioning of dry matter in shoots and roots, shoot: root ratio. Secondly, to identify the appropriate supply regimes for tomato plants for subsequent experiments.

II. MATERIALS AND METHODS

2.1 Growth conditions of tomato plants

Seeds of tomato plants (Lycopersicon esculentum) Mill. cultivar Moneymaker were incubated in darkness on moist filter paper for 6 days at 25 oC to promote germination. Germinated seeds were transplanted individually (1 plant per pot) to black plastic pots (13.5 × 13.5 × 13 cm) filled with vermiculite (Medium size) (East Riding Horticulture) and then grown in greenhouse conditions for 6 weeks.

2.2 Nitrogen nutrient treatments

Plants were watered with 40% Long Ashton Nutrient Solution (pH 6) modified to contain different concentrations of nitrogen as NO3- (supplied as KNO3) or NH4+ (supplied as (NH4)2SO4). Plants were watered with 0.5, 1, 2, 4 and 8mM NO3- or 1, 2, 4, and 8mM NH4+. Nutrient solution (250 ml) was applied daily. Plants were kept moistened with water throughout the experimental period by standing each pot on a damp piece of capillary matting. Each treatment consisted of 8 replicate plants randomly distributed within the cabinet in the greenhouse.

2.3 Measurements of plant growth

The measurements of the plant height started fourteen days after transplanting and continued until the end of experiment at the rate of once a week. The relative growth rate (RGR) of the plants was calculated based on the change in shoot length per week over the growth period. For the measurement of the dry weight of shoots and roots, tomato plants were harvested six weeks after transplanting. At harvest, the fresh shoots and roots of tomato plants were separated at the crown region. Roots were washed to remove vermiculite residues, rinsed three times in tap water and then blotted dry. Both fresh shoots and roots of tomato plants were placed in separate envelopes and dried at 54 oC for two weeks.

2.4 Determination of total N and P content

On the day of harvesting (42 days post-transplantation), leaf material was harvested, placed in a paper envelope and dried at 54˚C for two weeks for determination of the total amount of nitrogen and phosphorus using the Kjeldahl method. This method converts all of the nitrogenous and phosphate-containing compounds in a tissue sample into ammonia and orthophosphate respectively via the Kjeldahl digestion technique (Allen, 1989). The amount of ammonium and phosphate can be then determined using a spectrophotometer (N is measured at 590nm and P at 690nm).

Approximately 30-100 mg of dry plant material per replicate was weighed and then placed in a 30 ml boiling tube. Boiling tubes and glass marbles large enough to stopper the end of each tube were acid washed prior to use. Five ml of acid solution (3 parts conc. sulphuric acid and 1 part salicylic acid) and then 1 level spatula of catalyst (1 part copper sulphate to 9 parts lithium sulphate) were added to each sample. A marble was placed on each tube to allow reflux of the acid solution during the digestion and prevent the sample from drying out. The samples were heated at 370-390 oC for 8 hours in a fume hood. Once cooled the samples were diluted with distilled water to a total volume of 50 ml. After thorough mixing 15 ml was used for N and P analysis. Samples were analysed for ammonium and orthophosphate via flow injection analysis (Tecator Flow injection analysis system). Recovery of a certified reference hay powder was used for both N and P.

2.5 Statistical analyses

Analysis of variance (ANOVA) was used to determine statistically significant differences between the measurements (Minitab 13.3; Minitab Inc., State College, PA, USA).

III. RESULTS

3.3.1 The impact of nitrogen form and supply rate on the growth of tomato plants.

The effect of varying nitrogen supply rate, supplied as nitrate or ammonium, on the growth of tomato plants is shown in Figure 1.1. Plant height was greatest when plants were supplied with 8 mM nitrate - lower concentrations of nitrate resulted in progressively smaller plants (Fig. 1.1 A). The relative growth rate (RGR) of plants grown on nitrate was greatest 3 weeks after transplantation and then declined (Fig 1.1 B). This peak in RGR was observed in all nitrate treatments. The greater the RGR at week three, the larger the eventual size of the plant. In contrast, plants grew less well when supplied with ammonium and toxicity effects were apparent at the highest concentration (8 mM) supplied. The RGR was much more constant throughout the course of the experiment and the height of the plants at the end of the experiment did not vary as much as in nitrate-fed plants (Fig.1.1 C, D).

Figure 1.2 shows pictures of the plants taken at the end of the experiment, 6 weeks after transplantation. The observed plant growth at the end of the experiment was consistent with the measurements of the plant height made throughout the experiment. Plants supplied with 8 mM nitrate were large and bushy

with well developed root systems whilst those grown on 0.5 mM and 1 mM nitrate were much smaller and had less developed root systems. Although a gradation in size was apparent in plants supplied with ammonium, the differences were less marked and there was a less striking effect on root development.

Figure 1.3 shows the dry weight of these plants and the root: shoot ratio (RSR). When nitrogen was supplied as nitrate, the dry weight of shoots and roots was greater than that of tomato plants that had been supplied with ammonium (Fig. 1.3 A & B). An increase in nitrogen supply rate of both forms (nitrate and ammonium) was associated with an increase in shoots of tomato plants, except at the highest concentration (8 mM) of ammonium. The roots of tomato plants were also affected by nitrogen form and supply rate (Fig 1.2 C, D). The highest dry weight of roots was recorded in plants fed with 8 mM nitrate (Fig.1.3 A), while the lowest dry weight of roots was found in plants fed with 8 mM ammonium (Fig.1.3 B). The RSR of plants supplied with nitrate was highly responsive to concentration.

The RSR was greatest in plants supplied with 0.5 mM nitrate (RSR = 0.356 +/- 0.017) and was least in plants fed with 8 mM nitrate (0.164 +/- 0.008). .In contrast, the RSR was much less responsive to ammonium supply rate - there was no statistically significant difference in RSR of plants supplied with 1, 2 or 4 mM ammonium and was only reduced in plants fed 8 mM ammonium where toxicity effects were apparent.

1.7 The total content of nitrogen and phosphorus in tomato leave

The total nitrogen and phosphorus content of leaves of tomato plants grown at different supply rates of nitrate or ammonium are presented in Figure 1.4. There was no significant difference in the total nitrogen content of tomato leaves supplied with 0.5 mM, 1 mM and 2 mM nitrate; however a significant increase in nitrogen content was observed when the supply rate of nitrate was increased to 4 mM or 8 mM (Fig 1.4 A). The impact of increasing supply rates of ammonium on total content of nitrogen in tomato leaves is presented in Figure 1.4 B. Again, the total foliar content of nitrogen increased with an increase in the supply rate of ammonium; however the highest content of nitrogen was recorded in tomato leaves fed with 8 mM ammonium. There was no big difference in total content of nitrogen in tomato leaves fed with 1 mM of nitrate or ammonium (~ 17 and ~20 mg g-1 dry weight of leaves) respectively, also ~ 28 mg g-1 in tomato leaves treated with 4 mM nitrate or ammonium (Fig 1.4 A,B).

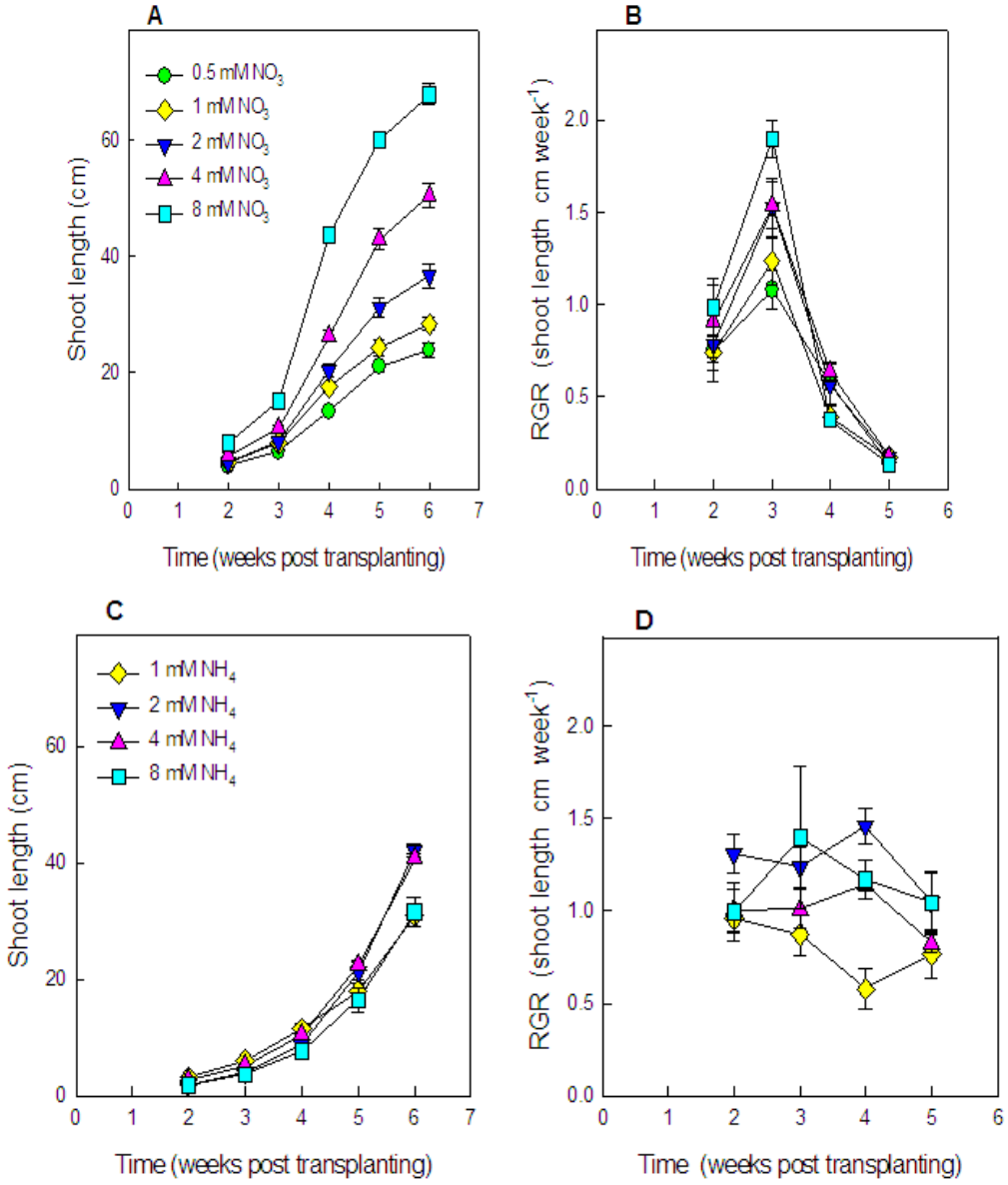
No significant differences in the total content of P were found in tomato leaves grown with different supply rates of nitrate (Fig 1.4 C); however, the total content of P differed significantly in tomato leaves fed with ammonium (Fig 1.4 D). The phosphorus content of tomato leaves declined with increased supply rates of ammonium, except at the highest supply rate of ammonium (8 mM) when toxicity was apparent and the total content of phosphorus increased (Fig 1.4 D).

IV. DISCUSSION

4.1 The impact of nitrogen form and supply rate on the growth of tomato plants:

In order to understand the impact of nitrogen form and supply rates on the growth of tomato plants, plants were grown with different supply rates of nitrogen in the form of nitrate or ammonium and various measurements of plant growth and development made. Increasing supply rates of nitrate from 0.5 mM to 8 mM led to a marked increase in plant growth.

Plant height and dry matter accumulation increased over the entire range of nitrate supply rates examined. This finding is consistent with nitrogen as a major determining factor of plant growth and development (Schortemeyer *et al.*, 1997; Trapani *et al.*, 1999). Although measurements of relative growth rate peaked at 3 weeks after transplantation and then declined, these measurements were made on plant height and did not account for the development of side branches which were numerous in 8 mM nitrate-fed plants. This is consistent with an abundant nitrogen supply increasing the number of meristems produced by plants and their growth, thus encouraging shoot formation and growth in most plants (Lawlor *et al.*, 1988; Lawlor *et al.*, 1989). In contrast, plants supplied with nitrogen in the form of ammonium showed a more complex growth response. Increasing supply rates of ammonium from 1 mM to 4 mM led to an increase in plant growth, although the RGR was relatively uniform throughout the study period. However, plants supplied with 8 mM ammonium were smaller than those supplied with 4 mM ammonium.


A
0.5 mM NO3
1 mM NO3
2 mM NO3
4 mM NO3
8 mM NO3
Shoot length (cm)
Time (weeks post transplanting)
B
RGR (shoot length cm week-1)
Time (weeks post transplanting)
C
1 mM NH4
2 mM NH4
4 mM NH4
8 mM NH4
Shoot length (cm)
Time (weeks post transplanting)
D
RGR (shoot length cm week-1)
Time (weeks post transplanting)

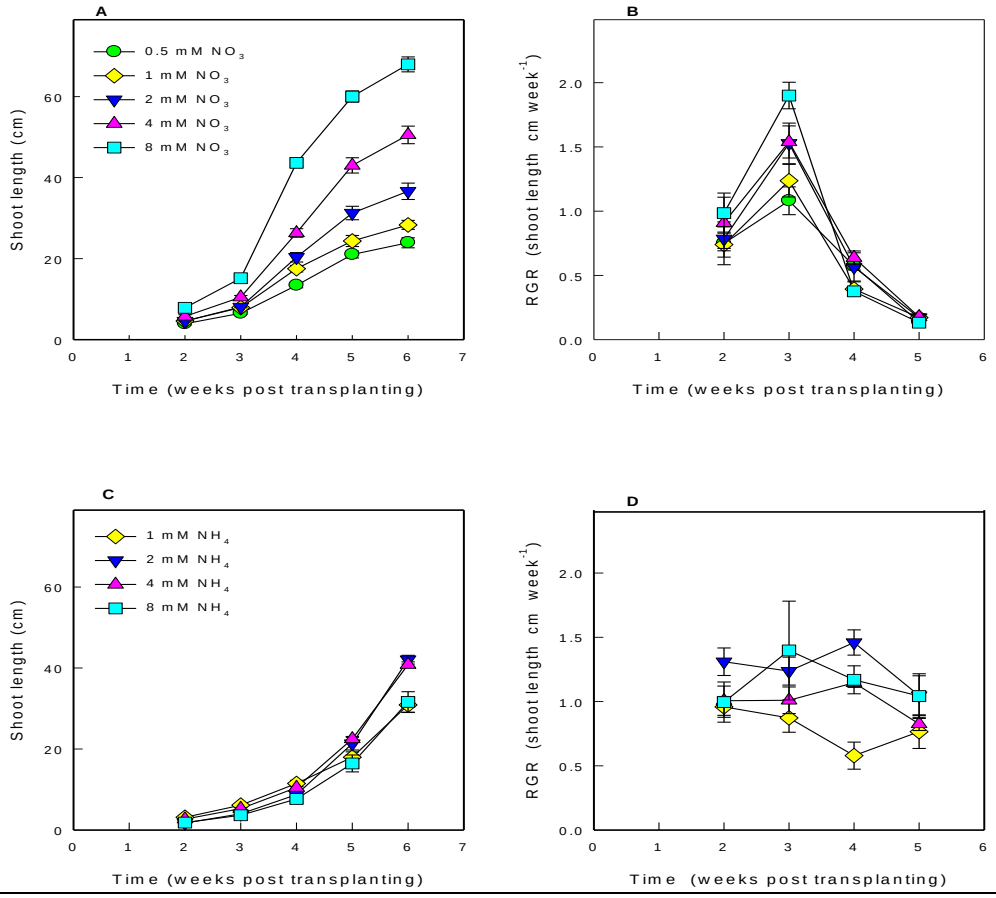
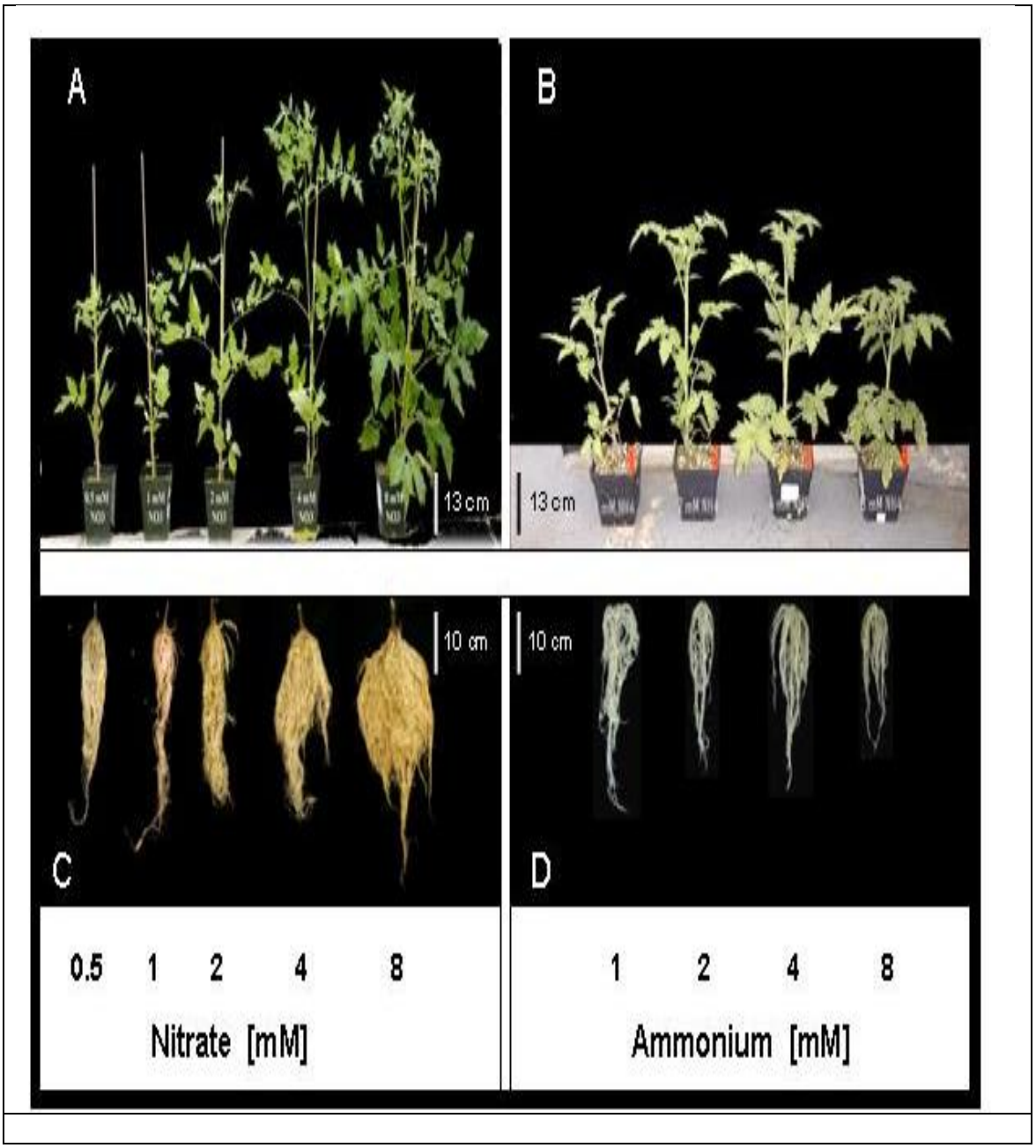


Fig 1.1: The effect of nitrogen form and supply rate on the shoot length of tomato plants. Tomato plants were grown at different supply rates of nitrogen supplied as either nitrate (A&C) ammonium (B&D). Data are means of 8 replicates ±SE.


A
13 cm
B
13 cm
10 cm
10 cm
C
D
0.5 1 2 4 8
Nitrate [mM]
1 2 4 8
Ammonium [mM]

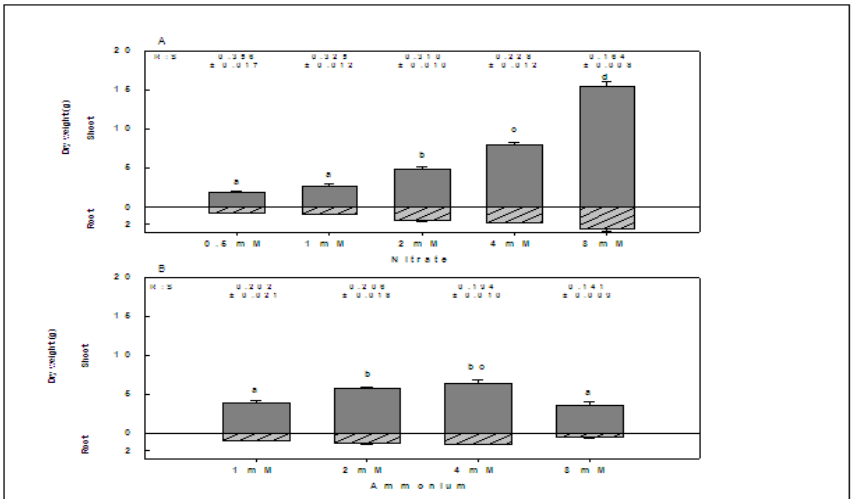


Fig 1.3: The effect of nitrogen form and supply rate on the growth of tomato plants. Dry weight of shoots, roots and root: shoot ratio of tomato plants that have been grown at different supply rates of nitrogen supplied as either nitrate (A) or ammonium (B).Data are means of eight replicates ± SE. Bars sharing the same letter code within the same group do not differ significantly from each other (Tukey's multiple comparison test, $p < 0.001$).

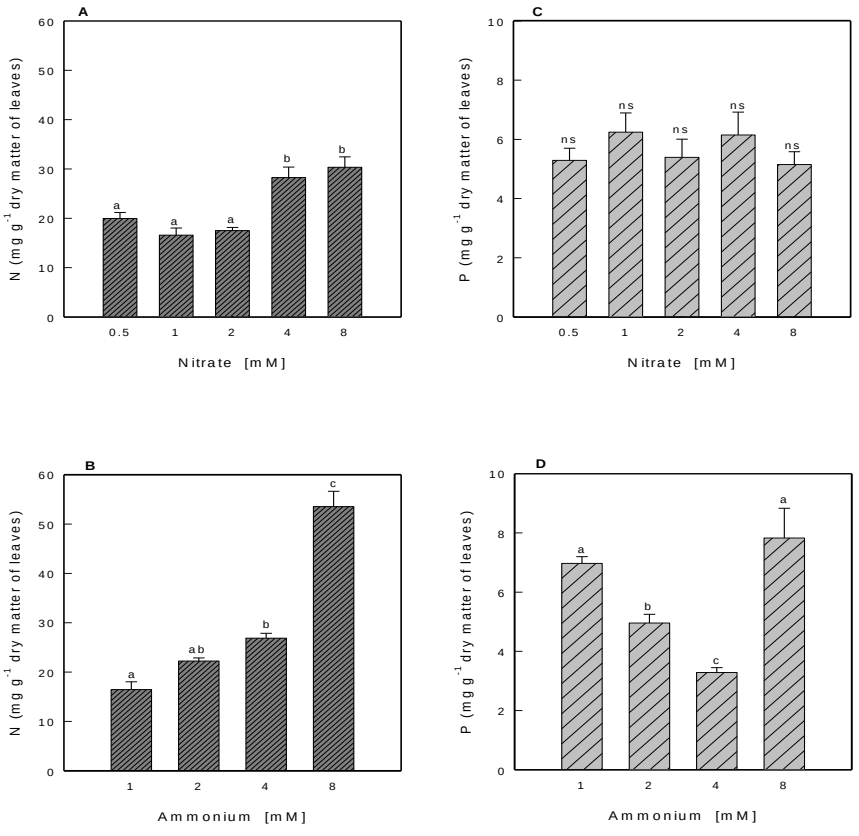


Fig 1.4: Total content of nitrogen and phosphorus of leaves of tomato plants grown at different supply rates of nitrogen in form of nitrate (A&C) or ammonium (B&D) respectively. Data are means of eight replicates ±SE. Bars sharing the same letter code within the same group did not differ significantly from each other. Bars marked with (ns) are not significantly different. (Tukey's multiple comparison test, (A) df = 4, f=15.39 and p<0.001, (B) df=3, f=60.09 and p < 0.001, (C) df=4, f=0.75 and p < 0.567 (D) df=3, f=14.20 and p < 0.001).

Toxicity caused by ammonium nutrition observed in many plant species is thought to occur for several reasons including proton extrusion that is associated with ammonium uptake, cytosolic pH disturbances, displacement of crucial cations such as K^+ and Mg^{2+}, shifts in plant carbohydrate status (Kronzucker *et al.*, 2001) and the high energetic cost of pumping NH_4^+ back out of cells (Britto *et al.*, 2001; Britto and Herbert, 2002). It has been demonstrated that high ammonium supply impairs the growth of plants compared to nitrate–grown plants (van Beusiichem *et al.*, 1988; Cramer and Lewis, 1993). This finding is consistent with the results in this chapter that tomato plants grew best when nitrogen was supplied in the form of nitrate and that high supply rate of ammonium (8mM) were toxic to tomato plants.

In addition to plant growth, the root:shoot ratio (RSR) was significantly affected by nitrogen form and supply rate. The RSR of plants was greatest at low nitrogen supply rates although tomato plants were much more responsive to nitrate than to ammonium. The well-documented increase in root to shoot ratio under nitrogen deficiency has been correlated to shift in endogenous phytohormone levels, with an increase in the abscisic acid and decrease in cytokinins in particular (Palmer *et al.*, 1996). The greater effect of nitrate on RSR compared with ammonium is consistent with nitrate acting as the principal signal influencing RSR as described in section 1.1.

As 8 mM am'high' nitrogen supply rates for further experiments. Plants grew to a comparable extent when supplied with these concentrations of nitrogen whether supplied as nitrate or ammonium. Plants supplied with high nitrogen were approximately twice the size of those supplied with low nitrogen. It is important to recognise, however, that plant growth at 'high' supply rates was still N limited as further increases in height and biomass accumulation could be achieved with greater supply rates of nitrate.

1.2 Did nitrogen form and supply rate alter the total content of N and P in tomato leaves?

In order to understand the impact of nitrogen form and supply rate on the total nitrogen and phosphorus content of tomato leaves, samples of six week old tomato leaves were harvested and the total content of N and P determined as described in section 2**.**2**.**3**.** The total content of nitrogen in leaves of tomato plants grown at 4 mM and 8 mM nitrate was greater (~1.5-fold) than that of leaves from plants grown at 0.5 - 2 mM nitrate. However, a much greater effect was observed in plants grown with different supply rates of ammonium. Whilst the N content of plants grown at 1, 2 and 4 mM ammonium increased with increasing supply rate, and were comparable to nitrate fed plants, the N content of plants supplied with 8 mM ammonium were much greater than in any other treatment. This finding might be interpreted as a result of increased nitrogen assimilation and monium resulted in toxicity effects, 1 mM and 4 mM nitrogen treatments were selected as 'low' and

subsequent growth in nitrate–grown plants. While the activity of nitrate reductase (NR) and other essential enzymes that are responsible for nitrogen assimilation in plants were clearly affected by different factors including the external supply of nitrogen (Claussen and Lenz, 1999; Stohr, 1999; Imai *et al.*, 2005), it can be assumed that nitrate-grown plants have been able to assimilate nitrate efficiently at a rate appropriate to support the observed growth. This is also likely to be true of plants supplied with 1 - 4 mM ammonium. However, at the 8 mM ammonium supply rate, the toxicity effects of high ammonium have inhibited plant growth leading to an accumulation of N within the foliar tissue. This increase may not be attributed to an increased activity of NR and other related enzymes of nitrogen assimilation but to the impact of ammonium toxicity on general metabolic processes of these plants.

Whilst there were no significant differences in the total content of P of tomato leaves supplied with different concentrations of nitrate, the P content of tomato plants supplied with ammonium declined with increasing N supply over the 1 - 4 mM range, but then increased significantly at the 8 mM supply rate. The latter can again be attributed to the toxicity of high ammonium supply rates limiting plant growth hence P taken up by the root system, albeit inefficiently, accumulated in the foliar tissues. The reduction in P content of tomato leaves supplied with 1 - 4 mM ammonium might be attributed to increased proton extrusion as a result of ammonium assimilation. The decreased pH of the medium would result in a decrease in the uptake of P by the plant roots. Such decreases in P content have been shown to have great effects on both the rate of leaf expansion and photosynthetic rate per leaf area of sunflower suggesting that phosphorus allowed more efficient use of supplied nitrogen (Lawlor, 1993; Rodraguez *et al.*, 1998; Zubillaga *et al.*, 2002).

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