MICHAELIS-MENTEN KINETICS: CALCULATION AND USE IN NUTRIENT UPTAKE MODELS

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(Received for publication 1 July 1994; revision 10 April 1995)

ABSTRACT

Uptake kinetic parameters for forest species are required in nutrient uptake models in order to accurately predict nutrient uptake by root systems. Several techniques have been used to calculate the kinetic parameters from depletion experiments. The objectives of this study were (1) to investigate four methods (Observed, Polynomial, Bhat, and Claassen and Barber) for determining Michaelis-Menten kinetics for potassium uptake by slash pine (*Pinus elliottii* Engelm. var. *elliottii*) seedlings, and (2) to determine the implications of using the values derived by the four methods on predicted potassium uptake using a nutrient uptake model. The four methods resulted in similar estimates for the kinetic parameters I_{max} and K_m; however, partial depletion curves gave unreasonable estimates of I_{max} and K_m using the observed and polynomial methods. Curve-fitting programs were useful in smoothing the data in order to calculate the kinetic parameters. Simulations of potassium uptake were also similar between each method except for the kinetic parameters from the partial depletion curves, suggesting that depletion curves should approach some low concentration in order to properly calculate I_{max} and K_m parameters.

Keywords: Michaelis-Menten Kinetics; nutrient depletion curves; uptake models; roots; *Pinus elliottii*.

INTRODUCTION

Mechanistic models of nutrient uptake describe the processes of nutrient transport and supply in the soil and ion absorption at the root surface (Barber 1995). Ion absorption at the root surface has been described mathematically by using one of several boundary conditions: a zero sink term where the concentration of the nutrient always remains zero at the soil-root interface (Hoffland *et al.* 1990); a constant influx where uptake is proportional to the ion concentration at the root surface using α, the root absorption coefficient (Nye & Tinker 1977); or an influx which is a function of the ion concentration at the root surface (Epstein & Hagan 1952). The latter approach, known as the Michaelis-Menten Kinetics, has been used frequently in nutrient uptake models and is described (Barber 1995) as:

$$I_n = \frac{I_{max} * (C_l - C_{min})}{K_m + (C_l - C_{min})} \quad (1)$$

where I_n is the ion influx (pmol/cm^2/s), I_{max} is the maximum ion influx (pmol/cm^2/s), K_m is the Michaelis constant (μmol/cm^3) and represents the solution concentration at one-half I_{max}, C_l is the ion concentration in solution (μmol/cm^3), and C_{min} is the ion concentration (μmol/cm^3) at which I_n is zero or no net influx.

Michaelis-Menten kinetic parameters have been determined on excised roots in solution cultures (Shock & Williams 1984), intact root systems growing in hydroponic cultures using the depletion method developed by Claassen & Barber (1974), or intact root systems growing in solutions where nutrient concentrations are kept constant during the growing period (Wild *et al.* 1979; Mullins & Edwards 1989). The latter method, however, has received some criticism in that this procedure may not adequately represent soil solution conditions in the rhizosphere; hence, the depletion method may be more representative of conditions in the field. Although few kinetic parameters have been determined for commercial forest tree species, most have been determined by the depletion method (Beck 1979; Van Rees *et al.* 1990; Payn 1991; Gillespie & Pope 1990; Kelly & Barber 1991; Kelly *et al.* 1992). Depletion data have been analysed by researchers from both agronomic and forestry fields by a variety of methods; however, these methods have not been compared nor has their resulting use in nutrient uptake models been discussed.

The objectives, therefore, of this study were to (1) compare four methods of calculating the Michaelis-Menten kinetic parameters for potassium uptake by *Pinus elliottii* seedlings, and (2) discuss the implications of the four methods for potassium uptake as predicted by the Barber-Cushman uptake model (Oates & Barber 1987).

MATERIAL AND METHODS

Depletion Experiments

One-year-old *P. elliottii* seedlings excavated from a tree nursery were grown in an aerated hydroponic solution culture in the greenhouse. Nutrient solutions (pH 4.5) consisted of 90 μM ammonium-nitrogen, 77 μM potassium, 65 μM calcium, 20 μM nitrate-nitrogen and phosphate-phosphorus, 10 μM magnesium and sulphate-sulphur, 0.24 μM borate-boron, 0.20 μM iron, 0.02 μM manganese and zinc, and 0.005 μM molybdate-molybdenum and copper. Solutions were changed every 4 days during the 6-week growth period. Forty-eight hours prior to each depletion experiment, the seedlings were placed in a nutrient solution lacking potassium.

Three experiments were conducted where five seedlings were placed in a pot containing an aerated nutrient solution. Marriotte flasks filled with distilled water were connected to each pot in order to maintain a constant solution volume. Solutions were sampled (5 ml) hourly during a 10-hour period (Expt 1 and 3) and periodically over a 33-hour period (Expt 2) to determine the rates of potassium depletion from solution (Claassen & Barber 1974). Depletion studies were replicated twice in each experiment with initial solution concentrations, pot volumes, and root lengths as listed in Table 1. The differences in initial potassium contents and degree of potassium depletion were chosen to test the four methods.

Analysis of Depletion Data

Potassium solution concentration *v.* time data was analysed by four different methods.

TABLE 1–Experimental parameters for the depletion curves.

Expt	Rep.	Initial concentration (μM)	Pot volume (ℓ)	Root surface area (cm^2)
1	1	14	1.2	617
	2	14	1.2	527
2	1	43	2.0	1706
	2	42	2.2	1839
3	1	46	1.4	467
	2	46	1.4	559

Observed data method

Slopes (absolute value) were calculated between each of the sampling times using the raw data from the depletion curves. The slopes and potassium concentrations at the midpoint of the calculated slopes were used in the Lineweaver-Burk plot to calculate the kinetic parameters. The Lineweaver-Burk plot transforms the Michaelis-Menten curve to a straight line as follows (Lineweaver & Burk 1934):

$$\frac{1}{I_n} = \frac{1}{I_{max}} + \frac{K_m}{I_{max}} \frac{1}{C_l} \quad (2)$$

The slopes (I_n) and concentrations (C_l) were substituted into Eqn 2 and linear regression analysis was used to determine I_{max} and K_m from the intercept and slope of this relationship.

3° polynomial method

Beck had previously used a 3° polynomial curve to calculate kinetic parameters. The observed data in this study were fitted to a 3° polynomial by a least squares procedure (Cricket Graph) in order to smooth the depletion curve. Since concentrations from individual sampling times can vary (i.e., increase or decrease), smoothing the data ensures that slopes along the curve are continually decreasing with time. The 3° polynomial equation was then differentiated to determine the slopes at the individual sampling times. The slopes and solution concentrations were used in the Lineweaver-Burk plot to determine I_{max} and K_m as described previously.

Bhat method

The observed data were fitted with a non-linear least squares program (SAS Institute 1982) to the equation developed by Bhat (1983):

$$C_l = a + \frac{b + ct}{1 + dt + et^2} \quad (3)$$

where t is time and a, b, c, d, and e are constants. The fitted curve was differentiated (Eqn 2; Bhat 1983) to calculate the slopes for use in the Lineweaver-Burk plot as described previously.

Claassen & Barber method (C & B)

The observed data were smoothed by a cubic spline function and fitted by a least squares procedure to the rate equation of Claassen & Barber (1974):

$$\frac{\partial C_l}{\partial t} = \frac{-L}{v}\left[\frac{I_{max} * C_l}{K_m + C_l}\right] - E \quad (4)$$

where L is root length (cm), v is solution volume (L), and E is nutrient efflux from the root (pmol/cm^2/s). The least squares procedure calculated the I_{max} and K_m values directly.

Model Simulations

The Michaelis-Menten kinetic parameters derived by the four methods were used in the nutrient uptake model developed by Barber & Cushman (1981) to determine the implications of using each of the four methods to estimate potassium uptake. The other model parameters for the potassium uptake simulations were obtained from the study by Van Rees *et al.* (1990) and Kelly *et al.* (1992). Although the study by Kelly *et al.* (1992) was conducted on *Pinus taeda* L., the model parameters are still valid for investigating the effect of different Michaelis-Menten parameters on potassium uptake for this study. An uptake period of 10 days was used in all simulations.

Statistics

Values of I_{max} and K_m and potassium uptake predictions from the uptake model were compared among the four methods by analysis of variance and means tested by LSDs at p=0.05 (SAS Institute 1982).

RESULTS AND DISCUSSION

Depletion Experiments

Potassium in solution was depleted or nearly depleted for Expt 1 and 2; however, potassium was depleted to about half of its initial content in the pots for Expt 3 (Fig. 1). The curve fitting by the 3° polynomial and Bhat equations was quite satisfactory with coefficients of determination >0.98. Problems did occur in Expt 1, however, where both curve-fitting programs resulted in negative concentrations at the end of the depletion experiment due to the low measured potassium concentrations. Hence, slopes were not calculated at these negative concentrations and these data points were removed from the analysis. The curves from the Bhat method for Expt 1 and 2 did not decrease for the first two sampling times. Hence, slopes were zero and these points had to be removed from the analysis as well. Therefore, curve-fitting procedures should be visually checked to determine how well the curves fit the data.

Values of I_{max} using the four methods for Expt 1 and 2 were not significantly different from each other (p=0.05) and varied from 1.12 to 1.51 pmol/cm^2/s (Table 2). The observed and 3° polynomial methods in Expt 3, however, had I_{max} values 7 to 11 times higher than the Bhat and C & B methods (Table 2), but these methods were not significantly different from one other (p=0.05). The trend in K_m was similar to that of I_{max} and K_m values in Expt 3 for the observed data and 3° polynomial methods were also considerably larger than for the Bhat and C & B methods. Values of K_m, however, were not significantly different between the four methods (p=0.05) for any of the experiments.

Due to the nature of the 3° polynomial function, differentiation of the equations produced slopes that first increased with time and then decreased with time. Thus the polynomial

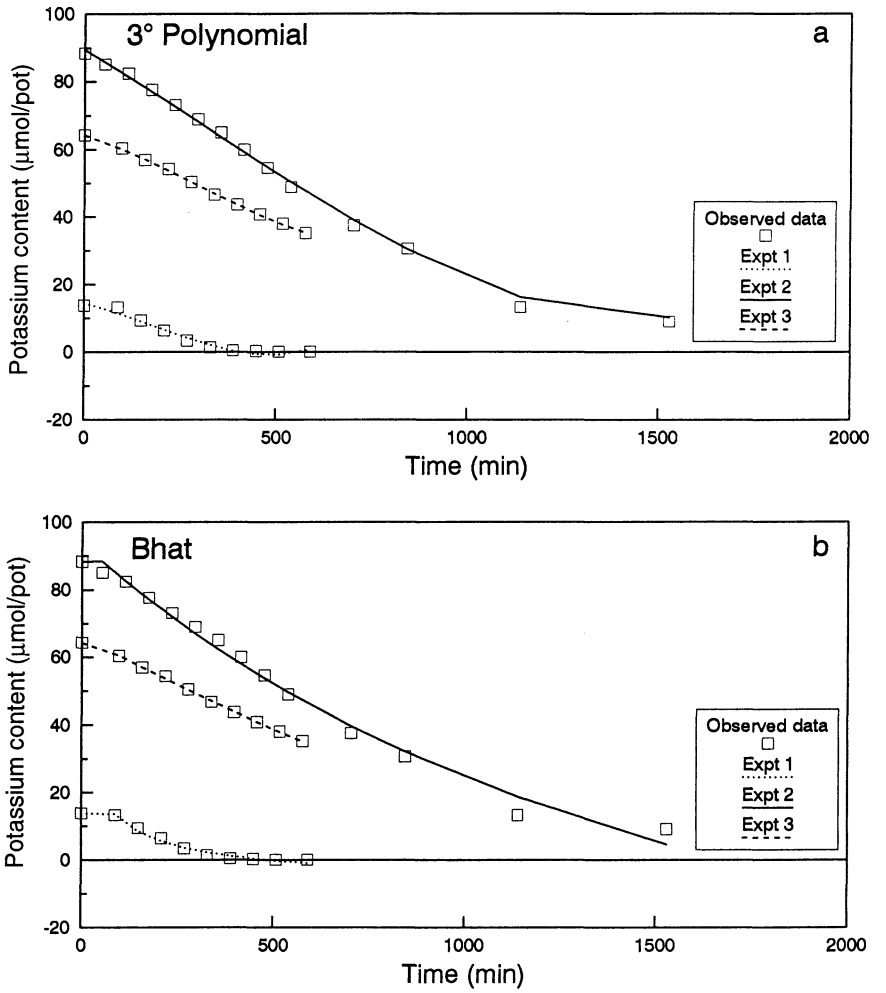

FIG. 1–Potassium depletion curves for one replication of each experiment fitted (a) by the 3° polynomial method and (b) by the Bhat method.

TABLE 2–Michaelis-Menten kinetic parameters determined by the four different methods. Values are means ± standard deviation.

Method	Expt 1		Expt 2		Expt 3	
	I_{max} (pmol/cm²/s)	K_m (µM)	I_{max} (pmol/cm²/s)	K_m (µM)	I_{max} (pmol/cm²/s)	K_m (µM)
Observed data	1.25±0.57	2.09±1.43	1.15±0.23	7.30±0.85	25.0±20.7	374±354
3° polynomial	1.34±0.23	1.13±0.85	1.12±0.11	9.05±5.21	21.1±3.8	281±104
Bhat	1.33±0.20	1.57±0.60	1.24±0.05	14.08±8.40	3.06±0.31	14.18±11.44
C & B	1.51±0.08	0.76±0.35	1.38±0.20	6.28±1.49	2.16±0.05	2.05*

* K_m for one replication was negative

function made it more difficult to determine when K_m was one-half of I_{max} owing to the cyclic nature of the slopes. Hence, the slopes at the beginning of the depletion curve were removed in order to determine I_{max} and K_m from the regression of the Lineweaver-Burk plot.

Although the various methods provided similar estimates of the I_{max} and K_m values for all experiments, Expt 3 did result in kinetic values that were an order of magnitude higher for the observed and 3° polynomial data than the other two methods. These I_{max} and K_m values for potassium are higher than those reported in the literature (Eissenstat & Van Rees 1994) and would appear to be unreasonable, especially since K_m values were higher than the initial solution concentrations. In addition, K_m for one of the replications in Expt 3 was negative using the C & B method. Depletion curves for other forest species that have been only partially depleted have also been reported, and in one study no K_m value could be calculated (Kelly & Barber 1991). Difficulties in determining kinetic parameters from partially depleted solutions suggest that nutrient concentrations should be depleted to a sufficiently low concentration in order to analyse the data by any of the four methods. Decreasing the concentration in solution to some low level is also advantageous as that concentration has been used to determine the C_{min} value which represents the concentration where net nutrient influx is 0 (Bhat 1981). Concentrations approaching zero, however, can result in negative concentrations with some curve-fitting programs.

Depletion curves are not always smooth declining curves and any scatter in the data will be reflected in the Lineweaver-Burk plots to calculate the kinetic parameters. As an example, the slopes calculated from the observed data method for Expt 2 resulted in a wide scatter of the data points in the Lineweaver-Burk plot with the resulting regression producing an I_{max} value of 0.92 pmol/cm^2/s and K_m of 8.1 μM ($r^2 = 0.37$). Fitting the curve by the Bhat method and then using the fitted concentrations in the observed data approach resulted in I_{max} and K_m values of 1.48 pmol/cm^2/s and 31.3 μM ($r^2 = 0.96$), respectively. Hence, depletion data should first be curve fitted so that each successive sampling point is less than the previous data point, thereby resulting in improved regression analysis for Lineweaver-Burk plots.

Nutrient Uptake Simulations

Predicted uptake of potassium using the data from either Van Rees *et al.* (1990) or Kelly *et al.* (1992) was not significantly different between the four methods for Expt 1 and 2 (Table 3). Simulations using kinetic data from Expt 3, however, were different from each other with the observed data and 3° polynomial methods generally predicting higher potassium uptake than the Bhat and C & B methods (Table 3). Sensitivity analysis by Kelly *et al.* (1992) found that changing I_{max} greatly influenced the predicted uptake of potassium, and the order of magnitude difference in I_{max} and K_m from Expt 3 resulted in significant differences in uptake between the four methods. Van Rees *et al.* (1990), however, had shown that predicted uptake of potassium was not sensitive to a 0.25 to 2 times change in the I_{max} and K_m values in a sensitivity analysis, yet Expt 3 showed significant differences in uptake between the four methods. Although I_{max} and K_m values varied by an order of magnitude between the four methods, the higher potassium uptake from the observed and 3° methods may not be valid since the kinetic values (particularly K_m) appeared to be unrealistic.

To date there is still a paucity of kinetic parameters for forest tree species with all measurements being conducted in hydroponic cultures with young seedlings. Information

TABLE 3–Predicted potassium uptake with the Barber-Cushman model using the Michaelis-Menten parameters determined by the four methods. Other model uptake parameters were obtained from Van Rees *et al.* (1990) and Kelly *et al.* (1992).

Method	Van Rees model data*			Kelly model data†		
	Expt 1 (mmol)	Expt 2 (mmol)	Expt 3 (mmol)	Expt 1 (mmol)	Expt 2 (mmol)	Expt 3 (mmol)
Observed data	1.51a‡	1.80a	6.56a	1.54a	1.84a	12.22ab
3° polynomial	1.19a	1.66a	7.23a	1.21a	1.70a	15.98a
Bhat	1.43a	1.93a	4.31ab	1.45a	2.00a	4.65bc
C & B	1.15a	2.12a	2.69a	1.16a	2.17a	2.73c

* D_e = 6.8E-7 cm^2/s, b = 6.26, C_{li} = 0.203 $\mu mol/cm^3$, v_0 = 3.79E-6 cm/s, r_1 = 2.59 cm, r_0 = 0.046 cm, L_0 = 41.3 cm, k = 4.55E-4 cm/s, C_{min} = 0.001 $\mu mol/cm^3$, t = 8640000 s

† D_e = 3.29E-6 cm^2/s, b = 10.55, C_{li} = 0.27 $\mu mol/cm^3$, v_0 = 5.66E-7 cm/s, r_1 = 2.0 cm, r_0 = 0.035 cm, L_0 = 285 cm, k = 1.62E-4 cm/s, C_{min} = 0.001 $\mu mol/cm^3$, t = 8640000 s

‡ Means followed by same letter within a column are not significantly different (p=0.05)

is still needed, however, for the lifespan of the tree species as well as for kinetic parameters measured *in situ* for trees growing in forest soils if researchers are to understand the processes of nutrient movement and uptake in forest ecosystems.

CONCLUSIONS

Generally, all methods resulted in similar values in the kinetic parameters when the concentration of potassium in solution was almost completely depleted. When potassium in solution was only partially depleted (i.e., less than half of initial amount), kinetic parameters were an order of magnitude higher for the observed data and 3° polynomial methods than the Bhat and C & B methods, suggesting that depletion experiments be carried out for a sufficient period of time in order to deplete the nutrient in solution. Thus, accurate estimates of the kinetic parameters from the various methods are also dependent on reasonably good depletion curves.

The four methods also varied in ease of determining the kinetic values. Calculating slopes from the observed data was the easiest approach and did not require any specialised programs. The depletion data, however, did not always decrease in a systematic fashion and it is recommended that a curve-fitting program be used to smooth the data before calculating the slopes for use in Lineweaver-Burk plots. The Barber Eqn was time consuming because the initial estimates of the parameters had to be quite close to the actual values in order for the program to converge.

REFERENCES

BARBER, S.A. 1995: "Soil Nutrient Bioavailability: A Mechanistic Approach". 2nd edition. John Wiley & Sons, New York.

BARBER, S.A.; CUSHMAN, J.H. 1981: Nitrogen uptake model for agronomic crops. Pp.382–409 *in* Iskander, K.K. (Ed.) "Modeling Waste Water Renovation—Land Treatment". Wiley-Interscience, New York.

BECK, R.H. 1979: Potassium and ammonium uptake kinetics of slash pine and corn. Ph.D.Dissertation, Purdue University, Abstract No.7926349.

BHAT, K.K.S. 1981: Nutrient inflows into apple roots. I. ^{32}P-orthophosphate uptake from solution by M.9 rootstocks and Worcester Pearman seedlings. *Plant Cell Environment 4*: 297–302.

——1983: Nutrient inflows into apple roots. *Plant Soil 71*: 371–80.

CLAASSEN, N.; BARBER, S.A. 1974: A method for characterizing the relation between nutrient concentration and flux into roots of intact plants. *Plant Physiology 54*: 564–8.

EISSENSTAT, D.M.; VAN REES, K.C.J. 1994: The growth and function of pine roots. Pp.76–91 *in* Gholz, H.L.; Linder, S.; McMurtrie, R.E. (Ed.) "Environmental Constraints on the Structure and Productivity of Pine Forest Ecosystems: A Comparative Analysis". *Ecological Bulletin No.43*.

EPSTEIN, E.; HAGAN, C.E. 1952: A kinetic study of the absorption of alkali cations by barley roots. *Plant Physiology 27*: 457–74.

GILLESPIE, A.R.; POPE, P.E. 1990: Rhizosphere acidification increases phosphorus recovery of black locust: II. Model predictions and measured recovery. *Soil Science Society of America Journal 54*: 538–41.

HOFFLAND, E.; BLOEMHOF, H.S.; LEFFELAAR, P.A.; FINDENEGG, G.R.; NELEMANS, J.A. 1990: Simulation of nutrient uptake by a growing root system considering increasing root density and inter-root competition. Pp.9–15 *in* van Beusichem, M.L. (Ed.) "Plant Nutrition—Physiology and Applications". Kluwer Academic Publishers.

KELLY, J.M.; BARBER, S.A. 1991: Magnesium uptake kinetics in loblolly pine seedlings. *Plant Soil 134*: 227–32.

KELLY, J.M.; BARBER, S.A.; EDWARDS, G.S. 1992: Modeling magnesium, phosphorus and potassium uptake by loblolly pine seedlings using a Barber-Cushman approach. *Plant Soil 139*: 209–18.

LINEWEAVER, H.; BURK, D. 1934: The determination of enzyme dissociation constants. *Journal of the American Chemical Society 56*: 658.

MULLINS, G.L.; EDWARDS, J.H. 1989: A comparison of two methods for measuring potassium influx kinetics by intact corn seedlings. *Journal of Plant Nutrition 12*: 485–96.

NYE, P.H.; TINKER, P.B. 1977: "Solute Movement in the Soil-root Systems". University of California Press, Berkeley.

OATES, K.; BARBER, S.A. 1987: Nutrient uptake: A microcomputer program to predict nutrient absorption from soil by roots. *Journal of Agronomy Educ. 16*: 65–8.

PAYN, T.W. 1991: The effects of magnesium fertiliser and grass on the nutrition and growth of *P. radiata* planted on pumice soils in the central North Island of New Zealand. Ph.D. Dissertation, University of Canterbury, Christchurch.

SAS INSTITUTE 1982: "SAS User's Guide: Statistics". SAS Institute, Cary, NC.

SHOCK, C.C.; WILLIAMS, W.A. 1984: Sulfate uptake kinetics of three annual range species. *Agronomy Journal 76*: 35–40.

VAN REES, K.C.J.; COMERFORD, N.B.; McFEE, W.W. 1990: Modeling potassium uptake by slash pine seedlings from low-potassium supplying soils. *Soil Science Society of America Journal 54*: 1413–21.

WILD, A.; WOODHOUSE, P.J.; HOPPER, J.J. 1979: A comparison between the uptake of potassium by plants from solutions of constant potassium concentration and during depletion. *Journal of Experimental Botany 30*: 697–704.