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Simultaneous Study of NH_4^+, Na^+, Mg^{2+}, Ca^{2+}, NO_3^-, SO_4^{2-}, (NO_2^-), $H_2PO_4^-$ and Cl^- Uptake by Intact Winter Wheat Seedlings in a Single Depletion Experiment

I. MATEJOVIČ

Research Institute of Plant Production,
Bratislavská cesta 122, 921 68 Piešťany, Czechoslovakia

Abstract. Simultaneous uptake of NH_4^+, Na^+, Mg^{2+}, Ca^{2+}, NO_3^-, SO_4^{2-}, (NO_2^-), $H_2PO_4^-$ and Cl^- ions by N-limited winter wheat seedlings (*Triticum aestivum* L., cv. Regina) in a single depletion experiment was investigated. Individual ion species in uptake solution samples were determined using capillary isotachophoresis. The operating systems used are described in detail. Processing of obtained concentration data allow to construct time curves of individual ions concentration in uptake solution, time curves of accumulative uptake, and curves for uptake rate versus time and uptake rate versus external concentration. From these curves there is possible to reveal a more complex picture of behaviour of individual ions during uptake process as well as to assess interactions among them. Attention was paid to the points of intersection of time curves of NO_3^- and NH_4^+ uptake rates. These points characterized by equal uptake rates may be considered as limits for preference of given ion species under given experimental conditions.

Additional index words: efflux, influx, isotachophoresis.

Sufficient evidences for genetic control of nutrients uptake have been shown in many plant species and varieties (Siddiqi and Glass 1983, Perby and Jensén 1983, 1986, Bloom and Finazzo 1986). These genetically controlled differences of plants in their ability to acquire the nutrients and in nutrients utilization efficiency under the conditions given may be important for selection or breeding of low input varieties and for varieties adaptable for specific ecological and growth conditions (Dambroth and El Bassam 1988). According to Klimashevsky (1988), agrochemically promising or energetically optimal varieties, respectively, are characterized by higher uptakes of N, P and K from soil as well as by higher rates of nutrients utilization in organic synthesis in root cells. These facts motivated several scientists to develop the screening techniques that would allow the selection of cultivars with desirable nutrient characteristics using plants under reasonable nutrient and environmental conditions (Perby and Jensén 1983, 1986, Jensén and Perby 1986, Woodend *et al.* 1986). However, a simple screening techniques allowing more complex study of simultaneous uptake of several nutrients as well as their interactions during

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the uptake process is still not available. Even if some studies have been dealing with simultaneous uptake of more nutrients in a single experiment, their determination needs several different analytical methods which makes them both rather complicated and more expensive.

More simple solution of this problem may offer modern analytical methods such as high performance liquid chromatography (based on ion-exchange principle, *i.e.* ion chromatography) and capillary isotachophoresis. Their generally accepted advantages such as possibility of simultaneous determination of several analytes in one run with high sensitivity and precision, minimum or no sample pretreatment and a small amount of sample required for analysis are the assumptions for their successful application in the study of nutrients uptake by solution depletion technique. Well-defined uptake solution as no much differing molar ratios of macro-components allowing to eliminate matrix effect are note-worthy too. Small amount of sample required for analysis can allow to perform experiments in micro-scale or on individual plant level, respectively.

Simultaneous uptake of nitrogen-nutrients (NO_3^-, NO_2^- and NH_4^+) using high performance liquid chromatography (HPLC) was already described (Goyal and Huffaker 1986a), but the potential of this method is much higher (see Cheam and Chau 1987) and could be very perspective in this field of study. Previously reported suitability of capillary isotachophoresis for the study of simultaneous uptake of NH_4^+, Na^+, Mg^{2+} and Ca^{2+} cations and NO_3^-, SO_4^-, (NO_2^-) and $H_2PO_4^-$ anions (Matejovič and Polonský 1988) is in presented work extended on determination of chlorides. Also more attention is given to processing and interpretation of obtained data, especially for NO_3^- and NH_4^+ uptake by N-limited intact winter wheat seedlings.

MATERIAL AND METHODS

Seeds of winter wheat (*Triticum aestivum* L., cv. Regina) were germinated in the dark on filter paper soaked with 0.2 mM $CaSO_4$ solution at 24 °C. After two days germinating seedlings were transferred to mesh flowing on 0.2 mM $CaSO_4$ solution. The seedlings grew in climate room with regime 14 h day 10 h night at the temperature 26/16 °C, relative humidity 55–70 % and irradiance 1 400 μmol m^{-2} s^{-1}. A day before the uptake experiment the nutrient solution was replaced. On the 7th day the groups of 15 plants were transferred to batches containing 50 ml of uptake solution composed of (in mM) 0.4 KCl, 0.3 $CaSO_4$, 0.2 K_2HPO_4, 0.3 $MgSO_4$, 0.6 NH_4NO_3, 0.2 NaCl and 0.02 Fe(III) Na salt of EDTA. The uptake solutions were continuously aerated. The samples of 1 ml volume were taken up in one hour intervals. Uptake experiment lasted 10 h and was replicated three times.

Abbreviations used: BALA – β-alanine; BICINE – N,N-bis hydroxyethyl glycine; EACA – ε-aminocaproic acid; HEC – hydroxyethyl-cellulose; TRIS – tris hydroxymethyl aminomethane.

TABLE 1

Operating systems and working conditions. Operating systems: I determination of NO_3^-, SO_4^{2-}, NO_2^- and $H_2PO_4^-$; II determination of Cl^-, SO_4^{2-}, NO_2^- and $H_2PO_4^-$; III determination of NH_4^+, Na^+, Mg^{2+} and Ca^{2+}.

Parameter	I	II	III
Solvent	H_2O	H_2O	H_2O
Leading ion	Cl^-	NO_3^-	K^+
Concentration [mM]	10	10	10
Counter ion	$BALA^+$	$EACA^+$	$BICINE^-$
Co-counter ion	Mg^{2+}	Cd^{2+}	–
Concentration [mM]	2.5	2.5	–
Additive	HEC (0.2 %)	HEC (0.2 %)	HEC (0.2 %)
pH of leading electrolyte	3.55	4.53	8.60
Terminating ion	citrate	citrate	$TRIS^+$
Concentration [mM]	5	5	5
Counter ion	–	–	acetate
pH of terminating electrolyte	–	–	5.12
Driving current [μA]			
Preseparation column	300	200	200
Analytical column	40	40	30
Analysis time [min]	20–23	20–23	22–25
Sample volume [μl]	40	20	25

For analyses of uptake solution samples the isotachophoretic analyser with column coupling technique ZKI 001 (ÚRVJT VVZPJT, Spišská Nová Ves, Czechoslovakia) was employed. NH_4^+, Na^+, Mg^{2+} and Ca^{2+} cations and NO_3^-, SO_4^{2-}, NO_2^- and $H_2PO_4^-$ anions were determined in operating systems described previously (Matejovič and Polonský 1988). Chlorides were determined using complexforming properties of Cd^{2+} with halides (Boček *et al.* 1977), but originally proposed non-buffering operating system was modified to a buffering one in which EACA as counter ion and Cd^{2+} as a co-counter ion were used. Compositions of operating systems and working conditions are given in Table 1.

The relationships $c = c(t)$ for concentration of given ion in uptake solution c_i and $Q = Q(t)$ for depleted amount of given ion Q_i by plants in corresponding time t_i were approximated via least squares method using orthogonal polynomials (Ralston 1978). First derivative of function $Q = Q(t)$ gives the uptake rate. The changes of solution volume due to sampling were considered. All uptake rates and accumulative uptakes are referred to fresh mass of the roots.

RESULTS AND DISCUSSION

Isotachophoretic analysis

Isotachopherograms in Fig. 1 show analyses of uptake solution at the beginning and at the end of uptake experiment. $H_2PO_4^-$ and SO_4^{2-} anions were determined in operating system I as their determination in mentioned system was more sensitive than in operating system II in which only Cl^- anions were determined. Uptake solution samples of 1 ml volume were sufficient for three parallel determinations of NH_4^+, Na^+, Mg^{2+}, Ca^{2+}, NO_3^-, SO_4^{2-}, NO_2^-, $H_2PO_4^-$ and Cl^- ions. Relative standard deviations of determinations were in most cases less than 2 %. To obtain reproducible results in operating system III, the change of terminating electrolyte between analyses was necessary. Applied operating systems proved to be suitable for determination of above mentioned ions in concentration range 0.04 – 0.60 mM.

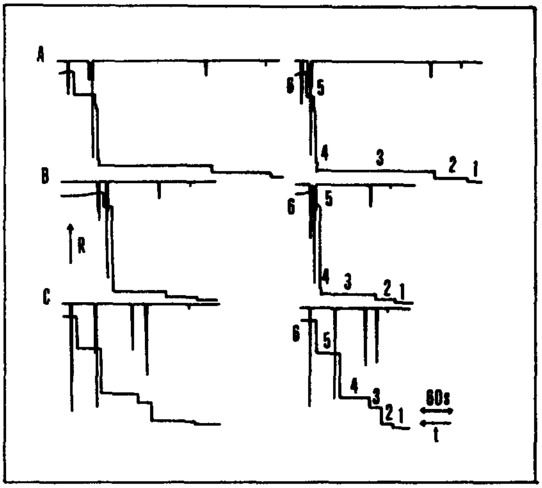

Fig. 1. Isotachopherograms of uptake solution at the beginning (left side) and at the end (right side) of the uptake experiment. A (operating system I): 1 Cl^-, 2 NO_3^-, 3 SO_4^{2-}, 4 NO_2^-, 5 $H_2PO_4^-$, 6 citrate. B (operating system II): 1 NO_3^-, 2 Cl^-, 3 SO_4^{2-}, 4 NO_2^-, 5 $H_2PO_4^-$, 6 citrate. C (operating system III): 1 K^+, 2 Na^+, 4 Mg^{2+}, 5 Ca^{2+}, 6 $TRIS^+$. R resistance, t time. Composition of operating systems and working conditions are given in Table 1.

In the isotachopherograms given in Figs. 1A and 1B appearance of NO_2^- zone is evident. Similar phenomenon has been already observed and ascribed to either the activity of nitrate-reductase or assumed to be consequence of possible damage of root tissues or contamination (Matejovič and Polonský 1988). In effort to explain this phenomenon. NO_2^- ions were not included into uptake solution. However, presented results do not allow to decide whether physiological process or artifact is in question as the NO_2^- zone has been visible only sporadically and under given conditions of analysis only near to detection limit (about 0.015 mM).

Uptake experiment

From the time courses of individual ions concentration in uptake solution in Fig. 2 rapid decreasing of NH_4^+, Cl^- concentrations, with lag periode for NO_3^- and in less extent for $H_2PO_4^-$ is evident. However, it may be noted, that these depletion curves characterize rather specifity of experiment design than physiological point of view as the concentration changes can be affected by gradual decreasing of solution volume due to sampling. At this point time courses of

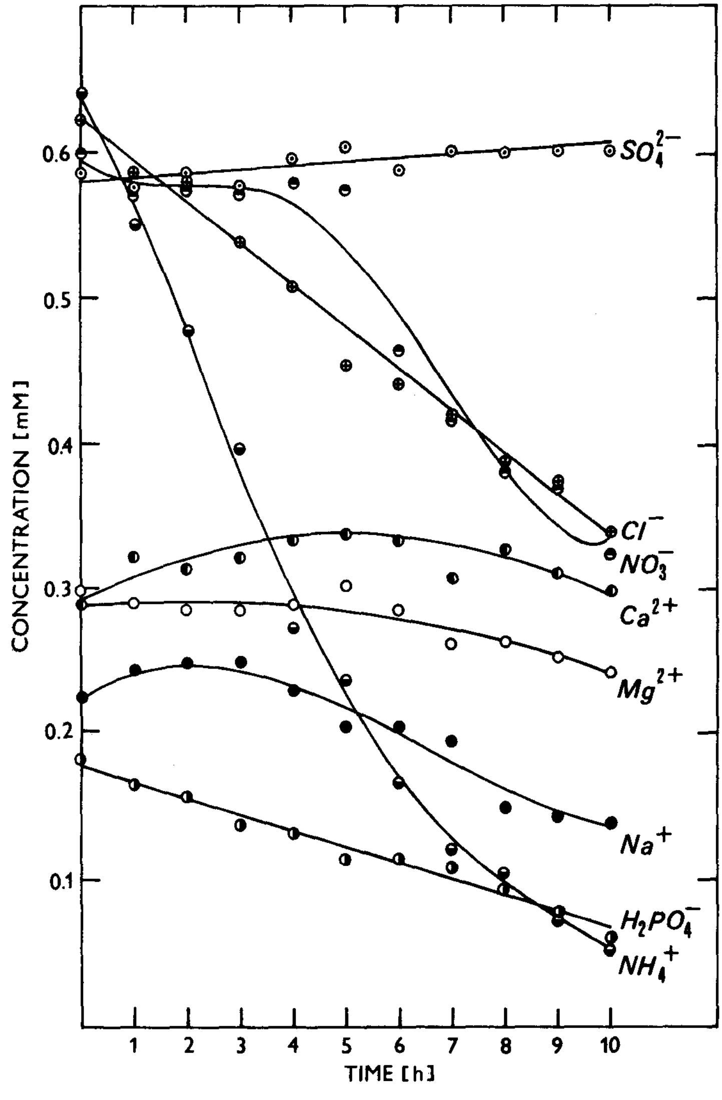

Fig. 2. Ion concentrations in uptake solution as functions of uptake time. Only one replication is shown.

accumulative uptake (Fig. 3) are more important. From these it results that NH_4^+ and NO_3^- uptakes were characterized by tendency to saturation. $H_2PO_4^-$ and Cl^- uptakes as well as SO_4^{2-} efflux by linear courses.

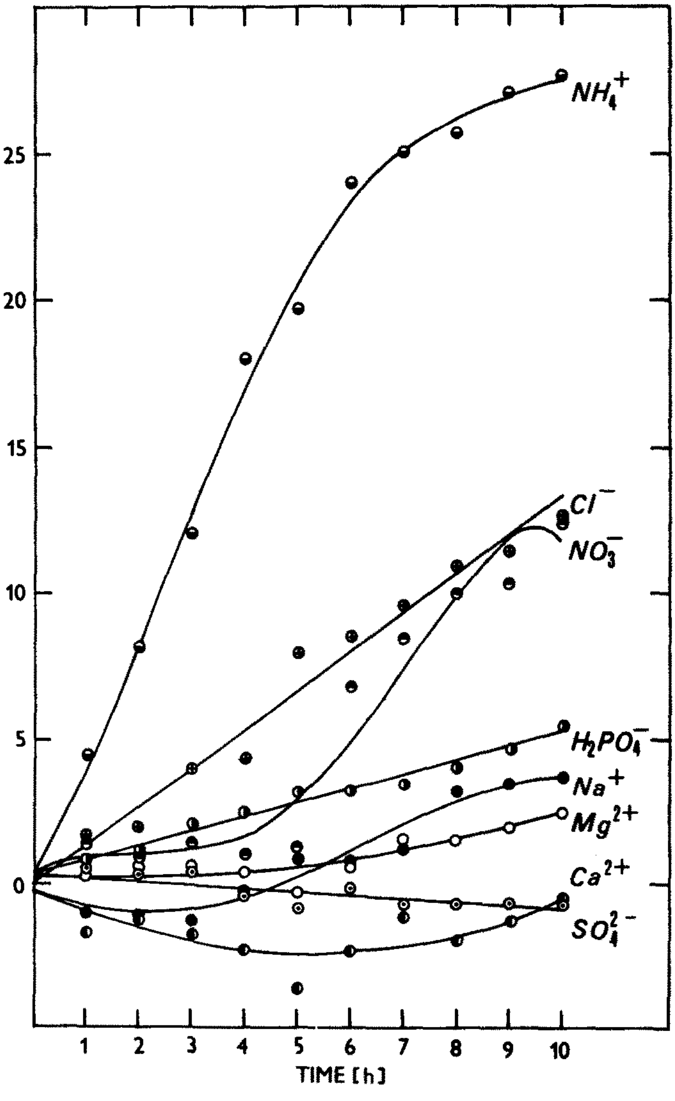

Fig. 3. Accumulative ion uptakes as functions of uptake time. Only one replication is shown.

Efflux was observed for Ca^{2+} and SO_4^{2-} ions which were exclusively dominant in growth history of seedlings and for Na^+ ions, but there were substantial differences in their courses. While SO_4^{2-} efflux increased during experiment linearly, initial Ca^{2+} and Na^+ efflux (maximum values 3.97 ± 0.27 and 0.95 ± 0.19 µmol g^{-1}, respectively) passed to influx. The magnitude of Ca^{2+}

TABLE 2

Accumulative ion uptakes at the end of experiment. Results are mean values of three replications.

Ion	Accumulative uptake [μmol g^{-1}]	
	Calculated	Observed
NH_4^+	27.91 ± 0.66	27.86 ± 0.88
Na^+	4.51 ± 0.68	4.24 ± 0.47
Mg^{2+}	2.29 ± 0.20	2.22 ± 0.30
Ca^{2+}	–1.87 ± 1.09	–2.01 ± 1.08
Cl^-	13.28 ± 0.14	12.60 ± 0.44
NO_3^-	12.93 ± 0.81	13.10 ± 0.73
SO_4^{2-}	–0.89 ± 0.03	–0.79 ± 0.11
$H_2PO_4^-$	5.31 ± 0.56	5.35 ± 0.55

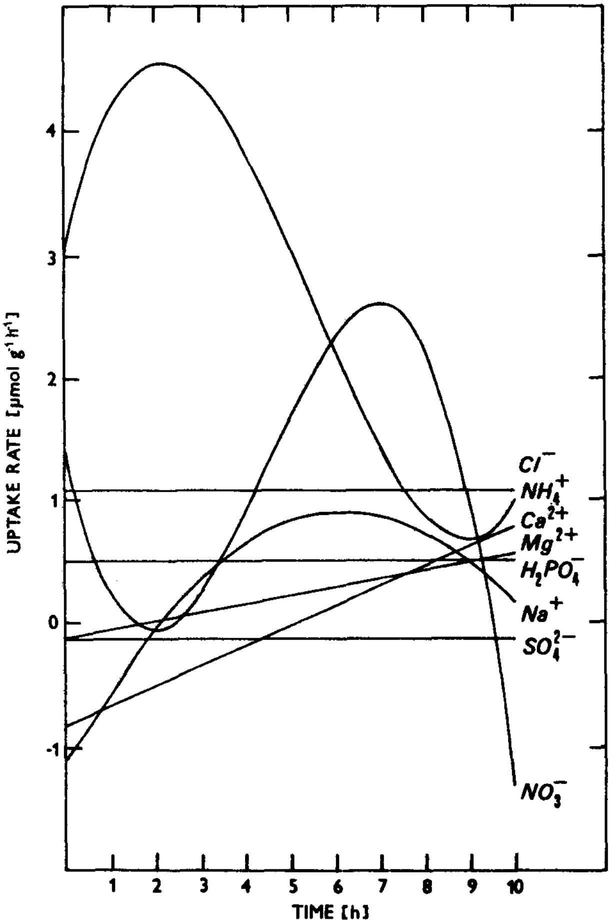

Fig. 4. Rates of ion uptake as functions of uptake time. Only one replication is shown.

efflux can be affected by stimulative effect of NH_4^+ ions (Siddiqi and Glass 1984).

Agreement between calculated and observed accumulative uptakes of ions species at the end of experiment (Table 2) indicates suitability of estimated polynomials fitting through experimental data.

Cl^- and $H_2PO_4^-$ uptake rates as well as the rate of SO_4^{2-} efflux were time independent (Fig. 4) and thereby also independent on concentration of these ions in uptake solution. Following values were obtained: 1.32 ± 0.06, 0.49 ± 0.03 and 0.11 ± 0.01 μmol g^{-1} h^{-1}. Mg^{2+} and Ca^{2+} uptake rates were increasing linearly with time and near the end of experiment they reached values 0.51 ± 0.06 and 0.60 ± 0.18 μmol g^{-1} h^{-1}.

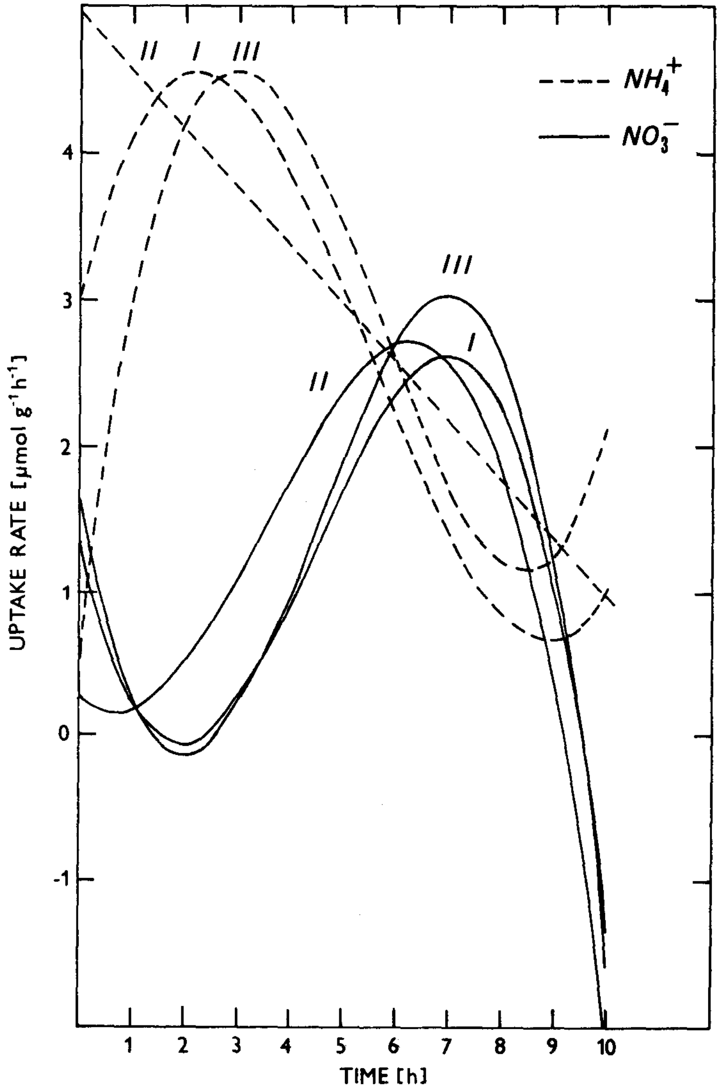

Fig. 5. NO_3^- and NH_4^+ uptake rates as functions of uptake time. All replications are shown.

Clarkson and Warner (1979) reported that in whole range of temperatures to which roots are generally exposed in nature, NH_4^+ uptake in ryegrass exceeded that of NO_3^-. Similarly in our experiment both the accumulative uptake and uptake rate of NH_4^+ ions in N-limited wheat seedlings were higher than those of NO_3^- even if their start concentrations were approximately equal. Big differences were observed in uptake rates and accumulative uptakes in favour of NH_4^+, especially, in first hours of experiment (see Figs 3 and 4) when for NO_3^- lag phase was typical. Minotti *et al.* (1969) for wheat and Morgan and Jackson (1988) for wheat, oat and barley also reported increasing of NH_4^+ uptake with increasing N-deprivation. The initial low NO_3^- uptake in comparison to that of NH_4^+ may be explained by different induction periods for NO_3^- and NH_4^+ transport mechanisms (Goyal and Huffaker 1986b) and lower NO_3^- accumulative uptake also by inhibitory effect of NH_4^+ (Bloom and Finazzo 1986, Ingemarsson *et al.* 1987) and SO_4^{2-} and Cl^- ions (Aslam *et al.* 1984) on NO_3^- uptake. The Cl^- ions were accumulated approximately in the same extent as NO_3^- (Table 2).

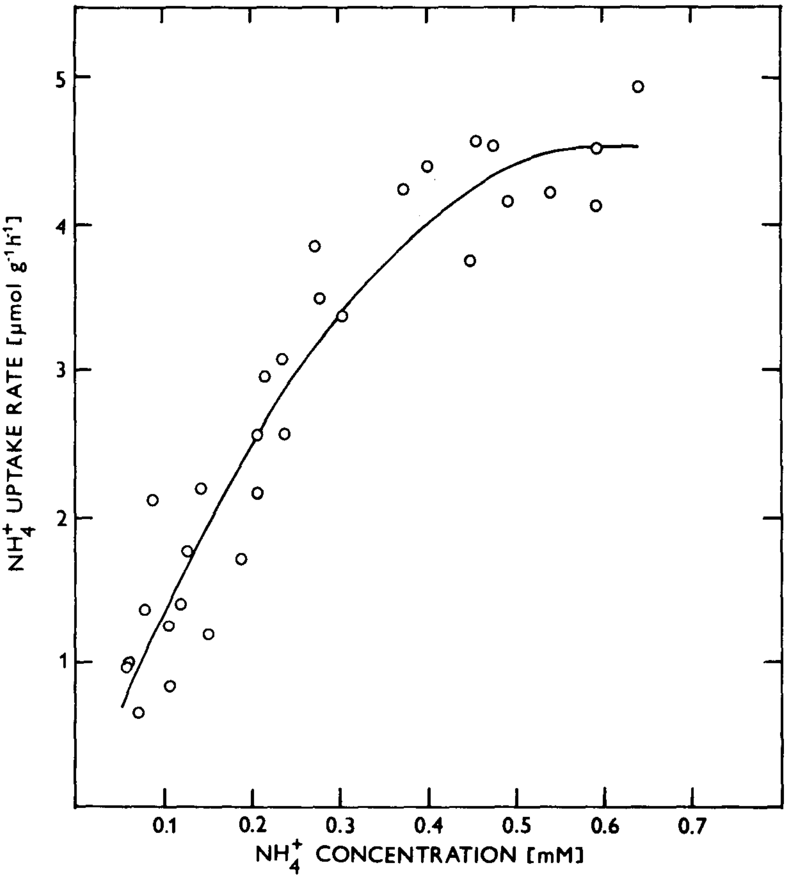

Fig. 6. NH_4^+ uptake rate as a function of NH_4^+ concentration in uptake solution. Best fitted curve through data of all replications is shown.

From the physiological point of view the intersection points of time curves of uptake rates may be important (see Fig. 4). For example, for NO_3^- and NH_4^+ ions two intersection points were typical (see Fig. 5): The first at time $t_1 = 5.84 \pm 0.10$ h and the second at $t_2 = 8.78 \pm 0.57$ h. These points are characterized by equal NO_3^- and NH_4^+ uptake rates ($v_1 = 2.53 \pm 0.20$ and $v_2 = 1.23 \pm 0.54$ µmol g^{-1} h^{-1}, respectively). Corresponding ions concentrations in external solutions were as follows: $c_{1,NH_4^+} = 0.20 \pm 0.04$ and $c_{1,NO_3^-} =$ $= 0.48 \pm 0.03$ mM (ratio $c_{1,NO_3^-}/c_{1,NH_4^+} \doteq 2.4$), and $c_{2,NH_4^+} = 0.11 \pm 0.03$ and $c_{2,NO_3^-} = 0.37 \pm 0.05$ mM (ratio $c_{2,NO_3^-}/c_{2,NH_4^+} \doteq 3.4$). These points may be considered as the limits for preference or for increasing or decreasing demand of plants for given ion species under experimental conditions given.

Maximum NH_4^+ and NO_3^- uptake rates estimated from seeking for maximum of functions uptake rate versus time (Fig. 5) were as follows: $v_{max,NH_4^+} =$ $= 4.69 \pm 0.23$ (corresponding time $t_{v_{max,NH_4^+}} = 1.73 \pm 1.55$ and concentration $c_{v_{max,NH_4^+}} = 0.52 \pm 0.12$) and $v_{max,NO_3^-} = 2.84 \pm 0.17$ µmol g^{-1} h^{-1} ($t_{v_{max,NO_3^-}} =$ $= 6.87 \pm 0.32$ h and $c_{v_{max,NO_3^-}} = 0.42 \pm 0.01$ mM). The best fitted curve of NH_4^+ uptake rate as a function of external NH_4^+ concentration through data of all replication is shown in Fig. 6. It seems to be saturable like in enzyme kinetics. *Via* linearization according to Hanes the following values of kinetic parameters were determined: $V_{max} = 7.16 \pm 1.14$ and $K_m = 0.43 \pm 0.09$ (mean of three replications) and from fitted curve $V_{max} = 9.24 \pm 0.44$ µmol g^{-1} h^{-1} and $K_m = 0.56 - 0.05$ mM, respectively. Values of V_{max} considerably differ from those of v_{max,NH_4^+}. However, both parameters must be differently interpreted. V_{max} is a limit case extrapolated to infinity concentration whereas v_{max} is really observed in given concentration range and is independent of any proposed uptake model (for review see Jensén *et al.* 1987).

Concluding remarks

Uptake of relatively large number of ions species using the same plants group can be investigated in a single depletion experiment using capillary isotachophoresis for their determination in uptake solution samples. Well defined objective together with well designed experiment may reveal more detailed picture of uptake of most macro-nutrients including possibility to assess interactions among them during uptake process. The intersection points of time courses of uptake rates may be of physiological importance. Kinetic parameters such as actually observed maximum uptake rate v_{max}, corresponding concentration $c_{v_{max}}$ and time $t_{v_{max}}$ are independent of any proposed uptake kinetics model as their values are obtained only via approximate method. We suppose that above mentioned possibilities together with another nutrient related characteristics such as, for example, relative growth rate, utilization efficiency, *etc.*, would be helpful in study of varietal differences in plant nutrition or for selection of cultivars with desirable nutrient related characteristics and for specific agro-ecological conditions.

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