

The Velocities of Ion Transport Into and Through the Xylem of Roots

FINDINGS WITH A TWO-POINT APPLICATION PULSE-CHASE TECHNIQUE¹

Received for publication April 11, 1973

EMANUEL EPSTEIN AND J. D. NORLYN

Department of Soils and Plant Nutrition, University of California, Davis, California 95616

ABSTRACT

Excised corn roots, *Zea mays*, had radioactively labeled solution applied at two points along their length, for 1 minute, and were then kept in dilute, unlabeled nutrient solution. During this "chase" period, exudate was collected at 1-minute intervals, and its content of radioions was determined. Two pulses of label appeared in succession, originating at the points of application near the cut end of the root and farther from the cut end, respectively. Calculation yields the velocity at which the ions moved radially across the root into the xylem (v_r) and the velocity at which they moved longitudinally within the xylem (v_l). For Rb^+ labeled with ^{86}Rb , v_r was 1.8 and v_l , 35 cm/hr. For Br^- labeled with ^{82}Br , v_r was 1.4 and v_l , 103 cm/hr.

Mineral ions from the external medium first enter the cells of roots by traversing the plasmalemma of an epidermal or cortical cell. After radial movement through the symplast into the stele, ions leave the symplast by crossing the plasmalemma of a living stelar cell into extracellular wall space and the vessels, in which they then move upward toward the shoot. Fully functional vessel elements are devoid of protoplasts and therefore vessels offer minimal resistance to the flow of the ascending solution. In contrast, the radial pathway includes passage from cell to cell within the symplast, and entry into it and exit from it across plasma membranes. In this work the speed of the radial movement of ions to the xylem of corn roots is compared with the speed of their subsequent flow within the vessels.

MATERIALS AND METHODS

Culture of the corn seedlings, *Zea mays* cv. DeKalb 805, was as described earlier (8), except that KCl was substituted for KNO_3 in order to preload the roots with Cl^- as well as with K^+ . On the 9th day (counting from the day the seeds were allowed to germinate), a root approximately 25 cm long was excised, and a length of polyethylene tubing was attached to the cut end. The tubing was flared at one end to facilitate insertion of the root and make for a good fit between root and tubing (Fig. 1 of ref. 8). The excised root was then returned to the nutrient

solution for an equilibration period of 1 hr, during which it exuded into the polyethylene tubing. The solution was kept at 30 C and was aerated.

The localized application of the experimental solution for radioactive pulse-labeling was as follows. The polyethylene tubing was cut off flush with the cut end of the root, and the root was inserted into the assembly, a top view of which is shown in Figure 1. Only the end of the root with its polyethylene ring protruded above the plastic sponge-lined clamp shown.

The root was rinsed with 0.5 mM $CaSO_4$ solution, and the entire assembly was put in the jig shown in Figure 2. It permits the precisely localized application of a solution containing radioactively labeled ions by means of felt bands 1 cm wide soaked in the labeling solution. Application was at two points along the root simultaneously, lasted 1 min, and was discontinued by removal of the root assembly from the jig and repeated rinsing of the root. The root assembly was then placed into a beaker containing unlabeled nutrient solution of the same composition as that in which the root was kept before. The solution was kept at 30 C and aerated, as before.

During this "chase" period, exudate was collected at 1-min intervals by touching 18×18 mm squares of Whatman No. 5 filter paper to the top of the root. This removed the droplet of exudate which had formed there. The squares of filter paper were then dropped into vials for counting in a Packard liquid scintillation spectrometer (3).

The time course of the appearance of the radiopulses in the xylem exudate and of their subsequent attenuation was plotted. There were two pulses for each experiment with a given root, corresponding, respectively, to the applications at the position near the cut end of the root and the one farther from it.

The interval between application of the pulses and their appearance in the exudate (measured at the peaks of the pulses) was designated m for the short distance pulse and n for the long distance pulse. We then have

$$t_r + t_l = m \quad (1)$$

$$t_r + ft_l = n \quad (2)$$

where t_r is the time it took the pulses to travel radially into the xylem, in minutes, t_l the time it took the short distance pulse to travel longitudinally in the xylem from the point of application to the cut end, and f the factor, long distance (from the cut end)/short distance. Subtracting equation 1 from equation 2:

$$t_l(f - 1) = n - m \quad (3)$$

from which t_l can be calculated. Substituting the value of t_l

¹This work was supported by a grant from the National Science Foundation.

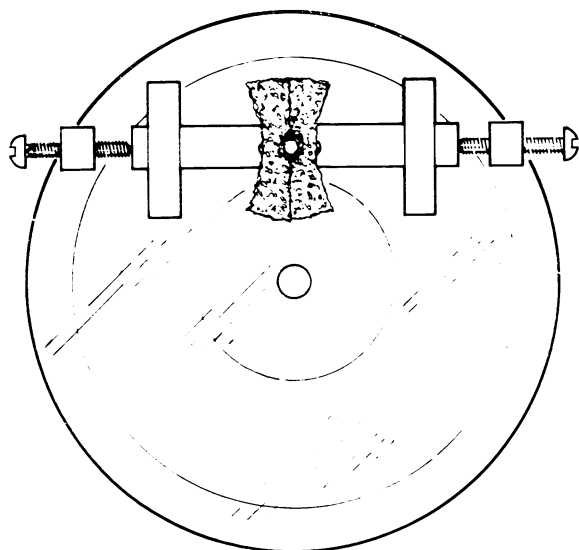


FIG. 1. Top view of the assembly by which an excised root is held and positioned during an experiment. An adjustable clamp is mounted on a Lucite cover 10 cm in diameter, made to fit onto a 400-ml beaker. The clamp consists of two bars, each moving in a yoke. The distance between the bars is adjustable by means of two set screws. The root is held by a slit piece of plastic sponge liner between the bars.

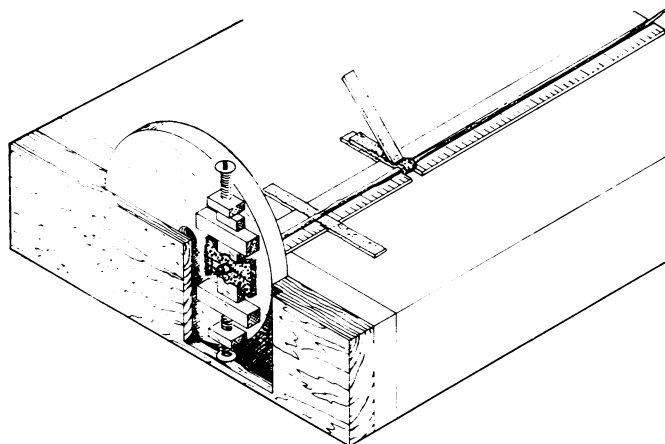


FIG. 2. Jig for localized application of labeled experimental solution. The assembly shown in Fig. 1 is in place. The root is in position in the groove of a plastic ruler which has two gaps 1 cm wide in which lie felt bands soaked in labeled experimental solution. One band is shown lying flat, as both are initially when the root assembly is put in the jig. The bands are then immediately looped over the root and held in place with a plastic spatula, as shown for the second position. Application is for 1 min simultaneously at both positions, whereupon the root is rinsed four times with dilute unlabeled nutrient solution of the same composition as that in which it had been kept before. The root assembly is then put on a 400-ml beaker containing unlabeled nutrient solution for the chase period during which samples of exudate are collected at 1-min intervals.

in equation 1 yields t_r , so that the time it took the pulse to traverse distance r and to travel distance l (both of which are known), and hence the respective velocities, can be calculated.

For the calculations, the distances of the two application points from the cut end need to be known. They were obtained as follows. After the experiment, the root was cut with a multi-blade device into 0.24-cm segments, and the radioactivity of the segments was determined and plotted as a function of the

distance from the cut end. There were two peaks, with essentially no radioactivity between them. The distances of these peaks from the cut end of the root were used in the calculations outlined above.

RESULTS

Figure 3 shows the result of an experiment in which the solution applied locally for 1 min consisted of 0.5 mM RbCl labeled with ^{86}Rb (and 0.5 mM CaSO_4). The two peaks were timed at 6 and 16 min, respectively, and the distances of the two points of application from the cut end of the root were 3.6 and 10.6 cm, respectively. By means of equations 3 and 1 we get 5.5 min for t_r , the time of longitudinal travel of the short distance pulse, and 0.85 min for t_r , the time of the radial movement across the root into the xylem. The velocities of movement were 41.9 cm/hr longitudinally and 3.5 cm/hr radially, the latter value being based on a radial distance of 0.5 mm.

Figure 4 shows the result of a similar experiment in which the labeling solution was 0.6 mM KBr labeled with ^{82}Br (and 0.5 mM CaSO_4). The calculated velocities were 117 cm/hr longitudinally and 0.98 cm/hr radially.

Table I summarizes results of experiments with Rb^+ and Br^- . The velocities of the radial movement of the two ions were similar, but Br^- traveled longitudinally within the xylem about three times faster than did Rb^+ . The ratios, longitudinal velocity/radial velocity, were 19/1 and 73/1 for Rb^+ and Br^- , respectively.

DISCUSSION

Validity of the Method. The experimental method is straightforward, but in calculating the results a major assumption is made, the validity of which is not obvious. We assume that the velocity at which ions travel radially across the root into the xylem is the same at two points along the root several centimeters apart (equations 1 and 2, "Materials and Methods"). The assumption would be very doubtful if any applications had been made near the tip, where the xylem vessels are not yet differentiated or not yet mature. However, all applications were at least 10 cm from the tip. Over the distance that applications were made, microscopic examination revealed no major differences in number, size, and degree of maturation of

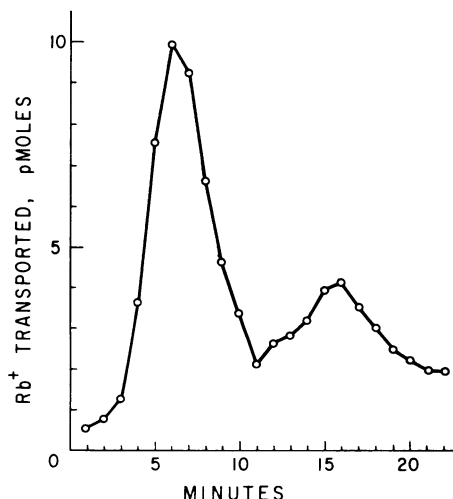


FIG. 3. Pulses of Rb^+ labeled with ^{86}Rb . The experimental solution contained 0.5 mM labeled RbCl and 0.5 mM CaSO_4 . The points of application were 3.6 and 10.6 cm from the cut end of the root.

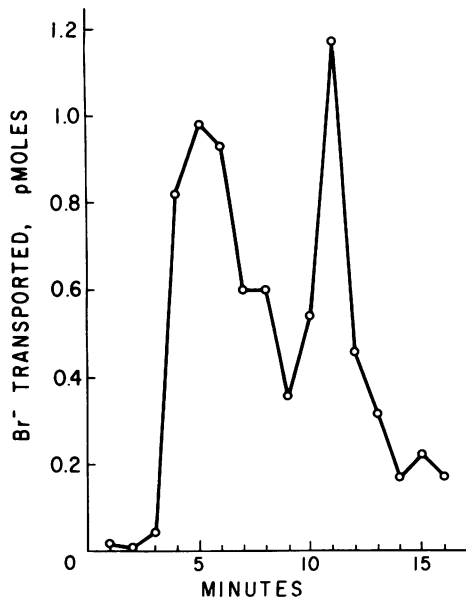


FIG. 4. Pulses of Br⁻ labeled with ⁸²Br. The experimental solution contained 0.6 mM labeled KBr and 0.5 mM CaSO₄. The points of application were 3.8 and 15.5 cm from the cut end of the root.

Table I. Summary of Velocities of Movement of Rubidium and Bromide through Corn Roots

The solutions used for pulse-labeling contained 0.5 mM CaSO₄ and either 0.5 mM RbCl labeled with ⁸⁶Rb or 0.6 mM KBr labeled with ⁸²Br. Application was for 1 min, and the length of root exposed at each point of application was 1 cm. The values are means ± confidence interval at 95%.

Ion	No. of Experiments	Longitudinal Velocity (v _l)	Radial Velocity (v _r)
		cm/hr	
Rb ⁺	8	35.25 ± 3.29	1.81 ± 0.85
Br ⁻	5	103.00 ± 10.58	1.41 ± 0.34

the cells. Anatomical observation, then, revealed no features that would tend to invalidate the assumption mentioned.

Rates of absorption of ions may vary along the root, in terms of amounts absorbed per unit time per unit length of root at different positions along the root (6, 7, and unpublished findings from the present investigation). Is this evidence against our assumption that the velocity of lateral movement of the ions across the root, in terms of cm/hr, is the same at two different positions along the root? We think not. We did experiments like those reported, but at higher concentrations (up to 50 mM). The amounts of ions absorbed at the point of application during the 1-min pulse were much higher at the higher concentration, but the velocities of lateral movement into the xylem were similar, whether the experiment was done at a low or a high concentration.

We also obtained essentially the same radial speed in experiments in which we varied the positions at which the two applications were made. The velocities, v_r, on this evidence do not depend to a significant degree on the particular points along the root where the applications are made.

There is evidence, not yet firm, of diurnal variations. The experiment shown in Figure 3 on Rb⁺ transport was done in the

morning, and resulted in a relatively high value for the velocity of radial transport (3.5 cm/hr), as described under "Results." The values for experiments done in the afternoon tended to be lower. The results given in Table I are means.

Interpretation of Results. In calculating the velocity, v_r, at which the ions progress across the root, we used the radius of the root as the length of the pathway. The radius was chosen as the nominal length of the radial pathway on the basis that any ion from the external solution which is eventually transported into the exudate enters the root volume at the surface of the epidermis and makes its way to a xylem vessel near the center of the root. No detailed assumptions are made as to what happens along that route. Some ions may be absorbed by a cell of the epidermis and then proceed within the symplast toward the stele and the xylem; others may diffuse within the "outer" (cell wall) space far into the cortex before being transported into the symplast, across the plasmalemma of a cortical cell. The degree of tortuosity of the path, once the ions have entered the symplast, is another unknown quantity. Without further assumptions, therefore, the radius of the root was taken as the length of the radial pathway, but the above discussion makes clear that this value is only a rough approximation of the length of the true symplastic pathway, which may be shorter or longer than the value used.

The radial velocities, v_r, were similar for Rb⁺ and Br⁻, but the longitudinal velocity, v_l, was about three times higher for Br⁻ than for Rb⁺. If movement up the xylem were simply a flow of a solution in a pipe the velocities would be expected to be the same for both ions. The slower speed of progression of the cation suggests that it gets involved in a chromatographic exchange migration on negatively charged sites of the cell walls of vessels (2). This interpretation gains force from unpublished findings that a divalent cation, Ca²⁺, which would be expected to have a higher affinity for negative sites than the monovalent Rb⁺, moved at a still slower speed (9.1 cm/hr).

Comparisons with Other Findings. How do the velocities of ion movement measured by means of the technique described here compare with previous results? There are no comparable prior experiments in which the velocities of both the radial and the longitudinal components of ion movements through roots were determined. Flow through the xylem of transpiring plants may be extremely rapid; for trees, Zimmermann and Brown (11) list values ranging from 1 to 44 m/hr, and still higher values have been reported. With a less refined technique than the present one, Läuchli *et al.* (9) estimated the longitudinal velocity of transport of Cl⁻ in exuding excised corn roots to be between 75 and 250 cm/hr—similar to the slowest rates reported for transpiring trees.

Since we are dealing with an osmotically driven movement of a solution through conduits it is of interest to note that the velocities measured for flow in the xylem of exuding roots closely correspond to those of another longitudinal flow which, according to the best available evidence, is osmotically driven: the movement of solutes in the phloem (4, 5).

As already pointed out, there seem to be no prior determinations of the velocity of lateral ion transfer into the xylem of roots. If we take the present results to reflect the velocity of symplastic transport, to a first approximation, we may compare them with estimates of symplastic transport in other systems. Arisz and Wiersema (1) report velocities of 1 to 4 cm/hr for symplastic movement of chloride in leaf tissue of the aquatic angiosperm, *Vallisneria spiralis*. The magnitude is similar to the values determined in the present investigation. Tyree (10) has discussed theoretical aspects of the transport of small solutes through the symplast.

Acknowledgments—We thank D. L. Fredrickson for technical help, C. E. Franti for biometrical assistance, and A. R. Spurr for microscopic examination of the roots.

LITERATURE CITED

1. ARISZ, W. H. AND E. P. WIERSEMA. 1966. Symplasmatic long distance transport in *Vallisneria* plants investigated by means of autoradiograms. IA. Substances translocated. Proc. Koninkl. Nederl. Akad. Wetenschappen C 69: 223-241.
2. BELL, C. W. AND O. BIDDULPH. 1963. Translocation of calcium. Exchange versus mass flow. Plant Physiol. 38: 610-614.
3. BRAY, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. 1: 279-285.
4. Crafts, A. S. and C. E. Crisp. 1971. Phloem Transport in Plants. W. H. Freeman, San Francisco.
5. Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons, New York.
6. HODGES, T. K. AND Y. VAADIA. 1964. Uptake and transport of radiochloride and tritiated water by various zones of onion roots of different chloride status. Plant Physiol. 39: 104-108.
7. KRAMER, P. J. 1969. Plant and Soil Water Relationships: A Modern Synthesis. McGraw-Hill, New York pp. 223-225.
8. LÄUCHLI, A. AND E. EPSTEIN. 1971. Lateral transport of ions into the xylem of corn roots. I. Kinetics and energetics. Plant Physiol. 48: 111-117.
9. LÄUCHLI, A., A. R. SPURR, AND E. EPSTEIN. 1971. Lateral transport of ions into the xylem of corn roots. II. Evaluation of a stellar pump. Plant Physiol. 48: 118-124.
10. TYREE, M. T. 1970. The symplast concept: a general theory of symplastic transport according to the thermodynamics of irreversible processes. J. Theor. Biol. 26: 181-214.
11. ZIMMERMANN, M. H. AND C. L. BROWN. 1971. Trees: Structure and Function. Springer-Verlag, New York.