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

II. The flows of cations, chloride and abscisic acid

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SUMMARY

Following a precultivation with pedospheric nitrogen nutrition, *Ricinus* plants were supplied with nitrogen solely by spraying nitrate or ammonium solution onto the leaves during the experimental period. The chemical composition of tissues, xylem and phloem exudates was determined and on the basis of the previously determined nitrogen flows (Peuke *et al.*, *New Phytologist* (1998), **138**, 657–687) the flows of potassium, sodium, magnesium, calcium, chloride and ABA were modelled. These data, which permit quantification of net-uptake, transport in xylem and phloem, and utilization in shoot and root, were compared with results obtained in plants with pedospherically-supplied nitrate or ammonium and data in the literature. Although the overall effects on the chemical composition of supplying ammonium to the leaves were not as pronounced as in pedospherically supplied plants, there were some typical responses of plants fed with ammonium (ammonium syndrome). In particular, in ammonium-sprayed plants uptake and transport of magnesium decreased and chloride uptake was increased compared with nitrate-sprayed plants. Furthermore, acropetal ABA transport in the xylem in ammonium-sprayed *Ricinus* was threefold higher than in nitrate-sprayed plants. Additionally, concentrations of anions were more or less increased in tissues, particularly in the roots, and transport fluids. The overall signal from ammonium-sprayed leaves without a direct effect of ammonium ions on uptake and transport systems in the root is discussed.

Key words: *Ricinus communis* L., foliar N application, cations, anions, ABA, phloem transport, xylem transport.

INTRODUCTION

Nitrogen is the most important mineral nutrient for plants. On a dry matter basis the principal elements in higher plants are carbon, hydrogen, oxygen, nitrogen, potassium, sulphur and phosphorus. In autotrophic higher plants carbon is taken up from the atmosphere, whilst mineral nutrients are usually taken up by the roots. Shoot organs, however, can take up mineral nutrients as well (Raven, 1988; Pearson & Stewart, 1993; Marschner, 1995). In contrast to the above-mentioned elements, nitrogen, which is taken up at the greatest rates of all minerals, is available for plants as either the anion nitrate or the cation ammonium. The use of nitrate or ammonium as an N-source may have fundamental consequences for the growth, development and metabolism of the plants (Haynes & Goh, 1978; Runge, 1983; Pearson & Stewart, 1993; Marschner, 1995). One such consequence is the charge balance in the plants. If ammonium is taken up there is a deficiency in negative charge. Van Beusichem, Kirkby & Baas (1988) calculated an excess of cation over anion uptake in ammonium-fed *Ricinus* and the opposite effect under nitrate supply. Indeed, when ammonium was taken up, lower concentrations of cations but higher concentrations of anions were found in tissues (Allen & Smith, 1986; Arnozis & Findenegg, 1986; van Beusichem *et al.*, 1988; Pearson & Stewart, 1993; Lang & Kaiser, 1994).

A direct effect of ammonium on cation uptake systems has been assumed (Haynes & Goh, 1978; Lee & Ayling, 1993). Of particular interest are interactions between ammonium and potassium uptake (Scherer, MacKown & Leggett, 1984; Wang, Siddiqi & Glass, 1996; Smart & Bloom, 1998) and between ammonium and nitrate uptake (Lee & Drew, 1989; Jackson & Volk, 1995; Smart & Bloom, 1998). Furthermore, as a result of different ion uptake, the nitrogen source also has a profound influence on the composition of the transport fluids

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in plant xylem and phloem (Allen & Smith, 1986; Arnozis & Findenegg, 1986; Shelp, 1987; van Beusichem *et al.*, 1988; Engels & Marschner, 1993; Peuke & Jeschke, 1995).

The effects of nutrition with ammonium are also termed 'the ammonium syndrome' (Mehrer & Mohr, 1989). This includes the acidification of the rhizosphere, the decrease of cation uptake, increase of anion content, changes in metabolism and with the final result of lower growth and even toxic effects.

It has also been shown in the past that nitrogen nutrition affects plant hormone relations. Nitrogen deficiency inhibits auxin formation (Witt, 1964; the old literature on N-nutrition and auxins is cited therein), gibberellin synthesis in potatoes (Krauss & Marschner, 1982) and cytokinin formation in different plants (Salama & Wareing, 1979; Sattelmacher & Marschner, 1978). Gao, Molosugi & Sugiura (1992) observed an increase of cytokinins in young apple trees when supplied with ammonium. Many papers have been published investigating the connections between nitrogen nutrition and abscisic acid and in most cases ABA was slightly accumulated under nitrogen deficiency. Peuke, Jeschke & Hartung (1994*b*, most of references on ABA and N-nutrition are cited therein) showed a decrease in ABA under N deficiency. Only when ammonium was the sole N source did ABA accumulation in root tissues and in the xylem sap increase significantly.

Effects of nitrate or ammonium nutrition on the transport of elements and ions in *Ricinus* have been reported (Peuke & Jeschke, 1993): when ammonium was used as N-source growth and uptake of cations was inhibited and the transport of potassium, sodium and calcium, but not of magnesium, to the shoot was decreased (for transport in maize see also Engels & Marschner, 1993); by contrast, the flow of chloride was increased, and the transport of carbon and nitrogen in the phloem was enhanced by ammonium supply to the roots. In a previous paper on *Ricinus* supplied with nitrate or ammonium by foliar spraying we showed the effects on growth, and the uptake, flow and assimilation of nitrogen and carbon within the whole plant (Peuke *et al.*, 1998).

In the present paper, which represents the second part of this experiment, we have investigated the effects on the chemical composition of transport fluids and tissues, and on the flows of cations, chloride and ABA in whole *Ricinus* plants. We want to investigate whether the use of ammonium even in small amounts supplied to the leaves, excluding direct effects of ammonium in the rhizosphere, can cause shifts in the ionic composition in xylem, phloem and plant organs and the flows of elements in the whole plant. The chemical composition of the transport fluids was investigated particularly with respect to potential signals between root and the shoot. This also includes ABA as a root to shoot

signal. The results are compared with plants that were pedospherically supplied with N.

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 each to 5-l pots filled with quartz sand, and the plants were supplied daily with an excess of nutrient solution, which contained 1 mM nitrate as N-source (Peuke & Jeschke, 1993). Additionally the shoots of the population were sprayed daily, one half with 20 mM KNO_3, the other half with 10 mM $(NH_4)_2SO_4$. Twenty-eight days after sowing, the rhizosphere was separated from the shoot by covering the pots with plastic foil, and the nutrient solution applied to the roots nitrate was replaced by KCl and $CaCl_2$. The nitrogen was applied by spraying the nitrate or ammonium solution onto the leaves. For further details of the cultivation procedure see Peuke *et al.* (1998).

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 lamps (300–500 µmol photon m^{-2} s^{-1}).

Harvesting and analysis

Nine plants of each treatment were harvested 41 d after sowing and divided into roots, stems, petioles, leaf laminae of the primary and the following leaves. Every plant part was carefully washed with water before chemical analysis. A second harvest was performed in the same way 10 d later. The period between days 41 and 51 after sowing represents the experimental period. During these 10 d, xylem and phloem saps were collected from the hypocotyl. All procedures of harvesting of plant material, extraction, collecting xylem and phloem saps, and chemical determinations have been described in detail (Peuke & Jeschke, 1993; Peuke, Hartung & Jeschke, 1994*a*). Carbon and nitrogen in dry matter were analysed using a CHN analyser (CHN-O-Rapid, Heräus, Hanau, Germany). Amino acids in the transport fluids were determined using an amino acid analyser (Biotronik Co., Maintal, Germany). Cations were measured by atomic absorption spectrometry (FMD 3, Carl Zeiss, Oberkochen, Germany) and anions by anion chromatography (Anionenchromatograph, Biotronik Co., Maintal, Germany).

The flows of ions were modelled on nitrogen flows published previously (Peuke *et al.*, 1998) according to the method of Pate, Layzell & McNeil (1979), Jeschke, Atkins & Pate (1985) and Jeshcke & Pate (1991). To make the flows comparable with earlier experiments they were calculated on the mean f. wt during the experimental period. The values of flows

Table 1. *Ion and element concentrations in xylem exudates of* Ricinus communis *41–51 d after sowing; the plants were sprayed with 20 mM nitrate or ammonium solution as sole N-source or supplied pedospherically with a nutrient solution containing 1 mM nitrate or ammonium*

	Sprayed		Pedospheric	
	NO_3^-	NH_4^+	NO_3^-	NH_4^+
K^+	5.21 ± 2.43	5.74 ± 2.52	8.29 ± 1.02	6.80 ± 0.90
Na^+	0.15 ± 0.06	0.13 ± 0.07	0.24 ± 0.04	0.50 ± 0.10
Mg^{2+}	0.97 ± 0.13	0.97 ± 0.32	2.23 ± 0.33	1.88 ± 0.16
Ca^{2+}	1.18 ± 0.20	1.25 ± 0.44	3.39 ± 0.68	1.57 ± 0.10
NH_4^+	0.41 ± 0.41	0.52 ± 0.22	0.31 ± 0.03	0.35 ± 0.03
Sum^+ (meq)	10.07 ± 3.04	10.84 ± 3.93	20.09 ± 2.78	14.55 ± 1.20
Cl^-	0.74 ± 0.20	0.85 ± 0.18	0.76 ± 0.09	3.85 ± 0.65
NO_3^-	0.70 ± 0.39	0.12 ± 0.02	13.86 ± 2.67	1.76 ± 0.89
$H_2PO_4^-$	1.81 ± 0.39	1.62 ± 0.43	1.12 ± 0.25	1.18 ± 0.16
SO_4^{2-}	0.75 ± 0.43	1.51 ± 0.23	1.40 ± 0.66	2.29 ± 0.27
Malate	1.00 ± 0.97	2.67 ± 0.80	0.30 ± 0.10	0.00 ± 0.00
Sum^- (meq)	6.75 ± 2.85	10.97 ± 2.43	19.15 ± 2.90	11.37 ± 1.26
N_{tot}	1.43 ± 0.23	1.54 ± 0.67	18.43 ± 3.39	15.86 ± 1.77
C_{tot}	5.13 ± 4.59	13.33 ± 4.13	12.16 ± 2.43	35.52 ± 3.84
AA-N	0.32 ± 0.24	0.90 ± 0.44	4.27 ± 0.98	13.88 ± 1.51
ABA	0.85 ± 0.17	0.73 ± 0.11	0.053 ± 0.001	0.266 ± 0.001

All values indicate mM except for ABA which are μM.

Data of pedospheric supplied plants were taken from Peuke & Jeschke, 1993, 1995. Confidence limits are given as $\pm (P = 0.95)$. AA-N, amino acid and amide nitrogen; N_{tot} and C_{tot}, total concentration of nitrogen or carbon.

Table 2. *Ion and element concentrations in phloem exudates of* Ricinus communis *41–51 d after sowing; the plants were sprayed with 20 mM nitrate or ammonium solution as sole N-source or were supplied pedospherically with a nutrient solution containing 1 mM nitrate or ammonium*

	Sprayed		Pedospheric	
	NO_3^-	NH_4^+	NO_3^-	NH_4^+
K^+	72.74 ± 3.39	81.25 ± 3.11	69.54 ± 5.85	49.64 ± 5.05
Na^+	0.82 ± 0.05	0.74 ± 0.07	1.25 ± 0.13	0.95 ± 0.19
Mg^{2+}	3.78 ± 0.25	4.02 ± 0.20	3.58 ± 0.31	4.06 ± 0.31
Ca^{2+}	1.37 ± 0.17	1.52 ± 0.22	1.10 ± 0.18	0.99 ± 0.20
NH_4^+	1.55 ± 0.05	1.62 ± 0.06	2.03 ± 0.29	1.90 ± 0.43
Sum^+ (meq)	85.41 ± 4.02	94.69 ± 3.46	82.16 ± 6.52	62.59 ± 5.38
Cl^-	10.37 ± 0.48	12.48 ± 0.95	4.34 ± 0.42	16.23 ± 1.26
NO_3^-	0.27 ± 0.07	0.36 ± 0.15	0.66 ± 0.25	0.20 ± 0.07
$H_2PO_4^-$	9.29 ± 1.13	10.22 ± 1.63	2.84 ± 0.61	3.79 ± 0.62
SO_4^{2-}	1.28 ± 0.19	3.25 ± 0.58	0.57 ± 0.09	0.92 ± 0.16
Malate	10.19 ± 1.26	12.73 ± 1.76	7.83 ± 2.43	0.81 ± 0.15
Sum^- (meq)	42.87 ± 4.12	54.72 ± 5.85	22.30 ± 4.65	23.42 ± 2.10
N_{tot}	37.39 ± 4.91	42.11 ± 5.00	73.78 ± 12.43	125.36 ± 10.99
C_{tot}	6100 ± 214	6117 ± 208	4796 ± 328	6234 ± 661
AA-N	35.57 ± 4.85	40.12 ± 4.95	71.09 ± 12.42	123.26 ± 11.15
ABA	3.20 ± 0.19	3.04 ± 0.32	1.53 ± 0.30	2.76 ± 0.73

All values indicate mM except for ABA which are μM.

Data of pedospheric supplied plants were taken from Peuke & Jeschke, 1993, 1995. Confidence limits are given as $\pm (P = 0.95)$. AA-N, amino acid and amide nitrogen; N_{tot} and C_{tot}, total concentration of nitrogen or carbon.

Table 3. *Element and ion concentration in roots, stems plus petioles, and leaves of* Ricinus communis *after spraying an ammonium or nitrate solution to the leaves*

	Nitrate			Ammonium		
	Root	Stem + petioles	Leaves	Root	Stem + petioles	Leaves
N	55.5 ± 8.3	85.3 ± 4.2	348.4 ± 21.9	54.7 ± 5.0	89.9 ± 9.7	414.4 ± 17.7
K^+	89.9 ± 11.4	68.8 ± 5.7	84.8 ± 8.4	87.5 ± 10.8	85.6 ± 7.0	116.0 ± 11.8
Na^+	4.5 ± 1.5	0.9 ± 0.2	0.5 ± 0.0	5.8 ± 1.0	0.9 ± 0.2	0.5 ± 0.1
Mg^{2+}	16.0 ± 2.2	17.0 ± 1.6	25.5 ± 2.5	16.4 ± 1.9	16.2 ± 1.2	29.0 ± 1.7
Ca^{2+}	14.4 ± 1.8	44.2 ± 4.4	95.4 ± 27.6	15.3 ± 1.4	40.5 ± 4.6	81.8 ± 13.1
Cl^-	44.9 ± 4.7	25.4 ± 4.3	16.0 ± 2.6	66.2 ± 15.2	33.8 ± 4.1	19.7 ± 1.8
NO_3^-	0.7 ± 0.6	0.5 ± 0.5	1.6 ± 0.8	0.11 ± 0.08	0.25 ± 0.1	0.19 ± 0.1
PO_4	14.8 ± 2.6	14.4 ± 1.3	8.1 ± 1.5	22.3 ± 5.4	14.3 ± 1.2	8.4 ± 1.2
SO_4	5.2 ± 1.1	5.2 ± 1.3	19.6 ± 5.2	10.8 ± 3.8	7.8 ± 1.4	71.3 ± 11.4
Malate	16.8 ± 4.0	13.5 ± 3.8	28.0 ± 9.7	14.4 ± 4.4	13.2 ± 3.4	14.3 ± 5.9
ABA	0.04 ± 0.01	0.84 ± 0.69	1.69 ± 0.29	0.02 ± 0.01	0.44 ± 0.09	1.23 ± 0.19

All values indicate μmol g^{-1} f. wt.

Data were obtained at the end of the experimental period, 51 d after sowing. Values are means $\pm$ SD; $n = 9$ for leaves and roots, $n = 3$ for stem plus petioles.
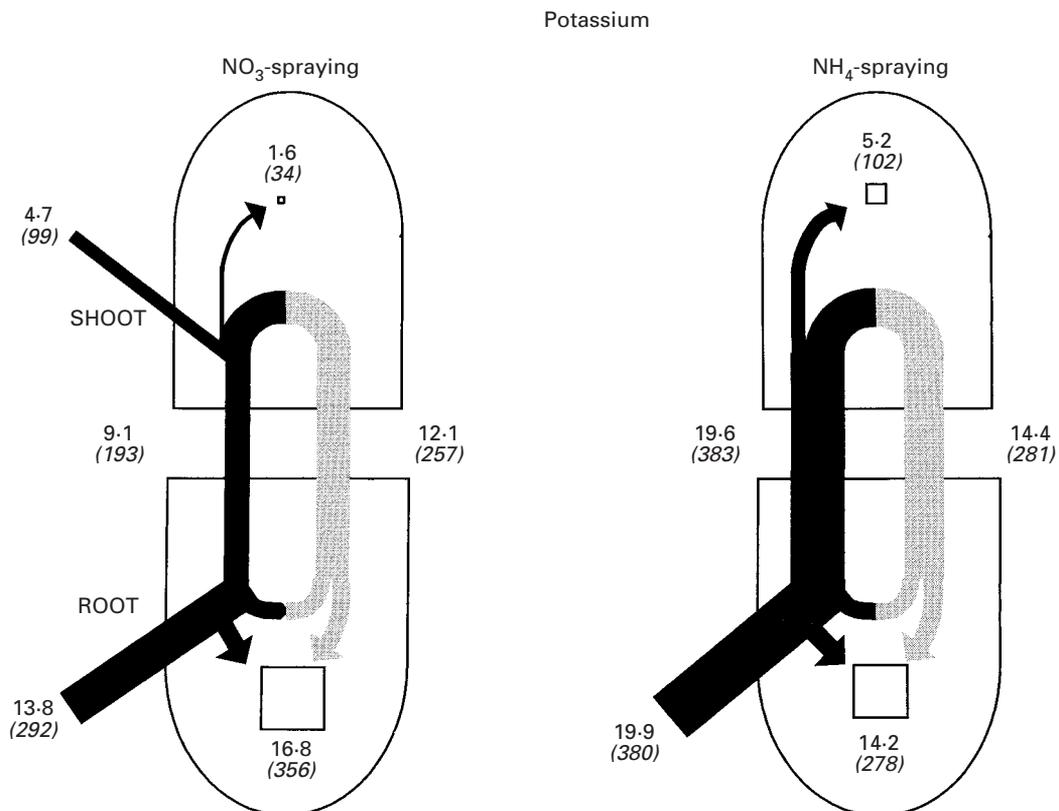

Figure 1. Flow profiles for uptake, transport and utilization of potassium in *Ricinus communis* sprayed with 20 mM nitrate or ammonium solution without further pedospheric nitrogen 41–51 d after sowing. Width of arrows (left, filled: flow in the xylem; right, grey: flow in the phloem) and length of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

are presented as μmol g^{-1} f. wt $(10 \text{ d})^{-1}$ and in parentheses and italics mmol per plant $(10 \text{ d})^{-1}$ (Figs 1, 3, 5, 7).

Abscisic acid analysis

Freeze-dried tissue samples were homogenized and extracted in 80 % (v/v) methanol. Extracts were passed through a Sep Pak® C_{18}-cartridge. Methanol was removed under reduced pressure and the aqueous residue partitioned three times against ethyl acetate at pH 3.0. The ethyl acetate of the combined organic fractions was removed under reduced pressure. The residue was taken up in TBS-buffer (Tris buffered saline; 150 mM NaCl, 1 mM $MgCl_2$, and 50 mM Tris; pH 7.8) and subjected to an immuno-

logical ABA assay (ELISA) as described previously (Mertens, Deus-Neumann & Weiler, 1985; Peuke *et al.*, 1994*b*). For phloem and xylem saps the Sep-Pak C_{18} purification step was omitted. The aqueous phase after partitioning against ethyl acetate was hydrolysed for 1 h at room temperature with 1 M NaOH. This fraction was acidified with concentrated HCl acid to pH 3, partitioned three times against ethyl acetate. The accuracy of the ELISA was verified for *Ricinus* in earlier investigations (Peuke *et al.*, 1994*b*). Recoveries of ABA during purification procedures were checked routinely using radioactive ABA and found to be > 95 %. The immuno-chemicals were generously supplied by Prof. Weiler, Ruhr Universität, Bochum, Germany.

Statistics

Determinations of ion content of the plant parts were obtained from nine plants for both harvests. Each plant part was analysed individually with exception of the petioles, where three plants were bulked. Each analysis consisted of two replicates of extraction and two or three independent measurements per extraction. For calculating the transport saps models for the xylem 20 or 25, and for the phloem 15 or 18, saps were analysed in nitrate or ammonium-sprayed plants, respectively.

RESULTS

Composition of xylem and phloem saps

No marked differences were found in the composition of cations in xylem exudates of *Ricinus* sprayed with nitrate or ammonium. The concentrations were lower than in pedospherically supplied plants, which also showed greater effects because of the N treatment (Table 1). The spectra of anions showed more effects of the treatments. In nitrate-sprayed plants, a sixfold higher nitrate concentration was found in the xylem exudate than in ammonium-sprayed ones, but this was only 5 % of the concentration of pedospherically supplied plants. The concentrations of sulphate and malate were about doubled in ammonium-sprayed plants. The total nitrogen concentration was the same in both treatments, but there was more amino and amide nitrogen in ammonium-sprayed plants which, with the addition of the malate content, meant that the carbon concentration was higher. Compared with pedospherically supplied plants the sprayed plants reached only 10 % of the nitrogen concentrations in the xylem. The ABA concentrations were the same in sprayed treatments but markedly higher than in root supplied plants.

The composition of the phloem saps, as for the xylem saps, was fairly similar for both sprayed
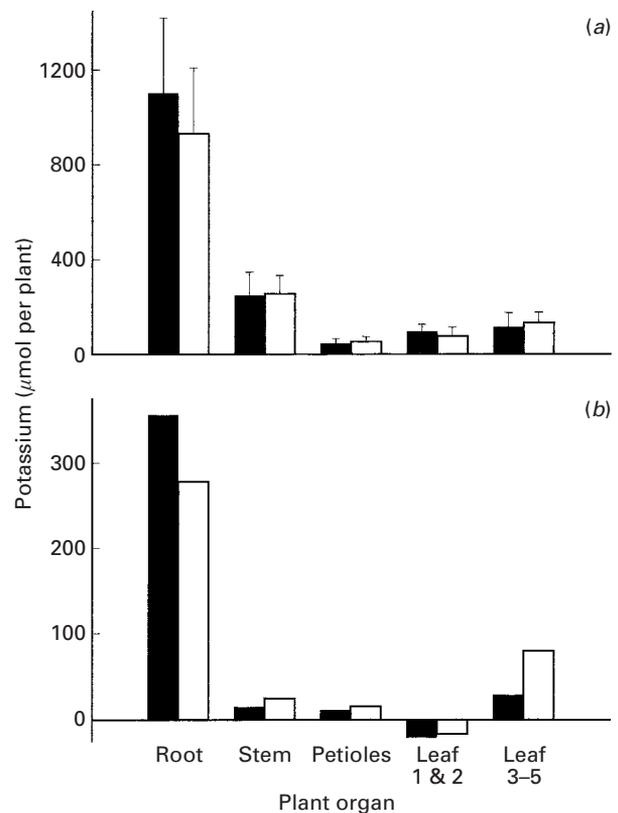

Figure 2. (*a*) Initial potassium content (41 d after sowing) and (*b*) the increment of potassium per plant in *Ricinus communis* during the experimental period 41–51 d after sowing. During the experimental period the plants were cultivated without pedospheric nitrogen, but the shoots were sprayed with 20 mM nitrate (■) or ammonium (□) solution. Bars, SD, $n = 9$.

treatments. Potassium and chloride were slightly increased in ammonium-sprayed plants, although sulphate concentration was more than twice as high in ammonium-treated than in nitrate-treated plants (Table 2). The concentrations of cations in the phloem of sprayed plants were in the same range as pedospherically supplied plants, whereas concentrations of anions were generally higher. Nitrogen concentration (which is similar to amino and amide nitrogen concentrations in phloem) was lower in the phloem of ammonium-sprayed *Ricinus* than in pedospherically supplied plants. However, the carbon concentrations were similar to plants supplied with ammonium to the roots, owing to the high sucrose concentrations. There were no differences for C, N and ABA between the sprayed treatments.

Composition of tissues

In ammonium-sprayed *Ricinus* the concentrations of nitrogen and potassium in leaves on a f. wt basis were *c*. 20 % and 36 % higher, respectively, than in nitrate-treated plants at the end of the experimental period (Table 3). The concentrations of other cations were similar. For anions, there were markedly higher
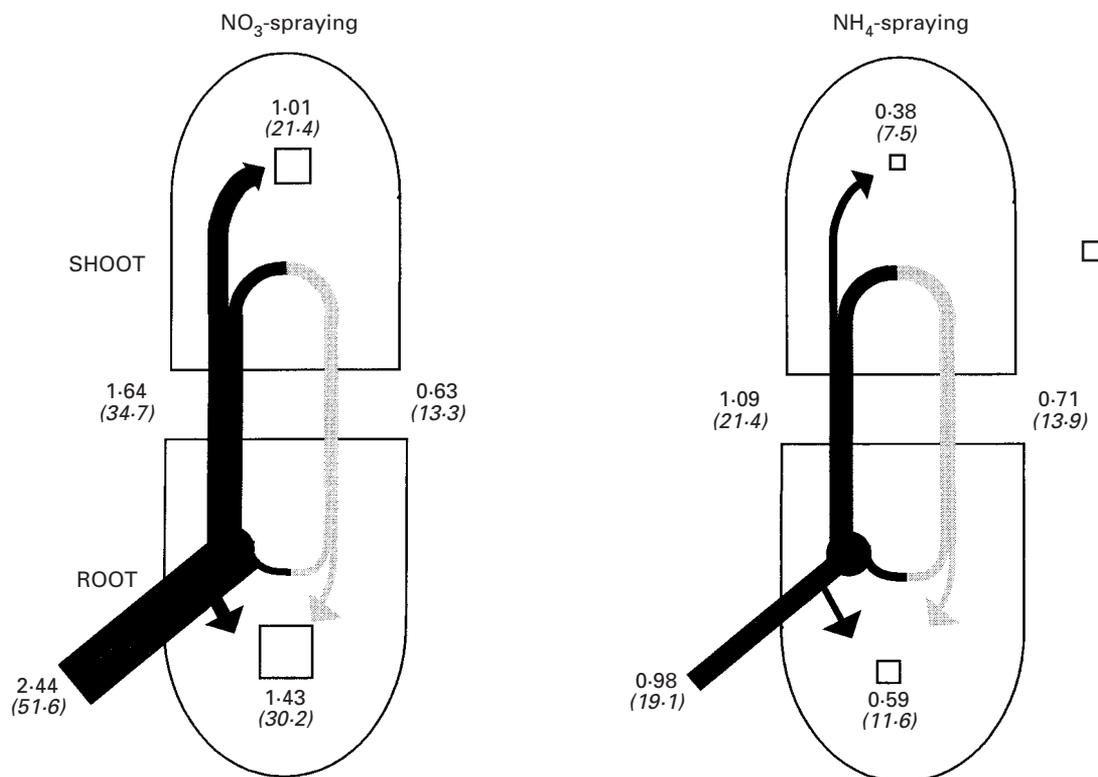
Magnesium

NO₃-spraying

NH₄-spraying

Figure 3. Flow profiles for uptake, transport and utilization of magnesium 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. Values indicate μmol g^{-1} f. wt (10 d)$^{-1}$; in parentheses mmol per plant (10 d)$^{-1}$.

root concentrations of chloride, phosphate and sulphate in ammonium-sprayed than in nitrate-sprayed plants. Additionally, the sulphate concentration in ammonium-sprayed leaves was increased 3.6-fold. On the other hand, in leaves of nitrate-sprayed *Ricinus* the concentrations of malate and nitrate were higher. There was a tendency for higher concentrations of ABA in tissues of nitrate-sprayed plants; the differences, however, were not significant.

Cation flows

For the evaluation of potassium flows in nitrate-sprayed plants we assumed that potassium was taken up stoichiometrically (1:1) with nitrate in the shoots, since a solution of 20 mM KNO₃ was sprayed. On the basis of this assumption one quarter of potassium was taken up by the shoots and the remaining uptake by the root was only 71 % of that in ammonium-sprayed plants, whereas total uptake (18.5 μmol g^{-1} f. wt (10 d)$^{-1}$) was only slightly decreased (95 %) (Fig. 1). Most of the potassium taken up by the roots was transported via the xylem to the shoots, to about twice the extent in ammonium-sprayed than in nitrate-sprayed plants. The transport rates in the phloem were similar in both treatments. Most of the

potassium was deposited in the roots, to the extent of 73 % in ammonium-sprayed plants and even 91 % in nitrate-sprayed plants (Fig. 2). Within the shoot most potassium was incorporated in leaves 3–5, whereas the primary leaves lost potassium. The shoot potassium gain was always slightly higher in ammonium-sprayed plants. Sodium was released to the rhizosphere during the experimental period in both treatments (negative sum of all increments) and the values of flows were similar (data not shown). Most of the release of sodium originated from the roots (95 % in both treatments).

Magnesium uptake was strongly decreased in ammonium-sprayed plants being only 40 % of that in nitrate-sprayed plants (Fig. 3). Most of the magnesium taken up was first transported to the shoots (67 % of 111 % in nitrate- or ammonium-sprayed plants, respectively). However, the phloem transport of magnesium was relatively high compared with that in the xylem and as a consequence the root was favoured in the partitioning of magnesium; *c.* 60 % was incorporated in the roots in both treatments. Per plant the gain of magnesium in roots was only a third as great in ammonium-sprayed than in nitrate-sprayed plants (Fig. 4), and in stems and petioles this was also markedly lower. Additionally the loss from the first two leaves was
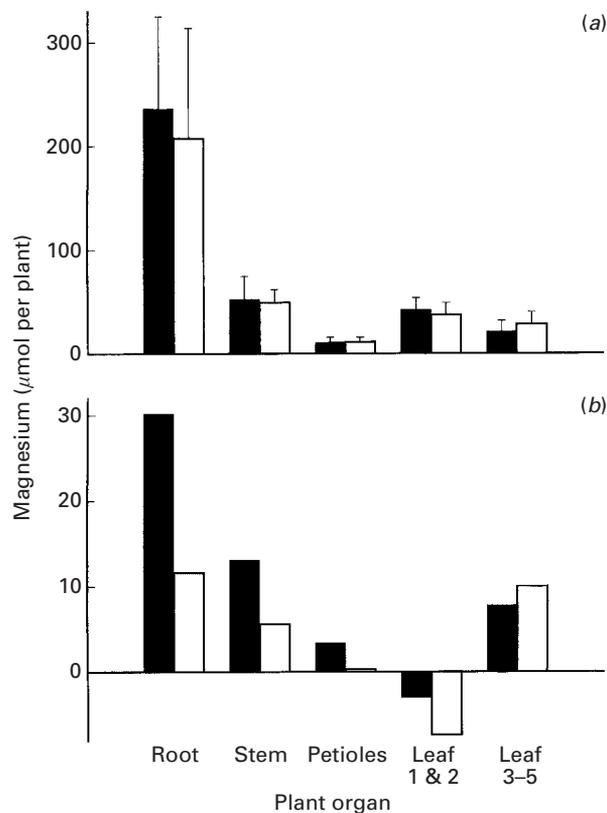
Figure 4. (*a*) Initial magnesium content (41 d after sowing) and (*b*) the increment of magnesium per plant in *Ricinus communis* during the experimental period 41–51 d after sowing. During the experimental period the plants were cultivated without pedospheric nitrogen, but the shoots were sprayed with 20 mM nitrate (■) or ammonium (□) solution. Bars, SD, *n* = 9.

higher in ammonium-sprayed plants. Only the incorporation of magnesium in leaves 3–5 was higher. In ammonium-sprayed plants the uptake of calcium was only slightly decreased (90 % of nitrate-sprayed plants, data not shown). Most of the calcium was retained in the roots; 71 % or 83 % of calcium was incorporated there in nitrate- or ammonium-sprayed plants, respectively. As expected for calcium, phloem transport was low.

Chloride flows

The applied treatments strongly affected the uptake, transport and incorporation of chloride in *Ricinus*. Ammonium-sprayed plants took up 2.5-fold more chloride than nitrate-sprayed plants (Fig. 5). Only 10–15 % of the newly taken up chloride was transported in the xylem and most of this was recycled back to the roots in the phloem. In nitrate-sprayed plants a net export of chloride from the shoots occurred by loss from petioles and primary leaves (Fig. 6). As a result, most chloride was incorporated into the roots, amounting to more than twice that in ammonium-sprayed *Ricinus*.

Abscisic acid flows

A strong root-to-shoot signal was found in the xylem of ammonium-sprayed plants. There was 4.5-fold more ABA transported than in nitrate-sprayed plants. In the phloem the transport rate was similar between the two treatments (Fig. 7). In ammonium-sprayed plants net ABA-biosynthesis was observed in roots whereas net catabolism occurred in shoots during the experimental period. Nitrate-sprayed plants, however, exhibited the opposite behavior. Additionally, the values for net-metabolism were 174–285 % higher in nitrate-sprayed plants. Significant deposition of ABA was found only in the shoots, here about twice as much ABA was accumulated in nitrate-sprayed plants (Fig. 8). Most ABA was incorporated into the newly developed leaves 3–5; whilst the primary leaves lost ABA.

DISCUSSION

Induction of ammonium syndrome by foliar application

After foliar application of ammonium, characteristics of the ammonium syndrome were induced, although roots were not affected by ammonium either directly or indirectly.

The ionic composition of tissues as well as of xylem and phloem exudates in *Ricinus* were less affected by foliar spraying than by the pedospheric supply of ammonium vs. nitrate. The effects on anions were stronger than on cations. The first reason for this observation may be related to the relatively low rates of foliar uptake of these ions (3–7 %, Peuke *et al.*, 1998) compared with application via the roots (Peuke & Jeschke, 1993). The second reason was that before the treatments and the experimental period started the plants were supplied with the same nutrient solution by the roots. This can be seen in the initial content of ions at the beginning of the experimental period (Figs 2, 4, 6). Nevertheless, there were some remarkably interesting effects from spraying ammonium or nitrate which were typical for the actions of the two different N-sources.

The differences in cation composition were low, perhaps because of the efficient pH-stat mechanism in the leaves (malate degradation, Peuke *et al.*, 1998) which might partly compensate for a cation excess from the supply of ammonium. The impact of ammonium on the composition of anions was more pronounced. For xylem sap this has been observed previously in pedospherically supplied *Ricinus* (Allen & Smith, 1986; van Beusichem *et al.*, 1988; Peuke & Jeshke, 1995; see Table 1) and in maize (Engels & Marschner, 1993). However, the increase in sulphate must be discussed with caution, since $(NH_4)_2SO_4$
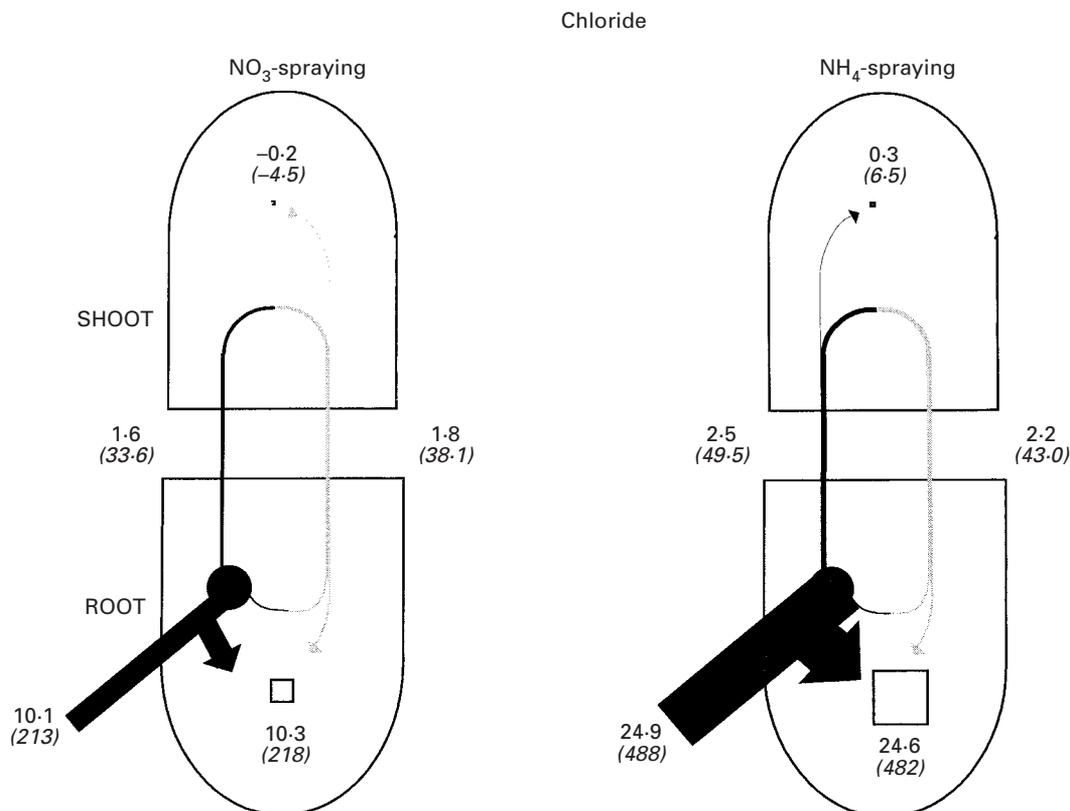
Chloride

NO₃-spraying

NH₄-spraying

Figure 5. Flow profiles for uptake, transport and utilization of chloride 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. Values indicate μmol g^{-1} f. wt (10 d)$^{-1}$; in parentheses, mmol per plant (10 d)$^{-1}$.

was sprayed and therefore supplied in addition to that taken up by the roots. Conversely, this effect was not observed for potassium in KNO₃-sprayed plants. The supply of sprayed potassium (from KNO₃) seemed to decrease the rate of uptake of this ion by the roots; if our assumptions are correct a higher foliar uptake of potassium can be assumed because of the chemical nature of the cuticle (Peuke *et al.*, 1998). The uptake of potassium was less affected by the nature of N-source than were other cations, agreeing with our previous work (Peuke & Jeschke, 1993). Contrary observations for potassium have been made. In the present experiment as well as in castor bean supplied pedospherically (Allen & Smith, 1986; Peuke & Jeschke, 1993) potassium concentrations in the tissues were less influenced by the N-source. By contrast potassium content was strongly diminished in another experiment in *Ricinus* of van Beusichem *et al.* (1988) after ammonium supply. Little impact on potassium was found also in barley (Lang & Kaiser, 1994) and sorghum, but a large decrease was reported in beet (Arnozis & Findenegg, 1986).

In pedospherically supplied plants a decrease in uptake of divalent cations was commonly observed in response to ammonium (Arnozis & Findenegg, 1986; van Beusichem *et al.*, 1988; Peuke & Jeschke, 1993; Lang & Kaiser, 1994). The uptake of the

divalent cations was also inhibited in ammonium-sprayed plants compared with nitrate-treated ones. For calcium this impact was smaller (ammonium-treated were 90 % of nitrate-treated). However, the effect was more pronounced (40 %) for magnesium (Fig. 3) in sprayed plants in relation to the pedospheric supply of ammonium in *Ricinus* (54 % or 55 %, respectively, Peuke & Jeschke, 1993; Table 4). Additionally, the chloride uptake was enhanced in ammonium-sprayed plants (24 %; Fig. 5), as found previously in plants supplied with ammonium via the roots compared with nitrate-fed plants (632 %; Peuke & Jeschke, 1993; Table 4). Nitrate limitation, however, also resulted in stimulation of chloride uptake (Peuke *et al.*, 1994*a*). The partitioning of the ions was strongly influenced by the N-limitation of the plants (Peuke *et al.*, 1998) which resulted in higher incorporation of elements, including ions, in the root (Duarte & Larsson, 1993; Peuke *et al.*, 1994*a*). The impact of ammonium on element partitioning in which the roots were favoured (Peuke & Jeschke, 1993) was confounded by the impact of nutrient deficiency. The release of sodium in both treatments (negative sum of all increments) pointed to a cation excess as well as to a nutrient limitation. Similar effects were found previously in salt-treated ammonium-fed and N-limited *Ricinus* (Peuke *et al.*, 1994*a*; Peuke & Jeschke, 1995; data not shown).
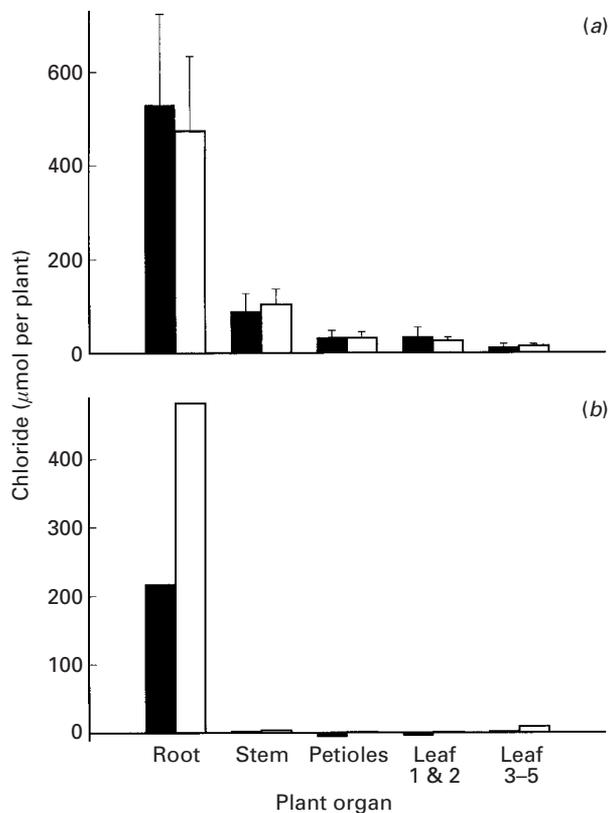
Figure 6. (*a*) Initial chloride content (41 d after sowing) and the increment of chloride per plant in *Ricinus communis* during the experimental period 41–51 d after sowing. During the experimental period the plants were cultivated without pedospheric nitrogen, but the shoots were sprayed with 20 mM nitrate (■) or ammonium (□) solution. Bars, SD, $n = 9$.

Arnozis & Findenegg (1986) concluded that the species-dependent site of nitrate reduction might be responsible for a sensitivity in cation composition due to ammonium nutrition. Plants in which nitrate reduction occurs mainly in the roots need no charge balance for nitrate transport in the xylem and so are less sensitive to ammonium. This agrees with the present results, since the exclusive N-assimilation in the shoots with a foliar supply of N (Peuke *et al.*, 1988) needed no charge balance of inorganic nitrogen in the xylem (see also Engels & Marschner, 1993). Furthermore, the differences in ionic uptake (Table 4) became obvious in the composition of the root tissue (Table 3, Figs 2, 4, 6).

Mechanisms for the effects of ammonium on ionic composition

With respect to cation uptake (magnesium and calcium), uptake of chloride and flow of ABA in the xylem, the ammonium-sprayed plants were affected in the same way as pedospherically ammonium-supplied plants compared with corresponding nitrate fed plants. Ammonium supply is known to inhibit the uptake of other ions (Haynes & Goh, 1978; Runge, 1983; Marschner, 1995), for example nitrate

(Lee & Drew, 1989; Jackson & Volk, 1995; Smart & Bloom, 1998) and potassium (Scherer *et al.*, 1984; Wang *et al.*, 1996; Smart & Bloom, 1998). However, the explanation for these effects is rather complicated, since it seems to be dependent on different conditions such as the ratio of concentrations, N-status, pretreatment (Jackson & Volk, 1992; Wang *et al.*, 1996; Smart & Bloom, 1998) and does not conform to a simple kinetic model (Scherer *et al.*, 1984; Lee & Drew, 1989). It was assumed that ammonium and potassium were transported by the same system (Lee & Ayling, 1993) or that the nitrate uptake is affected via inhibition of potassium uptake by ammonium (Jackson & Volk, 1995). However, potassium transport systems always have a high selectivity for potassium over ammonium (Maathuis & Sanders, 1996), although in the present experiment ammonium was not available in the rhizosphere to affect uptake of ions directly. Engels & Marschner (1993) assumed that ammonium decreased the concentration of cations in the tissues by internal demand rather than through uptake by the roots. Our results confirm the conclusion in the literature that the impact of ammonium on the uptake of other ions is not via a simple kinetic mechanism, but rather in a complex way at the whole plant level.

Signal of ammonium and root–shoot communication

The question arises of what the plants sense and which are the signals between root and shoot in the case of ammonium nutrition. The application of ammonium to root or shoot affected the other organ. In *Ricinus*, ABA was found to be a strong root to shoot signal when ammonium was supplied to the roots (Peuke *et al.*, 1994*b*). A similar result was found in the present experiment, even though no ammonium could reach the root either by the rhizosphere or by phloem transport. Furthermore both root systems were supplied with the same nutrient solution. When we compare data of Peuke *et al.* (1994*b*, nitrogen applied to roots) with the data of this paper (foliar spraying) we see an increase in the net synthesis of ABA and accumulation in the leaves of sprayed plants indicating that nitrate treatment stimulated ABA formation. The data of this paper demonstrate also that ABA accumulation is greater when leaves have been sprayed with nitrate. Recent findings show that one step of ABA biosynthesis, an oxidation of an aldehyde (either the ABA-aldehyde; Walker-Simmons, Kudrna & Warner, 1989; or the xanthoxal, Lee & Milborrow, 1997) requires the molybdenum cofactor which is also an important component of nitrate reductase (NR). When NR is induced by nitrate treatment an increased activity of the molybdo-aldehyde oxidase would be expected. Additionally, Scholten *et al.* (1985) observed that an *Arabidopsis* line (G1), with strongly reduced NR
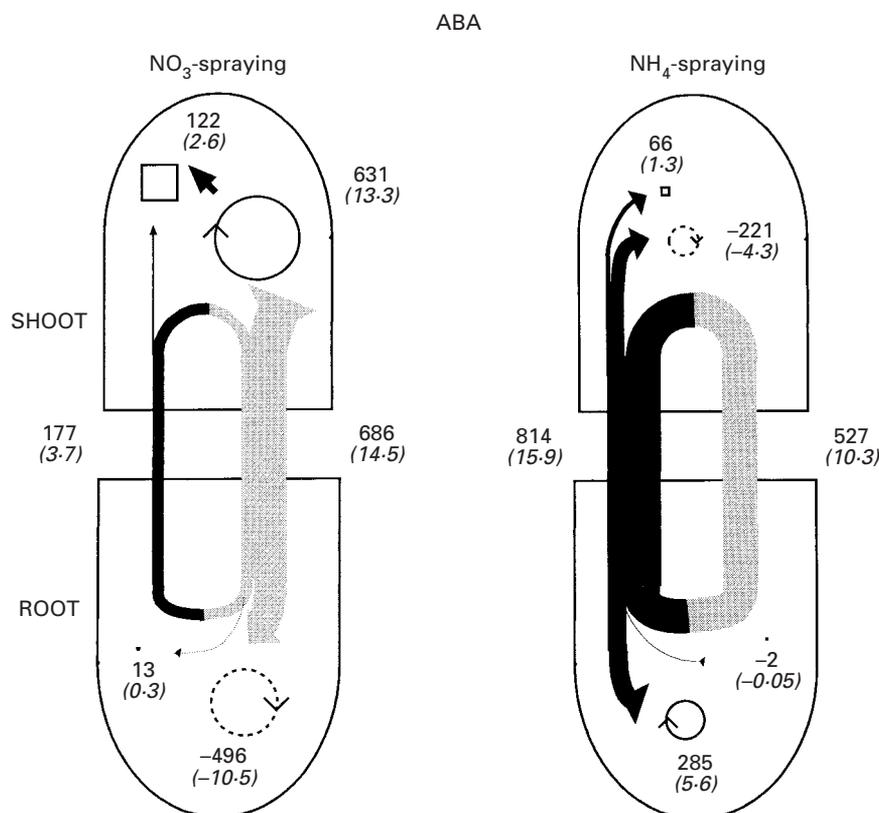
ABA

Figure 7. Flow profile for transport, net metabolism and deposition of ABA by whole plants of *Ricinus communis* sprayed with 20 mM nitrate or ammonium solution without further pedospheric nitrogen over the experimental period 41–51 d after sowing. Circles indicate net metabolism, synthesis (——, positive values) or degradation (–––––, negative values) of ABA. Values indicate pmol g^{-1} f. wt $(10 d)^{-1}$; in parenthesis, nmol $plant^{-1}$ $(10 d)^{-1}$. 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.

Table 4. *Uptake, xylem and phloem flows of either foliar or pedospherically ammonium-supplied* Ricinus communis *plants 41–51 d after sowing expressed as % of nitrate-supplied plants*

	Pedospheric supply			Foliar supply		
	Uptake (%)	Xylem flow (%)	Phloem flow (%)	Uptake (%)	Xylem flow (%)	Phloem flow (%)
K^+	66	70	75	105	215	119
Na^+	53	57	80	109*	100	93
Mg^{2+}	55	73	130	42	66	113
Ca^{2+}	54	50	100	90	63	113
Cl^-	632	482	417	247	158	122
ABA	—	460	77	—	332	192

Data from pedospheric supplied plants were taken from Peuke & Jeschke, 1993, * net-release of sodium.

activity that could be restored by addition of an excess of molybdenum, exhibited wilting characteristics very similar to ABA-deficient mutants. Similarly, Whenham *et al.* (1989) observed that ABA together with metabolites phaseic acid and dihydrophaseic acid accumulated in leaves of kale in field experiments when nitrate supply was enhanced.

Additionally Peuke *et al.* (1994*b*) were able to show that net ABA biosynthesis in roots and leaves of castor bean was increased under optimal nitrate nutrition. Our present observations as well as the findings in the literature are in agreement with our conclusions.
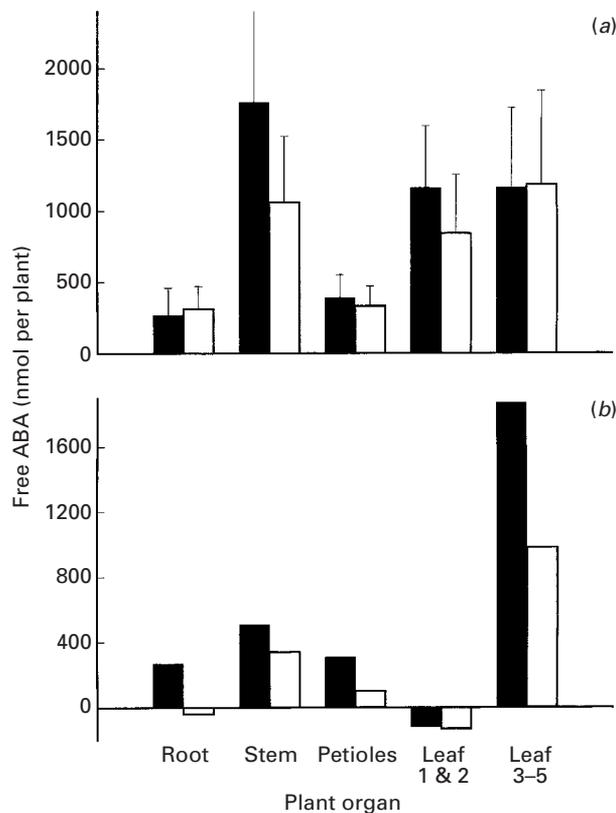
In the present experiment nitrate was withdrawn

Figure 8. (*a*) Initial ABA content (41 d after sowing) and (*b*) the increment of ABA 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, SD, $n = 9$.

from the rhizosphere before the experimental period and ammonium was not pedospherically supplied. A direct effect of ammonium on membranous uptake systems with respect to inhibited cation uptake or enhanced anion uptake as well as to the root borne ABA-signal to the shoot, can be excluded, since ammonium could not reach the roots during the experimental period. The rhizosphere was rigorously separated from the shoot environment and there was little transport of ammonium via phloem. Thus, a different condition must be assumed in an ammonium-supplied plant, and a signal in the xylem in case of pedospheric supply or a signal in the phloem of foliar supplied plants must be present. Unfortunately, so far no indication of the identity of the signal can be derived from the elemental (including ionic) composition of the phloem, as in both sprayed treatments they were similar (Table 2). This has previously been observed in *Ricinus* pedospherically supplied with nitrate or ammonium (Allen & Smith, 1986; van Beusichem *et al.*, 1988; Peuke & Jeschke, 1995) and in broccoli (Shelp, 1987). Therefore we have to postulate an unidentified signal between shoot and root in the case of ammonium spraying.

CONCLUSION

In conclusion we observed that some of the effects of the ammonium syndrome can also be induced by foliar application. Direct contact and effect of ammonium on the roots was avoided. An effect of the ammonium ion on uptake systems of other ions, a less effective use of carbon or an acidification of the rhizosphere by ammonium uptake (see also Peuke *et al.*, 1998) can be excluded as a means by which ammonium nutrition is influenced. The signal between root and shoot and vice versa remains unclear on the basis of the main determined solutes in xylem and phloem. An unidentified specific signal must be assumed. And the general question is still unanswered: what does a plant sense during the ammonium syndrome, the presence of ammonium or the absence of nitrate?

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