AMMONIUM UPTAKE FROM DILUTE SOLUTIONS BY *PINUS RADIATA* SEEDLINGS

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ABSTRACT

The rate of ammonium ion uptake by seven-week-old seedlings of **Pinus radiata** from complete nutrient solutions, when described in terms of the carrier hypothesis and the analogous theory of enzyme kinetics, had a Michaelis-Menten constant (K_M) of 15.3 μM/litre with a standard error of the mean equal to 5.6 μM/litre. Although uptake rates at concentrations up to 110 μM/litre were in accord with the Michaelis-Menten equation, anomalously high rates of uptake were observed at 230 μM/litre.

INTRODUCTION

The "carrier" theory of ion uptake (Epstein and Hagen, 1952) leads directly to a simple model relating ion concentration to ion uptake. This theory attributes the absorption of inorganic salts by plant roots to ion binding carriers within the cell membranes. It is assumed that the carriers are metabolically generated compounds which facilitate ion absorption in a manner similar to the promotion of an irreversible chemical reaction by enzymes. An algebraic description of such an enzyme reaction is provided by the Michaelis-Menten equation. Substituting ion uptake rate in place of chemical reaction rate, the equation becomes:

$$v = \frac{[s]\ V_{max}}{[s] + K_M}$$

where

v = ion uptake rate
$[s]$ = concentration of ion in solution
V_{max} = a constant: the "maximum" uptake rate
K_M = Michaelis-Menten constant (the value of $[s]$ where $v = 1/2\ V_{max}$)

The parameter K_M is of particular importance since the magnitude of the solute concentration, $[s]$, in relation to K_M determines the potential for increased uptake rates: for example, if $[s]$ is considerably greater than K_M, any further increase in $[s]$ will result in only slightly increased uptake rates (Fig. 1). Further discussions of uptake mechanisms may be found in Fried and Broeshart (1967) and Epstein (1972).

The present study was undertaken to quantify the Michaelis-Menten relationship, if applicable, for the uptake of the ammonium form of nitrogen by seedlings of *Pinus radiata* D.Don.

MATERIALS AND METHODS

Seedlings

Seed of *Pinus radiata* from Nelson, New Zealand, was germinated in unwashed river sand in a greenhouse. For the following 19 days, the sand was kept moist with a complete nutrient solution containing both ammonium and nitrate. Pairs of seedlings were then removed from the sand and mounted in 1.8-litre jars containing one-tenth strength Long Ashton ammonium-type nutrient solution (Hewitt, 1969) modified to include micro nutrients and additional phosphorus. The solutions were continually aerated and renewed weekly. A more detailed account of the materials and the experiments is given by Flewelling (1977).

Ammonium Uptake (1)

Seven weeks after germination 30 pairs of vigorous seedlings were selected and transferred to thirty 0.91-litre jars containing the modified Long Ashton solution in which the ammonium sulphate concentration was adjusted so that the ammonium concentration was 31.4 μM/litre. These jars were placed in a controlled environment room with constant lighting (130 microEinsteins m^{-2} sec^{-1}, 400-700 nm), temperature (22.5° ± 1.5°C) and relative humidity (60%). An equilibration period of 1.25 hours followed; 10-ml samples of solution were collected from each of the jars which were then topped off with distilled water to maintain constant volumes. After two hours each jar was re-sampled so that a calibration rate of uptake could be determined for each pair of seedlings. The 30 pairs of seedlings were then transferred to another set of 30 jars, each containing one of five different solutions (6 replicates per solution) identical to the modified Long Ashton solution except that ammonium level varied from 16 to 253 μM/litre. Solution samples were collected from each jar .75 hours after the transfer, and again 1.4 to 3.0 hours later (the experimental uptake period). The jars with the higher ammonium concentrations were allowed the longer uptake periods.

Ammonium ion concentrations were determined on a Technicon Auto-analyzer II using the phenol hypochlorite reaction. Uptake rates were to be computed for both the calibration period and the experimental uptake period. Depletion experiments can provide direct estimates of the average uptake rate over a particular time period, but because uptake rates are related to concentration, and thus change during the course of the experiment, the uptake rate at a particular solute concentration is difficult to determine. Accordingly a time dependent form of the Michaelis-Menten equation was fitted to the time-concentration data for each pair of seedlings to obtain estimates of V_{max}, and also of K_M if intermediate samples had been taken. To obtain the estimated uptake rate V_{max} and K_M were subsequently entered in the Michaelis-Menten equation (Equation 1), together with the mean concentration for the treatment. The time (t) dependent form of the Michaelis-Menten equation for uptake from a closed container, derived by integrating Equation 1 over time, is:

$$t_2 - t_1 = \left[\frac{K_M}{V_{max}} \ln\left(\frac{S_2}{S_1}\right) + \frac{S_2 - S_1}{V_{max}}\right] [\text{solution volume}] \quad \text{(2)}$$

Ammonium Uptake (2)

The same thirty pairs of seedlings were used. There was, however, no calibration period, and during the six hour experiment the solutions were sampled every two hours.

Only two initial concentrations of ammonium were used: 7.2 and 114 μM/litre. These concentrations were chosen to minimise the expected variance of the estimate of K_M. The optimisation process used in selecting these concentrations involved a mathematical simulation of this experiment which incorporated estimates of K_M, the process variance and the measurement variance from the previous experiment.

The procedure for determining the ammonium uptake rate was identical to that used previously except that the additional determinations of concentration during the experiment provided additional data points from each pair of seedlings to be used in fitting Equation 2. The fitting was accomplished by minimising the sums of error squared in the concentration estimates.

Concentration Gradient

Four pairs of seedlings were placed in jars of nutrient solution with ammonium concentrations of 7 μM/litre; uptake rates were monitored as before. At the end of seven hours, root sap was extracted from the seedlings by placing the excised roots of each seedling in a pressure chamber, applying pressure and collecting the expressed sap (Riekerk, 1977) which was subsequently analysed for ammonium concentration. The experiment was performed under greenhouse conditions at a temperature of approximately 30°C.

RESULTS AND DISCUSSION

(1): A linear relationship was found between uptake rates in the experimental period and calibration period. This relationship was used to estimate the uptake rate each pair of seedlings would have had if the seedlings all had the same uptake rate in the calibration period. The adjusted experimental uptake rates are plotted in Fig. 1. The curve in this figure is a Michaelis-Menten curve arbitrarily chosen to approximate most of the data.

(2): Results, by treatment, are presented in Table 1. An analysis of covariance on ammonium uptake rate, with the dry weight of the shoots as the covariate, was used to adjust the treatment means. These adjusted mean uptake rates are also presented in Table 1. The estimate of K_M and standard error of the mean, based on the ammonium concentrations and the adjusted mean uptake rates, is:

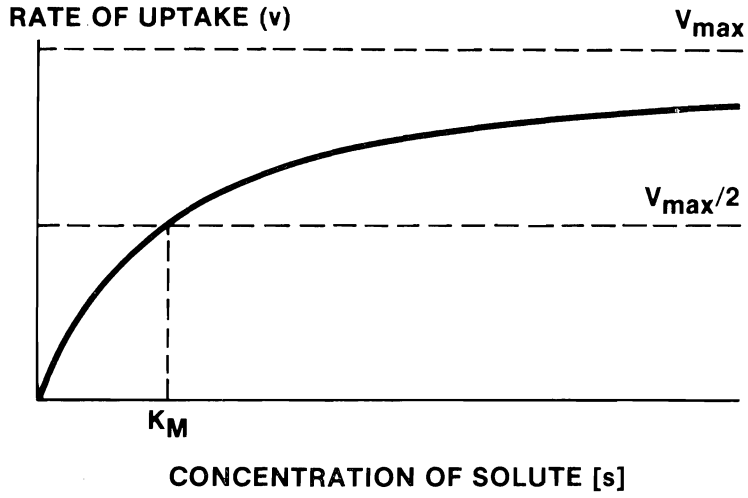
$$K_M = 15.3 \pm 5.6 \ \mu\text{M/litre} \quad (3)$$

TABLE 1—Mean results used in estimating K_M

Treatment	NH_4	rate Mean Uptake	of shoots Dry Weight	Adjusted Uptake rate Mean	S.E. of Mean of Adjusted
	(μM/litre)	(μM/hr)	(g)	(μM/hr)	(μM/hr)
Low	7.82	.60	.54	.63	.11
High	105.4	1.66	.58	1.63	.11

Gradient

The ammonium concentration for the nutrient solution was 7 μM/litre and for the root sap 407 μM/litre. The mechanism of ammonium uptake is an active process, as indicated by the high concentration gradient from the nutrient solution to the root



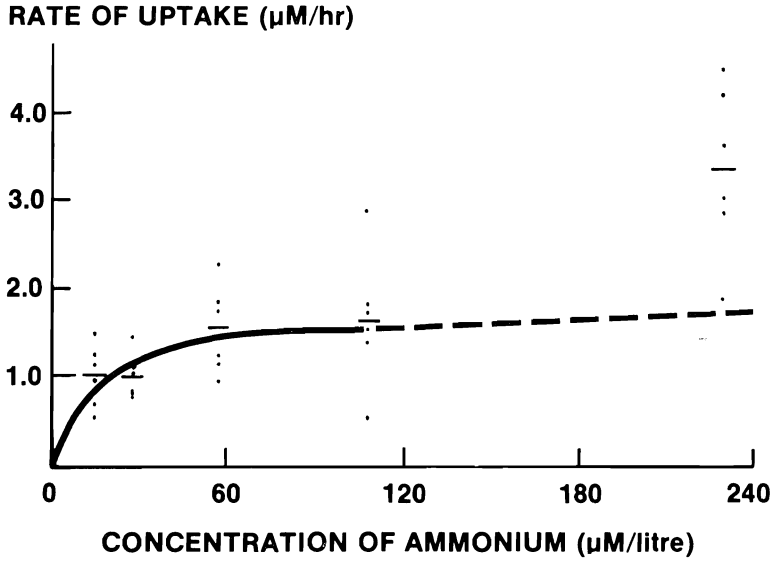


FIG. 1 (upper)—Michaelis-Menton uptake curve.
(lower)—Ammonium uptake rates adjusted by analysis of covariance versus concentration of ammonium in the first experiment (. data point; — treatment mean).

sap. While this is in agreement with the carrier hypothesis for uptake, some untested mechanism could also be responsible for the ammonium uptake.

The relationship between uptake rate and concentration of ammonium in the range of zero to 110 μM/litre is adequately described by the Michaelis-Menten equation. Anomalously high rates of uptake were observed at a concentration of 230 μM/litre. This could indicate a secondary uptake phase, but the literature indicates that any secondary phase probably starts at much higher concentrations (Epstein, 1972; Fried *et al.* 1965). The high variance in the data precludes any firm conclusion on this point.

The estimated value of K_M for ammonium uptake for *Pinus radiata* can serve as a useful guide in other situations, although it should be used with caution. This is necessary because the value of K_M may be affected by temperature, anions present and seasonal factors (Lycklama, 1963), and age (Edwards and Barber, 1976). Mycorrhizal fungi also influence uptake (Lamb and Richards, 1971) but their effect upon K_M has not been studied, while pH probably does not affect K_M (Becking, 1956).

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