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Effect of nitrate and ammonium on long distance transport in cucumber plants

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Abstract

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Cucumber plants (*Cucumis sativus* L. cv. 'Chinese Snake') were grown on culture solutions containing nitrate or ammonium as nitrogen source. The carbon and nitrogen fluxes from shoot to roots and vice versa have been investigated by analysis of plant material, xylem and phloem sap and by measurement of biomass production and root respiration. Despite the markedly reduced growth on ammonium, the C-flux from shoot to roots and root respiration were equivalent on a single plant basis for both treatments. Root respiration consumed about 30% of the carbon delivered through the phloem. Plants on ammonium used only 20% of the carbon for root biomass production, while 50% was recycled to the shoot through the xylem. In cucumbers fed with nitrate nearly 50% of the carbon went into root biomass, and only about 25% was recirculated to the shoot. From xylem sap analyses it became evident that plants fed with ammonium assimilated nitrogen in the roots and transported it to the shoot in organic form (bound to C-skeletons), while nitrate-fed plants transferred the nitrogen taken up mainly in unaltered form into the xylem. With ammonium nutrition, root biomass production and the high demand for C-skeletons used in ammonium assimilation compete for the phloem-derived carbon. The alterations in the long-distance transport must be considered as possible factors causing the weaker growth of ammonium-fed plants.

Key words: phloem – xylem – carbon flux – root respiration – *Cucumis sativus* – ammonium – nitrate – translocation

Introduction

Nitrate (anion) and ammonium (cation) are easily utilized by higher plants although the metabolism is strongly affected by the form of the inorganic nitrogen source (Kirkby and Mengel 1967). A markedly reduced biomass production was observed in cucumbers (Zhou and Collet 1983) and sunflowers (Weissmann 1964) when nitrogen was supplied exclusively as ammonium. These authors also reported that root growth was more severely affected than shoot growth.

A major portion of the nitrate taken up is generally translocated in unaltered form to the shoot, although the roots are capable of assimilating oxidized nitrogen (Dalling et al. 1972). Ammonium represents only a minor portion of the xylem nitrogen in plants grown on nutrient solutions with an ammonium concentration below 5 mM or with nitrate as nitrogen source (Kulaeva et al. 1957, Thomas et al. 1979). Rather high ammonium concentrations in the xylem sap were reported for plants fed with elevated ammonium concentrations during early vegetative growth (Zhou and Collet 1983, Kirkby and Mengel 1967). In healthy plants ammonium is mostly assimilated within the root system and translocated to the shoot in organic compounds (Bollard 1960).

Ammonium assimilation in the roots causes a large demand for C-skeletons which must be delivered through the phloem. The aim of the work presented here was to elucidate the influence of the nitrogen source on the partitioning of phloem-derived carbon into root respiration, root biomass production and recirculation through the xylem. A major goal was the quantitative description of nitrogen and carbon fluxes in xylem and phloem of cucumber plants grown on different nitrogen sources.

Material and Methods

Cucumber plants (*Cucumis sativus* L. cv. 'Chinese Snake') were grown for two weeks in quartz sand in a growth chamber. At day 14, similar sized plants were transferred to aerated nutrient solutions in 2 liter brown polyethylene containers, each container supporting two plants held by small plastic tubes. The nutrient solution was composed as described by Thomas et al. (1979) except for the iron, which was added as sequestren (0.07 g/liter), and the nitrogen concentration was 5 mM. At the beginning of the experiment the pH of the solutions was 5.3, never falling below 4.7 or increasing above 5.8 during the growth periods. The nutrient solutions were renewed in intervals of one week. From day 14 today 21 the solutions were diluted 1 : 4, during days 21–28 the dilution was 1 : 2 and after day 28 full strength nutrient solution was applied. After 21, 28 and 35 days of growth plants were harvested and divided into shoots and roots and each replicate was weighed.

Xylem sap samples were obtained by decapitating the plants about 1 cm above the root system. The exudate was removed by means of a syringe. Samples were collected at intervals of 5 minutes for 45 minutes, the first sample being discarded to avoid contamination originating from surrounding cells or phloem sap. Phloem sap samples were obtained from the same plants by collecting the bleeding sap deriving from the sieve tubes of the shoots. To prevent gelling of the phloem sap, the exudate was immediately transferred into 48% EtOH which precipitated the p-protein present. Then all samles were put into a boiling water bath for 5 minutes and centrifuged subsequently. If not otherwise stated, saps were collected at 12 noon in order to achieve comparable results.

Transpiration was determined by repeated weighing of the culture containers. The net uptake of mineral cations and nitrogen was estimated by the increments in the plant material. These results compared well with the data derived from the decrease in the external medium.

Chemical estimations were made on both the dried plant material and the bleeding saps. Potassium and calcium were estimated by atomic absorption spectrometry. The methods for the analyses on nitrogen compounds were based on those described by Thomas et al. (1979), except that free ammonium was detected by Nessler's reagent. Reduced nitrogen was measured with a microkjeldahl procedure. Organic carbon was detected as CO_2 in an infrared gas analyzer after oxidating with CuO.

The fluxes in the xylem were estimated by means of the Ca-accumulation in the shoots and the element ratios in the xylem sap, a procedure proposed by Armstrong and Kirkby (1979). The downward fluxes in the phloem were computed making use of the fact that the carbon flow consists mainly of three parts: a) root respiration, b) C-circulation and c) root biomass production.

Root respiration was derived from the CO_2-evolution by roots of intact plants held in sealed plastic containers, which were connected to an infrared gas analyzer. It was measured between the intervals of harvesting, i.e. at days 19, 26 and 33. The C-recirculation is identical with the upward flux of C through the xylem. The N-flux was calculated from the ratios of C to N in the deproteinized phloem sap and the absolute carbon requirements of the roots.

Results

The dry matter production was considerably lower in plants grown on ammonium than in those with nitrate as the sole nitrogen source (Fig. 1). Root growth was more affected by the form of the inorganic nitrogen than the dry matter accumulation in the shoots. Roots of ammonium-grown cucumbers were brown, short and sturdy, while nitrate-grown plants had long and ivory-coloured roots. Except for a yellowish border around the older leaves, no macroscopic symptoms of ammonium toxicity were detected in shoots.

The carbon fluxes through the phloem on a single plant basis were comparable for both treatments during the period from day 21–28, but during the following week more carbon was delivered to the roots of ammonium-grown plants (Fig. 2). No major effects of the nitrogen source on root respiration were observed, although plants with nitrate grew faster. The percentage of phloem-derived carbon utilized for root respiration decreased during the two intervals of investigation from 35 to 24%. A factor of 2 between the treatments was observed for the percentage of carbon flowing into the root biomass, which represented a very important sink in nitrate-fed plants. On the other hand, a large amount of the carbon translocated to the roots returned to the shoot in ammonium plants (about 50%), while this percentage remained below 30% for plants fed with nitrate.

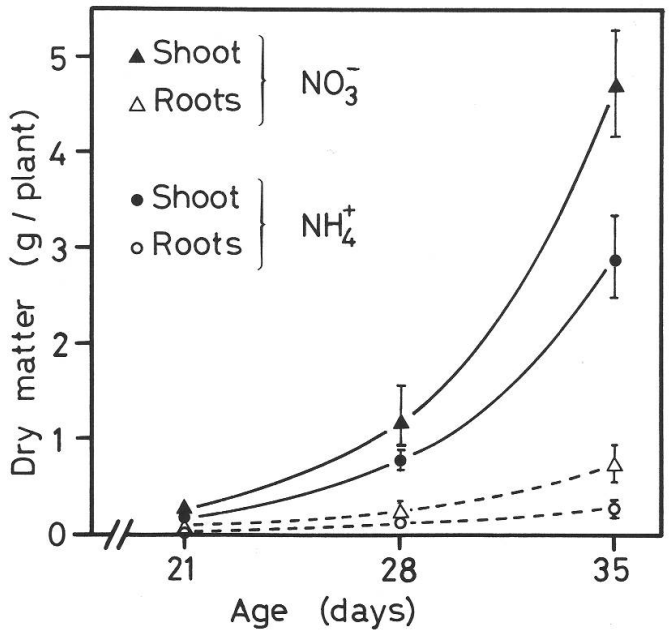

Fig. 1. Dry matter production in the roots and shoots of cucumber plants grown on nitrate or ammonium. The symbols represent the means of 5 replicates (each containing 2 plants). Standard deviations are shown where exceeding the symbol size.

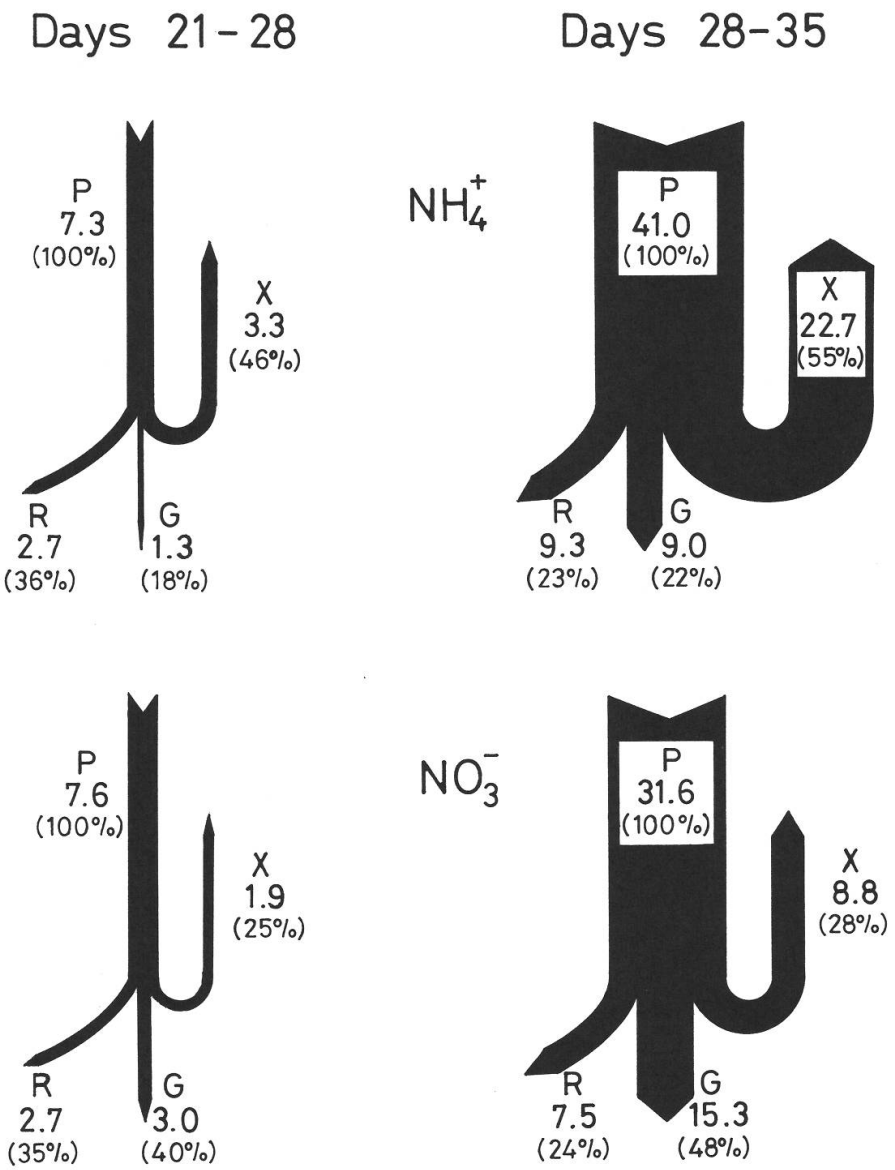

Fig. 2. Carbon fluxes in the roots of cucumber plants grown on nitrate or ammonium. The fluxes through the phloem (P), through the xylem (X), into the root growth (G) and to the root respiration (R) are expressed in mmoles carbon per plant and per week. The percentages of the total carbon delivered through the phloem to the root system are shown in brackets. The diagrams are based on analyses of five replicates (containing two plants each).

The carbon concentration in the xylem sap was 2.5 to 6 times higher in ammonium-treated plants, but the total nitrogen (nitrate plus reduced nitrogen) remained in the same range (Tab. 1). More than 80% of the xylem nitrogen is present in inorganic form in nitrate-fed plants. When ammonium was the nitrogen source, high concentrations of reduced nitrogen (mainly amino groups, essentially no free ammonium) were translocated through the xylem. Considering the fact that in amino acids several C-atoms per amino group are present, a high percentage of the xylem carbon in ammonium-fed plants presumably formed the C-skeletons for organic N-compounds. Potassium concentrations were relatively stable, while calcium was 3 to 6 times more concentrated in

Tab. 1. Composition of xylem sap during vegetative growth collected at 12 noon

Age (d)	N source	Concentration (μmol/ml)						
		K	Ca	C	$N_{red.}$	NO_3^-	Amino-groups	NH_4^+
21	Ammonium	15.59	1.55	198.55	23.23	<0.2	14.31	1.60
	Nitrate	16.59	7.96	41.48	6.20	27.46	2.83	0.25
28	Ammonium	11.74	1.03	121.46	30.49	<0.2	17.10	0.92
	Nitrate	12.90	5.91	19.32	3.24	18.10	1.97	0.25
35	Ammonium	15.04	1.36	113.27	22.95	<0.2	11.64	1.18
	Nitrate	13.25	4.71	43.41	2.98	16.77	1.69	0.28

Tab. 2. Composition of phloem sap during vegetative growth collected at 12 noon

Age (d)	N source	Concentration (μmol/ml)			
		K	C	$N_{red.}$	Amino-groups
21	Ammonium	91.21	1422	145.42	82.63
	Nitrate	86.89	1315	134.00	78.82
28	Ammonium	109.53	1604	137.95	55.63
	Nitrate	101.75	1333	108.94	61.20
35	Ammonium	84.08	1459	145.50	46.23
	Nitrate	89.42	971	105.83	41.39

the xylem of plants fed with nitrate. The composition of the phloem sap was more affected by plant age than by the nitrogen source (Tab. 2). No major changes were observed for the concentrations of potassium, carbon and reduced nitrogen, while the free amino groups in the phloem decreased by about 50% from day 21 to day 35.

The flow rates in absolute units wer lower in the xylem of ammonium-treated cucumbers (Tab. 3). This difference is similar to that observed for the biomass production. Despite the depressed growth rate of ammonium-fed cucumbers, the transport rates in the phloem were similar for both treatments.

In contrast to carbon, the inorganic nitrogen taken up from the nutrient medium must be considered as a major flux of nitrogen into the roots (Fig. 3). The nitrogen uptake from nitrate medium was about twice the rate observed in plants on ammonium. The flux from the external medium into the plants was affected by the inorganic nitrogen source in a similar way as the biomass production (Figs. 1 and 3). The nitrogen flux into root growth was considerably lower in plants grown on ammonium and was consistent with the situation found for carbon fluxes (Figs. 2 and 3). Compared with the other fluxes, root growth represented only a minor sink for nitrogen. The delivery of nitrogen through the phloem to the roots in absolute units was slightly lower in nitrate-treated plants. Regardless of the differing results obtained with two methods of calcu-

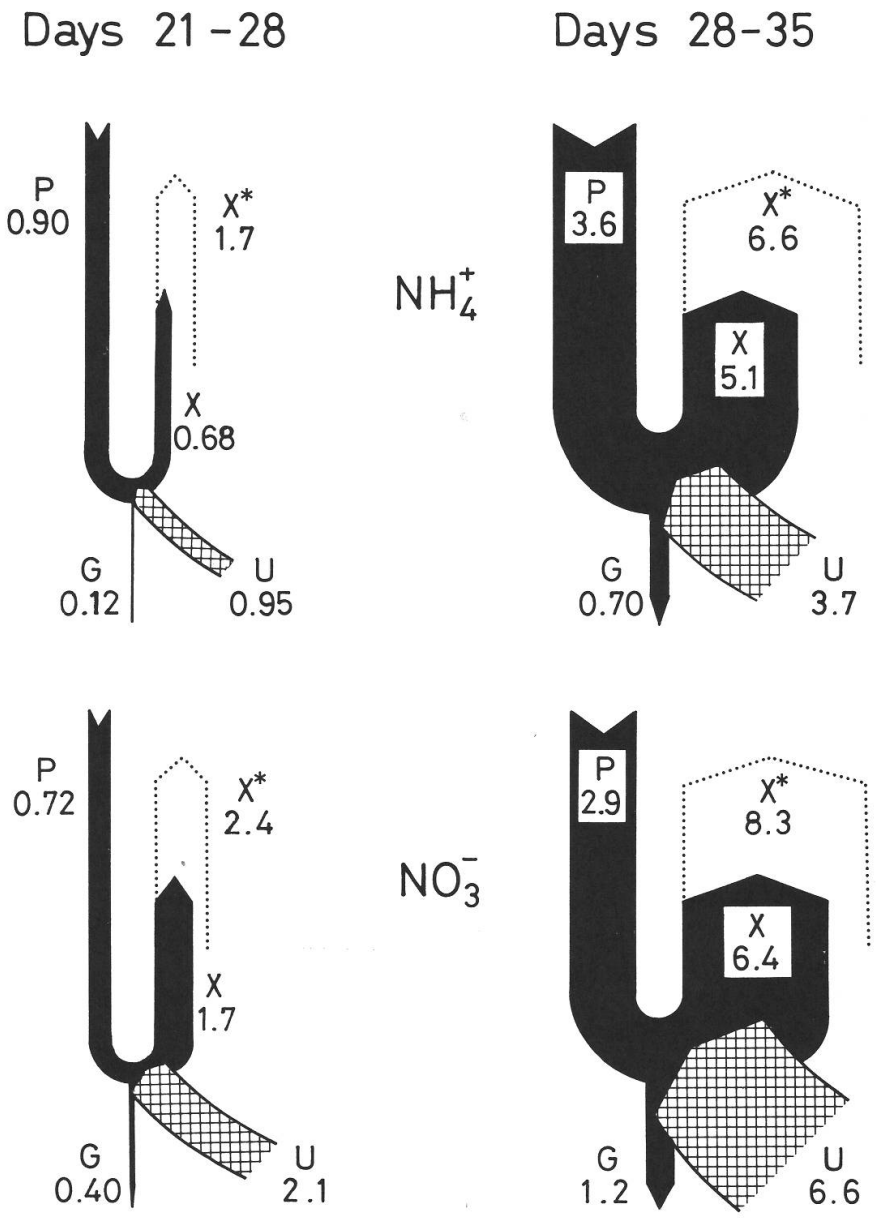

Fig. 3. Nitrogen fluxes in the roots of cucumber plants grown on nitrate or ammonium. The fluxes through the phloem (P), through the xylem (X) and (X*), into the root growth (G) and from the external medium into the roots (U) are expressed in mmoles per plant and per week. X represents the N-flux through the xylem calculated from element ratios in the xylem sap and the Ca increments in the shoots. X* represents the sum of the nitrogen delivered through the phloem to the roots plus the increment of nitrogen into the shoot. The diagrams are based on analyses of five replicates (containing two plants each).

lation, the transport through the xylem into the shoot was a major nitrogen flux in the root system.

The nitrogen fluxes in the xylem calculated from the net nitrogen accumulation in the shoots and the downward transport in the phloem (dotted arrows in Fig. 3) were especially in younger plants considerably higher than claculated from the nitrogen/calcium ratios in the xylem (solid arrows in Fig. 3). This discrepancy became less impor-

Tab. 3. Transport rates in xylem und phloem. The flow in the xylem is derived from measurements of transpiration. The phloem flow was calculated from C-concentration in the sap and the absolute C requirements of the roots. Saps were analysed at the beginning and the end of the intervals and the means were used for calculations.

Interval	N source	Flow rates (ml/plant)	
		Xylem	Phloem
21–28	Ammonium	143.72	4.86
	Nitrate	163.28	5.74
28–35	Ammonium	427.57	26.97
	Nitrate	582.73	27.44

Tab. 4. Diurnal changes in the composition of xylem and phloem sap at day 28

Time	N source	Xylem sap (μmol/ml)				Phloem (μmol/ml)		
		K	Ca	C	$N_{red.}$	K	C	$N_{red.}$
08.00	Ammonium	16.31	0.62	204.2	31.81	105.6	1517	168.2
	Nitrate	8.27	5.90	230.3	8.40	105.1	1814	191.9
12.00	Ammonium	27.93	1.46	179.7	26.34	122.5	1998	187.2
	Nitrate	12.62	6.17	45.4	7.80	135.8	1568	208.9
17.00	Ammonium	10.93	0.51	191.1	48.20	103.8	1517	172.9
	Nitrate	11.39	5.39	39.3	7.16	110.3	1504	209.9
22.00	Ammonium	19.41	1.47	267.0	64.17	118.5	1979	178.7
	Nitrate	12.80	6.02	25.8	5.84	119.3	1362	175.8

tant in older plants. An upward flow in the phloem of younger plants as reported by Martin (1971) or diurnal changes in the sap composition (Tab. 4) may cause this uncertainty concerning the nitrogen fluxes in the xylem. These discrepancies do not affect the main conclusions drawn above, but they should be considered in future experiments.

Discussion

From the results mentioned above it becomes evident that nitrate and ammonium, in addition to their function as external nitrogen sources, also act as regulators for the metabolism and for the long distance transport in higher plants. Especially in ammonium-grown cucumbers, which show a markedly reduced biomass production, impressive quantities of nitrogen and carbon are exchanged between the root system and the shoot. Not only the N-translocation, but also the fluxes of carbon and inorganic cations are influenced by the nitrogen source. Pristupa and Kursanov (1957) concluded from

comparative studies with N-fed and N-deprived pumpkin plants that the descending flow of assimilates is closely related to the nitrogen absorbing activity of the roots. Our results are consistent with this observation. With ammonium as the sole nitrogen source the cucumbers are in a comparable situation to legumes living in symbiosis with N_2-fixing bacteria: Herridge and Pate (1977) found that in nodulated cowpeas the N-assimilating system (nodules) respired 43% of the carbon delivered and returned 51% to the shoot, data which agree well with those obtained for ammonium-fed cucumbers.

The fluxes of nutrients and assimilates between the roots and the shoots should also be considered as long distance signals within the intact plant. Altered source/sink relations for a particular element may influence the metabolism in various organs. Quality and quantity of the nitrogen available become important in this context, since the transport rates in xylem and phloem are influenced by the nitrogen supply. The application of nitrate and ammonium can be used as a tool to modify root/shoot interactions in a physiological manner.

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