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Kinetics of NH_4^+ Influx in Spruce[1]

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Influxes of $^{13}NH_4^+$ across the root plasmalemma were measured in intact seedlings of *Picea glauca* (Moench) Voss. Two kinetically distinct uptake systems for NH_4^+ were identified. In N-deprived plants, a Michaelis-Menten-type high-affinity transport system (HATS) operated in a 2.5 to 350 μM range of external NH_4^+ concentration ($[NH_4^+]_o$). The V_{max} of this HATS was 1.9 to 2.4 μmol g^{-1} h^{-1}, and the K_m was 20 to 40 μM. At $[NH_4^+]_o$ from 500 μM to 50 mM, a linear low-affinity system (LATS) was apparent. Both HATS and LATS were constitutive. A time-dependence study of NH_4^+ influx in previously N-deprived seedlings revealed a small transient increase of NH_4^+ influx after 24 h of exposure to 100 μM $[NH_4^+]_o$. This was followed by a decline of influx to a steady-state value after 4 d. In seedlings exposed to 100 μM external NO_3^- concentration for 3 d, the V_{max} for NH_4^+ uptake by HATS was increased approximately 30% compared to that found in N-deprived seedlings, whereas LATS was down-regulated. The present study defines the much higher uptake capacity for NH_4^+ than for NO_3^- in seedlings of this species.

Early studies of NH_4^+ uptake in corn (Becking, 1956; van den Honert and Hooymans, 1961), wheat (Tromp, 1962), and ryegrass (Lycklama, 1963) established that net uptake of NH_4^+ could be described using the Michaelis-Menten formalism of enzyme kinetics. The existence of such a Michaelis-Menten-type uptake system, operating at $[NH_4^+]_o \leq 1$ mM, was later confirmed in barley (Bloom and Chapin, 1981; Mäck and Tischner, 1994), corn (Vale et al., 1988), wheat (Goyal and Huffaker, 1986; Botelia et al., 1994), rice (Youngdahl et al., 1982; Wang et al., 1993b), *Lemna* (Ullrich et al., 1984), tomato (Smart and Bloom, 1988; Kosola and Bloom, 1994), *Phalaris*, and *Glyceria* (Brix et al., 1994), as well as in several algal systems (see Glass and Siddiqi, 1995, for refs.). This saturable uptake component has been termed the HATS for NH_4^+ (Wang et al., 1993b). K_m values for this system in the various species studied commonly range from approximately 10 to 170 μM (Glass and Siddiqi, 1995), but values as low as 1.6 μM have been reported (Brix et al., 1994). At higher $[NH_4^+]_o$ ($\geq$500 μM), the operation of a linear system was also observed in barley (Mäck and Tischner, 1994), corn (Vale et al., 1988), rice (Wang et al., 1993b), and *Lemna* (Ullrich et al., 1984). This linear system has been referred to as the LATS for NH_4^+ and was found to be additive to the saturable HATS component (Wang et al., 1993b). In one instance with soybean, however, a multiphasic NH_4^+-uptake system was reported to operate over the entire range of $[NH_4^+]_o$, with three distinct saturable phases but no linear component (Joseph et al., 1975).

In previous studies (Kronzucker et al., 1995a, 1995b, 1995c, 1995e), we established a marked preference for NH_4^+ over NO_3^- in white spruce (*Picea glauca* [Moench] Voss.), and we discussed its ecological significance as well as practical implications in deforestation and reforestation in boreal forests (cf. Kronzucker et al., 1995b). Yet, in conifers, a detailed kinetic characterization of NH_4^+ uptake is lacking. We are aware of only one study, which reported kinetic parameters for *net* NH_4^+ uptake in Douglas-fir (Kamminga-van Wijk and Prins, 1993). The purpose of the present study was to kinetically define NH_4^+ influx into seedling roots of a conifer species. We used nonmycorrhizal white spruce as a model system, since our laboratory has previously examined the kinetics of NO_3^- influx (Kronzucker et al., 1995d) as well as other aspects of NO_3^- and NH_4^+ fluxes and metabolism in this system (Kronzucker et al., 1995a, 1995b, 1995c, 1995e). The radiotracer ^{13}N was used to conduct direct measurements of NH_4^+ influx as a function of $[NH_4^+]_o$. Moreover, the time profile of the response of NH_4^+ influx to external NH_4^+ provision was recorded, and changes in kinetic parameters of NH_4^+ influx with previous exposure of seedling roots to external NO_3^- were examined. The present study further defines the pronounced preference for NH_4^+ over NO_3^- in spruce.

MATERIALS AND METHODS

Plant Growth Conditions

Seedlings of white spruce (*Picea glauca* [Moench] Voss., provenance 29170, from the Prince George region in British Columbia, Canada) were used. A detailed description of growth conditions was given by Kronzucker et al. (1995d). In brief, seedlings were grown in an outdoor nursery for a minimum of 3 months in a peat:perlite (3:1) mixture; a slow-release fertilizer was added during the first 2 months and no further fertilization occurred in the final month prior to transfer. Then seedlings were transferred to hydroponic culture for a period of 3 weeks in indoor growth chambers. The tanks contained one-tenth strength N-free modified Johnson's solution. The detailed solution compo-

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Abbreviations: HATS, high-affinity transport system; LATS, low-affinity transport system; $[NH_4^+]_o$, external NH_4^+ concentration; $[NO_3^-]_o$, external NO_3^- concentration.

sition was given by Kronzucker et al. (1995b, 1995c). Seedling roots maintained in hydroponic solution were nonmycorrhizal, as determined by microscopic examination. NH_4^+ was withheld completely from growth tanks, except in time-dependence experiments, in which 100 μM NH_4^+ was added in the form of $(NH_4)_2SO_4$ 1 to 7 d prior to influx measurements (see below for details). In some experiments, seedlings were exposed to 100 μM external NO_3^- [added as $Ca(NO_3)_2$] for 3 d prior to measuring NH_4^+ influx.

Solutions were checked daily for [K^+] (using a model 443 flame photometer; Instrumentation Laboratory, Lexington, MA) and for pH using a microprocessor-based pocket-size pH meter (pH Testr2 model 59000–20; Cole Parmer, Chicago, IL). $[NH_4^+]_o$ was measured according to the method of Solorzano (1969) (using a PU 8820 UV/visible spectrophotometer; Philips, Eindhoven, The Netherlands). To buffer against pH changes caused by plant uptake processes, powdered $CaCO_3$ was added to the tanks (pH was kept at 6.5 ± 0.3), and solutions were replaced every 3 d.

All seedlings were maintained in a 16-h/8-h photoperiod, at 70% RH, and at 20 ± 2°C. A photon flux of approximately 250 μmol m^{-2} s^{-1} measured at plant level (with an LI-189 light meter and LI-190SA quantum sensor; Li-Cor, Lincoln, NE) was provided by fluorescent lamps (VITA-LITE/DURO-TEST, Bergen, NJ).

Influx Measurements

The radiotracer ^{13}N (9.96 min half-life) was produced by the University of British Columbia (Vancouver, Canada) cyclotron facility (Tri-University Meson Facility). Proton irradiation of a water target was used to generate the isotope. This procedure provides $^{13}NO_3^-$ with high radiochemical purity (Kronzucker et al., 1995b). An irradiated solution of approximately 700 to 740 MBq was supplied in sealed 20-mL glass vials. Procedures for the removal of radiocontaminants and conversion of $^{13}NO_3^-$ to $^{13}NH_4^+$ were as described in detail elsewhere (Kronzucker et al., 1995a, 1995b, 1995c, 1995e).

Following the purification and chemical conversion steps, the $^{13}NH_4^+$-containing solution was added to a volume of 4 to 6 L of vigorously stirred uptake solution. The chemical composition of the uptake solution was identical with the growth solution in the hydroponic tanks (see above; Kronzucker et al., 1995b). The uptake solution was contained in a pump-operated delivery system that was designed for work with large volumes of radioactive solutions (Kronzucker et al., 1995d). Individual 500-mL plastic vessels behind lead shielding contained NH_4^+ at the desired concentrations (see "Data Analysis").

Seedlings were transferred from the hydroponic growth tanks to prewash solutions in 1-L vessels for 5 min prior to immersion of the roots of intact seedlings in the labeled uptake solutions. This protocol minimized perturbation and allowed plant roots to equilibrate to the exact solution temperature and to the solution composition used during influx. After the prewash, seedlings were transferred to the uptake vessels for 10 min. Immediately following the 10-min isotope loading, roots were dipped into nonlabeled solutions for 5 s to minimize carryover of label by the root surface to the desorption solution. Roots were then postwashed in identical but unlabeled solution for a period of 3 min to desorb $^{13}NH_4^+$ contained in the free space. The duration of these steps was based on earlier determinations of half-lives of exchange for NH_4^+ for the root surface, the Donnan free space, and the cytoplasm in efflux experiments (Kronzucker et al., 1995c, 1995e). An exposure time of 10 min to $^{13}NH_4^+$ was chosen, since the contribution of tracer efflux from the cytoplasm can be expected to be negligible during this time (Kronzucker et al., 1995d). Moreover, if labeling times ≤5 min were used, apparent influx values were obtained that were up to 1.7 times higher than rates calculated from 5- or 10-min labeling experiments (Fig. 1; Wang et al., 1993a). Whether these overestimates at short labeling times are the result of errors introduced by small amounts of cell-wall-bound tracer, which could become appreciable when multiplied by 30 or 60 to yield flux values on a per-hour basis (Siddiqi et al., 1989), or whether they are possibly due to reabsorption of tracer into the cytoplasm during the proportionately longer desorption is unclear at this time.

Following desorption, seedling roots were excised from the shoots, the roots were spun in a low-speed centrifuge for 30 s to remove surface liquid, and the fresh weights of roots and shoots were determined. The plant organs were then placed separately into 20-mL scintillation vials, and the radioactivities of roots and shoots were determined in a Packard γ-counter (Minaxi δ, Auto-γ 5000 Series), measuring the 511-kilo-electron-volt positron-electron annihilation radiation generated by recombination of ambient electrons and β^+ particles emitted from ^{13}N. NH_4^+ fluxes were calculated using the value for specific activity (^{13}N/[^{13}N + ^{14}N]) of the loading solution and the total fresh root weight of each seedling and expressed in μmol g^{-1} (fresh weight) h^{-1}.

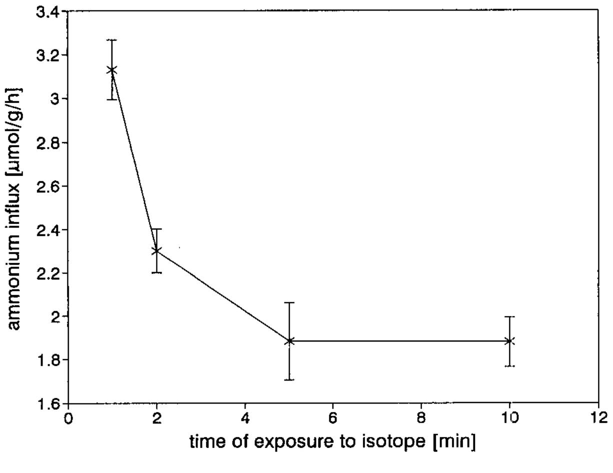

Figure 1. Apparent NH_4^+ influx into roots of white spruce as a function of time of exposure to the radiotracer $^{13}NH_4^+$ (100 μM $[NH_4^+]_o$ was added as the carrier). Seedlings were cultivated in N-free solutions for 3 weeks (N deprived) prior to flux determinations. Data are means ± SE ($n \geq 9$).

Data Analysis

All experiments were replicated at least three times. Each experimental treatment consisted of three seedling samples (minimum root mass was 3 g fresh weight per sample). Data from several experiments were pooled ($n \geq 9$) for calculations of means and SEs. These values were used for plotting representative time-dependence curves and uptake isotherms as well as for calculating V_{max} and K_m values. Four separate data-transformation methods (Cornish-Bowden and Wharton, 1988), based on the Michaelis-Menten formalism, were used to obtain V_{max} (as a measure of maximal unidirectional NH_4^+ influx) and K_m estimates for the saturable isotherm components in the present study: (a) the linear transformation according to Lineweaver-Burk: $1/v = K_m/V_{max} \times 1/[NH_4^+]_o + 1/V_{max}$; (b) the linear transformation according to Eadie-Hofstee: $v = V_{max} - K_m \times v/[NH_4^+]_o$; (c) the linear transformation according to Hanes-Wolf: $[NH_4^+]_o/v = K_m/V_{max} + [NH_4^+]_o/V_{max}$; and (d) the least-squares method by Cornish-Bowden: $K_m = (\Sigma\, v^2 \times \Sigma\, v/[NH_4^+]_o - \Sigma\, v^2/[NH_4^+]_o \times \Sigma\, v)/(\Sigma\, v^2/[NH_4^+]_o^2 \times \Sigma\, v - \Sigma\, v^2/[NH_4^+]_o \times \Sigma\, v/[NH_4^+]_o)$; $V_{max} = (\Sigma\, v^2/[NH_4^+]_o^2 \times \Sigma\, v^2 - (\Sigma\, v^2/[NH_4^+]_o)^2)/(\Sigma\, v^2/[NH_4^+]_o^2 \times \Sigma\, v - \Sigma\, v^2/[NH_4^+]_o \times \Sigma\, v/[NH_4^+]_o)$, where v is the unidirectional influx of NH_4^+ at a given value of $[NH_4^+]_o$; and Σ is "sum of."

No specific fit was preferred over another for the Michaelis-Menten phases in isotherm plots (see also Kronzucker et al., 1995d). To avoid bias, data points in isotherms were instead connected directly.

Student's *t* test was used to examine the slopes and *y* intercepts of Hanes-Wolf transformations for significant differences of regression lines for the HATS in N-deprived seedlings and in seedlings previously exposed to NO_3^- for 3 d.

RESULTS

Time Profile of NH_4^+ Influx

Measured NH_4^+ influx was substantially higher in seedlings that had been deprived of N for a period of 3 weeks than in those maintained under steady-state conditions of external NH_4^+ supply (Fig. 2). Upon first exposure of seedling roots to 100 μM $[NH_4^+]_o$, influx was 1.6 μmol g^{-1} h^{-1} and declined to a steady value of approximately 1 μmol g^{-1} h^{-1} after approximately 4 d of continued exposure to the same concentration of external NH_4^+. This steady rate of influx was maintained until 7 d of exposure, the longest exposure period in our experiments. Only at d 1 did the plants exhibit a rate of influx that was approximately 12% higher than the initial rate measured in the N-deprived state. However, this increase was transient, and influx measured at d 2 was already below the initial rate.

Concentration-Dependence of NH_4^+ Influx

In N-deprived seedlings, a single saturable NH_4^+-influx system was apparent in the concentration range from 2.5 to 350 μM $[NH_4^+]_o$ (Fig. 3). At 500 μM $[NH_4^+]_o$, influx had increased 20 to 30% beyond the saturated rate, and at 1 mM $[NH_4^+]_o$ influx was 2 to 3 times that rate. The saturable low-concentration system was constitutive and conformed to Michaelis-Menten kinetics. The kinetic parameters of V_{max} and K_m for this influx system were determined by several data-transformation methods as well as a least-squares method. The estimates for K_m ranged from 19.8 to 41 μM, whereas V_{max} estimates were between 1.86 and 2.44 μmol g^{-1} h^{-1} (Table I). The lowest estimates for both K_m and V_{max} were obtained by Lineweaver-Burk transformations, whereas Hanes-Wolf transformations consistently yielded estimates biased toward higher values. Notwithstanding a commonly lower r^2 value in regressions of Eadie-Hofstee plots compared to either Lineweaver-Burk or Hanes-Wolf plots (Table I), the Eadie-Hofstee estimates were closest to those arrived at by the least-squares method according to Cornish-Bowden and Wharton.

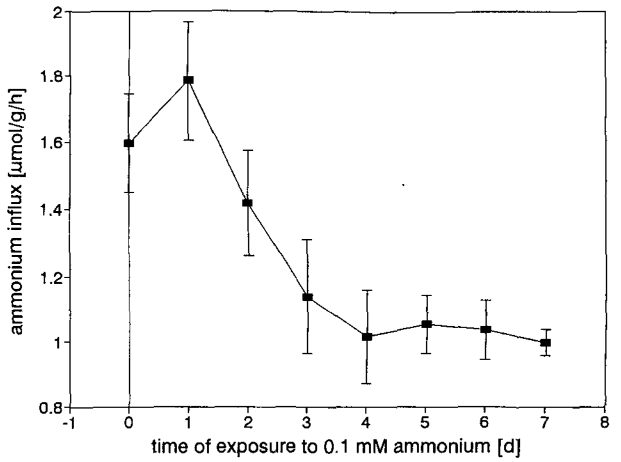

Figure 2. NH_4^+ influx into roots of white spruce as a function of exposure to external NH_4^+ for up to 7 d. Seedlings were cultivated in N-free solutions for 3 weeks and then supplied with 100 μM $[NH_4^+]_o$ for the indicated time periods and during the 10-min flux measurements. Data are means ± SE ($n \geq 9$).

At $[NH_4^+]_o \geq 350$ μM, a separate constitutive high-concentration influx system was evident. The system showed linearity up to 50 mM $[NH_4^+]_o$ (Fig. 4).

In seedlings that had been previously exposed to external NO_3^- for 3 d, influx was also resolved into two kinetically distinct systems, a Michaelis-Menten-type low-concentration system and a linear high-concentration system. However, influx in the low-concentration range (up to 500 μM $[NH_4^+]_o$) was 20 to 40% higher in NO_3^--supplied as opposed to N-deprived plants. A comparison of isotherms for plants receiving these different pretreatments is shown in Figure 5. The difference for V_{max} was highly significant ($P \leq 0.005$), as illustrated for Hanes-Wolf-transformed data (Fig. 6). By contrast, influx beyond 1 mM $[NH_4^+]_o$ was substantially lower in NO_3^--supplied than in N-deprived plants (data not shown). At 1 mM $[NH_4^+]_o$, influx, after subtraction of the respective values for V_{max} of the low-concentration systems, was 2.7 times larger in N-deprived than in NO_3^--supplied seedlings (2.56 compared to 0.96 μmol g^{-1} h^{-1}, respectively).

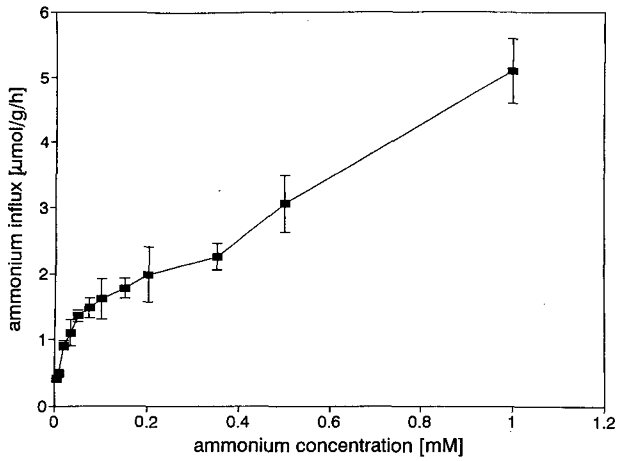

Figure 3. NH_4^+ influx into roots of N-deprived white spruce seedlings as a function of $[NH_4^+]_o$ in the low-concentration range (2.5 µM to 1 mM). Data are means ± SE ($n \geq 9$).

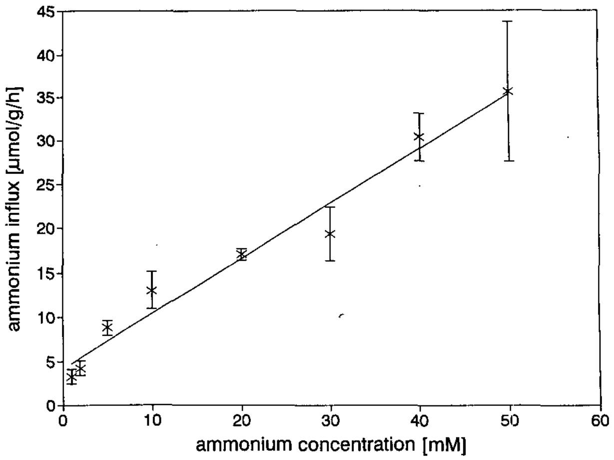

Figure 4. NH_4^+ influx into roots of N-deprived white spruce seedlings as a function of $[NH_4^+]_o$ in the high-concentration range (1–50 mM). A linear regression line has been fitted ($r^2 = 0.96$). Data are means ± SE ($n \geq 9$).

DISCUSSION

Enhanced NH_4^+ Influx in N-Deprived Plants

Rates of NH_4^+ uptake in spruce seedlings first exposed to external NH_4^+ were 1.6 times larger than steady-state rates of uptake (Fig. 2). This finding is in agreement with several other studies in higher-plant species (Ivanko and Ingversen, 1971; Lee and Rudge, 1986; Morgan and Jackson 1988a, 1988b; Henriksen et al., 1992; Jackson and Volk, 1992; Mäck and Tischner, 1994). Typically, initial fluxes are in the order of 2 to 3 times steady rates (Bowman et al., 1988; Lee et al., 1992). Similar responses have been documented for potassium, phosphate, sulfate, chloride, and bromide when plants were deprived of these ions (cf. Lee and Rudge, 1986, for refs.). With continued exposure to NH_4^+, NH_4^+ influx declined with time. Gradual repression of NH_4^+ uptake was most likely due to a buildup of negative feedback effectors such as cytoplasmic and/or vacuolar NH_4^+ and assimilation products (Ullrich et al., 1984; Lee et al., 1992; Wang et al., 1993b; Kronzucker et al., 1995c). Likewise, the initially high rate of uptake is probably due to release from negative feedback in N-deprived plants as well as initially low efflux of NH_4^+. It has been suggested that the initially high rate of uptake corresponds to the filling of the apparent free space (Mäck and Tischner, 1994). However, the pronounced temperature dependence of this flux (Mäck and Tischner, 1994) is at variance with this hypothesis, as is its disappearance in N-pretreated plants, regardless of whether N was supplied as NO_3^- (Volk and Jackson, 1992) or as NH_4^+ (Volk and Jackson, 1992; Mäck and Tischner, 1994).

Table I. *K_m and V_{max} values for the HATS for NH_4^+ in roots of white spruce as estimated by different mathematical methods*

Seedlings were cultivated hydroponically without N for 3 weeks and exposed to external NH_4^+ only during the 10-min influx period. An influx isotherm constructed from data pooled from several experiments was used as the basis for calculation of the kinetic parameters (see "Data Analysis"). –, Not applicable.

Calculation Method	K_m	V_{max}	r^2
	µM	µmol g^{-1} h^{-1}	
Lineweaver-Burk	19.79	1.86	0.93
Eadie-Hofstee	24.42	2.07	0.86
Hanes-Wolf	41.00	2.44	0.99
Cornish-Bowden	25.81	2.14	–

Steady-State NH_4^+ Influx

Following the initial maximum, a steady rate of uptake is commonly achieved within 5 to 10 h of NH_4^+ resupply in cereals and soybean (Goyal and Huffaker, 1986; Morgan and Jackson, 1988b; Jackson and Volk, 1992; Mäck and Tischner, 1994). However, in spruce, the decline in influx to approximately 1 µmol g^{-1} h^{-1} required 3 to 4 d of exposure to external NH_4^+. A slower and smaller response to changes in N supply compared to other higher-plant spe-

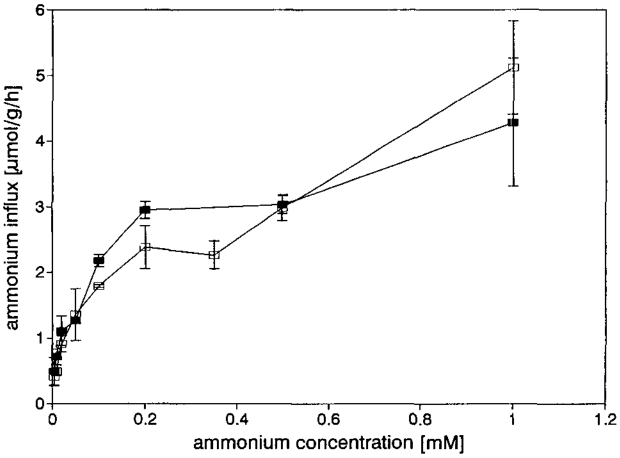

Figure 5. Overlay plot of NH_4^+ influx in the low-concentration range (2.5 µM to 1 mM) for N-deprived white spruce seedlings (☐) and seedlings that were exposed to 100 µM $[NO_3^-]_o$ for 3 d prior to NH_4^+-flux measurements (■). Data are means ± SE ($n \geq 9$).

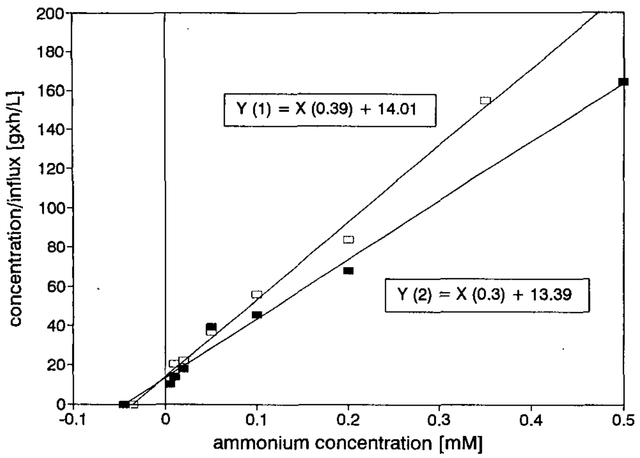

Figure 6. Hanes-Wolf transformation of the data for high-affinity NH_4^+ transport of N-deprived (1) and NO_3^--supplied (2) white spruce seedlings (Fig. 5) in the 2.5 to 500 μM range of $[NH_4^+]_o$. Regression lines and linear equations are included for the two data sets (see text). The slopes of the two lines ($1/V_{max}$) were significantly different as evaluated by Student's *t* test ($P \leq 0.005$), whereas the intercepts with the *y* axis (K_m/V_{max}) were not significantly different.

cies was also reported for the induction of NO_3^- transport in spruce (Kronzucker et al., 1995a).

Is NH_4^+ Uptake Inducible by External NH_4^+?

A transient peak of NH_4^+ influx was apparent after 24 h of exposure to external NH_4^+, before fluxes decreased to a steady level (Fig. 2). In several other studies, such a transient increase in NH_4^+ uptake was noted in the first hours of resupplying NH_4^+ (Goyal and Huffaker, 1986; Morgan and Jackson, 1988a; Jackson and Volk, 1992; Mäck and Tischner, 1994). In these systems, NH_4^+ uptake reached a new maximum after approximately 5 h. This observation has been interpreted as an "induction" by external NH_4^+ of NH_4^+ uptake, analogous to the induction of NO_3^- uptake by external NO_3^- (cf. Kronzucker et al., 1995a). However, the enhancement of NH_4^+ uptake is typically considerably smaller than the corresponding induction of NO_3^- uptake, where up to 30-fold increases have been observed in some species (Siddiqi et al., 1989; see also Kronzucker et al., 1995a, for refs.). Moreover, Jackson and Volk (1992) have demonstrated that exposure to NO_3^- also evoked the transient increase of NH_4^+ uptake. Therefore, the response is not specific for NH_4^+, in contrast to the induction of NO_3^- uptake by external NO_3^- (cf. Kronzucker et al., 1995a). Wang (1994) also showed that K^+ influx was enhanced in rice as NH_4^+ influx reached its "inductive" peak.

We consider it more plausible that plants transiently respond positively to the increased N availability, before negative feedback takes effect (see above). In this regard it is interesting that concentrations of Gln, a popular candidate for the negative feedback effector (Clarkson and Lüttge, 1991), actually increase from low to maximal levels within 5 to 10 h following NH_4^+ resupply to previously N-deprived cereals (Amâncio and Santos, 1992; Lee and Lewis, 1994). In conifers, this accumulation of Gln is more gradual, and maximal Gln levels are commonly seen after 24 h (Lavoie et al., 1992; Vézina et al., 1992). The present evidence therefore appears to argue against the NH_4^+ induction hypothesis and rather for a transiently improved N status prior to the establishment of negative-feedback control as an explanation for the transient flux increase.

Concentration Dependence of NH_4^+ Influx (Transport Systems)

Compared to the large number of higher-plant influx studies for other macronutrient ions, such as NO_3^- (Kronzucker et al., 1995d), the kinetics of NH_4^+ uptake have remained understudied (Wang et al., 1993b; Kosola and Bloom, 1994). Moreover, with only a few exceptions (Glass and Siddiqi, 1995), previous kinetic studies of NH_4^+ uptake have been largely based on depletion of NH_4^+ from solution. Data obtained by such experiments fail to distinguish between influx and efflux and may be confounded by altered N status, diurnal changes, or other factors occurring during relatively long measurement times. The application of Michaelis-Menten kinetics is then questionable (Kronzucker et al., 1995d), and derived kinetic values have descriptive rather than mechanistic meaning.

The kinetic pattern of NH_4^+ influx in white spruce was clearly resolved into two distinct phases. Influx displayed saturable kinetics at $[NH_4^+]_o \leq 500$ μM (Figs. 3 and 5). The system could be described using the Michaelis-Menten formalism (Table I), and calculated K_m and V_{max} values are within the range of reported literature values (Glass and Siddiqi, 1995). In accordance with other studies it has been termed the HATS for NH_4^+ (Wang et al., 1993b). Since it was fully expressed in N-deprived seedlings (Fig. 3), HATS was constitutive in white spruce and responded to tissue N status. This was evident as a 30% increase in V_{max} when seedlings had been exposed to 100 μM NO_3^- for 3 d prior to NH_4^+-influx determinations (Fig. 5). Statistically, this increase was highly significant (Fig. 6). Similar to the transient peak of NH_4^+ influx that was observed in our time-dependence study (see above), this flux increase is believed to result from an improved N status of the plants after NO_3^- provision compared to the N-deprived state. Negative feedback upon NH_4^+ influx, which is evident when N is resupplied in the form of NH_4^+ (Fig. 2; Kronzucker et al., 1995c), was not observed when N was administered in the form of NO_3^-. The reasons for this difference may be complicated. First, less N is acquired from NO_3^- than from NH_4^+ (Kronzucker et al., 1995a, 1995b, 1995c, 1995d). Second, amino acid profiles differ markedly depending on N source (Lavoie et al., 1992; Vézina et al., 1992). Third, the compartmentation of NO_3^- and NH_4^+ assimilation may be quite different.

At $[NH_4^+]_o \geq 500$ μM, an additional NH_4^+-influx system was evident in white spruce. Like HATS, it was constitutive (Figs. 3 and 4) but followed a linear pattern, with no indication of saturation even at 50 mM $[NH_4^+]_o$ (Fig. 4). A similar linear high-concentration system has also been documented in *Lemna* (Ullrich et al., 1984) as well as in several cereal species (Vale et al., 1988; Wang et al., 1993b; Mäck and Tischner, 1994) and has been termed the LATS for

NH_4^+. Unlike HATS, LATS in spruce was down-regulated in NO_3^--supplied as opposed to N-deprived seedlings (Fig. 5). This down-regulation was so pronounced that already at 1 mM $[NH_4^+]_o$ NH_4^+ influx was lower in seedlings that had been exposed to 100 μM $[NO_3^-]_o$ for 3 d than in N-deprived ones. This difference was visible even without subtracting the V_{max} values for the HATS (Fig. 5). This is similar to what has been reported for NO_3^- in barley and spruce (Siddiqi et al., 1990; Kronzucker et al., 1995d) but different from the results for NH_4^+ in rice reported by Wang et al. (1993b), who found that LATS activity was increased by pretreatment at 1 mM $[NH_4^+]_o$. Since cytosolic NH_4^+ or amino acids cannot be expected to increase substantially upon NO_3^- feeding (see above; Kronzucker et al., 1995a), the nature of the negative-feedback signal acting on LATS is unclear. Possibly, shoot-to-root communication plays a role but does not affect HATS, at least not at this "early" stage of N resupply.

Preference for NH_4^+ over NO_3^- in Spruce

NH_4^+ influx in white spruce was substantially higher than NO_3^- influx measured under comparable conditions (cf. Kronzucker et al., 1995a, 1995b, 1995c, 1995d). When influx was determined in N-deprived seedlings upon first exposure to either NH_4^+ or NO_3^-, V_{max} for NH_4^+ influx was approximately 20 times larger than that for NO_3^- influx (cf. Kronzucker et al., 1995d). We established previously that white spruce seedlings require 3 d of exposure at 100 μM $[NO_3^-]_o$ before an inductive influx maximum is reached (Kronzucker et al., 1995a, 1995d). For this reason, we also pretreated seedling roots at 100 μM $[NO_3^-]_o$ for 3 d in the present study to ensure full induction of NO_3^- transport. V_{max} values in these NO_3^--induced plants was approximately 3 μmol g^{-1} h^{-1} for NH_4^+ influx compared to approximately 0.7 μmol g^{-1} h^{-1} for NO_3^- influx, whereas K_m values were only marginally different (cf. Kronzucker et al., 1995d). A preference of similar magnitude for NH_4^+ over NO_3^- in conifers has been documented by net-flux studies (for refs., see Lavoie et al., 1992; Kronzucker et al., 1995b, 1995c). We have shown that the discrimination against NO_3^- at the level of transport also extends to the capacity for NO_3^- reduction and subsequent metabolism (Kronzucker et al., 1995a, 1995b). It is well established that many conifers exhibit a better growth response on NH_4^+ than on NO_3^- (McFee and Stone, 1968; Van den Driessche 1971; see also Lavoie et al., 1992, for refs.). However, silvicultural practice in many parts of North America involves outplanting of conifers like white spruce onto disturbed sites, where NO_3^- is the predominant N source and NH_4^+ is in short supply (see Kronzucker et al., 1995b, for refs.). For physiological reasons, reforestation problems with such species on disturbed sites may have to be anticipated.

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