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Transpiration, Potassium Uptake and Flow in Tobacco as Affected by Nitrogen Forms and Nutrient Levels

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• *Background and Aims* Ammonium can result in toxicity symptoms in many plants when it is supplied as the sole source of N. In this work, influences of different nitrogen forms at two levels (2 and 15 mM N) on growth, water relations and uptake and flow of potassium were studied in plants of *Nicotiana tabacum* 'K 326'.
• *Methods* Xylem sap from different leaves was collected from 106-d-old tobacco plants cultured in quartz sand by application of pressure to the root system. Whole-shoot transpiration for each of the treatments was measured on a daily basis by weight determination.
• *Key Results* Total replacement of NO_3^- N by NH_4^+-N caused a substantial decrease in dry weight gain, even when plants grew under nutrient deficiency. Increasing nutrient concentration resulted in a greater net dry weight gain when nitrogen was supplied as NO_3^- or NH_4NO_3, but resulted in little change when nitrogen was supplied as NH_4^+. NH_4^+- N as the sole N-source also caused reduction in transpiration rate, changes in plant WUE (which depended on the nutrient levels) and a decrease in potassium uptake. However, the amount of xylem-transported potassium in the plants fed with NH_4^+ was not reduced: it was 457 % or 596 % of the potassium currently taken up at low or high nutrient level, respectively, indicating a massive export from leaves and cycling of potassium in the phloem.
• *Conclusions* Ammonium reduces leaf stomatal conductance of tobacco plants. The flow and partitioning of potassium in tobacco plants can be changed, depending on the nitrogen forms and nutrient levels.

Key words: Carbon-isotope discrimination ($\delta^{13}C$), *Nicotiana tabacum*, nitrogen forms, nutrient levels, potassium uptake, potassium flow, transpiration, water use efficiency.

INTRODUCTION

Ammonium (NH_4^+) and nitrate (NO_3^-) are two main forms of nitrogen (N) that can be absorbed by higher plants from the natural environment. Because the two forms are fundamentally different in charge, they have quite different effects on the physiological and metabolic processes of plants (Haynes and Goh, 1978; Marschner, 1995). Although NH_4^+ is an intermediate in many metabolic reactions, it can result in toxicity symptoms in many plants when it is supplied as the sole nitrogen source (Britto and Kronzucker, 2002).

One of the inhibitory effects of NH_4^+ on plant growth is its influence on water relations. It has been reported that NH_4^+- N caused an increase in stomatal conductance in white clover (Høgh-Jensen and Schjoerring, 1997), and enhanced the transpiration rate in alfalfa (Khan *et al*., 1994) and tomatoes (Lugert *et al*., 2001). In both cases, leaf water and osmotic potential was lower than that of plants grown under NO_3^- conditions (Ashraf, 1999). However, in *Phaseolus vulgaris*, NH_4^+ was found to decrease stomatal conductance and reduce root water uptake (Guo *et al*., 2002). Other research using *Beta vulgaris* indicated that the N-source had no effect on stomatal conductance, although use of NH_4^+-N did reduce water uptake in muskmelon and sugar beet (Raab and Terry, 1994; Adler *et al*., 1996), with a slight reduction in leaf conductance in castor beans (Peuke *et al*., 1994*a*). The dual-affinity nitrate transporter gene *AtNRT1.1* (*CHL1*) is expressed not only in roots, but also in guard cells of *Arabidopsis thaliana* plants and functions as a NO_3^- transporter, and it has been demonstrated that *CHL1* supports stomatal function in the presence of NO_3^- (Guo *et al*., 2003). The results imply that NO_3^- plays a role in stomatal opening, since in the absence of NO_3^- stomatal aperture and transpiration are reduced.

NH_4^+-N nutrition also causes a decrease in water use efficiency (WUE) in tomatoes (Lugert *et al*., 2001; Claussen, 2002), white clover (Høgh-Jensen and Schjoerring, 1997), *Phaseolus vulgaris* and *Ricinus communis* (Raven *et al*., 1992), but increases WUE in wheat (Morgan, 1986; Yin and Raven, 1998). It seems that the influence of NH_4^+ on water relations varies depending on the plant species and the experimental conditions. Given these variable responses, it would be useful to determine within species how water relations behave when plants grow under various nutritional regimes and supplied with different nitrogen forms. Stable carbon isotope discrimination ($\delta^{13}C$) is a useful method to study plant photosynthesis (Farquhar *et al*., 1989). It is known that variations in the $^{13}C/^{12}C$ ratio of the organic carbon of plants are a result of genetic and environmental factors (Farquhar *et al*., 1989; Raven *et al*., 1992). N-sources, for example, can affect plant $\delta^{13}C$ by influencing plant WUE and carbon assimilation (Yin and Raven, 1998). A common finding is that a lower WUE is correlated with a more negative $\delta^{13}C$ value (Farquhar *et al*., 1989; Raven and Farquhar, 1990; Raven *et al*., 1992; Yin and Raven, 1997, 1998).

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NH_4^+-N has been found to cause a strong inhibition in potassium uptake by plants (Allen and Raven, 1987). Potassium, as a macro-element in the cells of higher plants, exhibits high mobility. Its flow, however, is quite different depending on plant type (Armstrong and Kirkby, 1979; Jeschke *et al*., 1987; Jiang *et al*., 2001) and can be changed by stress. Under saline conditions, for example, plants have been found to have higher potassium cycling rates compared with plants grown under normal conditions. This phenomenon may contribute to the plant's ability to adjust to saline stress (Jeschke *et al*., 1987; Jeschke and Pate, 1991). Plants infected by a parasitic angiosperm (Hibberd *et al*., 1999) or that have been decapitated (Jiang *et al*., 2001) have also shown changes in potassium flow and cycling patterns within them. However, the extent to which translocation in the xylem and circulation of potassium is altered by the supply of N in different forms under varying nutrient conditions is not clear.

The present experiments were carried out to address these questions. The influence of different N-forms at two nutrient levels on the growth, water relations, and potassium uptake and flow in tobacco plants was investigated to better understand the underlying mechanisms of the inhibitory effects of NH_4^+-N on plant growth.

MATERIALS AND METHODS

Plant cultivation

Tobacco seeds (*Nicotiana tabacum* L. 'K 326') were germinated in a mixture consisting of 60 % (w/w) peat culture substrate, 20 % (w/w) ground maize stalk and 20 % (w/w) perlite, and grown in a seedbed in a naturally illuminated greenhouse for 65 d. The plants were then washed with tap water to remove all substrate from the roots, and transferred to 2·3 L pots (one plant per pot) containing quartz sand (0·25–0·5 mm in diameter). They were supplied initially with a half-strength nutrient solution as used by Walch-Liu *et al*. (2000). The solution consisted of (in mM for full strength): KH_2PO_4 0·5, $MgSO_4$ 1·2, $CaCl_2$ 2·0, KNO_3 2, H_3BO_3 $1{\cdot}0 \times 10^{-2}$, $MnSO_4$ 5×10^{-4}, $ZnSO_4$ 5×10^{-4}, $CuSO_4$ 1×10^{-4}, $(NH_4)_6Mo_7O_{24}$ 1×10^{-5}, Fe-EDTA $1{\cdot}5 \times 10^{-2}$. After 2 d a full strength solution was provided. The pots were watered every morning with an excess of the nutrient solution, a hole in the bottom of the pot allowing drainage. Plants were grown in a growth room with a 14-h photoperiod. The photosynthetically active radiation at the surface of the pots was 220–270 mmol m^{-2} s^{-1} provided by reflector sunlight dysprosium lamps (DDF 400, Nanjing, China). The first harvest was made 106 d after sowing and 41 d after transferring to constant environmental conditions; the second harvest was performed 10 d later.

Treatments and harvest procedures

For harvest, the plants were divided into seven groups of five plants, each of similar size and development. One group was used for the first harvest, the other six groups for the second harvest. On the day of the first harvest, the remaining six groups of plants were treated with either a low (as used by Walch-Liu *et al*., 2000) or high (Hoagland solution) concentration of nutrient solution combined with different forms of nitrogen (NO_3^-, NH_4^+ and NH_4NO_3) as shown in Table 1.

TABLE 1. *Macronutrient composition of the solutions with low or high concentration of nutrients combined with different N-forms (in mM)*

	Treatment					
Composition	Low NH_4^+	Low NO_3^-	Low NH_4NO_3	High NH_4^+	High NO_3^-	High NH_4NO_3
KH_2PO_4	0·5	0·5	0·5	1	1	1
KNO_3		2			5	
NH_4NO_3			1			7·5
$(NH_4)_2SO_4$	1			7·5		
$Ca(NO_3)_2$					5	
$CaCl_2$	2·0	2·0	2·0	5		5
$MgSO_4$	1·2	1·2	1·2	2	2	2
K_2SO_4	1		1	2·5		2·5

In addition, the media with low concentration of nutrients contained the same micronutrient as mentioned in the section 'Plant cultivation'. The high concentration of nutrients contained the following micronutrients (mM): H_3BO_3 $4{\cdot}6 \times 10^{-2}$, $ZnSO_4.7H_2O$ $7{\cdot}6 \times 10^{-4}$, $CuSO_4.5H_2O$ $3{\cdot}2 \times 10^{-4}$, $(NH_4)_6MoO_{24}$ $1{\cdot}6 \times 10^{-5}$, $MnCl_2.4H_2O$ $9{\cdot}0 \times 10^{-3}$, FeEDTA $3{\cdot}7 \times 10^{-2}$.

Plant leaves were numbered in ascending order, starting with the lowest mature leaf, which was designed as leaf 1. Smaller leaves, which had already senesced, were removed. At the first harvest, the youngest unfolded leaf was number 9, and the top part of the plant above this was incorporated into the upper leaves because of its small size. At harvest, the plants were separated into the lower, middle and upper stratum of three leaves each, and also into roots, stem and top. For the second harvest the top included new leaves that had grown out above leaf 9. Roots were washed free of sand with tapwater. The root and the three strata of leaves were divided into two lateral symmetrical parts: one was kept at −20 °C until analysis of tissue NH_4^+ and NO_3^- contents, and the other was dried (70 °C) for determination of ions present in different organs with Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP) (Perkin Elmer 3300 DV, USA).

For the measurement of tissue NH_4^+ and NO_3^- contents, samples were homogenized with distilled water and centrifuged. The residue was washed twice with distilled water by repeating the homogenization/centrifugation procedure. The combined supernatants were used for analysis of tissue NH_4^+ and NO_3^- contents with a TRAACS-2000 autoanalyser (Bran+Luebbe, Germany).

Measurement of transpiration

Whole-shoot transpiration was measured on a daily basis by weighing five pots of each of the treatments at the beginning of the light period, after the daily addition of nutrient solution and draining, and at the end of the light period (08:00 h, 10:00 h and 22:00 h, respectively). The transpiration between 08:00 h and 10:00 h was calculated by averaging the amount of transpiration between 10:00 h and 22:00 h. Corrections were applied for water loss from

pots without plants. In all cases, evaporation from the pots was minimized by covering the exposed surface with a layer of filter paper.

Collection of xylem sap

For collection of xylem sap, plants were grown in special pots in order to apply pressure to the root system (Seel and Jeschke, 1999), but were otherwise treated the same as the plants for harvest. The xylem sap collection procedure was essentially the same as described by Jeschke and Pate (1991). Samples were taken from leaf numbers 2, 5 and 8 (counting up from the base). In brief, approximately midway along the length of the leaf an incision was made into the midrib. The cut surface was washed carefully with distilled water and a Teflon tube attached. Pressure was applied to both the sand substrate and the roots of the treated plants. After slowly applying pressure, xylem sap started to exude from the midrib after reaching a balancing pressure (Seel and Jeschke, 1999), and sap was collected 50 kPa above this pressure. The first exudate was discarded to avoid contamination from damaged cells. Xylem sap was also collected from the stem base of the pressurized plants, either from an incision into the stem (Hibberd *et al*., 1999) or by inserting a syringe needle into the stem until it reached the xylem vessels. Xylem sap was kept on ice during collection and stored at −20 °C before analysis. After appropriate dilution, ions were analysed directly using ICP (PE 3300 DV, USA).

Measurement of carbon isotope discrimination ($\delta^{13}C$)

Leaves (including leaf veins and petioles) were dried at 70 °C and ground into a fine powder. They were then treated and analysed for carbon isotope composition using a mass spectrometer (Delta S, Finnigan MAT, Germany) according to the method described by Farquhar *et al*. (1989), with three internal replications. The $^{13}C/^{12}C$ ratios of the samples were expressed as $\delta^{13}C$ relative to the isotope composition of the fossil belemnite standard from the Pee Dee Formation (PDB).

Estimation of the net flows of potassium through xylem and phloem in the whole plant

Net flows of potassium within plants were estimated using the method described by Armstrong and Kirkby (1979). Based on the assumption that nutrients were transported solely through xylem and phloem, Ca^{2+} could only be transported apically through the xylem and had no mobility in the phloem.

The net xylem flow of Ca^{2+}, $J_{Ca,x}$, transported to various plant parts during a given period was the same of that of the net increment of Ca^{2+}, ΔCa, in the same position during the same phase, where

$$J_{Ca,x} = \Delta Ca$$

The content of K^+ and calcium had a stable ratio in the xylem during relatively stable growth conditions. The $[K/Ca]_x$ ratio was obtained by determining the content of these two elements in the xylem sap collected.

For a given period, the net xylem flow of potassium, $J_{K,x}$, towards a site was calculated from the net increment of Ca^{2+}, ΔCa, and the ratio of potassium and calcium, $[K/Ca]_x$, in the xylem by:

$$J_{K,x} = \Delta Ca[K/Ca]_x$$

The amount of potassium exported through the phloem, $J_{K,p}$, was equal to the difference between the measured potassium increment, ΔK, in each organ and the net xylem import according to:

$$J_{K,p} = \Delta K - J_{K,x}$$

A positive difference indicated net phloem import, while a negative difference implied net phloem export from an organ. Working progressively along the plant from the root to the leaves and top, the net flows of potassium within the whole plant were obtained as shown in Figs 3 and 4. The differences between the quantities of potassium translocated in the phloem and in the xylem also allowed the estimation of transfer processes between these translocation streams.

Statistical treatment

Dry weight increments were obtained from the five replicates of each treatment at the first and second harvest. All further analyses were made with five individual samples for each organ. For statistical analysis of the data, SAS for Windows (version 6.12) was used (SAS, 1987). Differences between data in all tables were tested with ANOVA.

RESULTS

Plant growth and development

At low nutrient levels, all plants treated with the three forms of nitrogen showed symptoms of N deficiency, such as thin leaves and light-green lower leaf colour, and small plant size at the second harvest compared with those grown at higher N-levels. However, the growth of the NH_4^+-fed plants was more inhibited than those of either NO_3- or NH_4NO_3-fed plants. The total net increment in dry weight of the NH_4^+-treated plants was 23·5 % and 22·7 % lower in comparison with that of the NO_3- and NH_4NO_3-fed plants, respectively. Increasing nutrient concentration resulted in a greater net dry weight gain when nitrogen was supplied as NO_3^- or NH_4NO_3, but resulted in little change when nitrogen was supplied as NH_4^+. The total net dry weight gain of the NH_4^+-treated plants was 38·8 % and 47·9 % lower than that of the NO_3- and NH_4NO_3-fed plants, respectively (Table 2). Under the NO_3^- and NH_4NO_3 treatments at both nutrient-levels, the plant top (including new leaves and upper leaves) contributed the most to the dry weight increment, while under the NH_4^+ treatment, the upper and middle leaves were the main sites for assimilate deposition. In spite of the inhibited growth of the plants treated with different N-forms at low nutrient-level during the study period, their root net increments were not reduced in comparison with the respective

TABLE 2. *Initial values and net increments in dry weights of component organs and of whole tobacco plants under different treatments over a 10 d study period*

Position	Initial DW (g organ^{-1})	Net increase in DW (g organ^{-1})					
		Low concentration			High concentration		
		NH_4^+	NO_3^-	NH_4NO_3	NH_4^+	NO_3^-	NH_4NO_3
Top + new leaves	0·43 ± 0·05	0·53^{bc}	0·95^{b}	0·79^{bc}	0·42^{c}	2·05^{a}	2·21^{a}
Upper leaves	0·48 ± 0·08	0·77^{c}	1·15^{b}	1·16^{b}	0·80^{c}	1·49^{a}	1·61^{a}
Middle leaves	0·43 ± 0·15	0·81^{ab}	0·79^{ab}	0·88^{a}	0·84^{ab}	0·71^{b}	0·90^{a}
Lower leaves	0·58 ± 0·15	0·40^{a}	0·44^{a}	0·53^{a}	0·53^{a}	0·39^{a}	0·51^{a}
Stem	0·38 ± 0·18	0·64^{b}	0·69^{ab}	0·54^{b}	0·62^{b}	0·71^{ab}	0·84^{a}
Roots	2·30 ± 0·52	0·62^{bc}	0·90^{abc}	0·98^{ab}	0·57^{c}	0·82^{abc}	1·18^{a}
Whole plant		3·77^{d}	4·93^{c}	4·88^{c}	3·78^{d}	6·18^{b}	7·26^{a}

Values in rows followed by the same letter are not significantly different ($P > 0·05$).

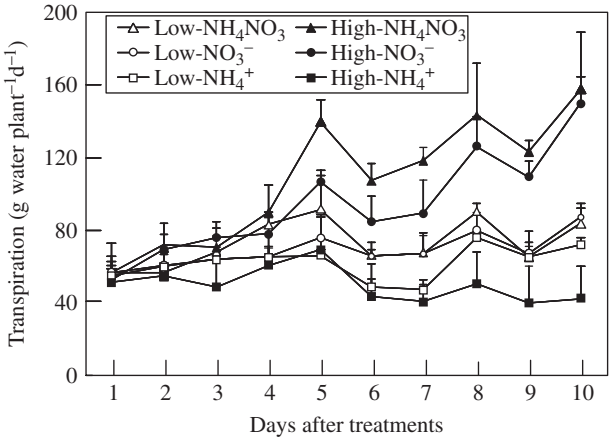

FIG. 1. Time-course of whole-shoot transpiration of tobacco plants as dependent on N-forms and nutrient. Bars denote s.e. of the mean, $n = 5$.

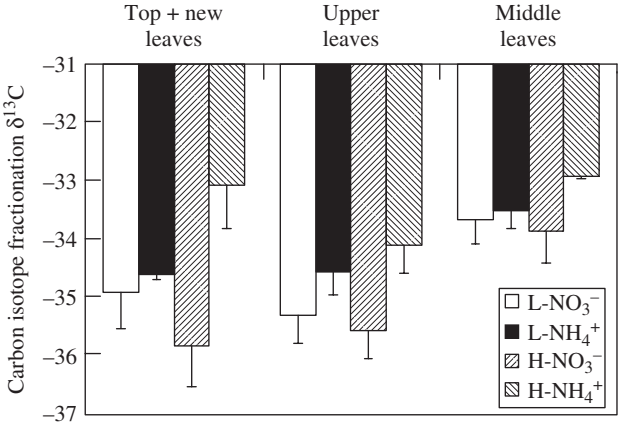

FIG. 2. Carbon isotope fractionation ($\delta^{13}C$, ‰) of leaf tissues of NH_4^+- and NO_3-fed plants at both low and high nutrient levels during a 10 d study period. L and H represent the low and high nutrient level, respectively. Bars denote s.e. of the mean, $n = 5$.

treatments at high nutrient-level (Table 2), resulting in a decreased shoot/total dry weight ratio.

Total transpiration and leaf $\delta^{13}C$

When a high level of nutrients was supplied, the shoot transpiration of the NO_3- and NH_4NO_3-fed plants increased soon after the beginning of the treatment, remaining stable until the end of the experiment. Shoot transpiration declined during the same time for plants fed with NH_4^+ (Fig. 1). At low nutrient level, the shoot transpiration of the NO_3- and NH_4NO_3-treated plants did not increase as much as that of the plants supplied with the same N-forms at higher nutrient level, but was still higher than that of the NH_4^+-fed plants.

At the end of the experiment, samples collected from the middle and upper leaf strata and top and new leaves of the NH_4^+- and NO_3-fed plants were used to compare their stable carbon isotopes $\delta^{13}C$. Tissue $\delta^{13}C$ in the NH_4^+ treatment was less negative (smaller absolute value) than that in the NO_3^- treatment at either nutrient level; especially in the top and new leaves under high nutrient level (Fig. 2).

NH_4^+ and NO_3^- contents in leaf tissues and roots

As shown in Table 3, no significant differences in NH_4^+ and NO_3^- content was observed in the leaves and roots of the plants treated with three N-forms at low nutrient level. At high nutrient level, however, the NO_3^- content in the upper and middle leaves of the NH_4^+-fed plants was substantially lower than that of the NO_3- and NH_4NO_3-treated plants, and NO_3^- could not be detected in lower leaves and roots. The NH_4^+ content, on the other hand, was obviously higher in the upper leaves and roots of both NH_4^+-fed (NH_4^+ and NH_4NO_3) plants than that of the NO_3-fed plants.

Effect of N supply on contents and changes in potassium

The changes in net potassium increment as affected by different N-forms and nutrient levels were similar to, but clearly more dramatic than those in net dry weight gain. The total net uptake of potassium in NH_4^+-fed plants was markedly smaller than that in the NO_3- or NH_4NO_3-fed plants at both nutrient levels (Table 4). The greatest increments of

TABLE 3. *Contents of NH_4^+ and NO_3^- in different leaves and roots of tobacco plants grown under different nutrient concentrations and nitrogen forms over a 10 d study period (mmol organ^{-1})*

Position	Treatment					
	Low NH_4^+	Low NO_3^-	Low NH_4NO_3	High NH_4^+	High NO_3^-	High NH_4NO_3
NH_4^+ content						
Upper leaves	2·65^{b}	2·71^{b}	2·37^{b}	35·17^{a}	4·34^{b}	23·25^{a}
Middle leaves	1·81^{b}	1·29^{b}	1·68^{b}	16·41^{a}	6·81^{ab}	12·75^{ab}
Lower leaves	1·81^{a}	0·92^{a}	2·01^{a}	3·64^{a}	1·30^{a}	2·22^{a}
Roots	12·55^{bc}	4·52^{c}	3·78^{c}	21·90^{ab}	6·18^{c}	28·32^{a}
NO_3^- content						
Upper leaves	–	–	–	0·70^{c}	231·87^{a}	96·96^{b}
Middle leaves	–	–	–	1·77^{b}	58·88^{a}	40·84^{a}
Lower leaves	–	–	–	–	20·49^{a}	21·97^{a}
Roots	–	–	–	–	52·92^{a}	24·06^{b}

Values in rows followed by the same letter are not significantly different ($P > 0·05$). – indicates not detected.

potassium were found in the rapidly growing organs, i.e. top and new leaves, and upper leaves of the NO_3- and NH_4NO_3-fed plants, and top and new leaves, and upper and middle leaves of the NH_4^+-fed plants. Substantial increments were also found in the middle leaves, stem and roots of the NO_3- and NH_4NO_3-treated plants, while that in stem and roots of the NH_4^+-treated plants was much less at both nutrient levels during the study period. Again, as with dry weight gain, the total potassium K^+ contents in the NO_3- and NH_4NO_3-treated plants increased when potassium supply was elevated. The potassium content of the NH_4^+-fed plants was relatively unchanged under the same conditions.

Water use efficiency of plants

The WUE is presented as net increase in plant dry weight per unit water used (g DW increase kg^{-1} H_2O) during the experimental period. The WUE of the NO_3- and NH_4NO_3-fed plants was almost same under both nutrient levels, but that of the low-NH_4^+-fed plants was the lowest and that of the high-NH_4^+-fed plants was the highest among the treatments (Table 5).

Estimation of net flows of potassium within tobacco plants

As shown in Figs 3 and 4, in spite of the inhibited K^+ uptake in the NH_4^+-fed plants, K^+ transport in the xylem was still more than that in the NO_3-fed plants. The highest amount of the xylem-transported potassium was found in the NH_4NO_3-treated plants at both nutrient levels. The amount of the xylem-transported potassium was much more than accounted for by uptake, and was higher at high nutrient level than that at low nutrient level. Phloem retranslocation of potassium from shoot to root contributed to the xylem-transported potassium. The amount of the phloem-retranslocated potassium in the NH_4^+-fed plants was four times more than that of the total uptake under high nutrient levels and 2·6 times more under low levels.

The phloem-recycled potassium from shoot to roots came mainly from leaves (Figs 3, 4). The proportion of potassium exported via the phloem to that imported via the xylem of a leaf depended on leaf age, nutrient levels and N-forms. In plants fed with a high level of NO_3-N, for example, it was 45 %, 70 % and 78 % in the upper, middle and lower leaves, respectively; while under high NH_4^+-N conditions, it was increased to 60 %, 92 % and 94 %, respectively. Also at low nutrient level, the value was 35 %, 68 % and 85 % respectively in the NO_3-fed plants, and 42 %, 89 % and 91 % in the NH_4^+-fed plants.

DISCUSSION

Effect on plant growth

The reported symptoms of NH_4^+ toxicity generally appeared with external NH_4^+ concentrations above 0·1–0·5 mM (reviewed in Britto and Kronzucker, 2002). In spite of the inhibited growth under low nutrient levels (2 mM N), as represented by lower net dry weight gain (Table 2), lower shoot/total dry weight ratio and obvious N-deficient symptoms (photographs not shown), plant growth was further suppressed when NH_4^+ was supplied as the sole N-source. Increasing nutrient concentration (Hoagland, 15 mM N) resulted in a greater net dry weight increment when nitrogen was supplied as NO_3^- or NH_4NO_3, but produced no change when nitrogen was supplied as NH_4^+ (Table 2). These results could not simply be explained by NH_4^+ toxicity. In the present study, the same amount of NH_4^+ was found in the leaf tissues and roots of the plants grown under the low nutrient level with different N-forms. Furthermore, at the high nutrient level, a similar NH_4^+ content was detected in the tissues of both NH_4^+- and NH_4NO_3-fed plants (Table 3): the latter were able to grow well and even obtained the highest net increment in dry weight (Table 2). As shown in Table 3, a large amount of NO_3-N was also present in the tissues of the NH_4NO_3-fed plants grown under the high nutrient level but not in the NH_4^+-fed plants. These results support the conclusion that co-provision of NO_3^- can not only alleviate NH_4^+ toxicity, but may also induce a synergistic growth response that can surpass the maximal growth rates produced by either N-source alone (Britto and Kronzucker, 2002). As long as there was NO_3^-, NH_4^+ did not inhibit plant growth (Walch-Liu *et al*., 2000). One of the important roles of NO_3^- is as a signal to stimulate (or optimize) a multitude of biochemical responses (Tischner, 2000; Britto and Kronzucker, 2002).

Transpiration and water use efficiency

NO_3-N and NH_4NO_3 had similar effects on plant transpiration, while NH_4^+-N caused a marked reduction in transpiration (Fig. 1), although all of the treated plants were watered daily and showed no signs of water stress. The present results do not agree with the observation that use of NH_4^+-N increased leaf transpiration in alfalfa (Khan *et al*., 1994), tomato (Lugert *et al*., 2001), maize and wheat (Lewis *et al*., 1989), and increased stomatal conductance of white clover (Høgh-Jensen and Schjoerring, 1997). Although

TABLE 4. *Initial values and net increments in K^+ contents of component organs and of whole tobacco plants under different treatments over a 10 d study period*

Position	Initial values (mmol organ^{-1})	Net K^+ increment (mmol organ^{-1})					
		Low concentration			High concentration		
		NH_4^+	NO_3^-	NH_4NO_3	NH_4^+	NO_3^-	NH_4NO_3
Top + new leaves	0·49 ± 0·07	0·52^{c}	1·37^{b}	1·08^{bc}	0·46^{c}	3·05^{a}	3·37^{a}
Upper leaves	0·53 ± 0·06	0·68^{c}	1·56^{b}	1·65^{b}	0·81^{c}	2·29^{a}	2·45^{a}
Middle leaves	0·39 ± 0·12	0·52^{c}	0·88^{b}	0·96^{b}	0·60^{c}	1·01^{b}	1·36^{a}
Lower leaves	0·37 ± 0·06	0·25^{d}	0·41^{bc}	0·49^{ab}	0·33^{cd}	0·50^{ab}	0·58^{a}
Stem	0·17 ± 0·06	0·18^{d}	0·68^{b}	0·49^{bc}	0·25^{cd}	1·02^{a}	1·14^{a}
Roots	1·96 ± 0·21	0·15^{b}	0·88^{a}	1·00^{a}	0·11^{b}	0·92^{a}	1·06^{a}
Whole plant		2·30^{d}	5·78^{c}	5·67^{c}	2·56^{d}	8·81^{b}	9·97^{a}

Values in rows followed by the same letter are not significantly different ($P > 0·05$).

TABLE 5. *Water use efficiency (g DW increase kg^{-1} H_2O) in different treatments over a 10 d study period*

	Low concentration			High concentration		
	NH_4^+	NO_3^-	NH_4NO_3	NH_4^+	NO_3^-	NH_4NO_3
WUE	5·69	6·49	6·12	6·89	5·89	6·07

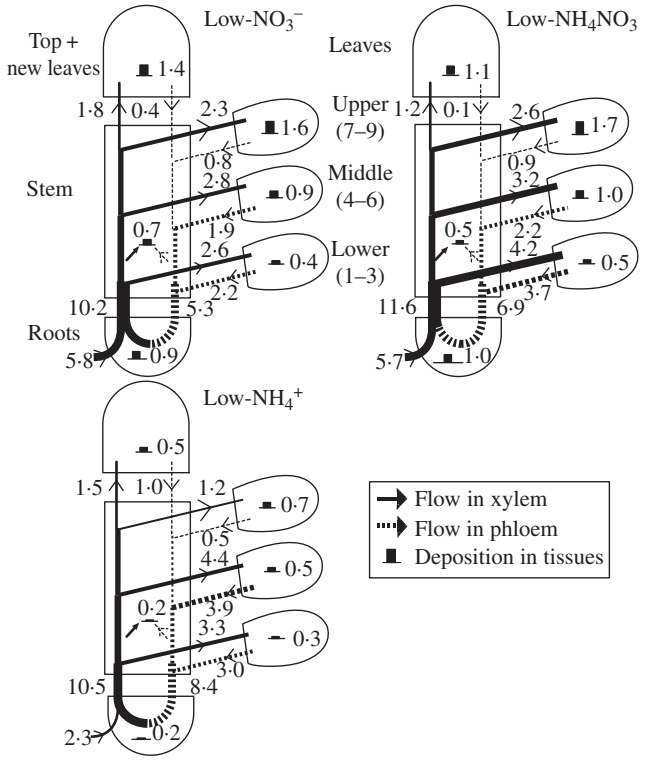

FIG. 3. Flow profiles for uptake, transport and utilization of K^+ in tobacco plants supplied with a low nutrient concentration (2·5 mM K and 2 mM N) over a 10 d experimental period, starting 106 d after sowing. The values of K^+ deposition and the statistical significance are given in Table 3. The width of arrows and the height of histograms are drawn in proportion to the net flows and deposition of K^+. The numbers indicate the values of uptake, transport and utilization (mmol K^+ per plant over the 10 d study period).

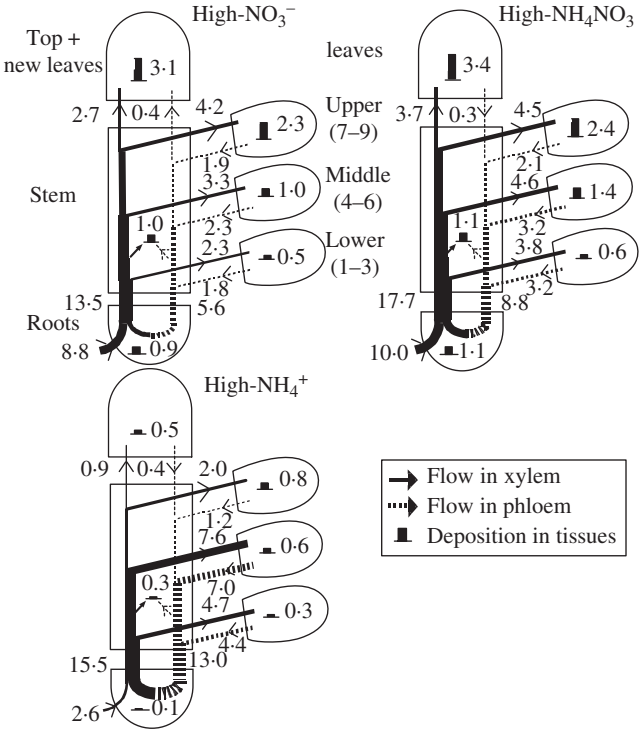

FIG. 4. Flow profiles for uptake, transport and utilization of K^+ in tobacco plants supplied with a high nutrient concentration (6 mM K and 15 mM N) over a 10 d experimental period, starting 106 d after sowing. The values of K^+ deposition and the statistical significance are given in Table 3. The width of arrows and the height of histograms are drawn in proportion to the net flows and deposition of K^+. The numbers indicate the values of uptake, transport and utilization (mmol K^+ per plant over the 10 d study period).

NH_4^+-treated plants have smaller leaf area (Khan *et al.*, 1994; Raab and Terry, 1994) and lower leaf area ratio (Magalhaes and Wilcox, 1984), these could not be the reasons to explain the reduced transpiration in the present study. At the second harvest, the low − NH_4^+-fed shoots increased in dry weight by 3·15 g, 1·64 times more than that at the first harvest, and daily transpiration increased by only 33 %. The high-NH_4^+-fed shoot increased in dry weight by 3·21 g, 1·67 times more than that at the first harvest, while daily

transpiration was reduced by 13 % compared with the first harvest (Fig. 1). Furthermore, results of carbon isotope discrimination for different leaves of the NO_3- and NH_4^+-fed plants indicated a reduced stomatal conductance in the NH_4^+-supplied plants, especially in the top and new leaves, and upper leaves, meaning that the stomata in the leaves were at least partly closed. Also in the NO_3-fed plants, a smaller stomatal conductance at the low nutrient level than that at the high nutrient level was observed (Fig. 2). The results suggest that the reduced leaf transpiration in the NH_4^+-treatment was not due to the reduced leaf area, but due to reduced stomatal conductance (Guo *et al*., 2002). There is some evidence that ABA may be responsible for the reduced stomatal conductance in leaves of the NH_4^+-fed plants, since elevated biosynthesis of ABA and an obvious increase in the xylem and phloem flow of ABA in NH_4^+-fed plants have been observed in comparison with NO_3-grown plants in castor beans (Peuke *et al*., 1994*b*; Jeschke and Hartung, 2000). On the other hand, Guo *et al*. (2003) provided evidence for a role of NO_3^- in stomatal opening, and demonstrated by using *chl1* mutants of *A. thaliana* that the reduced stomatal aperture is not the result of effects on ABA responses. There is also an early report that supplies evidence that KCl supports stomatal opening as well as KNO_3 in *Vicia* epidermal strips (Humble and Hsiao, 1969).

N-sources can influence plant WUE and $\delta^{13}C$ value. A common finding is that a lower WUE is correlated with a more negative $\delta^{13}C$ value (Farquhar *et al*., 1989; Raven and Farquhar, 1990; Raven *et al*., 1992; Yin and Raven, 1997, 1998). Different nutrient levels may also influence WUE of plants. In winter oilseed rape (Brück *et al*., 2001) and sweet potato plants (Kelm *et al*., 2001), a low N level led to lower WUE, since N stress led to increased transpiration per unit leaf area and decreased WUE (Kelm *et al*., 2001). In the present study, however, opposite results were obtained. The WUE of the low-NO_3- and low-NH_4NO_3-fed plants was higher than that of the high-NO_3- and high-NH_4NO_3-fed plants (Table 5). By comparison of the transpiration rate on a leaf dry weight basis at the second harvest, it was found that the transpiration rate for low-NH_4^+-, low-NO_3- and low-NH_4NO_3-fed plants was 18·6, and 17·8 g d^{-1} g^{-1} leaf dry weight, respectively, and for high-NH_4^+-, high-NO_3- and high-NH_4NO_3-fed plants it was 10·6, 25·0 and 23·9 g d^{-1} g^{-1} leaf dry weight, respectively (calculated based on the results in Table 2 and Fig. 1), showing a decreased transpiration rate for the low-NO_3- and low-NH_4NO_3-fed plants compared with that for the high-NO_3- and high-NH_4NO_3-fed plants. The WUE of the NO_3- or NH_4NO_3-fed plants was almost same under both nutrient levels (Table 5), which was in agreement with previous results for wheat (Yin and Raven, 1998) and peanut (Hubick, 1990). The WUE of the low-NH_4^+-fed plants was the lowest and that of the high-NH_4^+-fed plants the highest among the treatments. Apart from the negative influence on leaf stomatal conductance discussed above, the effect of NH_4^+-N on root water uptake rate relative to assimilation of carbon dioxide might also contribute to the difference in WUE under both nutrient levels. At the second harvest, the water uptake rate on a root dry weight basis for low-NH_4^+-, low-NO_3- and low-NH_4NO_3-fed plants was 76, 70 and 62 mL d^{-1} g^{-1} dry weight, respectively; i.e. the low-NH_4^+-fed plants had the highest water uptake rate. For high-NH_4^+-, high-NO_3- and high-NH_4NO_3-fed plants the water uptake rate on a root dry weight basis was 45, 127 and 106 mL d^{-1} g^{-1} dry weight, respectively; i.e. the high-NH_4^+-fed plants had the lowest water uptake rate, and the lowest leaf transpiration (Fig. 1).

Uptake, flow and partitioning of potassium

There was no difference in the amount of K^+ taken up by NO_3- and NH_4NO_3-fed plants at the low nutrient level (2·5 mM potassium). By increasing the nutrient concentration (6 mM K; where the concentration of potassium supplied was 1·4 times more), potassium uptake increased by only 52 % and 75 % in the NO_3- and NH_4NO_3-fed plants, respectively (Table 4). This is in agreement with the proposed theory that cycling of potassium in plants can act as an important signal for feedback control of nutrient uptake (Drew *et al*., 1990; Engels and Marschner, 1992). However, when NO_3-N was replaced by NH_4^+-N, potassium uptake was substantially reduced, and it was and unchanged when the potassium supply was increased (high nutrient level). This is consistent with earlier results from castor bean (Van Beusichem *et al*., 1988) and wheat (Wang and Below, 1998), because of the strong inhibition of potassium uptake by NH_4^+-N (Marschner, 1995; Britto and Kronzucker, 2002).

NH_4^+-N not only inhibited potassium uptake, but also markedly influenced its flow and partitioning within plants. In the NH_4^+-fed plants grown under both nutrient levels, the amount of potassium transported in the xylem was almost the same compared with the NO_3- and NH_4NO_3-fed plants. It was 457 % or 596 % of the potassium currently taken up at low or high nutrient level, respectively (Figs 3, 4), a situation that can be explained only by massive export from leaves and retranslocation of potassium in the phloem. Retranslocation of potassium from the shoot via the phloem and cycling through the roots also took place in the NO_3- and NH_4NO_3-fed plants (Figs 3, 4). It contributed to the potassium transported in the xylem by 52 %, 59 % and 80 % in the low-NO_3-, low-NH_4NO_3- and low-NH_4^+-fed plants, respectively, and 41 %, 50 % and 84 % in the high-NO_3-, high-NH_4NO_3- and high-NH_4^+-fed plants, respectively. Thus, retranslocation of potassium in the phloem of NH_4^+-treated plants exceeded that of the NH_4NO_3- and NO_3-fed plants.

The results from the present study indicate that total replacement of NO_3-N by NH_4^+-N caused a substantial decrease in dry weight gain, even when plants were grown under nutrient deficiency. This suggests that the inhibitory effect of NH_4^+-N on plant growth might not be directly caused by ammonium toxicity. In addition, NH_4^+-N as the sole N-source caused a reduction in transpiration rate, changes in plant WUE (depending on the concentration of nutrient solution) and a decrease in potassium uptake. This leads to a massive export of K^+ from leaves and recycling in the phloem. The results highlight the high degree of reutilization by retranslocation via the phloem, and provide further evidence that the partitioning and retranslocation of potassium in plants can be changed, depending on

environmental conditions (Jeschke *et al*., 1987; Jeschke and Pate, 1991; Hibberd *et al*., 1999; Jiang *et al*., 2001).

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