

Studies of Root Function in *Zea mays*

IV. EFFECTS OF APPLIED PRESSURE ON THE HYDRAULIC CONDUCTIVITY AND VOLUME FLOW THROUGH THE EXCISED ROOT

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ABSTRACT

The volume flux, J_v , and the osmotic driving force, $\sigma\Delta\pi$, across excised root systems of *Zea mays* were measured as a function of ΔP , the hydrostatic pressure difference applied across the root, using the pressure jump method previously described (Miller DM 1980 Can J Bot 58: 351–360). J_v varied from 5.3% to 142% of its value in intact transpiring plants as a result of the application of pressure differences from -2.4 to 2.4 bar. The calculated hydraulic conductivity was 5.9×10^{-4} cubic centimeters per second per bar per gram root and was independent of pressure. A model of root function similar to those appearing in the literature failed to provide quantitative accord with the data. A proposed model, which includes the effect of volume flux on the distribution of solutes in the symplasm, predicts accurately J_v , $\Delta\pi$, and the xylem solute concentration as a function of ΔP .

Several authors (2–5, 10) have devised models of root functions in an attempt to predict J_v , the rate of exudation from excised root systems as a function of ΔP , the hydrostatic pressure difference between the outside surface of the root, P_o , and the xylem vessels, P_x , ($\Delta P = P_o - P_x$). These models were tested against data obtained from the earlier literature as reviewed by Fiscus (3) together with more recent observations obtained by him (4). In all this work, the applied pressures were positive, since P_x was held at atmospheric pressure, while J_v was increased by raising P_o .

The present work extends these data in two ways. First, not only were measurements of J_v made as before by increasing P_o , the pressure outside the root, but in addition, by raising the xylem pressure P_x above P_o , measurements of low values of J_v at negative ΔP were obtained. Second, with each measurement of J_v , a corresponding measurement of the osmotic driving force within the root was obtained. These observations were made using an apparatus previously described (6), which allows the recording against time of either J_v under a fixed pressure gradient ΔP , or the root pressure, P_x , when the flow rate, J_v , is zero. In addition, the apparatus has the ability to determine how great an increase in P_x is just necessary to bring about an instantaneous termination of flow. The procedure was to establish a pressure drop across the root system and then to record the volume flow rate, J_v , once it had become steady. This should obey the well known expression:

$$J_v = L_p(\Delta P - \sigma\Delta\pi) = L_p(P_o - P_x - \sigma\Delta\pi) \quad (1)$$

where L_p is the hydraulic conductivity of the root system, σ is the reflection coefficient of transported ions, and $\Delta\pi$ is the sum

of all osmotic pressure gradients across the root. Next, the pressure at the cut end of the root was increased by a series of pressure jumps until one was found which rendered J_v zero momentarily. If this critical pressure jump is ΔP_x^o , then expression 1 becomes

$$J_v = L_p(P_o - (P_x + \Delta P_x^o) - \sigma\Delta\pi) = 0$$

or

$$\Delta P_x^o = (P_o - P_x - \sigma\Delta\pi) \quad (2)$$

and since P_o and P_x are known, the osmotic driving force $\sigma\Delta\pi$ can be obtained. Furthermore, it is unlikely that any changes in concentration gradients within the root system will occur during transition between the flowing and nonflowing states, since the application of the pressure jump required a time interval of less than 0.3 s. Thus, the driving force which ΔP_x^o nullifies, must be the same one which caused the volume flow, J_v , immediately before its application, and it should therefore be valid to rewrite expression 1 as $J_v = L_p\Delta P_x^o$, and to calculate the hydraulic conductivity as $L_p = J_v/\Delta P_x^o$. The value of L_p can be found by this method for each applied pressure and is furthermore independent of the value of σ .

It will be shown that the osmotic driving force across the root becomes larger at high flow rates and thus opposes volume flow through the root. Furthermore, since the magnitude of this force is greater than the maximum which could be generated by differences between the concentration of ions in the exudate and the outside solution (even when the former is zero), further concentration gradients must be present within the root. A model in which such gradients are generated by the flow is described and shown to predict J_v , $\Delta\pi$, and the xylem solute concentration, X , as a function of ΔP .

MATERIALS AND METHODS

Hybrid sweet corn (119 Seneca 60) was grown as before (9), with roots supported by glass beads confined in growth tubes. These were excised and sealed to the apparatus previously described (6) which had been altered so as to allow both positive and negative pressure differences to be applied across the root (9).

Lower rates of volume flow were measured by the automatic syringe described earlier (6). This consisted of a tube leading exudate from the root which terminated in a mercury filled U-tube manometer. As exudate entered the tube, the mercury was forced up one arm of the manometer and, in so doing, activated a commercial level-sensor attached to it. This in turn energized a motor driven syringe, which withdrew exudate from the tube (to which it was connected), thus lowering the mercury level until the sensor (and motor) was deactivated. By a continuous

repetition of these events, the volume of exudate entering the tube was exactly compensated for by an increase in syringe volume. The syringe motor, while operating, rotated the shaft of a potentiometer whose sliding contact potential was, as a consequence, directly proportional to the syringe volume. Thus, a recording of this potential provided a plot of the exudation volume against time, the slope of which equalled the exudation flow rate. An example of one such recording is shown in Figure 1.

At higher external pressures, however, the syringe motor was too slow to match the rate of exudation, and volume rates had to be measured manually. For this procedure, valve C (Ref. 6; Fig. 2) was closed and a timer started when the level sensor indicated that the mercury in the U-tube had reached the control point. The syringe volume was increased manually by a predetermined amount causing the mercury to drop below the control point and the timer stopped when it again reached this point. The volume increase divided by the measured time equals the flow rate. Examples of this type of measurement are plotted in Figure 2.

As was shown previously (6), the exudation rate was constant, only briefly following excision, and then declined to about 90% of the original value after 1 h. Since the rate of decline increased after this, measurements were confined to the 1st h. Furthermore, all readings at pressure differences other than zero were bracketed by readings made at zero pressure before and after it, as shown for the flow rate in Figures 1 and 2. Thus, by dividing the value of each reading obtained under pressure, by the average of the zero pressure readings obtained immediately before and after it, all measurements were normalized to those at zero applied pressure. Each root system was used to obtain readings at two or three different non-zero pressures and a sufficient number of root systems was employed to provide 10 to 12 replicates at each pressure.

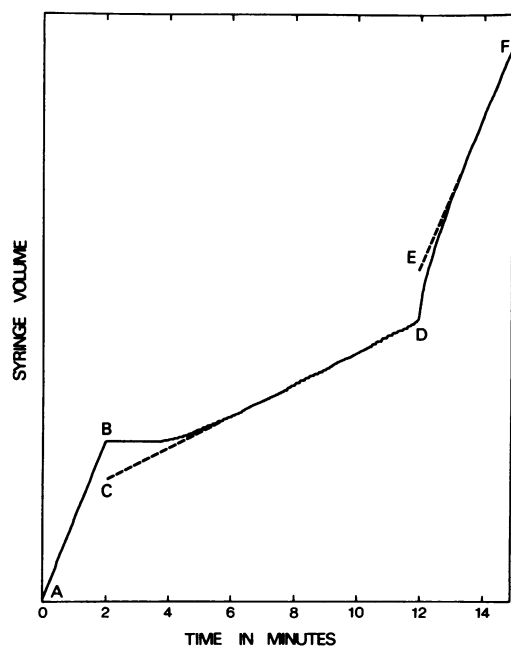


FIG. 1. Example of the measurement of a normalized flow rate at lower applied pressures. The solid line is a photograph of a recording—made by the automatic syringe—of the exudate volume as a function of time. A pressure of 2.0 bar was applied to the cut end of the root at B (i.e. $\Delta P = -2.0$ bar) and reduced to zero again at D. The broken lines are extensions of the linear portions of the curve. The normalized flow rate is the slope of CD divided by the average of the slopes of AB and EF.

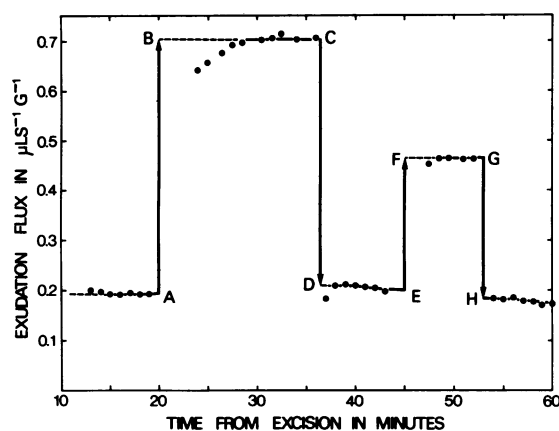


FIG. 2. Example of the measurements of normalized flow rates at higher applied pressures. The points are plots of flow rates measured manually as a function of time. Pressures P_o , applied to the external solution was 0 at A, D, E, and H; 2.4 bar from B to C and 1.6 bar from F to G. The broken lines are drawn through points whose measurements appear to have stabilized following a change in pressure. The normalized flow rate is $(B + C)/(A + D)$ at 2.4 and $(F + G)/(E + H)$ at 1.6 bar.

The transpiration rate of whole plants was measured by determining the water lost from a reservoir supplying six plants, during the last half of the 16-h light period. Correction for evaporation was made by subtracting the water lost under similar conditions, but without plants. This measurement was made, both under normal growing conditions in a controlled environment room (C.E.R. [6]) and in a greenhouse under full mid-June sunlight, using solution grown plants raised under these conditions.

Root weights were obtained following centrifugation (6). Osmotic pressures were obtained using an Advanced Instruments (Newton Highlands, MA) freezing point depression osmometer. Both standards supplied by the manufacturer and those made by weighing oven-dried NaCl were employed.

All experiments were performed on roots held at 30°C.

RESULTS

The volume flux, J_v , is usually found by dividing the rate of volume flowing through a barrier, by the area of the barrier. The root system is a highly branched complex structure however, making it difficult to assign an unambiguous area to it, even though its dimensions are well known (7). To avoid this difficulty here, the flow rates obtained were divided by the root weight, providing the flux with units of volume time⁻¹ mass⁻¹. Since the root density (mass volume⁻¹) is close to one (7), the flux could also have been expressed as time⁻¹, but the former convention, used in the previous papers in this series, will be used here.

Seventy-two root systems were employed in this work. For each, the first measurements were made under zero pressure difference as soon as possible after excision (within 5–10 min). Averaging these results provided the following absolute values at zero pressure difference (together with standard deviations): flow rate, $J_v = 0.236 \pm 0.065 \mu\text{l s}^{-1}(\text{g root})^{-1}$; pressure jump, $\Delta P_x^o = 0.402 \pm 0.102$ bar and the initial rate of pressure increase following the pressure jump (6), $(dP_x/dt)_o = 0.122 \pm 0.035$ bar s⁻¹.

With each root, the initial zero pressure measurements were followed by two or three further measurements under pressure, alternating, of course, with readings at zero pressure. These were converted to relative values, as mentioned above, by dividing them by the mean of the two zero pressure readings made before and after them. Ten to twelve such readings, each made on a different root, were combined to give each of the average relative values listed in Table I. Finally, multiplying these latter values

Table I. Normalized Values of Volume Flow, J_v , Pressure Jump, ΔP_x^o , and Initial Rate of Pressure Increase, $(dP/dt)_o$, Measured at Various Pressures

P_o , the pressure applied to the outside solution, and P_x , the pressure applied to the cut end of the root, are in bars above atmospheric (which is taken as zero). The values of J_v , ΔP_x^o and $(dP/dt)_o$ are relative to those measured at $P_x = P_o = 0$ (for the same root) and are thus unitless. Numbers in brackets, SD.

P_o	P_x	J_v	ΔP_x^o	$(dP/dt)_o$
bar		relative units		
0	2.4	0.114 (0.043)	0.122 (0.061)	0.241 (0.074)
0	2.0	0.173 (0.072)	0.153 (0.072)	0.285 (0.122)
0	1.6	0.315 (0.047)	0.324 (0.086)	0.427 (0.131)
0	1.2	0.420 (0.068)	0.400 (0.068)	0.551 (0.150)
0	0.8	0.582 (0.034)	0.623 (0.101)	0.663 (0.164)
0	0.4	0.754 (0.047)	0.746 (0.130)	0.842 (0.142)
0	0	1.000	1.000	1.000
0.4	0	1.259 (0.039)	1.326 (0.128)	1.226 (0.133)
0.8	0	1.625 (0.106)	1.685 (0.170)	1.593 (0.315)
1.2	0	1.904 (0.121)	1.777 (0.385)	2.144 (0.385)
1.6	0	2.341 (0.427)	2.450 (0.413)	2.621 (0.335)
2.0	0	2.669 (0.248)	2.967 (0.801)	3.245 (0.896)
2.4	0	3.060 (0.498)	3.165 (0.572)	3.403 (0.782)

Table II. Root Parameters as a Function of the Applied Pressure

$\Delta P = P_o - P_x$, where P_o is the pressure at the outer surface of the root and P_x that in the xylem. The $\Delta P = 0$ values of the volume flow, J_v , the pressure jump, ΔP_x^o , and the initial rate of pressure rise dP_x/dt_o (all underlined) were absolute values found by averaging measurements made on 72 roots immediately following excision. The remaining values were calculated by multiplying the latter figures by the ratios in Table I. The hydraulic conductivities were calculated from the ratio $L_p = J_v/\Delta P_x^o$ for each applied pressure. The osmotic pressure gradient found by freezing point depression was $\Delta\pi_f = \pi_o - \pi_f$ where π_o is the osmotic pressure of the outer solution (0.5 bar) and π_f is that of the xylem exudate reported previously (9). The osmotic pressure gradient found by direct pressure measurement was $\Delta\pi_p = (\Delta P - \Delta P_x^o)/\sigma$ (from equation 2).

ΔP	J_v	ΔP_x^o	$(dP_x/dt)_o$	L_p	$\Delta\pi_f$	$\Delta\pi_p$
bar	$\mu l s^{-1} g^{-1}$	bar	bar s^{-1}	$\mu l s^{-1} bar^{-1} g^{-1}$		bar
-2.4	0.027	0.049	0.026	0.551	-2.81	-2.88
-2.0	0.041	0.062	0.035	0.661	-2.62	-2.43
-1.6	0.074	0.130	0.052	0.569	-2.23	-2.03
-1.2	0.099	0.161	0.067	0.615	-1.84	-1.60
-0.8	0.137	0.250	0.081	0.548	-1.56	-1.24
-0.4	0.178	0.300	0.130	0.593	-1.20	-0.82
0.0	<u>0.236</u>	<u>0.402</u>	<u>0.122</u>	0.587	-1.12	-0.473
0.4	0.297	0.533	0.150	0.557	-0.76	-0.156
0.8	0.384	0.677	0.194	0.567	-0.68	0.145
1.2	0.449	0.714	0.262	0.629	-0.453	0.572
1.6	0.552	0.985	0.320	0.560	-0.475	0.724
2.0	0.630	1.193	0.396	0.528	-0.337	0.949
2.4	0.722	1.272	0.415	0.568	-0.266	1.327

by the absolute values found at zero pressure (previous paragraph) gave the absolute values for each applied pressure listed in Table II. This procedure was followed in the hope of obtaining results which are relatively free from effects due to aging of the excised roots.

The pressure jump, ΔP_x^o , reported here is somewhat smaller than that reported earlier (6). This difference probably arises from the change in medium, since NH_4^+ , a rapidly penetrating ion, was present in the first medium but not in the one used here.

The root weights averaged 2.4 ± 0.4 g. The correlation coefficient between weight and initial J_v at zero pressure was -0.08 showing that the volume flux was virtually independent of root size. This is important since it justifies the comparison of flow rates measured with roots of different sizes on a 'per weight' basis.

The transpiration rate was found to be $0.51 \pm 0.4 \mu l s^{-1}$ (g root) $^{-1}$ in the C.E.R. and $1.2 \pm 0.1 \mu l s^{-1}$ (g root) $^{-1}$ under direct sunlight.

DISCUSSION

Equation 1 is basic to any discussion of the flow of water through roots. It contains the coefficients σ and L_p , however, which must first be evaluated. In the previous publication (9), it was shown that, for the roots used in this work, the reflection coefficient, σ , is equal to 0.85. The hydraulic conductivity was calculated using the present data, and the results listed in Table II. These show that L_p has only a slight dependency on ΔP (correlation coefficient = -0.3). Thus, L_p will be assumed to be constant at all pressures and will be assigned that value found for it at zero pressure (where there was the largest number of determinations) of $0.59 \pm 0.16 \mu l s^{-1} bar^{-1}$ (g root) $^{-1}$. Column 6 of Table II is a list of the osmotic pressure (o.p.) differences at each applied pressure, $\Delta\pi_f = \pi_o - \pi_f$, where π_o is the o.p. of the outside solution (0.5 bar) and π_f is the o.p. for the exudates collected at the various applied pressures (determined previously [9] by freezing point depression). Finally, in column 7, $\Delta\pi_p$, the

o.p. differences as calculated from the pressure jump data using equation 2 are given.

The goal of previous workers (2-5, 10) was to devise a model of root function which would accurately predict the volume flow for any positive value of the applied pressure. The model which emerged considered the root symplasm to act essentially as a single membrane separating the xylem compartment from the outside solution. In this model, flow results not only from the application of a hydraulic pressure across the root but also from a concentration difference generated by active pumping of solutes across the membrane at a rate J_s . These workers assumed a constant value for J_s , which is valid when the volume flow rate is high (as was the case for their data) but as J_v approaches zero, as in the present work, this assumption predicts that the root pressure will become infinite. Actual measurements have shown, however, that with zero flow rate the root pressure rises rapidly at first but more slowly with time, finally approaching a limiting value of 4.21 bar above the outside solution (6, 8, 9).

Net transport in any biological system is the difference between the fluxes F_{in} and F_{out} . These unidirectional fluxes are frequently seen to obey Michaelis-Menten kinetics so that the transport equation can be written as

$$\frac{dn}{dt} = F_{in} - F_{out} = \frac{V_{max}^o C_o}{K_m^o + C_o} - \frac{V_{max}^i C_1}{K_m^i + C_1} \quad (3)$$

where dn/dt is the number of solute molecules passing through a unit area of membrane per unit time, F_{in} and F_{out} are the individual fluxes, V_{max}^o , K_m^o , and C_o are maximum rate constant inward, affinity constant, and concentration at the outer membrane surface and V_{max}^i , K_m^i , and C_1 are their counterparts at the inner membrane surface. Since the volume of solution surrounding the root was much larger than the root itself, C_o , and hence F_{in} , was constant for all experiments reported here. The values of K_m and V_{max} have been determined for a number of facilitated transport systems which are not active. In active systems, however, it is usually found that K_m^i is so large as to exceed any value of C_1 attainable and therefore $F_{out} \approx kC_1$ where $k = V_{max}^i/K_m^i$. Equation 3 thus reduces to

$$\frac{dn}{dt} = F_{in} - kC_1 \quad (4)$$

from which we can conclude that transport will occur into the root (*i.e.* dn/dt will be positive) as long as $kC_1 \ll F_{in}$, and furthermore C_1 will tend to rise until $F_{in} = kC_1$. That this happens in the root is demonstrated by the recordings of the rise in osmotic pressure of the xylem which results when exudation is prevented (6, 8, 9). This pressure is proportional to the concentration of solutes transported to the xylem, which can be seen to approach a maximum value with time. Thus, if the value of the xylem concentration for which transport ultimately becomes zero (at infinite time) is X_∞ , equation 4 can be written

$$J_s = \frac{dn}{dt} = k(X_\infty - X) \quad (5)$$

where X is the total concentration of solute in the xylem at any time t . This expression states that active transport will operate in such a direction as to make $X = X_\infty$ and further predicts that a plot of J_s against X should be a straight line of slope $-k$ and intercept kX_∞ . Plots of this type have been shown to be linear for individual ions (9) and should therefore be valid for the total concentration (X).

Another prediction which previous theories make is that, since they are based on a two compartment model, the total osmotic pressure drop across the membrane $\Delta\pi_f$ should simply be the difference between the xylem o.p., π_f , and the outside o.p., π_o , and this should therefore be equal to $\Delta\pi_p$. It can readily be seen

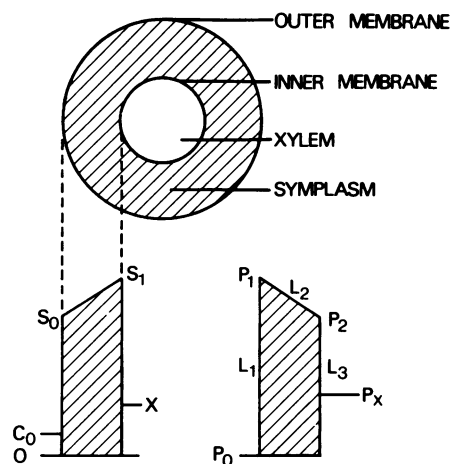


FIG. 3. Top, Simplified cross section of a root branch. Bottom, Concentration (left) and pressure profiles predicted by the model for flow under a positive applied pressure. The shaded areas represent the symplasm which is bounded on each side by a membrane. C_o is the concentration of solutes in the outer solution, S_o and S_1 are the concentrations on the symplasmic side of the outer and inner membranes, and X is that in the xylem. P_o to P_x are pressures at the locations indicated, and L_1 to L_3 are the hydraulic conductivities as specified in the text.

by comparing the last two columns in Table II that this is only true at the lowest flow rates, beyond which increasing J_v brings increasing discrepancies, to as high as -1.6 bar. This would seem to indicate that as a result of flow, other osmotic pressure gradients which oppose the flow must be present somewhere in the system. These probably arise as a result of the flow sweeping solutes away from the membranes separating the symplasm from the outer solution and piling them up against the membranes separating the symplasm from the xylem (1). The result of this phenomenon acting in a simplified model of the root would be to establish the concentration and pressure profiles shown in Figure 3. To test the validity of this model, equations relating J_v and X to ΔP must be derived and their predicted values compared to those observed. To do this, expressions describing transport of water and solute across the outer membrane, the symplasm, the inner membrane, and the xylem will be derived separately and then combined. First, however, certain generalizations concerning transport must be made.

In the preceding report (9), the six major inorganic ions present in the outer solution were found at higher concentration in the cell sap than in the outer solution and must therefore have been actively transported across the outer membrane. The nitrate ion was then partially converted by metabolism to other species (mainly NH_4^+ and amino acids) which were transferred together with unchanged NO_3^- and the other inorganic ions into the xylem. The final equilibrium concentration of many of these species in the xylem was different from that in the symplasm, showing that active forces are at work during transport through the inner membrane as well. Their total concentration on the other hand, proved to be roughly the same in both compartments. This was due to the fact that many of the actively transported ions, such as H^+ and the amino acids, were at low concentration, while those showing a deficit such as K^+ , were partially replaced by other ions (NH_4^+). Thus, if we consider only the total number of moles of solute passing through the root, or in other words treat all chemical species as a single solute, transport through the outer membrane can be considered active and that through the inner membrane nonactive, but facilitated.

We may now proceed to the derivation of the flux equation region by region as follows.

The Outer Membrane. Letting S_o be the solute concentration

and P_1 the pressure at the symplasmic (inner) surface of the outer membrane (see Fig. 3), the osmotic pressure difference across the outer membrane can be written as $\Delta\pi = \sigma RT(C_o - S_o)$ and expression 1 becomes

$$J_v = L_1[P_o - P_1 - \sigma RT(C_o - S_o)]$$

where L_1 is the membrane hydraulic conductivity (per g root). Following the same arguments used to derive equation 5, the active solute transport should then be given by

$$J_a = K_1(S_\infty - S_o)$$

where K_1 is the transport rate constant (per g root), and S_∞ is the highest level of concentration to which the active transport system can raise the solute. In addition there will be transport due to solvent drag which has the form (1) $(1 - \sigma)\bar{C}J_v$ where \bar{C} is the mean concentration of solute in the membrane, $\approx (C_o + S_o)/2$. Adding this to J_a gives the total solute flux.

$$J_s = K_1(S_\infty - S_o) + \beta(C_o + S_o)J_v$$

where $\beta = (1 - \sigma)/2$.

The Symplasm. Solutes in this region will be carried inward by the flow at the rate $S \cdot J_v$, where S is the solute concentration at a distance r from the center of the root, and by diffusion at the rate $AfD(\partial S/\partial r)$ where A is the wall area of a cylinder of radius r , f is the fraction of this area open to diffusion (*i.e.* as plasmodesmata or cell walls), and D is the diffusion coefficient. (Note that the diffusion term is positive since flow is defined as positive in the direction of decreasing r). If the total length of the root is L , so that $A = 2\pi rL$, the total solute transport at r will be the sum of the mass flow and diffusion processes, or

$$J_s = S \cdot J_v + 2\pi rLfD\left(\frac{\partial S}{\partial r}\right)$$

which is similar to Dainty's "convection-opposed-by-diffusion" equation (1). Since mass must be conserved, J_s and J_v must be constant for all values of r . Thus, by rearranging and integrating this equation between the limits $S = S_o$ and S_1 and $r = r_o$ and r_1 (the outer and inner radius of the symplasm), the solute flux is found to be

$$J_s = \frac{(S_1 - ES_o)J_v}{(1 - E)}$$

where $E = \text{EXP}(K_2J_v)$ and $K_2 = [\ln(r_o/r_1)]/2\pi rLfD$. The volume flow expression may be found by integrating

$$J_v = 2\pi rL_s\left(\frac{\partial P}{\partial r}\right)$$

where L_s is the local hydraulic conductivity of the symplasm, (or apoplasm, depending on the route taken by the water) between the limits P_1 and P_2 and r_o and r_1 , to give

$$J_v = L_2(P_1 - P_2)$$

where $L_2 = 2\pi rL_s/\ln(r_o/r_1)$, the overall hydraulic conductivity of the symplasm.

The Inner Membrane. Since this membrane is not considered to be active, transport will operate in such a way as to equilibrate the concentrations on both sides of the membrane, so that (again including the solvent drag term)

$$J_s = K_3(S_1 - X) + \beta(S_1 + X)J_v$$

with K_3 being the transport rate constant. If L_3 is the hydraulic conductivity of this membrane, the volume flow will be

$$J_v = L_3[P_2 - P_x - \sigma RT(S_1 - X)]$$

The Xylem. Here, since the volume flow is J_v , solutes will be

carried along at the rate $J_s = X \cdot J_v$.

The General Flux Equations. Since in this study we are considering steady state or time-independent fluxes and since matter must be conserved, the volume and amount of solute flowing through all regions must be equal. Thus, by equating the J_s and J_v terms for all regions, the following expressions are obtained:

$$S_o = A_1 - A_2X, \quad S_1 = A_3X,$$

$$X = EA_1/(EA_2 + A_3 + E - 1) \quad (6)$$

where $A_1 = (K_1S_\infty + \beta C_o J_v)/(K_1 - \beta J_v)$, $A_2 = J_v/(K_1 - \beta J_v)$,

$$A_3 = (K_3 + (1 - \beta)J_v)/(K_3 + \beta J_v) \text{ and}$$

$$J_v = L_p[P_o - P_x - \sigma RT(C_o - S_o + S_1 - X)] \quad (7)$$

with $1/L_p = 1/L_1 + 1/L_2 + 1/L_3$. These expressions, which are implicit in J_v , can be further combined to a single equation in the form of a polynomial. Such an equation, however, is too complex to provide any information about the fluxes by inspection and furthermore, still being implicit in J_v , must be solved by a successive approximation method. Since this method can be applied as readily to equations 6 and 7 (see "Appendix"), no further algebraic reduction is warranted.

Solution of these equations requires values for the rate constants K_1 , K_2 , and K_3 as well as the concentration S_∞ . The latter can be obtained by the following argument. When exudate flow is stopped, or $J_v = 0$, solute continues to move until a steady state is reached as indicated by the fact that the pressure becomes constant at its highest value. At this point, the xylem concentration has reached its maximum value X_∞ and equation 6 (which applies only to the steady state) predicts that when $J_v = 0$, $X = S_1 = S_o = S_\infty = X_\infty$. According to previous work (Ref. 9; Table II), the maximum osmotic pressure of the xylem sap was 5.45 bar which is equivalent to a concentration $X_\infty = 216$ ideal $\mu\text{osmol cm}^{-3}$.

The three rate constants can next be evaluated by fitting the J_v versus ΔP data listed in Table II to equations 6 and 7 by a least squares method. This was accomplished by introducing various values for K_1 , K_2 , and K_3 into the equations, solving for J_v at each of the applied pressures, then determining the root mean square differences between the calculated and observed volume flux. The values of the rate constants resulting in the lowest errors are those considered to be closest to the true values. These are listed in Table III together with the observed and calculated volume fluxes. The excellent fit which results (Fig. 4A) is hardly surprising when considering the degree of freedom afforded by adjusting the three coefficients K_1 , K_2 , and K_3 .

A real test of the model, however, is provided by a comparison of the total concentration gradient across the root derived from the above calculations and listed in Table III as ΔC (CALC) to that measured more directly by the pressure jump technique. ΔC is not simply $C_o - X$ as in the model of Fiscus and others, but is the sum of the gradients across the outer membrane ($C_o - S_o$) and the inner membrane ($S_1 - X$), and it is just this that the pressure jump technique measures, as the following argument shows. Consider the volume flux through a root under an applied pressure $\Delta P = P_o - P_x$ as described by equation 7. If ΔP_x° is the sudden increase in the xylem pressure P_x necessary to reduce J_v to zero momentarily (the pressure jump), it follows, from equation 7, that $\Delta C = C_o - S_o + S_1 - X = (\Delta P - \Delta P_x^\circ)/\sigma RT$. Values for ΔC calculated from the data in Table II in this way are listed in Table III as observed values and plotted in Figure 4B. These show good agreement with values calculated from the equations and provide strong support for the model. It should be stressed furthermore, that the values for ΔC and J_v found at each applied pressure, were derived from totally independent measurements, those of ΔP_x° (from which ΔC (OBS) were calculated) being made immediately after the flux measurements, J_v , but com-

Table III. Comparison of Calculated (CALC) and Observed (OBS) Values

$\Delta P = (P_o - P_x)$ is the applied pressure in bars. S_o , S_i , X , and ΔC are concentrations in ideal $\mu\text{osmol cm}^{-3}$. J_v is the volume flux in $\mu\text{l s}^{-1}$ (g root) $^{-1}$ ($10^{-3} \text{ cm}^3 \text{ s}^{-1}$ (g root) $^{-1}$). The calculated values arise from the solution of equations 6 and 7 with $C_o = 20 \mu\text{osmol cm}^{-3}$, $RT = 0.0252 \text{ cm}^3 \text{ bar } \mu\text{mol}^{-1}$, $S_o = 216 \mu\text{osmol cm}^{-3}$, $L_p = 5.9 \times 10^{-4} \text{ s}^{-1} \text{ bar}^{-1}$ (g root) $^{-1}$, $K_1 = 4.2 \times 10^{-4} \text{ cm}^3 \text{ s}^{-1}$ (g root) $^{-1}$, $K_2 = 590 \text{ cm}^{-3} \text{ s}$ (g root) and $K_3 = 5.8 \times 10^{-5} \text{ cm}^3 \text{ s}^{-1}$ (g root) $^{-1}$. J_v (OBS) values are from Table II; X (OBS) values are the osmotic pressures reported previously (Table I, 9) expressed as concentrations (π_f/RT ideal $\mu\text{osmol cm}^{-3}$), while ΔC (OBS) = $\Delta\pi_f/RT$ (see Table II). ΔC (CALC) = $C_o - S_o + S_i - X$.

ΔP	S_o	S_i	X	X	J_v	J_v	ΔC	ΔC
(CALC)	(CALC)	(CALC)	(OBS)	(OBS)	(CALC)	(OBS)	(CALC)	(OBS)
-2.4	205.8	207.4	136.5	131.3	0.037	0.027	-114.9	-114.3
-2.0	203.5	206.2	120.1	123.9	0.052	0.041	-97.5	-96.3
-1.6	201.3	205.5	104.5	108.2	0.072	0.074	-80.3	-80.8
-1.2	199.1	205.6	90.2	93.0	0.098	0.099	-63.7	-63.5
-0.8	197.0	206.7	77.6	81.9	0.133	0.137	-47.8	-49.0
-0.4	194.8	209.0	66.9	67.5	0.178	0.178	-32.7	-32.7
0.0	192.7	212.6	58.4	64.4	0.234	0.236	-18.5	-18.8
0.4	190.4	217.3	52.0	50.0	0.301	0.297	-5.1	-6.2
0.8	187.9	222.9	47.3	46.7	0.376	0.384	7.7	5.7
1.2	185.3	229.1	43.9	37.8	0.457	0.449	19.9	22.7
1.6	182.3	235.6	41.5	38.7	0.543	0.552	31.8	28.7
2.0	179.1	242.2	39.7	33.2	0.632	0.630	43.4	37.7
2.4	175.5	248.7	38.4	30.4	0.724	0.722	54.8	52.7

pletely separate from them.

A third set of data for comparison is provided by the xylem concentrations X (CALC) and X (OBS) = $\pi_f/\sigma RT$ in Table III. Again, an excellent fit is obtained to a completely separate set of measurements (the freezing point depression data) which were made on a different set of roots and reported earlier (9).

The coefficients K_1 and K_3 are, in fact, permeability constants for the inner and outer membranes and may be expressed in more familiar units by dividing by the area of the membrane concerned. Thus, dividing by the epidermal surface area of 276 cm^2 (g root) $^{-1}$ (7), K_1 becomes $1.5 \times 10^{-6} \text{ cm s}^{-1}$. Similarly, if it is assumed that transport into the stelar apoplast occurs through the surface of all cells in this region, whose area is 670 cm^2 (g root) $^{-1}$ (7), K_3 becomes $8.7 \times 10^{-8} \text{ cm s}^{-1}$. Tyree (13) has summarized the values reported in the literature for the permeability of the plasmalemma of various cells to individual ions. These values (converted to the units used here by multiplying by RT) range from 2.5×10^{-7} to $5 \times 10^{-10} \text{ cm s}^{-1}$ showing that K_1 and K_3 are of reasonable magnitude. That they are at the top of the range might be expected, since K_1 and K_3 represent the total permeability of a number of ions, and furthermore, excised corn roots are noted for their high exudation rate relative to other species, many of which show no exudation without the application of pressure.

The apparent diffusion coefficient in the symplast can be shown (by rearranging the expression defining K_2 above) to be $fD = [\ln(r_o/r_i)]/2\pi LK_2$. If r_o and r_i are taken as the radius of the root and the stele, respectively, then, since the volume of the cortex is 0.80 times that of the root, and the root length $L = 4600 \text{ cm}$ (g root) $^{-1}$ (7), $fD = 5 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$. This means that if the open portion of the plasmodesmata is water filled, then D for solutes is $10^{-5} \text{ cm}^2 \text{ s}^{-1}$, and f , the fraction of the area of the cell walls occupied by this open portion will be 0.005. This is somewhat less than the value 0.009 estimated by Tyree (13) but greater than the 0.0001 to 0.0005 range given by Robards and Clarkson (11).

The plot of the J_v versus ΔP data in Table II approaches a straight line at high flow rates as it does for most excised roots

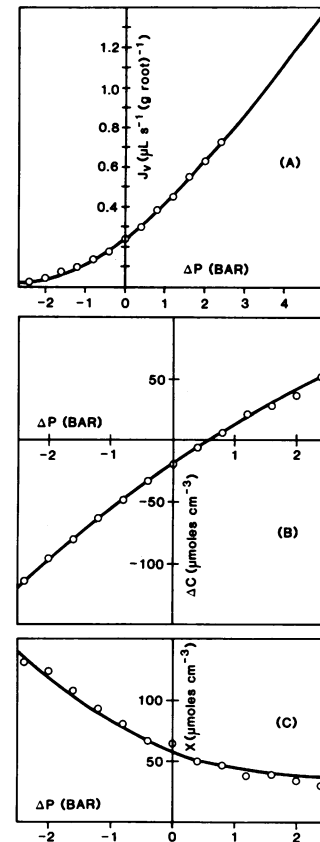


FIG. 4. Comparison of the data (O) to the computed values (curve). A, Volume flux versus applied pressure. B, Total concentration gradient (ΔC (OBS), Table III) versus applied pressure. C, the xylem concentration (X) versus applied pressure. The calculations in A have been extrapolated to 5 bar to show that they are linear over the range of pressures used by Fiscus (5) in his measurement of L_p .

(Fig. 4A). According to Fiscus (4), the slope of this line should equal L_p . This follows from equation 1 if it is assumed that, as the xylem concentration is reduced (diluted by an increasing water flux), $\Delta\pi$ approaches a constant value ($\sigma RT C_o$). The measurements reported here have shown, however, that $\Delta\pi$ does not approach a limiting value but continues to increase in a roughly linear manner (Fig. 4B). As a result, the driving force which brings about the flux ($\Delta P - \sigma\Delta\pi$) is over-estimated in the method of Fiscus, producing a value for L_p which is too low (by about 50% in the present case).

The present value of L_p ($0.59 \mu\text{l s}^{-1} \text{ bar}^{-1}$ (g root) $^{-1}$) is somewhat higher than that previously obtained by the same method ($0.41 \mu\text{l s}^{-1} \text{ bar}^{-1}$ (g root) $^{-1}$ [6]). This is probably due to the different growth media used in the two studies. Both these values are, however, within the range of the estimates obtained by the pressure relaxation technique (0.3 – $0.6 \mu\text{l s}^{-1} \text{ bar}^{-1}$ (g root) $^{-1}$ [9]). To compare these values with other methods necessitates a conversion of units, which in turn, requires that the location and area of the main barrier to water entry be known. Usually the plasmalemma of the outer surface of the epidermis is assigned this function and since its area is known to be 276 cm^2 (g root) $^{-1}$ (7), $L_p = 2.1 \times 10^{-6} \text{ cm s}^{-1} \text{ bar}^{-1}$. This is higher than the values reported by other workers but agrees with those found for individual root cells (12).

Stuedle and Jeschke (12) have objected to the use of the present apparatus because, as they claim, its high compressibility makes the pressure jump measurements difficult. The standard deviation for the value of ΔP_x^o found in the present study at $\Delta P = 0$ was $\pm 25\%$. This is similar to that found for J_v ($\pm 28\%$), but since

J_v can be measured with considerable accuracy, must be ascribed mainly to biological variation. This conclusion is born out by the fact that the values of L_p listed at the different pressures in Table II, each of which is calculated as the ratio of the average values of J_v and ΔP_x^o , and should therefore not be as subject to biological variations, show only a 6.4% SD from their overall average (0.580 ± 0.037). Thus in spite of these authors' misgivings, the pressure jump measurements appear to be a consistent body of data.

Steudle and Jeschke (12) also suggest that the root must supply a large amount of exudate to reach the maximum root pressure. This amount can be estimated from the data already reported. Using $0.78 \mu\text{l bar}^{-1}$ for $\Delta V/\Delta P$, the compressibility of the root-apparatus system (6) and $100 \mu\text{l}$ for V_x the xylem volume ($0.040 \text{ cm}^3 \text{ g}^{-1}$ [7] for a 2.5 g root), the apparent elastic modulus $\approx V_x \Delta P/\Delta V = 130 \text{ bar}$. As this is about the same as the average for the cells they used, the roots employed here should not have been taxed any more than were the cells of these workers. The root pressures measured by Steudle and Jeschke (12) were lower and much more variable (0.9–2.9 bar) than those reported in this work ($4.21 \pm 0.34 \text{ bar}$ [9]), suggesting that their roots, for which no special support during handling appears to have been provided, may have suffered slight but significant injuries. (See discussion of this point in the previous publication [9].) This would also explain their rather high water exchange half times (100–700 s) which are typical of a pump-and-leak system.

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APPENDIX

Simultaneous Solution of Equations 6 and 7. A successive approximation method, consisting of the following steps, was used:

An initial estimate of the volume flow, termed J_o , was made (the observed value of J_v was usually satisfactory) and employed in the solution of equation 6. This provided values for the concentrations which were substituted into equation 7 to give a new value, J_1 , for the flux. The initial estimate was increased by the factor i (usually set at 0.001) to give a new starting value $J_2 = (1 + i)J_o$ and this in turn used as before to provide new estimates of the concentrations leading to a second value, J_3 , for the flux as calculated from equation 7. An improved value for J_v is now

$$J_v = J_o - iJ_o(J_o - J_1)/(J_1 + J_2 - J_3 - J_o)$$

Letting J_o now equal this improved value, the above steps were repeated to provide a further correction. This procedure was repeated until the corrections became insignificant.