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Foliar application of nitrate or ammonium as sole nitrogen supply in *Ricinus communis*

I. Carbon and nitrogen uptake and inflows

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SUMMARY

Following a precultivation with pedospheric nitrogen nutrition, nitrate or ammonium solutions were supplied to the shoots of *Ricinus* plants by spraying (during the experimental period) resulting in an increase of biotic/organic and abiotic/inorganic particles on the surface, which significantly increased wetting of the leaf surfaces. The distribution of particles on the surface of sprayed leaves, in particular crystals around and in stomata, indicated the possible entry of nutrients via thin water films through the stomatal pores in addition to diffusion through the cuticle. Ammonium was taken up more readily than nitrate by the foliage, but both at relatively low rates which caused N limitation. Interestingly, the inorganic N, both in the form of nitrate and even ammonium, was entirely assimilated in the shoots; phloem transport of inorganic N to the root was negligible. The flows of malate, and the acidification of the apoplastic washing solution of leaves in ammonium-sprayed plants pointed to the role of metabolism of malate and excretion of protons in maintaining pH during ammonium assimilation in the shoot. Ammonium-sprayed plants incorporated the N in the same amounts in shoots and roots, only 38% of the shoot-borne N being recycled in the xylem. In nitrate-sprayed plants the root was not only favoured in N partitioning, but even a net export of previously incorporated N from the shoots occurred which reflected the N limitation. The N limitation also affected carbon metabolism, in particular the flows of C, incorporation in the shoot and photosynthesis, which were decreased when compared with data from recent experiments with pedospheric well fed *Ricinus*. However, there was little difference in C flows between nitrate and ammonium-sprayed plants with respect to respiration, C partitioning and, most interestingly, in relative stimulation of root growth. The loss of C from dark respiration of the shoots was high on a f. wt basis as well as in relative terms, owing to exclusive N assimilation in the shoot. In general the plants invested untargeted increases in root growth as a result of N limitation irrespective of the imposed artificial treatment which made the shoot the site of mineral N uptake.

Key words: *Ricinus communis* L., foliar application, carbon, nitrogen, nitrate, ammonium, phloem transport, xylem transport.

INTRODUCTION

In higher plants, inorganic nitrogen is usually taken up by the roots as nitrate and/or ammonium. Shoot organs, however, can take up mineral nutrients as well (Clarkson, Kuiper & Lüttge, 1986; Raven, 1988; Marschner, 1995). In agriculture, a common method of fertilization is the application of nutrients by spraying a solution onto the leaves (foliar nutrition). Gaseous air pollutants like NO_2, NH_3 and SO_2 can also diffuse into shoot tissue and can be taken up into the cells. Uptake of the nutrients into the leaves could occur either by penetration through the stomata, or by diffusion through the cuticle. Once in the leaves, the nutrients must be dissolved in the aqueous phase of the cell wall before uptake into

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the symplast can take place. Nutrients taken up by roots from the soil actually follow a similar route, since the supply of shoots with nutrients occurs via apoplastic transport in the xylem (after symplastic loading of nutrients at the endodermis). After reaching the shoots, mineral ions must be taken up once again from an apoplastic pool into the symplast.

There have been several investigations into the uptake and use of sulphurous and nitrogenous gases, but much less is known about foliar deposition of ions. Faller (1972) supplied shoots of plants with SO_2, H_2S, NO_2 and NH_3 as the only S or N sources. At certain gas concentrations he found 'normal growth' and transport of N and S to the roots. Ammonia was incorporated more sucessfully than NO_2. The uptake of atmospheric N by plants is of special interest with regard to increasing environmental pollution. For oxides of N this problem was reviewed by Wellburn (1990) and for ammonia by Pearson & Stewart (1993). In these papers the uptake and biochemical and physiological responses of nitrogenous gases are discussed.

Foliar uptake of N is not only supplementary, but can influence the N status of the whole plant. In spruce, NO_2 uptake could account for up to 40% of NO_3^- uptake in short-term and up to 15% in long-term experiments of the whole-plant N-budget (Muller, Touraine & Rennenberg, 1996). Garten & Hanson (1990) compared the foliar uptake of nitrate with that of ammonium in red maple and white oak by applying simulated rain, and found that ammonium uptake was greater. They later formulated a generalized model for HNO_3 uptake in leaves (Hanson & Garten, 1992).

A general problem in the uptake and assimilation of N by the shoot is the regulation of pH. In roots the problem can be solved by H^+ exchange with the rhizosphere. In the shoot the available exchange space (apoplastic space) is limited, and biochemical or transport mechanisms are needed (Raven & Smith, 1976; Raven, 1988). For example, Pearson & Soares (1995) assumed that plants having a greater capacity to reduce nitrate in the leaves are physiologically more competent at buffering acidic inputs from atmospheric pollution.

In previous studies we described quantitatively the fundamental difference between the two N sources nitrate and ammonium fed to roots, in regard to uptake and flow of nutrients within the whole plant (Peuke & Jeschke, 1993). Both N uptake and C uptake were slightly decreased in ammonium-fed plants, and as a consequence the growth was reduced. We have also investigated the site of inorganic-N assimilation (Peuke *et al.*, 1996). The reduction of nitrate takes place in roots as well as in shoots, but the proportions depend on nutritional conditions. With higher nitrate supplies more nitrate was reduced in the shoots. Ammonium, however, was almost completely assimilated in the roots. Consequently, roots had a higher demand for C if N assimilation was enhanced in the roots. This C was needed as an energy supply and to provide skeletons for amino acids. The transport of inorganic N in the phloem was always very low for both ions, leading to the conclusion that inorganic N transported to the shoots cannot be recycled from the shoots of *Ricinus*.

The question arose of how plants would deal with a situation where N is exclusively supplied by leaf nutrition without pedospheric N. Would the plants then assimilate all inorganic N in the shoots, even in the form of ammonium? Or would it be possible to transport nitrate or ammonium to the roots via the phloem, owing to high inorganic-N concentrations in the shoot? Additionally, a possible consequence might be a reduced C transport to the roots if N is only assimilated in the shoots.

In more detail, the questions refer to the site of N assimilation, maintenance of pH, the nature of the N directed into long-distance transport and the response of C metabolism under conditions of foliar application of N.

In an attempt to answer these questions, N supply during the experimental period was restricted to spraying a solution of nitrate or ammonium onto the leaves. Roots were not supplied with any N. Effects on C and N flows between roots and shoots, assimilation of N and pH relations, as well as changes in surface properties of sprayed leaves as an indication for the route of uptake were investigated.

MATERIALS AND METHODS

Plant cultivation

Seeds of *Ricinus communis* L. were germinated in vermiculite moistened with 0·5 mM $CaSO_4$. After 10 d uniform seedlings were transferred to 5-l pots filled with quartz sand. The plants were supplied daily to excess with a nutrient solution, which contained 1 mM nitrate as the N-source (Peuke & Jeschke, 1993). To adapt the plants to the spraying before the experimental period the shoots of one half of the population were sprayed daily with 20 mM KNO_3, the other half with 10 mM $(NH_4)_2SO_4$. Some of these sprayed solutions reached the quartz sand and were diluted there by the nutrient solution, but could by taken up by the roots. To achieve the sole foliar supply of N 28 d after sowing, the rhizosphere was separated from the shoot by covering the pots with plastic foil which was sealed to the hypocotyl with a layer of elastic silicone material. In the nutrient solution applied to the roots, KNO_3 and $Ca(NO_3)_2$ were replaced by KCl and $CaCl_2$. Nitrogen was applied from day 28 after sowing, up to the end of the experiment by spraying only the leaves twice daily with 20 mM KNO_3 or 10 mM $(NH_4)_2SO_4$. These high concentrations were chosen to obtain a supply as high as possible: preliminary experiments showed that concentrations higher than these were damaging. The plants were sprayed as long as they were totally wet at every treatment, so the exact amount of N applied could not be stated. After 40 d, 1 d before the experimental period, the cotyledons were removed.

The plants were cultivated in a glasshouse (15–25 °C; 45–70 % r.h.) with an artificial 16-h photoperiod provided by Osram® HQL 400 (300–500 μmol photons m^{-2} s^{-1}).

Harvesting and analysis

Forty-one d after sowing, nine plants from each treatment were harvested, and 10 d later a second harvest was performed. The plants were divided into roots, stems, petioles and leaf laminae of the primary and the secondary leaves, and every plant part was carefully washed with water before chemical analysis. During the experimental period (41–51 d after sowing) xylem and phloem saps were collected from the hypocotyl, and the respiration of the plant parts was measured. Carbon and N in the plant tissue were determined by a CHN-analyser (CHN-O-RAPID Heraeus, Hanau, Germany). C:N ratios in the transport fluids were calculated from the composition of solutes (amino acids and ammonia by an amino-acid analyser, and nitrate and malate by Anionenchromatograph, both from Biotronik Co., Maintal, Germany). Methods such as documentation of growth, harvesting of plant material, collecting xylem and phloem saps, extraction, chemical determinations and respiration measurements were as described in detail recently (Peuke & Jeschke, 1993; Peuke, Hartung & Jeschke, 1994).

The flows of C and N were modelled according to the method of Pate, Layzell & McNeil (1979), Jeschke, Atkins & Pate (1985) and Jeschke & Pate (1991). In this method the incremental data and the concentration relations of the two elements in the transport sap were combined on the basis of three assumptions:

(1) ion uptake occurred only through the roots;
(2) ions were returned to the roots solely by phloem transport;
(3) transport exchange took place by mass flow in xylem or phloem.

This resulted in the flows of N and C in phloem ($J_{N,P}$ and $J_{C,P}$) according to the following equation:

$$J_{N,P}/J_{C,P} = [N]_P/[C]_P.$$

The relation of the flow of N in the phloem to that of C is the same as that of the concentrations therein ($[N]_P/[C]_P$).

The increment of N in the shoot ($\Delta_{N,shoot}$) resulted from the difference of xylem ($J_{N,X}$) and phloem flow ($J_{N,P}$):

$$\Delta_{N,shoot} = J_{N,X} - J_{N,P}.$$

For C, the contribution of photosynthesis (C_{fix}) and respiration (C_{res}) must be included:

$$\Delta_{C,shoot} = J_{C,X} + C_{fix} - C_{res} - J_{C,P}.$$

The uptake of an element could be calculated by the sum of increments in all organs:

$$\text{Uptake} = \Delta_{shoot} + \Delta_{root}.$$

With these assumptions and equations, the flow of C in the phloem could be calculated:

$$\begin{aligned} J_{C,P} &= (([C]_P/[N]_P) \times (([C]_P/[N]_P) - ([C]_X/[N]_X))^{-1}) \\ &\quad \times (\Delta_{C,root} + C_{res} - ([C]_X/[N]_X) \\ &\quad \times (\Delta_{N,root} - N_{uptake})). \\ J_{N,P} &= J_{C,P} \times ([C]_P/[N]_P)^{-1}, \\ J_{N,X} &= \Delta_N - J_{N,P}, \\ J_{C,X} &= J_{N,X} \times ([C]_X/[N]_X). \end{aligned}$$

The flows of inorganic N and malate were modelled on the basis of the N flows (Peuke *et al.*, 1996). Since nitrate, ammonium and malate are very mobile and metabolizable ions, the values must be regarded as net flows including synthesis (not for nitrate) and degradation. To make the flows comparable with those in earlier experiments, they were calculated on the basis of the mean f. wt during the experimental period. The values are given as μmol g^{-1} f. wt $(10\ d)^{-1}$ and in parentheses as mmol per plant $(10\ d)^{-1}$.

Scanning electron microscopy

Upper and lower surfaces of *Ricinus* leaves treated with NO_3^-, NH_4^+ or water (control) were investigated by scanning electron microscopy. Samples of $5 \times 5\ mm^2$ were cut from the leaves with a razor blade, fixed by double-sided tape to aluminium holders and dried at −25 °C over silica gel for several weeks. Before scanning electron microscopic investigation (Zeiss DSM 962, Oberkochen, Germany) leaf surfaces were sputtered with gold, to a depth of *c.* 20 nm. Leaf surfaces were investigated using an acceleration voltage of 15 keV.

Contact angle measurements

Contact angles of the upper and lower surfaces of *Ricinus* leaves of the different treatments (NO_3^-, NH_4^+ and H_2O) were measured with a goniometer (Krüss, Hamburg, Germany) to an accuracy of ±1°. Droplets of 2 μl of aqueous solutions buffered at pH 3·0 (10^{-3} mol l^{-1} citric buffer, KOH) and pH 9·0 (10^{-3} mol l^{-1} borate, HCl) were placed on the leaf surfaces fixed to microscopic slides of glass. Contact angles were measured at two different pH values, since it was shown previously that contact angles measured on certain surfaces can decrease with increasing pH values (Schreiber, 1996)

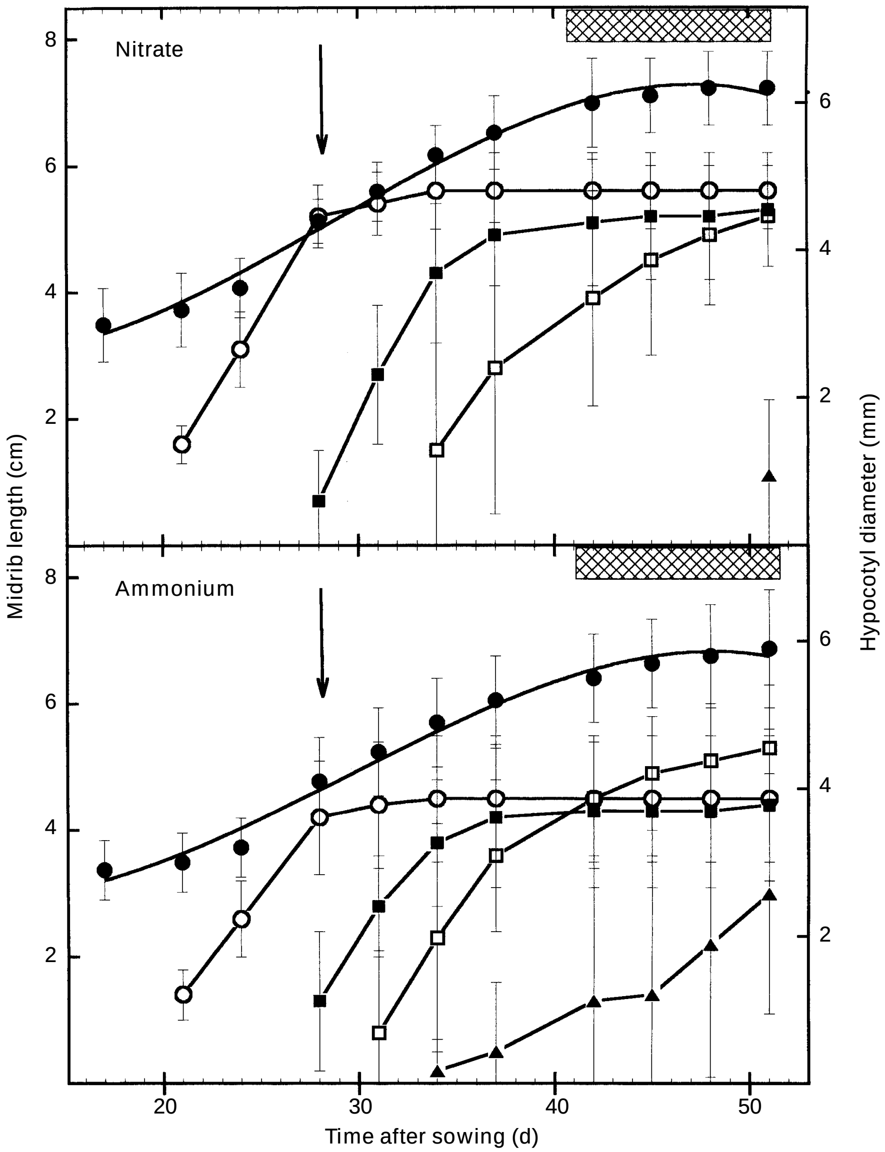

Figure 1. Change with time of hypocotyl diameter (●), midrib length of the first and second (○), third (■), fourth (□) and fifth (▲) leaves of *Ricinus communis*. The plants were cultivated in quartz sand supplied with a nutrient solution without nitrogen and the shoots were sprayed with either a nitrate (20 mM KNO_3) or an ammonium (10 mM $(NH_4)_2SO_4$) solution. The arrows indicate the time of withdrawal of nitrate from the rhizosphere and the bar at the top of the graphs shows the experimental period. The curve of hypocotyl diameter was fitted and the bars indicate SD of nine replicates.

Collection of apoplastic washing solution

The surfaces of the first two leaves or of leaves 3 and 4 were carefully rinsed with deionized water and dried with filter paper. The apoplastic washing solution was prepared by the method of Brune, Urbach & Dietz (1994), with a slight modification. The washed leaves were vacuum-infiltrated with 0·1 M sorbitol, then centrifuged at 1000 ***g*** for 5 min.

Statistics

Determinations of f. wt and d. wt and ion and element content of the plant parts were obtained from nine plants for both harvests. Each plant part was analysed individually with the exception of petioles, where three plants were bulked. Each analysis involved two extraction replicates and two or three independent measurements per extraction. In the case of transport saps, samples (for the xylem 20 or 25 and for the phloem 15 or 18) were analysed in nitrate-sprayed or ammonium-sprayed plants, respectively. Contact angles of 20 independent droplets were measured with each of the two pH values, on both leaf sides and for all four treatments. Results are given as means and the error bars indicate the 95 %-confidence intervals.

RESULTS

Plant growth and development

The first two leaves stopped growing immediately after withdrawal of N from the roots (28 d after

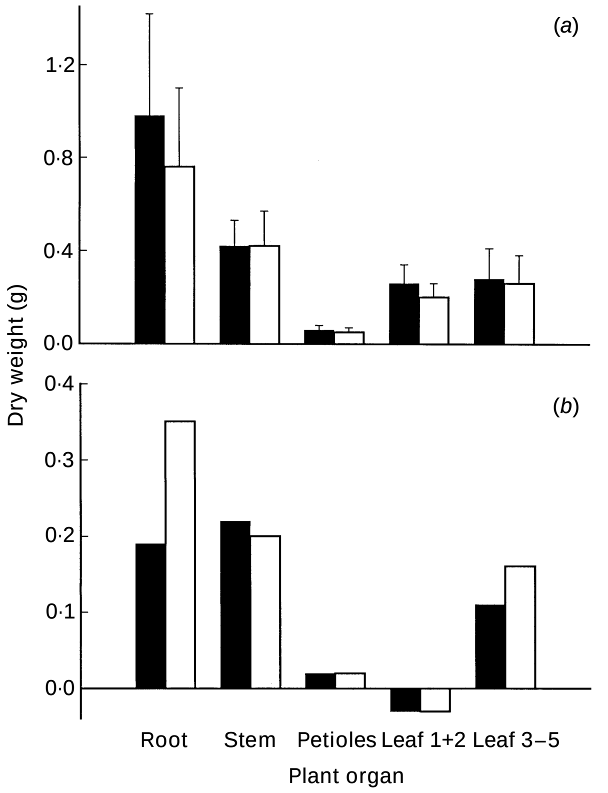

Figure 2. Initial d. wt (*a*) (41 d after sowing) and the increment of d. wt (*b*) per plant in *Ricinus communis* during the experimental period 41–51 d after sowing. During the experimental period the plants were cultivated without pedospheric nitrogen, but the shoots were sprayed with 20 mM nitrate (■) or ammonium (□) solution. Bars indicate SD of nine replicates.

sowing) and the growth of *Ricinus* was strongly reduced compared with plants where N was supplied to the roots (compare with Peuke & Jeschke (1993)). At this time the two primary leaves were larger in nitrate-sprayed than in ammonium-sprayed plants. The subsequent leaves (leaves 3–5) developed without pedospheric N, and were hence smaller than the first leaves under both conditions (Fig. 1). Only in ammonium-sprayed plants was leaf 4 larger than the earlier expanded leaves, and it nearly reached its fully expanded size at the end of the experimental period. In ammonium-fed plants, leaf 5 started to emerge before the experimental period, and was still growing at the end. By contrast, in nitrate-sprayed plants the development of leaves 4 and 5 was delayed. Leaf 4 was once again smaller than the primary leaves and leaf 5 had just started to emerge.

The biomass and the water content of the different plant parts at the end of the experimental period and the mean values were similar under both conditions (data not shown), the only difference being in the roots of ammonium-sprayed plants which were somewhat smaller than those of plants sprayed with nitrate. This difference was more obvious at the beginning of the experimental period (Fig. 2), since the highest d. wt increment was found in these roots.

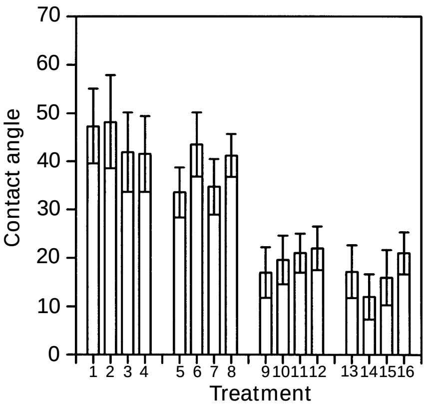

Figure 3. Contact angles of aqueous solutions buffered at pH 3 (10^{-3} mol l^{-1} citric buffer, KOH) and pH 9 (10^{-3} mol l^{-1} borate, HCl) on the upper (US) and lower surface (LS) of *Ricinus communis* leaves. The plants were cultivated in quartz sand and the leaves were sprayed with either a nitrate (20 mM KNO_3; NO_3) or an ammonium (10 mM $(NH_4)_2SO_4$; NH_4) solution. Controls were sprayed with water and supplied with a pedospheric nutrient solution containing 1 (K1) or 4 mM (K4) nitrate (Peuke & Jeschke, 1993; Peuke *et al.*, 1994). Error bars represent 95 % confidence intervals.1, US-pH 3-K1; 2, US-pH 9-K1; 3, LS-pH 3-K1; 4, LS-pH 9-K1; 5, US-pH 3-K4; 6, US-pH 9-K4; 7, LS-pH 3-K4; 8, LS-pH 9-K4;9, US-pH 3-NH_4; 10, US-pH 9-NH_4; 11, LS-pH 3-NH_4; 12, LS-pH 9-NH_4;13, US-pH 3-NO_3; 14, US-pH 9-NO_3; 15, LS-pH 3-NO_3;16, LS-pH 9-NO_3.

Stems and roots gained most biomass during the experiment; under both conditions the primary leaves even lost biomass and leaves 3–5 gained more mass in ammonium-sprayed plants.

Macroscopic and microscopic observations

At the beginning of the application of the nutrient solutions to the leaves, pronounced droplets formed on the leaf surfaces. During the first few days, however, leaf-surface wetting increased constantly. After *c.* 3 d leaves were completely wettable, since they were always covered after spraying by a thin film of the nutrient solution, which dried off in *c.* 1 h. Contact angles measured on the surfaces with buffered aqueous solutions ranged between 12–20° (Fig. 3), significantly smaller than those of water-treated controls (cultivation as reported by Peuke & Jeschke (1993); Peuke *et al.* (1994)) having contact angles of *c.* 40° (Fig. 3). Contact angles were independent of the pH of the aqueous solutions (Fig. 3).

Macroscopic changes like salt residues could not be observed on the treated leaf surfaces when the aqueous films had dried after spraying. Preliminary experiments revealed that spraying with very concentrated solutions (200 mM used to supply abundant N compared with 20 mM KNO_3 and 10 mM $(NH_4)_2SO_4$) caused salt crystals to form on, and necrosis of, the leaves. Scanning electron micro-

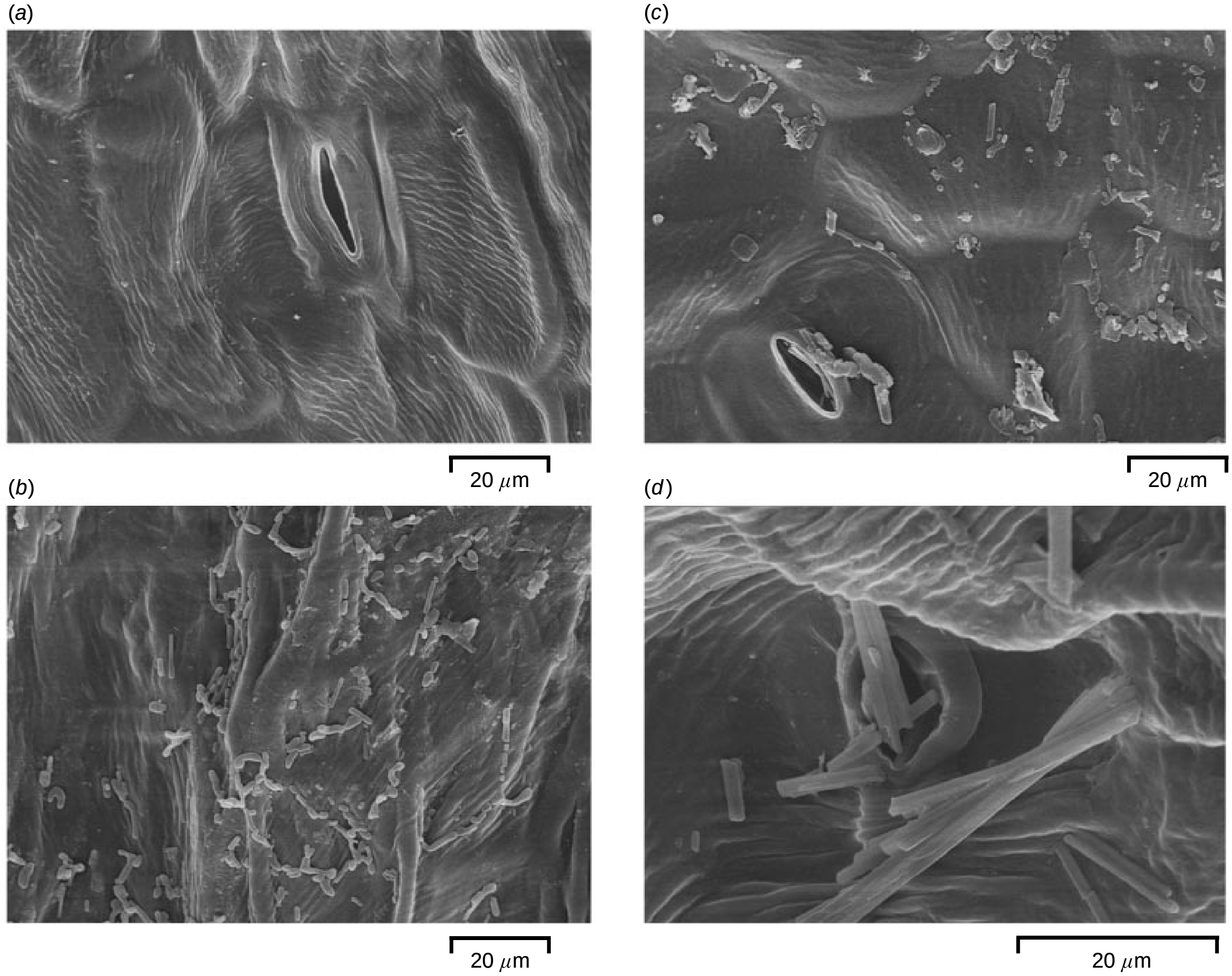

Figure 4. Scanning electron micrographs showing representative leaf surface regions of *Ricinus communis* after spraying with (*a*) water as control or either with (*b*) a nitrate (20 mM KNO_3) or (*c*, *d*) an ammonium (10 mM $(NH_4)_2SO_4$) solution. (*a*) Leaf surface with a stoma of an unsprayed control. The leaf surface is free of any depositions. (*b*) Surface of a leaf sprayed with a nitrate solution. A well developed epiphyllic microflora, dominated by a yeast, can be seen. (*c*) Surface of a leaf sprayed with an ammonium solution. Homogeneously distributed ammonium crystals are detectable. (*d*) Detail of a leaf surface sprayed with an ammonium solution showing the accumulation of the ammonium crystals within a stomatal pore and around the stoma.

graphs, however, showed high numbers of particles on the surfaces of sprayed leaves (Fig. 4*b*–*d*) than in controls (Fig. 4*a*). The observed effects were the same in nitrate-sprayed and ammonium-sprayed plants. Two different types of particles could be identified on treated leaves: epiphyllic microorganisms (Fig. 4*b*) and salt crystals (Fig. 4*c*, *d*). Salt crystals formed mainly next to stomata, very often within the stomatal pore (Fig. 4*d*). The property of increased wetting of the sprayed leaf surfaces could be removed by carefully rinsing them three to four times with deionized water.

Flows of carbon

The flows of C in the whole plant were similar under both experimental conditions (Fig. 5), nearly half of that gained in photosynthesis being transported to the roots in the phloem. The proportions of C in the roots and shoots used in dark respiration and for incorporation into biomass were similar. The recycling of C via the xylem was $< 1\,\%$ of the photosynthetic C gain. Increments of C and rates of photosynthesis were somewhat higher in ammonium-sprayed than in nitrate-sprayed plants, reflecting the greater growth. Within the shoot, the stem attracted most of the C, followed by the growing leaves 3–5 (Fig. 6). Here once again higher increments were found in ammonium-sprayed plants. The primary leaves even lost C under both conditions.

Flows of nitrogen and nitrogen assimilation

Plants sprayed with ammonium took up 84 % more N than did nitrate-sprayed plants (Fig. 7), the root and shoot incorporating similar proportions of the N supplied to the shoots. By contrast, in nitrate-sprayed plants a net loss of N from the shoot occurred, which was then incorporated into the root. Consequently the phloem flow was somewhat higher than in ammonium-sprayed plants, but in both

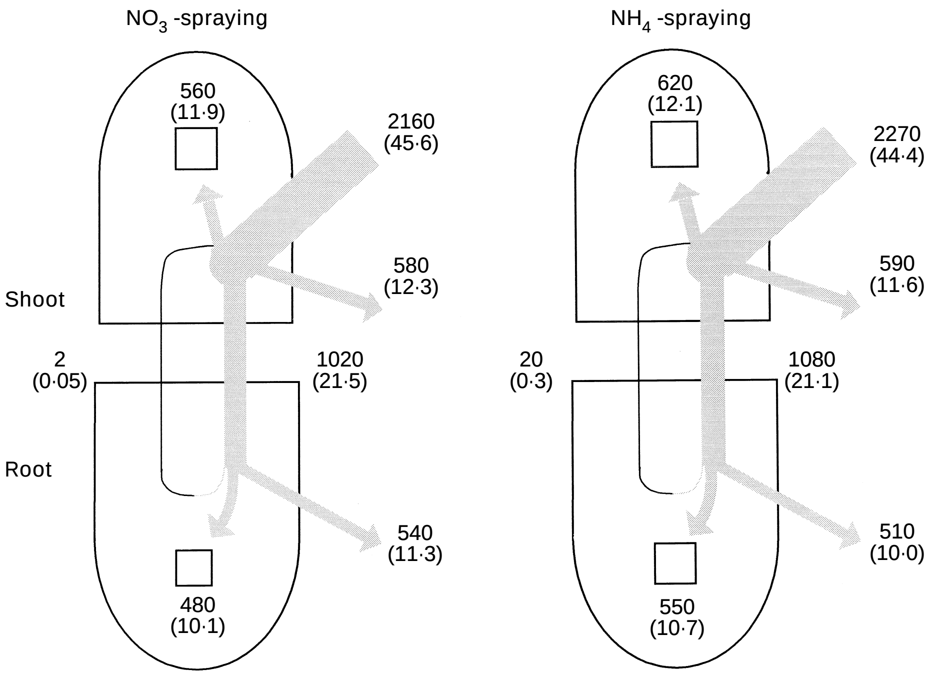

Figure 5. Flow profiles for uptake, transport and utilization of carbon in *Ricinus communis* sprayed with 20 mM nitrate or ammonium solution without further pedospheric nitrogen 41–51 d after sowing. Width of arrows (left, filled: flow in the xylem; right, grey: flow in the phloem) and length of squares (increment) are drawn proportional to uptake, flow and incorporation. The arrows leaving the shoot and root indicate the respiration. The numbers indicate μmol g^{-1} f. wt (10 d)$^{-1}$, in parentheses, mmol per plant (10 d)$^{-1}$.

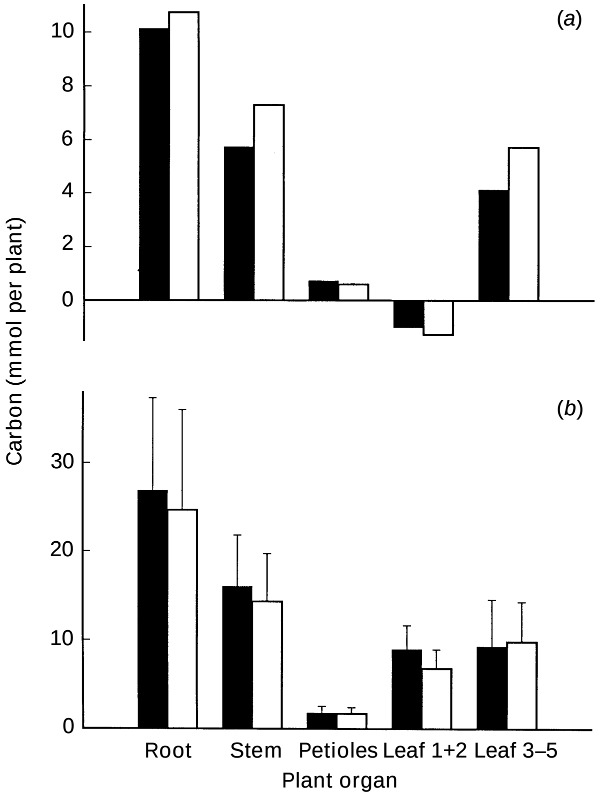

Figure 6. Increment of carbon per plant (*a*) and initial carbon content (41 d after sowing) (*b*) in *Ricinus communis* during the experimental period 41–51 d after sowing. During the experimental period the plants were cultivated without pedospheric nitrogen, but the shoots were sprayed with 20 mM nitrate (■) or ammonium (□) solution. Bars indicate SD of nine replicates.

treatments the flow of N in the phloem was higher than in the xylem, owing to low recycling of N in the xylem. However, the transport of N in the xylem was fivefold higher in ammonium-sprayed than in nitrate-treated plants.

Under nitrate nutrition the highest incorporation of N was found in the root, but when ammonium was sprayed, it was in the growing leaves (3–5) (Fig. 8). Under both conditions the primary leaves exported a large proportion of the acquired N. The proportion of N uptake between primary and secondary leaves was estimated by calculating the daily leaf area by the non-destructive method described by Jeschke *et al.* (1996) using the equation: $A = \alpha \times r^2$ (α, empirical factor; r, length of the middle rib, see also Figure 1) which allows the leaf area of intact plants to be followed. This approach allowed us to estimate that, in nitrate-sprayed plants, 50 % of the N was taken up by the primary leaves compared with only 39 % in ammonium-sprayed plants, so the adult primary leaves strongly exported N (11 μmol g^{-1} f. wt (10 d)$^{-1}$ in both treatments) including newly acquired and previously stored N.

Under both conditions the sprayed inorganic N was almost entirely assimilated into the shoots (Fig. 9). but little of either form was transported in the plants. Only 0·4 % (nitrate) or 3·2 % (ammonium) from the inorganic N taken up was transported in the phloem. Owing to a remobilization of stored nitrate and net generation of ammonium in the roots, transport of inorganic N was higher in the xylem than in the phloem. More of the ammonium was

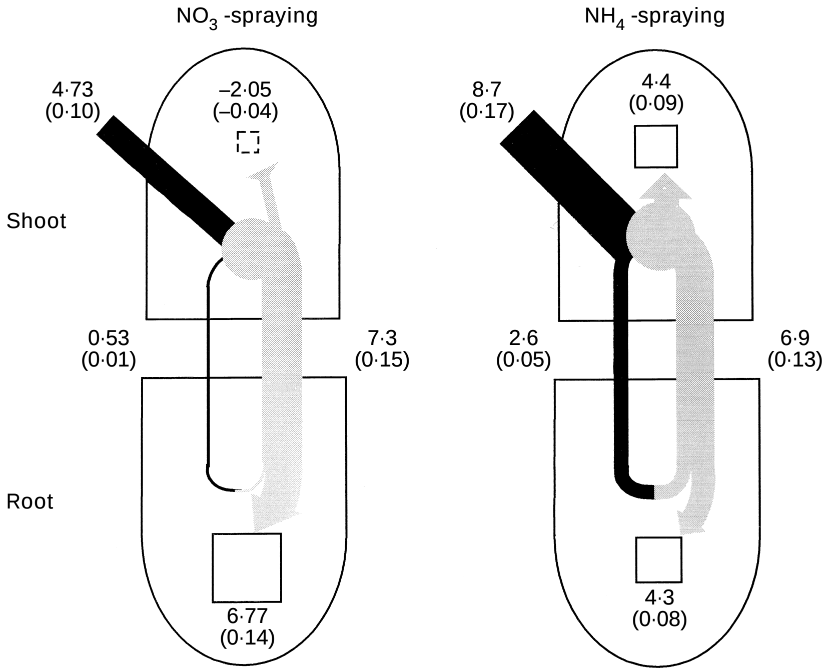

Figure 7. Flow profiles for uptake, transport and utilization of nitrogen in *Ricinus communis* sprayed with 20 mM nitrate or ammonium solution without further pedospheric nitrogen 41–51 d after sowing. Width of arrows (left, filled: flow in the xylem; right, grey: flow in the phloem) and length of squares (increment) are drawn proportional to uptake, flow and incorporation. The numbers indicate μmol g^{-1} f. wt (10 d)$^{-1}$, in parentheses, mmol per plant (10 d)$^{-1}$. Dashed lines and negative numbers indicate remobilization of previously stored elements.

transported (sixfold more in the phloem and eightfold more in the xylem) than nitrate (Fig. 9). Moreover, the incorporation of nitrate in nitrate-sprayed plants was lower than that of ammonium, which also reflects the lower uptake of sprayed nitrate.

Flows of malate and pH of apoplastic washing solution

The flows of malate in the phloem of sprayed plants were the same under both conditions (Fig. 10), but in ammonium-sprayed plants the malate was efficiently recycled by the xylem (6·8 times more than in the nitrate treatment). By contrast, previously stored malate was mainly mobilized in the shoot of nitrate-sprayed plants. In the shoots, malate was deposited and metabolized in both treatments. This was only possible because the plants were supplied before the experimental period with pedospheric nitrate, and during previous nitrate assimilation malate was accumulated. The remobilization of stored malate in the shoot was twofold and the metabolism almost eightfold higher in ammonium-sprayed than in nitrate-sprayed plants. The turnover of malate in the roots was lower; there was a net incorporation in both treatments (but only 50 % in ammonium-sprayed plants compared with nitrate-sprayed ones). In the nitrate treatment there was a small metabolism of malate in the root; in ammonium-sprayed plants malate was synthesized in the root.

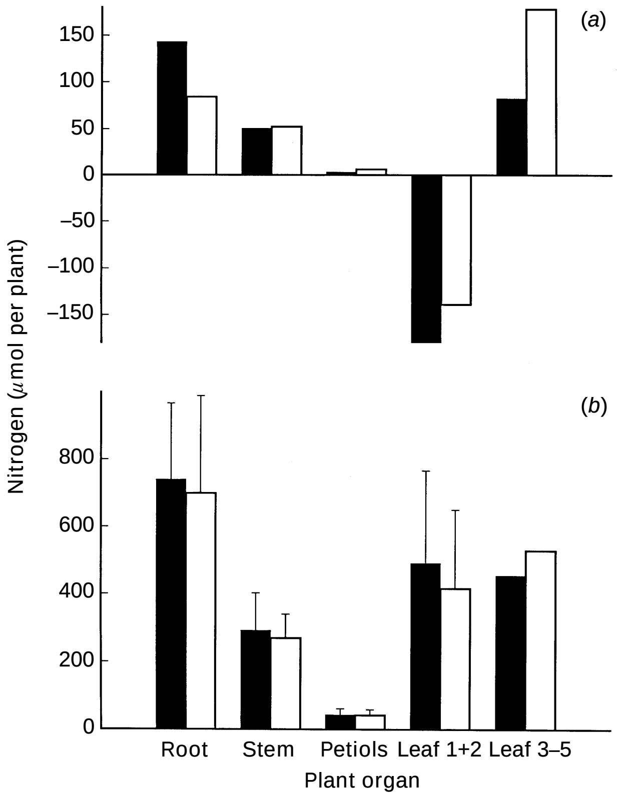

Figure 8. Increment of nitrogen per plant (*a*) and initial nitrogen content (41 d after sowing) (*b*) in *Ricinus communis* during the experimental period 41–51 d after sowing. During the experimental period the plants were cultivated without pedospheric nitrogen, but the shoots were sprayed with 20 mM nitrate (■) or ammonium (□) solution. Bars indicate SD of nine replicates.

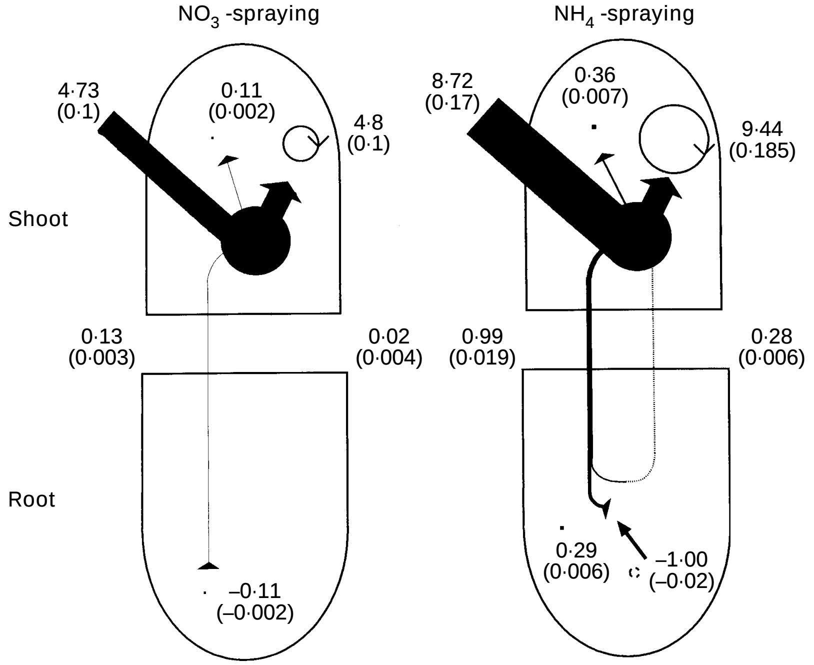

Figure 9. Flow profiles for uptake, transport and utilization of nitrate (in nitrate-sprayed plants, left) or ammonium (in ammonium-sprayed plants, right) in *Ricinus communis* sprayed with 20 mM nitrate or ammonium solution without further pedospheric nitrogen 41–51 d after sowing. Circles indicate metabolism; in the case of nitrate the reduction of nitrate, in the case of ammonium, assimilation (——) or ammonium production (– – – – and negative values). Width of arrows (left, filled: flow in the xylem; right, grey: flow in the phloem) and length of squares (increment) are drawn proportional to uptake, flow and incorporation. The numbers indicate μmol g^{-1} f. wt (10 d)$^{-1}$, in parentheses, mmol per plant (10 d)$^{-1}$.

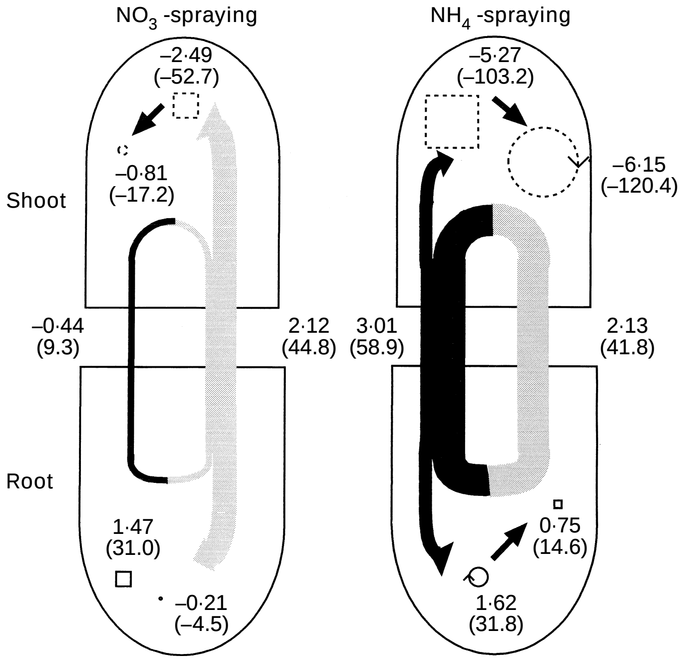

Figure 10. Flow profiles for transport, synthesis (○), metabolism (◌ and negative numbers), deposition (□) or remobilization (⬚ and negative numbers) of malate in *Ricinus communis* sprayed with 20 mM nitrate or ammonium solution without further pedospheric nitrogen 41–51 d after sowing. Width of arrows (left, filled: flow in the xylem; right, grey: flow in the phloem) and length of squares (increment) are drawn proportional to uptake, flow and incorporation. The numbers indicate μmol g^{-1} f. wt (10 d)$^{-1}$, in parentheses, mmol per plant (10 d)$^{-1}$.

The pH of the apoplastic washing solution of leaves ranged from 6·08 to 6·93 between the applied treatments (Table 1). It seemed slightly higher (*c.* 0·1 pH unit) in younger than in older leaves, when the plants were supplied with pedospheric N. An inverse and somewhat more pronounced (but stat-

Table 1. *pH of the apoplastic washing solution of the leaves of* Ricinus communis *51 d after sowing*

Application of nitrogen	Pedospheric		Foliage	
	1·0 mM NO_3^-	4·0 mM NO_3^-	NO_3^--spraying	NH_4^+-spraying
Leaf 1+2	6·70±0·11	6·83±0·08	6·71±0·23	6·39±0·23
Leaf 3+4	6·78±0·25	6·93±0·13	6·33±0·19	6·08±0·12
All leaves	6·74±0·18	6·88±0·11	6·52±0·28	6·24±0·23

The plants were supplied with pedospheric 1 mM and 4 mM nitrate or were sprayed with nitrate (20 mM KNO_3) or ammonium (10 mM $(NH_4)_2SO_4$) solution as sole N source. SD of three replicates are given as $\pm n = 3/6$.

istically not significant) effect was found in the sprayed plants (0·3–0·4 pH units). In plants with N supplied to the foliage the pH of the apoplastic washing solution of ammonium-plants was always > 0·2 pH units lower than in nitrate-sprayed plants.

DISCUSSION

Growth

At the beginning of the experimental period the plants sprayed with ammonium had a slightly lower biomass, mainly due to lower root weight, but in part, to smaller leaves, which might have been caused by the input of sprayed ammonium to the rhizosphere before it was covered with plastic foil. During the experiment, however, the growth of ammonium-sprayed plants was greater, the % increments of the initial d. wt being 52 % in ammonium-sprayed plants and 34 % in nitrate-sprayed plants. In particular, the d. wt of roots increased more on a relative basis (65 % compared with 42 %). This was caused by the better availability of N, since uptake was considerably higher in ammonium-sprayed than in nitrate-treated plants (*c*. 84 % more, Fig. 7).

The withdrawal of pedospheric N immediately stopped the growth of the developing primary leaves (Fig. 1). During further development, the pattern of growth was totally different from that in control plants fed continuously with pedospheric 1 mM nitrate (Peuke & Jeschke, 1993). In general, for both conditions, root growth was enhanced relative to that of the shoot, a phenomenon which points to N limitation. Indeed the root:shoot ratios were higher than with other nutritional conditions employed for growing *Ricinus* in previous experiments (Peuke & Jeschke, 1993; Peuke *et al.*, 1994). The plants invested highly in root growth regardless of the fact that the shoots were the sole source of mineral N in the present experiment. The conclusion to be drawn is that the plants responded to nutrient limitation irrespective of the site of N application and were unable to sense the site of N uptake.

Pathways of nitrogen uptake in leaves

Basically there are two possible routes of N uptake into the leaves: penetration through stomatal pores and diffusion through the cuticle. Hanson & Garten (1992) suggested a model for the uptake of HNO_3 via transcuticular or stomatal pathways. There is evidence that both routes might have significantly contributed to foliar uptake of N in the present experiments. In general, cations are transported much better through cuticles than anions (Tyree, Scherbatskoy & Tabor, 1990) because plant cuticles are negatively charged at pH values > 3·0 since there are free carboxylic groups in the cutin polymer (Schönherr & Bukovac, 1973). Consequently, negatively charged ions are largely excluded from cuticular diffusion owing to the Donnan potential. Thus, it was not surprising that N uptake was significantly higher in ammonium-sprayed leaves than in nitrate-sprayed leaves (Fig. 7), also observed by Garten & Hanson (1990). It seems that the positively charged ammonium was more easily transported across the cuticle than was the negatively charged nitrate.

Additionally, increased leaf-surface wetting after spraying (Fig. 3) and the accumulation of salt crystals in and around stomata after drying (Fig. 4) indicate that stomata might also have served as possible routes of N uptake. Recently, Burkhardt & Eiden (1994) suggested that thin water films on conifer needles are responsible to a large extent for the mass exchange between atmosphere and leaf. The formation of these films is due to an increased deposition of hygroscopic, atmospheric particles on the leaf surface. It is interesting to note that these particles were found to be deposited preferentially in the stomatal regions (Burkhardt, Peters & Crossley, 1995) as in this study (Fig. 4). It is argued that these hygroscopic depositions form a continuous connection between the leaf surface and the apoplast of the mesophyll (Burkhardt & Eiden, 1994). Foliar uptake of substances, as well as leaching of compounds from the leaf interior, is assumed to take place along these trans-stomatal water films and the same mechanism might also have contributed to the uptake of inorganic N into the leaves observed in this study. Nevertheless, it should be mentioned that direct evidence for the existence of trans-stomatal water films is still lacking. For ammonia, Sutton *et al*. (1995) stressed the importance of emission and recapture by plant cuticles and water layers. They postulated that NH_4^+ uptake might occur as a result of pH gradients between the leaf surface and apoplast.

Besides the occurrence of salt crystals on the leaf surface after the application of ammonium and nitrate solutions, we saw the increased development of an epiphyllic microflora on the surfaces of treated leaves (Fig. 4*b*). Presumably the growth of epiphytic micro-organisms was enhanced by the higher availability of nutrients after spraying. Recently, it was demonstrated with *Abies grandis* needles that increased leaf-surface wetting was mainly due to the increased occurrence of epiphyllic micro-organisms, whereas a change in the wax composition of the needles was not detectable (Schreiber, 1996). Similar results could be observed in this study, since the wax composition of *Ricinus communis* leaf surfaces was similar in controls and treated leaves (data not shown). Thus, it can be concluded that the increased wetting of the leaf surfaces of *Ricinus* after foliar nutrition might be due to the combined effects of salt crystals and epiphyllic micro-organisms.

Nitrogen

The N uptake in these experiments (4·7 or 8·7 μmol g^{-1} f. wt $(10\ d)^{-1}$) was in the range found in N-deficient plants in former experiments (5·1 μmol g^{-1} f. wt $(10\ d)^{-1}$, Peuke *et al*. (1994)) and again demonstrates N limitation. Ammonium was taken up more readily by the leaves than nitrate in the present experiment. Garten & Hanson (1990) found similar results. They exposed red maple and white oak leaves to simulated rain solutions containing ^{15}N-labelled nitrate or ammonium, and found that several-fold more nitrate than ammonium could be removed by washing with water. Faller (1972) observed that the ammonia was taken up from the atmosphere more readily than nitrogenous gases. Ammonia is dissolved in the apoplastic pool of the leaves as the reduced N-form ammonium, and NO_x in the oxidized forms nitrite and nitrate. The partitioning of N (and other elements) in the present experiment also showed the effects of N limitation, since the root was preferentially supplied and a net export from the shoot even occurred. In well fed plants *c*. 75 % or more of the N is usually incorporated into the shoot, for instance in *Ricinus* (Peuke & Jeschke, 1995; Jeschke *et al*., 1996) as well as in *Lupinus* (Pate *et al*., 1979; Jeschke *et al*., 1985) or in *Triticum* (Lambers *et al*., 1982; Larsson *et al*., 1991). Relatively more N was deposited in the roots in N-limited (Duarte & Larsson, 1993; Peuke *et al*., 1994) or P-limited plants (Jeschke *et al*., 1996). Lambers *et al*. (1982), observing increased translocation of N to the roots under N-limiting conditions, assumed that the N distribution between roots and shoots is adjusted in the shoot. This hypothesis is consistent with the low recycling of shoot-borne N in the xylem in the present experiment, since it was only this amount of N exported from the shoot which was essentially needed in the roots. Scheible *et al*. (1997) recently concluded that in tobacco the accumulation of nitrate leads to an inhibition of root growth, as one possible factor for regulation.

Nitrogen assimilation and consequences for carbon flows and pH relations

After application to the shoots, inorganic N was almost entirely assimilated in the shoots. As reported before (Marschner, 1995) inorganic-N transport in the phloem was low (0·3 % nitrate or 3·2 % ammonium of total uptake, Peuke *et al*. (1996)) possibly because of the absence of a transporter for loading into the phloem. Schobert & Komor (1992) fed *Ricinus* cotyledons with inorganic N and also found no loading of nitrate into the phloem and, in contrast to our results, no uptake of ammonium even into the leaves. As a consequence of restricted transport of inorganic N in the phloem, nitrate or ammonium could not themselves be exported from the shoot organs. Additionally, since ammonium is toxic to cells and cannot be stored in large quantities, a fast assimilation at the site of uptake (or production) is essential for growth and development.

Photosynthesis on a f. wt basis was lower in sprayed plants than in well fed plants (Peuke & Jeschke, 1995); for instance, only half that of *Ricinus* supplied with 1·0, 4·0 mM nitrate or 1·0 mM ammonium via the rooting medium (Peuke & Jeschke, 1993; Peuke *et al*., 1994). About 50 % of the photosynthetically fixed C was lost in the present experiment by root respiration and dark respiration of the shoot; the rest was incorporated to roughly the same extent in root and shoot, in ammonium-sprayed slightly more than in nitrate-sprayed plants, whilst in the former experiments on pedospheric nutrition, the shoot attracted most of the incorporated C (64–75 %, Jeschke & Pate (1991); Peuke & Jeschke (1993); Peuke *et al*. (1994)). The preferential partitioning into the shoot was similar in pea (78 %, Duarte & Larsson (1993)) and in wheat (91 %, Lambers *et al*. (1982). Both on a f. wt basis (590 μmol g^{-1} f. wt $(10\ d)^{-1}$, see Figure 5) and in relation to total photosynthesis (25 %), shoot respiration was higher in this experiment than in growth conditions in our past work (10–17 %, Peuke & Jeschke (1995)). This reflected the N assimilation in the shoots, particularly in ammonium-sprayed plants. On the other hand, larger amounts of nitrate, up to 25-fold more, were reduced in the shoots of *Ricinus* fed with pedospheric nitrate, (Peuke *et al.*, 1996). The flows and partitioning of C, however, were not only influenced by shoot N assimilation, but also by N limitation. Deficiency of nutrients generally favours the root in respect to partitioning and growth (Duarte & Larsson, 1993; Peuke *et al.*, 1994; Jeschke *et al.*, 1996). So in the present paper the question whether exclusive assimilation of inorganic N in the shoot reduces the allocation of C to the root, cannot be answered, since this effect was confounded by that of N limitation.

In growing plants, assimilation of NO_3^- generates an excess of 0·78 moles of OH^- whereas NH_4^+ produces an excess of 1·22 moles H^+ per mole N assimilated (Raven, 1988). The assimilation of sprayed nitrate in the shoots did not result in a proportional turnover of malate (Fig. 10) or in changes in the pH of apoplastic washing solution of the leaves (Table 1). Raven & Smith (1976) proposed a mechanism for pH homeostasis during nitrate assimilation in the shoot which involves synthesis of organic acids such as malate in the shoot followed by either storage in the shoot cells or transport to the roots. Our data are not consistent with this mechanism; in fact a remobilization and metabolism was even found. Compared with the rate of reduction of xylem-borne nitrate in pedospherically supplied *Ricinus* (Peuke **et al.**, 1996), rates of nitrate reduction were relatively low in the foliar-supplied plants (only 4% compared with pedospheric 4 mM nitrate). Consequently, the metabolic pressure on the plants to maintain pH homeostasis and to synthesize carbohydrates as a source of energy and C-skeletons was not very severe. By contrast, a high turnover of malate occurred in ammonium-sprayed plants (Fig. 10). The metabolism of malate, 85% of which was mobilized from previously stored malate (obviously during assimilation of pedospheric nitrate during the precultivation) consumed 12·3 μmol H^+ g^{-1} f. wt (10 d)$^{-1}$. Accordingly, the assimilation of 9·44 μmol NH_4^+ g^{-1} f. wt (10 d)$^{-1}$ will produce, according to Raven (1988), 11·5 H^+ μmol g^{-1} f. wt (10 d)$^{-1}$ (1·22 H^+ per NH_4^+ assimilated). Additionally, the leaf apoplast was acidified. The decrease of the apoplastic pH by 0·2 to 0·3 units estimated from pH measurements in the apoplastic washing solution (Table 1) corresponds to 0·3 μmol H^+ g^{-1} f. wt, assuming an apoplastic volume of 80 μl g^{-1} f. wt and a buffer capacity of 20 mM (Dietz, 1997). The effect of the supply of different N forms on the apoplastic pH differs between species. Mühling & Sattelmacher (1995) found no effect of soil-borne N form on the pH of the apoplast of *Phaseolus*, whilst in *Glycine max* the pedospheric supply of NH_4NO_3 decreased the pH more than nitrate nutrition (Kosegarten & Englisch, 1994). The species-dependent site of nitrate reduction (root or shoot) might have an important influence on the pH of the leaf apoplast. During ammonium assimilation in the shoot, the metabolism of malate and the excretion of protons to the apoplast seem to play a major role in pH-stat regulation in the present experiment.

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