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Influence of additional ammonium supply on some nutritional aspects in hydroponic rose plants

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SUMMARY

The influence of an additional supply of ammonium to a nitrate containing hydroponic solution on the mineral nutrition of greenhouse rose plants (*Rosa hybrida* cv. 'Lambada') has been investigated. The effect on NPK uptake, mineral contents in roots and leaves as well as nitrate reductase (NR) and glutamine synthetase (GS) activities were examined. The addition of ammonium in a nutrient solution containing nitrate produced a total nitrogen uptake increase during shoot elongation, while in the absence of ammonium, nitrate uptake was lower during shoot elongation. Adding ammonium to the hydroponics solution caused an increase of potassium uptake while ammonium absorption was constant. Phosphate uptake was always higher in combined nitrate plus ammonium treatment, likewise in this treatment the most relevant effect on plant mineral content was the increase of P concentration in the roots. In both treatments with or without NH_4^+, free nitrate was notably higher in the roots than in leaves, indicating that nitrate reduction in rose plants takes place mainly in the leaves. NR activity in the leaves was higher when ammonium was present, whereas the root GS activity was similar in both treatments. The influence of ammonium on phosphate uptake and the subsequent effects on transport of other ions and enzymatic activities are discussed.

INTRODUCTION

In contrast to other crops, roses do not experience antagonism between vegetative growth and production. The improvement of vegetative growth implies higher flower production; each shoot will develop a flower. Nitrogen has been described as an essential element for increasing the number of flowers and their quality (Agbaria *et al.* 1996). However, when nitrogen is applied only in nitrate form, it usually causes chlorosis (Laurie & Kiplinger 1944). The presence of ammonium in nutrient solutions is beneficial for hydroponic rose growth. It has been reported that applying 25% of total N requirements in the form of ammonium significantly increases greenhouse rose yield in terms of flower production (White & Richter 1973; Feigin *et al.* 1986). The radicular pH must be around 5 to 6 to ensure membrane stability and, as a result, nutrient absorption (Zieslin & Snir 1988; Zieslin & Abolitz 1994). Recent works using buffered solutions (either nitrate or ammonium based) showed that pH control achieved by balancing NH_4^+ and NO_3^- rather than ammonium content per se, could be the origin of improved plant responses to ammonium uptake (Pilbeam & Kirkby 1992; Cabrera & Evans 1995). It was suggested that the problems observed using ammonium solution (Woodson & Boodley 1982) could be due to pH changes instead of the ammonium content.

The present study uses a simplified hydroponics system and a single stem-shoot rose model to evaluate nitrate and ammonium uptake patterns and the effect of ammonium on the uptake of NPK during shoot elongation. The nitrate reductase and glutamine synthetase activities and mineral contents were evaluated as internal metabolic markers.

MATERIALS AND METHODS

Plant material

Eight-month-old rose plants cv. 'Lambada' were established in a simplified hydroponic system under glasshouse conditions. Plants were forced to produce

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a single shoot from their base by eliminating all bar one shoot and every axillary and flower bud from this shoot. The shoot length was measured every 2–5 days until the flower bud was developed and petal colours became visible, at which time leaf and root samples were analysed and enzyme activities determined. The elongation rate was calculated subtracting each measurement from the previous.

During the assay the temperature varied between 18 and 35 °C and the maximum photosynthetic flux density varied from 1000 to 300 μmol/m² per s. Plants were divided into 2 lots of five plants.

Treatments

Two treatments were applied: a nutrient solution containing 120 ppm NO_3-N (N treatment) and a nutrient solution containing 120 ppm NO_3-N plus 20 ppm NH_4-N (C treatment). Other elements in the nutrient solution were: PO_4-P 50 ppm; K^+ 90 ppm; Mg^{++} 35 ppm, Ca^{++} 43 ppm, SO_4 1300 ppm. Micro-elements were adjusted as described by Cid *et al.* (1995).

Each individual plant was cultured in 5 litres of nutrient solution that was renewed every 5 to 7 days. The pH was adjusted daily to 5·6 using sulphuric acid or calcium hydroxide. The ion uptake NPK was estimated by measuring the initial and final nutrient solution volumes and ion contents each time the solution was changed. A control hydroponic solution was set up to monitor potential nitrification.

Determination of mineral contents and enzymatic activities

Nitrate content in the nutrient solution was measured by reduction to nitrite (through a copper-cadmium column) and the nitrite quantified as the azo dye formed with sulfanilamide and N-1-napthylethylene-diamine at 540 nm. Ammonium content in the nutrient solution was measured by colorimetry of the ammonium salycilate complex (pH 12·8) at 660 nm. Phosphate content in the nutrient solution was analysed by colorimetry of the vanadomolibdo-phosphoric acid complex at 420 nm. Potassium content in the nutrient solution was measured by atomic absorption spectrophotometry adding caesium chloride to the solution to avoid background interference.

Mineral content (NPK) of leaves and roots were determined by the dry-ash method (Chapman & Pratt 1973). Free nitrate and ammonium were determined using the method described by Padgett & Leonard (1993). Briefly, 20 mg of dry leaf tissue were dispersed in 10 ml of distilled water for 60 min at 45 °C. After centrifugation the nitrate and ammonium content of the supernatant were analysed as described above as were phosphate and potassium.

Nitrate reductase (NR) and glutamine synthetase (GS) activities were measured according to the method described by Agbaria *et al.* (1996). Protein content was measured according to the method described by Bradford (1976).

Statistical data analysis

The statistical programme Systat 5.0 (SPSS Inc.) was used applying variance analysis (ANOVA) to groups of 4–5 replicates. Significant values were considered with $P < 0{\cdot}1$.

RESULTS AND DISCUSSION

Shoot elongation and nutrient uptake

No significant differences in shoot lengths or rates of elongation were found between treatments (Fig. 1), observations in accord with the results by Cabrera *et al.* (1995) for cv. 'Royalty'. Nitrogen uptake patterns depended on treatments during shoot elongation. In the N treatment, N uptake initially decreased during maximum shoot elongation rate but tended to swing upwards as growth slowed prior to flower formation (Fig. 2). With regard to this initial descent, Cabrera *et al.* (1995) suggested that this behaviour could be explained by photoassimilate competition between the stems and roots, wherein the roots lack the energy required for nitrate uptake.

In the C treatment, nitrate uptake and shoot length increased while the NH_4^+ uptake was fairly constant (Fig. 3*a* and 3*b*). Because no nitrification of NH_4^+ was found in the control hydroponic solution, we considered that any ammonium removed from the solution was absorbed by the plant. Nitrogen supplied in the form of ammonium is not only more readily absorbed by the roots but also can be directly

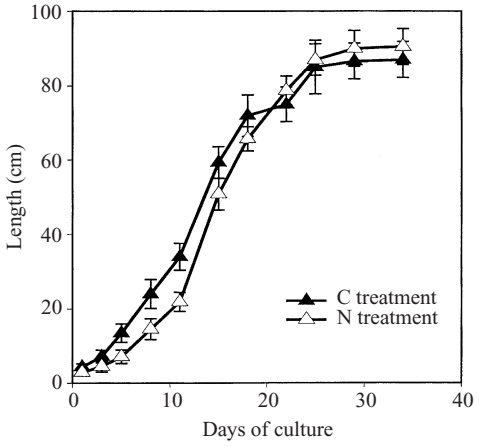

Fig. 1. Renewal shoot elongation in C (nitrate plus ammonium) and N (nitrate) treatments during the hydroponic culture (bars = S.E.).

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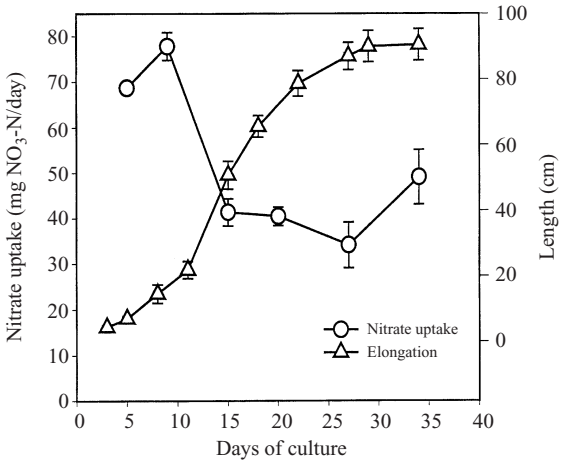

Fig. 2. Nitrate uptake in N treatment in relation to shoot elongation. Ion uptake was measured as described in Materials and Methods. Values represent five replicates (bars = S.E.).

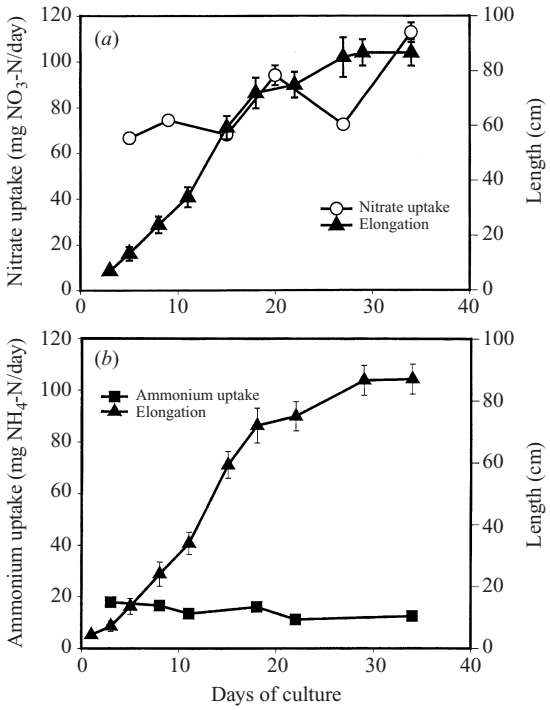

Fig. 3. (*a*) Nitrate, (*b*) Ammonium uptake in treatment C (nitrate plus ammonium) in relation to shoot elongation. Ion uptake was measured as described in Materials and Methods. Values represent five replicates (bars = S.E.).

incorporated into the protein synthesis process at a reduced energetic cost. Many plant species seedlings readily absorb more ammonium than nitrate at the beginning of their growth cycle (McKee 1962).

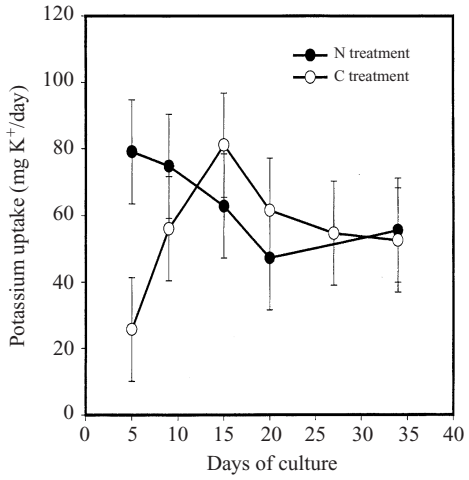

Fig. 4. Potassium uptake during the growth period. Ion uptake was measured as described in Materials and Methods. Values represent five replicates (bars = S.E.).

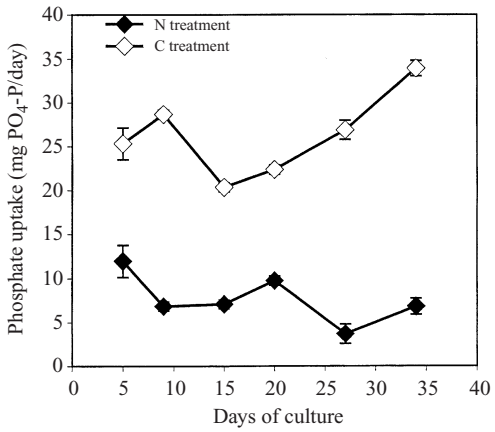

Fig. 5. Phosphate uptake during a growth cycle. Ion uptake was measured as described in Materials and Methods. Values represent five replicates (bars = S.E.).

Initially, at bud formation, K uptake was higher in the N treatment (79 mg/day) than in the C treatment (26 mg/day), but as shoot elongation progressed these values tended to converge, levelling out at 55 mg/day (Fig. 4). The absorption of ammonium at the beginning of the culture could favour the potassium uptake. The nitrate uptake increase during the shoot elongation would produce the decrease of potassium uptake at this stage.

Phosphate uptake was 2 to 5 times higher in the C treatment (Fig. 5), which could be due to PO_4/NH_4^+ synergy to balance charges. The absence of ammonium could produce a lack of phosphate in metabolic processes that involve phosphate in their chemical structure. However, it is very difficult to

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Table 1. *Leaf and root mineral contents. Samples were analysed when shoot elongation stopped and colour petal became apparent*

	Leaves		Roots	
	C	N	C	N
N (% FW)	1·97	1·76	2·72	2·31
P (% FW)	0·31	0·28	1·02	0·56*
K (% FW)	1·42	1·8	1·27	1·55
NO_3-N (‰ FW)	0·1	0·04	2·92†	2†
NH_4-N- (‰ FW)	0·07	0·08	0·29	0·12
NH_4^+/NO_3^- ratio	0·7	2*	0·09	0·06

* Significant values with respect to C treatment based on four replicates and $P < 0{\cdot}1$.
† Significant values with respect to leaf samples based on four replicates and $P < 0{\cdot}1$.

establish a relationship between any of these processes and the phosphate uptake because of the huge number of them present in the cells.

Mineral content

No significant differences were found in total N content of leaves or roots (Table 1). Leaf phosphorus content was similar in both treatments, but root phosphorus was lower in the N treatment as a logical consequence of the reduced PO_4-P uptake. Potassium in both leaves and roots was lower in the C treatment, due likely to NH_4^+/K^+ antagonism, as has been found in similar studies in tomato (Mills & Pokorny 1978). Free nitrate for roots was higher than that in leaves in both treatments, suggesting that nitrate reduction occurs basically in the leaves. This is consistent with previous experiments using ungrafted rose plants (Agbaria *et al.* 1996).

NR and GS activities

Leaf NR activity was considerably higher in the C treatment plants (Fig. 6). This is in agreement with work using cell suspension culture of cv. Paul's Scarlet where it was shown that ammonium promotes NR activity (Mohanty & Fletcher 1976). The low level of NR in the N treatment is probably an effect of the moment in which enzyme determination was carried out (at the end of shoot development). At this point, the reduced nitrate uptake at that point would result in a lack of enzymatic substrate. The low nitrate level would affect the enzyme de-repression/activation or could indirectly cause a low level of NR gene transcription (Caboche & Rouze 1990; Crawford & Arst 1993; Kaiser & Huber 1994). The higher free NH_4/free NO_3 ratio encountered in leaves in the N treatment could inhibit the enzyme, as occurs in other plants and microorganisms.

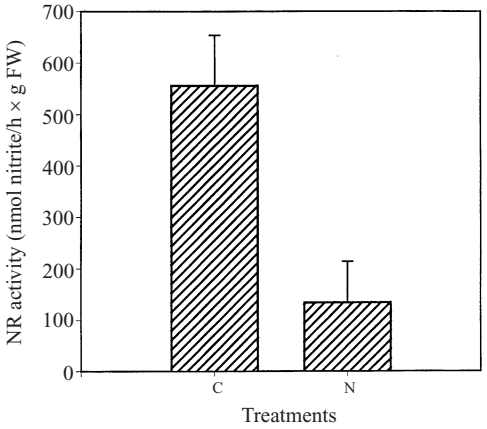

Fig. 6. Leaf nitrate reductase activity. Young leaves were analysed at the end of shoot elongation by the *in vivo* method as described by Agbaria *et al.* (1996). Values represent five replicates.

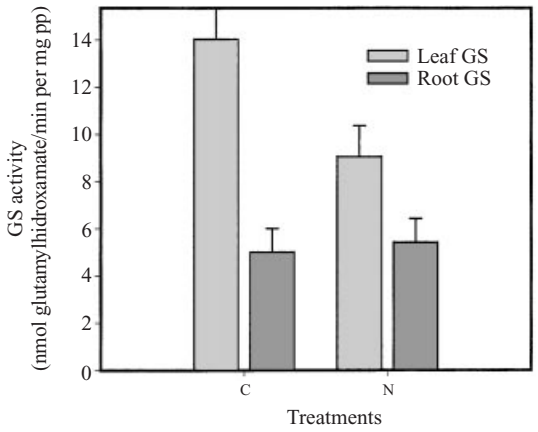

Fig. 7. Glutamine synthetase activity in leaves and roots. Young leaves and roots were analysed at the end of shoot elongation by *in vitro* method as described by Agbaria *et al.* (1996). Values represent an average of five measurements.

The leaf GS activity was slightly higher in the C treatment (Fig. 7), but no differences between treatments were found in roots; this suggests that the GS2 isoform in the chloroplasts was influenced or activated by ammonium.

CONCLUSIONS

The nitrogen sources used in nutrient solutions do not seem to improve shoot growth at least at low ammonium levels, but the uptake pattern observed depended on the treatment. The presence of ammonium implied a higher nitrogen uptake although it did not improve the developing shoot tissues. This nitrogen could be stored in the source shoot.

The presence of ammonium in C treatment has two main effects. With regard to mineral contents, it produced a decrease in potassium level and increased leaf nitrate reductase and leaf glutamine synthetase. The experimental model used for this study does not allow the observation of effects on flower production, but it is known that an ammonium percentage in nutrient solution is beneficial to rose production. In our study it is shown that NR and GS activities could be used as enzymatic markers of plant nutritional status.

Finally, the high level of free nitrate found in roots could indicate that the nitrate reduction is produced preferentially in leaves when they are own-rooted as described by Agbaria *et al*. (1996).

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REFERENCES

Agbaria, H., Heuer, B. & Zieslin, N. (1996). Shoot-root interaction effects on nitrate reductase and glutamine synthetase activities in rose (Rosa × hybrida cvs Ilseta and Mercedes) graftlings. *Journal of Plant Physiology* **149**, 559–563.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein dye binding. *Analytical Biochemistry* **72**, 248–254.

Caboche, M. & Rouze, P. (1990). Nitrate reductase: a target for molecular and cellular studies in higher plants. *Trends Genetics* **6**, 187–192.

Cabrera, R., Evans, R. Y. & Paul, J. L. (1995). Cyclic nitrogen uptake by greenhouse roses. *Scientia Horticulturae* **63**, 57–66.

Chapman, H. D. & Pratt, P. F. (1973). *Metodos de Analisis para Suelos, Plantas y Aguas*. Mexico: Ed. Triallas.

Cid, M. C., Socorro, A. R. & Zieslin, N. (1995). Changes in nutrient solution caused by volcanic cinder media of soilless greenhouse roses in the Canary Islands. *Acta Horticulturae* **424**, 107–110.

Crawford, N. M. & Arst, Jr., H. N. (1993). The molecular genetics of nitrate assimilation in fungi and plants. *Annual Review Genetic* **27**, 115–146.

Feigin, A., Ginzburg, C., Gilead, S. & Ackerman, A. (1986). Effect of NH_4/NO_3 ratio in nutrient solution on growth and yield of greenhouse roses. *Acta Horticulturae* **189**, 127–135.

Kaiser, W. M. & Huber, S. C. (1994). Post-translational regulation of nitrate reductase in higher plants. *Plant Physiology* **106**, 817–821.

Laurie, A. & Kiplinger, D. C. (1944). Culture of greenhouse rose. *Ohio Agricultural Experimental Station Bulletin* **654**, 1–9.

McKee, H. S. (1962). *Nitrogen Metabolism in Plants*. Oxford: Clarendon Press.

Mills, H. A. & Pokorny, F. A. (1978). The influence of nitrapyrin on N retention and tomato growth in sand-bark media. *Journal of American Society of Horticultural Sciences* **103**, 662–664.

Mohanty, B. & Fletcher, J. (1976). Ammonium influence on the growth and nitrate reductase activity of Paul's Scarlet rose suspension cultures. *Plant Physiology* **58**, 152–155.

Padgett, P. E. & Leonard, R. T. (1993). Contamination of ammonium-based nutrient solutions by nitrifying organisms and the conversion of ammonium to nitrate. *Plant Physiology* **101**, 141–146.

Pilbeam, D. J. & Kirkby, E. A. (1992). Some aspects of the utilisation of nitrate and ammonium by plants. In *Nitrogen Metabolism of Plants* (Eds K. Mengel & D. J. Pilbeam), pp. 55–70. Oxford: Oxford Publications.

White, J. W. & Ritcher, D. (1973). Supplementary fluorescent lighting and low moisture stress improve growth of greenhouse roses. *Journal of American Society of Horticultural Sciences* **98**, 605–607.

Woodson, W. R. & Boodley, J. W. (1982). Effects of nitrogen form and potassium concentration on growth, flowering and nitrogen utilization of greenhouse roses. *Journal of American Society of Horticultural Sciences* **107**, 275–278.

Zieslin, N. & Abolizt, M. (1994). Leakage of phenolic compounds from plant roots: effects of pH, Ca^{2+} and NaCl. *Scientia Horticulturae* **58**, 303–314.

Zieslin, N. & Snir, P. (1988). Responses of rose plants cv. Sonia and *R. indica* major to changes in pH and aeration of the root environment in hydroponic culture. *Scientia Horticulturae* **37**, 339–349.