

A Method for Characterizing the Relation between Nutrient Concentration and Flux into Roots of Intact Plants¹

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ABSTRACT

A method based on the rate of depletion of a nutrient from solution was developed to characterize nutrient flux of plant roots. Nutrient concentration of the solution was measured at a series of time intervals to describe the complete depletion curve. An integrated rate equation, based on a Michaelis-Menten model, was developed and fit to the data of the depletion curve using a least-square procedure. The equation contained values for V_{max} , the maximum rate of influx; Km , the Michaelis constant; and E , efflux, which were used to describe the relation between solution concentration and net influx rate. Models other than Michaelis-Menten could also be used. The method uses only one plant or group of plants to obtain data over a range of nutrient concentrations, is adapted particularly to the low concentration range, and measures the concentration below which net influx ceases. With this method the plant is in steady state absorption prior to the experiment and continues at this steady state until near the end of the experiment.

A procedure was also developed to measure uptake rate at constant concentration by adding nutrients to the pot at a constant rate that matched net influx into the root. This method also provides a means of measuring diurnal fluctuations in net influx rates.

Knowledge of the relation between the rate of ion absorption by plant roots and the concentration of the ion external to the root is important for doing plant nutrition studies, for investigating ion absorption mechanisms, and for evaluating mechanisms supplying nutrients to roots growing in soil (6, 10). Hence, a convenient procedure that mathematically describes the kinetics of ion absorption would be useful.

Ion absorption rate as related to ion concentration in the external solution has usually been measured using short term absorption by excised roots of isotopically labeled ions from solution (4) and long term absorption by intact plants from solutions maintained approximately at constant concentration (1). In the first, only ions with a convenient isotope for labeling can be studied and in both, separate measurements of uptake rate must be made for each of a graded series of ion con-

centrations in order to establish the relationship between rate of absorption and concentration of the ion in solution. Where rate of uptake by intact plants is measured over time periods of hours or days, it is often difficult to maintain low concentrations at a constant level.

This paper reports a method for characterizing the rate of ion absorption by the root over a range of ion concentration in solution using only one plant or group of plants. The observed data are evaluated mathematically using Michaelis-Menten kinetics so that values are obtained for V_{max} , the maximum influx and Km , the Michaelis constant, which is the concentration where influx is one-half of V_{max} , E , efflux, and c_0 , the ion concentration in solution below which net influx of the ion ceases. The method measures uptake by determining depletion of the ion from solution similar to that used by Olsen (11).

THEORY

When roots are in a solution containing salts, ions may move both into and out of the root. The terms we use to describe this ion movement are: influx, movement of ions from the external solution into the root; I , the rate of influx per unit length of root; efflux, the movement of ions out of the root into the external solution; E , the rate of efflux per unit length of root, and I_n , the net rate of influx per unit root length which is equal to $I - E$. The values of I , E , and I_n in this paper are in terms of root length; however, root surface area, or root weight could be used if desired.

When plants are grown in a solution of a constant volume, v , the decrease in ion content of the solution measures the net amount of the respective ion absorbed. The amount of ion in the nutrient solution, Q , is given by equation 1 where c is the ion concentration in solution.

$$Q = cv \quad (1)$$

A plot of Q versus time, t , gives a curve showing the depletion of the ion from solution resulting from plant absorption and is called the depletion curve. The net influx per pot at any point on this curve is given by $-dQ/dt$. The slope of the curve can be estimated by expressing Q as a function of t and calculating the derivative. A relation $Q = f(t)$ can be obtained by fitting the data using a parabolic spline function (3) or a cubic spline function (13). Both functions consist of a number of segments called splines fixed by the experimenter which describe the relation by a series of parabolic or cubic functions. In both, the function and the first derivative are continuous through all segments. In the cubic spline, the second derivative is also continuous. In the cubic spline program we used, the junctions of

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the segments, called knots, were shifted by the computer until the smallest residual sum of squares was obtained. In the parabolic spline program we used, the location of the knots was fixed by the experimenter. Both of these programs allows one to obtain a mathematical function that will fit the data points closely.

A different approach is to develop a rate equation that relates $-dQ/dt$ to the flux parameters and then integrate it to obtain $Q = f(t)$. This integrated rate equation can be fit to the experimental data of the depletion curve to estimate the parameters that characterize the ion-uptake kinetics. This procedure is used in chemistry to determine reaction order (2). The degree to which the proposed rate equation describes ion uptake kinetics will be indicated by how well the experimental data follow the curve predicted from its integration.

In this paper influx is expressed per unit of root length, L , hence:

$$I_n = -\frac{1}{L} \frac{dQ}{dt} \quad (2)$$

Also by definition:

$$I_n = I - E \quad (3)$$

To use the rate equation procedure we need to know the relation between I and c (which is Q/v) and E and c to substitute into equation 3. There is general agreement that for many ions, influx over the low concentration range (for K < 1 mM) follows Michaelis-Menten kinetics so this model was selected for I , hence:

$$I = \frac{V_{\max} c}{K_m + c} \quad (4)$$

Little is known about the relation of E and c . Michaelis-Menten kinetics assumes no efflux occurs by the carrier mechanism. We assume for K that E is a passive process along the electrochemical gradient (5, 12). Tentatively we have used a constant value for E and the reason for this will be discussed later.

By using a constant value for E , combining equations 2, 3, and 4, substituting Q/v for c as shown in equation 1, and rearranging, we obtain the expression describing the rate equation:

$$\frac{dQ}{dt} = -L \left[\frac{V_{\max} Q/v}{K_m + Q/v} - E \right] \quad (5)$$

Equation 5 can also be expressed in terms of c where v remains constant.

$$\frac{dc}{dt} = -\frac{L}{v} \left[\frac{V_{\max} c}{K_m + c} - E \right] \quad (6)$$

Integration of equation 5 gives a function that when fit to the experimental data by a least-squares procedure describes the depletion curve. Equation 5 was integrated numerically using a computer because the function obtained by analytical integration is not explicit in Q .

When depletion curves are conducted over time periods long enough to have appreciable root growth, the increase in L with t can be accounted for by using the expression:

$$L = L_0 e^{kt} \quad (7)$$

where L_0 is the root length at the start of the experiment and k , measured separately, is a constant describing the rate of L increase with t .

The described procedure requires the use of a computer to

integrate the rate equation and fit it to the data. Approximate values of I_n can be obtained by visual estimation of the slope of the tangent to the depletion curve at a series of points over the length of the curve.

MATERIALS AND METHODS

The proposed method was evaluated by measuring the net K influx of corn (*Zea mays* L.) seedlings. Corn kernels were placed in tap water-moistened paper towels for germination. After 5 days, 10 seedlings were transplanted into 2.8-liter plastic containers containing aerated nutrient solution of the following composition; 3.5 mM Ca, 1 mM Mg, 6 mM N, 0.5 mM K, 0.5 mM P, 1.5 mM S, 46 μ M B, 9 μ M Mn, 0.8 μ M Zn, 0.3 μ M Cu, 0.8 μ M Mo, and 75 μ M Fe as Fe-DTPA (diethylenetriaminepentaacetic acid). The solution pH was maintained between 5.5 and 6.5. The solution K was determined daily and renewed as required. The solutions were changed after the first 4 days and then at 2-day intervals. The plants were grown in a controlled climate chamber with a 16-hr day temperature of 28 C with 20,000 lux of light and an 8-hr night temperature of 22 C.

Determination of Depletion Curves. The seedlings were grown as described until 18 days old, and the solution was changed. On the morning of the 19th day the K in solution was adjusted to 190 μ M and the pH to 5.5. The solution in the pot was then sampled continuously using a peristaltic metering pump which removed 0.65 ml/min. The sampled solution was fed to tubes in a fraction collector set to change at 10-min intervals. The K removed in sampling was <0.2% of that absorbed by the plant, hence it could be ignored in calculation. Deionized water was added to the pot to maintain a constant solution volume. Samples were collected until 40 min after the K concentration in solution had reached a minimum. Several pots were run simultaneously using a multichannel metering pump for sampling.

With the procedure used, I_n values were determined with actively growing plants where K was being absorbed and translocated at a steady state rate of V_{\max} prior to and during the experiment until 2 hr before completion. Consequently, the K level of the roots would be similar to that termed "high salt" and would change very little during the experiment.

Potassium Uptake at Constant Concentration. Uptake rates at constant solution K concentration were measured by adding K to the pot at a constant rate that was less than the rate the roots absorbed K from the pot at V_{\max} . Solution was fed into the pot at 0.65 ml/min and solution for measuring the K level in the pot was removed at 0.65 ml/min. In this experiment two solutions with K concentrations of 1 mM and 2 mM were used. When the 1 mM K was added, the K level in the pot adjusted with time until a constant K concentration was reached where $I_n L$ for K equaled the rate of K addition. This gave one point for the relation between c and I_n . After the K concentration in the pot reached a constant level, the concentration of the solution being added was changed to 2 mM K and the K level in the pot increased to a new c where $I_n L$ for K equaled the new rate of addition. This gave a second point. In the same way, additional values were obtained in a separate experiment. Variations in solution volume within the pot were kept within 3% by adding water to replace that which had transpired.

This procedure can also be used to measure diurnal changes of K flux into the root by adding K at a constant rate over a period of 1 or 2 days and following the change in K concentration with time.

Analytical Measurements. At the completion of the experiments, the plants were harvested and root length was determined by the line-intercept procedure (8). K in the solution

Table I. Observed Values for K Content

The observed values are shown for the K content of a solution from which 10 18-day-old corn plants were absorbing K and the predicted values using parabolic spline and cubic spline functions and a Michaelis-Menten rate equation.

Time <i>min</i>	K Content of Solution			
	Observed	Predicted by		
		Parabolic spline	Cubic spline	Michaelis-Menten
	<i>μmoles/pot</i>			
0	537.1	541.5	536.0	533.3
10	479.8	477.6	482.0	476.9
20	422.5	417.8	419.0	421.5
30	354.5	362.6	357.2	367.3
40	307.9	311.2	306.7	314.5
50	265.0	263.8	265.9	263.5
60	223.4	218.3	221.6	214.9
70	172.6	174.5	173.0	169.2
80	128.9	132.3	128.2	127.4
90	87.4	92.7	91.9	90.6
100	73.0	61.3	63.6	60.2
110	27.2	38.9	42.3	37.2
120	36.5	25.1	26.9	21.8
130	7.2	18.7	16.6	12.7
140	21.5	13.9	10.3	8.0
150	7.2	10.0	7.1	5.6
160	8.6	7.0	6.0	4.5
170	3.6	4.7	7.0	4.1
180	2.9	2.6	5.6	3.7
190	2.9	2.7	3.3	3.7
200	3.6	3.7	-1.8	3.7
R ²		0.9987	0.9980	0.9983

samples collected was determined by flame-emission spectroscopy. Standard curves for several concentration ranges were used so that the relative precision of K measurement would be similar over the range of K levels found in the solution.

Fitting Curves to the Experimental Data. Curves were fit to the experimental data by using computer programs for fitting the parabolic and cubic spline (3, 13). The equations for the splines were differentiated to obtain I_n at various levels of c . The rate equation, equation 5, was integrated numerically and the experimental data were fit by coupling the numerical integration procedure to a nonlinear regression program. The output gave predicted values of Q and values of V_{max} , Km , and E . The values of V_{max} , Km , and E were substituted into the expanded form of equation 4:

$$I_n = \frac{V_{max} c}{Km + c} - E \quad (8)$$

to generate a curve of I_n versus c . The maximum I_n is $V_{max} - E$ and Km is equal to c where I equals one-half V_{max} and not where I_n equals one-half $V_{max} - E$.

RESULTS

The depletion curve procedure for measuring I_n versus c was evaluated by using corn seedlings growing in solution culture. The K contents in solution after various values of t are given in Table I. A parabolic spline, a cubic spline, and the integrated rate equation were fit to the data and the predicted values of solution K contents for each value of t are given in

Table I. The observed data and the curve fitted by the integrated rate equation procedure based on Michaelis-Menten kinetics are shown in Figure 1. The R^2 values, indicating the degree of fit were similar for all procedures. Hence, one method was as good as the other for describing the depletion curve.

Values for I_n were calculated for each value of t using the derivatives of the equations for the curves fitted by the parabolic and cubic spline procedures. Fitting the integrated rate equation to the depletion curve gave values of V_{max} of 4 pmoles $cm^{-1} sec^{-1}$, Km of 27.9 μM and E of 0.18 pmoles $cm^{-1} sec^{-1}$. These were used with equation 8 to give a curve of I_n versus c . This curve and points calculated using the parabolic spline and cubic spline curve-fitting procedures are shown in Figure 2.

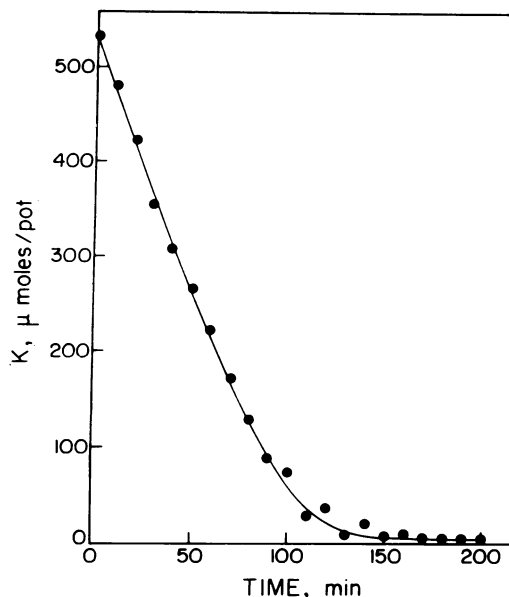


FIG. 1. Depletion of K from 2.8 liters of solution by 10 18-day-old corn plants with time. The points are observed values. The curve was fit using an expression based on Michaelis-Menten kinetics.

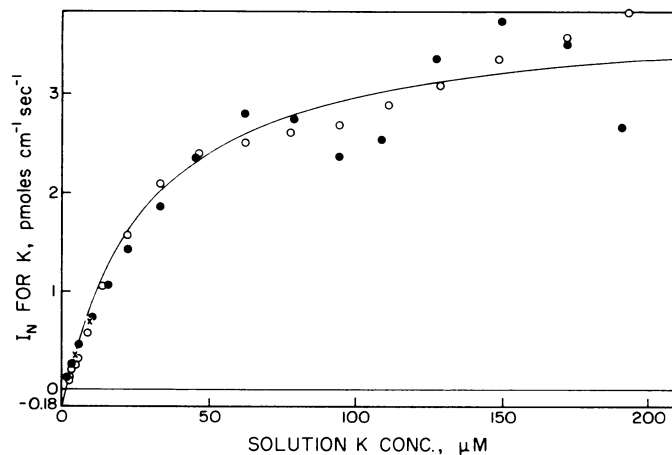


FIG. 2. Mean I_n of 18-day-old corn plants as a function of solution K concentration. The curve was calculated using Michaelis-Menten kinetics, the solid points were calculated using a cubic spline function, the circles parabolic spline, and the x's are values measured from uptake at constant concentration.

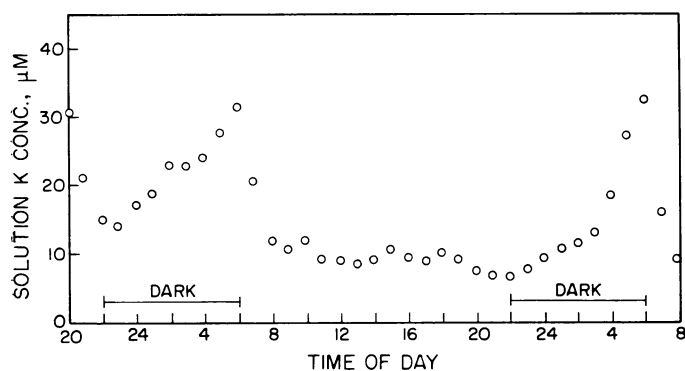


FIG. 3. Relation of solution K concentration with time where K was added at 54 $\mu\text{moles/hr}$ and 10 20-day-old corn plants were growing in 3 liters of nutrient solution.

The integrated rate equation procedure produces a smooth curve whereas the values obtained from curves fitted by the parabolic and cubic spline procedures produce I_n values that oscillate at the higher concentrations of solution K. The erratic predicted values at higher c are in the region where the depletion curve is almost linear. These methods of curve fitting follow deviations as a result of experimental error and small changes in the almost linear curve cause large changes in the derivatives. At levels below 50 μM K all three procedures gave similar results for I_n versus c .

Measuring Net K Influx at Constant Concentration. Net K influx was measured also where K was fed continuously into the nutrient solution at a constant rate of less than that removed by the plants absorbing at V_{max} . The solution K concentration adjusted with time until a constant c was reached where $I_n L$ equaled the rate of addition. Two values obtained in this way are shown as "x" in Figure 2. They agree with values obtained from the depletion curve. In a separate experiment, 17 values were measured over a range of 4 to 150 μM K. These values obtained at constant concentration were compared with values obtained from a depletion curve. The R^2 for correlation was 0.92 and the regression equation $I_n(\text{depletion}) = -0.162 + 0.871 I_n(\text{constant})$. There was agreement between the two methods.

The procedure for measuring K uptake from solutions at constant K concentration can also be used to measure diurnal fluctuations in rates of K absorption by roots. When K was fed into the solution in the pot at a constant rate and the K concentration in the solution monitored continuously, the data in Figure 3 were obtained. When the solution K increased, uptake rate was less than the rate of addition. When the K level decreased uptake was faster than the rate of addition. During the dark period, net K influx decreased, when the lights came on, it rapidly increased. The period between clock hours of 11 and 18 had a uniform influx rate and hence would be a desirable time to conduct depletion experiments.

It is sometimes difficult to predict the rate of K addition needed to measure I_n at constant solution concentration, particularly for values approaching V_{max} . A slightly different method can be used where K is added at a rate faster than the plant roots remove at $I_n = V_{\text{max}}$. A larger solution volume is added with the K and a similar larger volume removed continuously for sampling. A level of K in solution will be reached where net K influx plus that removed for sampling equals that added. For example, a 6 mM K solution was added at 1 ml/min. The K level of the solution in the pot being sampled at 1 ml/min reached a constant level of 4 mM. The net influx into the plant at 4 mM K was equal to the difference in the amount

of K added and the amount removed or 2 μmoles of K/min. Because the K solution concentration was much higher than with the depletion curve, the amount removed was an appreciable amount of that added.

The value of E was obtained by using a least-squares fit of the equation to the data. Little information on K efflux is available in the literature. Mengel (7) reported K efflux from barley (*Hordeum vulgare*) roots. We estimate E of 0.17 to 1.6 $\mu\text{moles g (fresh wt)}^{-1} \text{hr}^{-1}$ from his data. These are similar to the value we obtained of 0.55 $\mu\text{mole g}^{-1} \text{hr}^{-1}$.

DISCUSSION

The three curve-fitting procedures all fit curves closely to the data. The parabolic and cubic spline methods are very flexible and will fit almost any data. Because the integrated rate equation is not so flexible, the degree of fit indicates how well the model chosen describes net K influx. We chose the Michaelis-Menten kinetics model since it has been used widely. It gave a close fit, hence was a useful model for describing K flux of corn roots.

Metabolic uptake of K is generally considered to be by an active uptake mechanism that operates against an electrochemical gradient (5, 12) and hence efflux, which is in the opposite direction would be passive. We have used a constant value for E in our model. If E is passive it would not vary much with c for the conditions of our experiment for the following reasons: according to Nobel (9, equation 3-15, p 110) the passive flux of an ion across a membrane is proportional to the difference between external and internal concentrations, where the electrical potential is constant. For K, the internal concentration is large (approximately 100 mM) compared to the external concentration (in our experiments <0.2 mM), thus, changes in external concentration would have little effect on the difference between internal and external concentrations and hence E . Internal concentrations in our experiments would remain relatively constant where I is near V_{max} . During the last 2 hr when $I < V_{\text{max}}$, and assuming constant K translocation, we calculated that internal K concentration would decrease less than 15% in 2 hr. Using a constant E was a first approximation, other expressions could be used if a lack of fit of the data were observed.

The depletion curve procedure has the advantage that one plant or set of plants can be used in mathematically describing the curve of I_n versus c , hence, experimental error is reduced as compared to the use of separate pots for each level of c . With the Michaelis-Menten model, a continuous function is generated which is described by V_{max} , K_m , and E . This simplifies the reporting of the effect of environmental factors such as temperature, plant age, and light intensity on nutrient flux characteristics as well as allowing the evaluation of the effect of these variable factors on each of the constants. The procedure also allows for estimation of the concentration below which I_n of an ion ceases, a parameter about which little information exists.

The procedure is conceptually correct because the negative slope of the absorption curve is, without assumptions, the rate of net influx. Assumptions are made in the development of the rate equation since an expression must be known beforehand that describes the relation between net influx rate and solution concentration. In this paper, K influx was assumed to follow Michaelis-Menten kinetics and a constant efflux component was used. The model described K uptake by corn satisfactorily. In other research, satisfactory results were obtained using the same model to describe P uptake by corn of various ages. Results for N uptake by corn were not satisfactorily described by this model indicating that a different model may be necessary.

Because most active ion influx in the low concentration range may be described by Michaelis-Menten kinetics, lack of fit to the proposed model may result solely from E not remaining constant as c varies. Passive flux has been proposed in some cases to be through exchange or facilitative diffusion (9).

To get a reliable estimate of the parameters needed for calculating the relation of I_n versus c it is necessary to have (a) a good estimate of the near linear initial part of the depletion curve, (b) a good estimate of the near horizontal part of the depletion curve which gives the minimum concentration, and (c) a reasonable number of observations joining these segments. The experiment should have a plant to solution ratio such that it can be conducted over a 3 to 5 hr period and diurnal variations in I_n are minimized. We have found the following conditions resulted in suitable depletion curves: (a) an initial ion concentration 6 to 10 times Km ; (b) an initial rate of decrease in concentration of between 2 and 4 times the concentration of Km/hr , (c) sampling at 10-min intervals; (d) termination of the experiment 1 hr after the horizontal part of the depletion curve is reached.

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