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Effect of Time, Water Flow, and pH on Centripetal Passage of Radiophosphorus across Roots of Intact Plants[1]

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ABSTRACT

The effects of time, rate of the water flow, and ambient pH on centripetal passage of radiophosphorus across intact bean roots to the xylem were studied. Isotope which completed passage and entered the xylem stream, as well as amounts delivered to the plant top, served to measure centripetal passage.

Centripetal passage of radiophosphorus increased parabolically reaching a maximum after 1 hr and maintained this level during the 2nd hr. This pattern was consistent for all conditions studied. The curve suggested that passage did not progress as an abrupt front, but rather that it occurred through a phosphorus pool before reaching the xylem.

Differences in rate of water flow through test plants, accomplished by adjusting the humidity of the foliage environment, did not significantly affect centripetal passage of radiophosphorus. Water flow did, however, profoundly influence composition of the xylem stream by altering the solvent to isotope ratio.

Centripetal passage of radiophosphorus was not affected by solution pH in the acid range (pH 4.8, 5.2, 6.4), but was inhibited in the more alkaline range (pH 7.0, 7.5, 8.0). The similarity of these findings to those in the literature for phosphorus uptake by individual cells suggests that cell uptake may constitute the primary rate-limiting step in the over-all process of ion passage to the xylem.

The mechanics by which ions undergo centripetal passage to the root xylem have received increased scrutiny and progress has been reviewed periodically, most recently by Laties (14). Much of the experimental work employed detopped roots or root segments, where concentration of test ion in the exudate served as the measure of centripetal passage. Techniques were recently developed in this laboratory for determining centripetal passage of radiophosphorus across roots of intact functioning plants. They were employed to study the effects of time, rate of water flow, and ambient pH on centripetal passage of the isotope across bean roots.

MATERIALS AND METHODS

Test plants, *Phaseolus vulgaris* L. var. Red Wade, were grown to the proper stage of maturity in a controlled environment facility. Approximate settings for the 18-hr photoperiod were: temperature 28 C, relative humidity 55%, light 1500 ft-c at plant height. During the 6-hr dark period temperature was 24 C. Plants were started from seed on a damp blotter and transferred to an aerated solution supplied with all nutrients essential for rapid healthy development. Plants were taken for treatment 13 days from seed when the first trifoliate leaves were approximately 2 cm long.

The amount of radiophosphorus which reached the xylem sap following addition of the isotope to the root medium measured centripetal passage. Radioactivity of the sap was determined in a specially designed environment control system described in detail elsewhere (6) and shown diagrammatically in Figure 1. Each of three isolated compartments housed a distinct part of the test plant. Root and foliage compartments were held at rigidly controlled functional environments. The stem was held at 0 C to arrest metabolic accumulation of isotope in the stem.

Plants were taken about midpoint in the photoperiod and secured in the apparatus. Roots were submerged in 30 ml of the old growth solution, and the plants were allowed to equilibrate in the new environment for 30 min. The old solution was then replaced with 30 ml of 0.1 mM KH_2PO_4 containing ^{32}P as phosphoric acid at about 32 × 10^3 cpm/ml. Solutions also contained 0.3 mM $Ca(NO_3)_2$. The test solutions were renewed every 15 min to minimize depletion. Radioactivity of the chilled stem was recorded as a continuous graph over a 2-hr period following introduction of the radiophorphorus solution. All measurements were replicated at least 12 times.

Total radioactivity of the chilled stem (Z) represented isotope in the xylem sap (S) as well as temperature-insensitive building of isotope in the stem (X). X was a direct and linear function of S and time t according to the expression $X = 2.42\ St$. S was derived from the expression $S = Z/(2.42\ t + 1)$. These calculations were reviewed in an earlier report (6).

After the 2-hr uptake period, total isotope delivered to the plant top was determined. The stem was severed at a point slightly above the solution meniscus, and the plant top was dried, ashed, and radioactivity was determined.

RESULTS

Time-Course. Content of radiophosphorus in the xylem sap following introduction of the isotope is given in Figure 2a. Concentration increased parabolically, reaching a maximum after the 1st hr, and this maximum was maintained during the 2nd hr. Adjustment of the plant to the test conditions was not a factor since the time-course was similar whether the isotope was added immediately to the KH_2PO_4-$Ca(NO_3)_2$ solution or after a delay of as much as an hour.

The pattern for delivery of radiophosphorus to the plant top (Fig. 2b) confirmed that the sap readings accurately reflected centripetal passage of isotope. Delivery increased at an accel-

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erated rate during the 1st hr and followed a more linear rise thereafter. This is the anticipated pattern were the xylem sap data a reflection of centripetal passage.

Rate of Water Flow. Transpiration, and thus rate of water flow through test plants, was varied by adjusting the relative humidity in the foliage compartment to 30 or 80% for "high" and "low" rates of water flow, respectively. The effects of these treatments on radiophosphorus in the xylem sap are given in Figure 3a.

The basic time-course pattern described earlier was maintained in both treatments, but low water flow rate (high humidity) yielded a xylem sap considerably more concentrated than the high rate of flow (low humidity).

Rate of water flow had little effect on delivery of isotope to the plant top. Radiophosphorus in the top was statistically unchanged by the treatments (Fig. 3b). The data for the tops indicate that the differences in xylem sap composition noted above were not caused by changes in centripetal passage of isotope, but resulted instead from dilution of the sap by water.

Ambient pH. The pH of solutions in the previous experiments was held near 6.4 throughout the 2-hr test period. In the current tests, solutions were made acid or alkaline with 0.1 N HCl or NaOH, and the effect on centripetal passage of radiophosphorus was noted. Drifts in pH were minimized by the routine practice of renewing solutions at 15-min intervals. Also, each aliquot was checked twice during the period of its use with a Beckman Model 180 pH meter and adjusted as needed.

Results are given in Figure 4a. Solutions in the acid range (pH 4.8, 5.2, 6.4) had no effect on centripetal passage of radiophosphorus as reflected by the xylem sap composition. The more alkaline solutions (pH 7.0, 7.5, 8.0), however, markedly reduced centripetal passage of the isotope, the effect increasing with increasing pH.

It was demonstrated earlier that composition of the xylem sap could be altered by water flow through the plant as well as by centripetal passage of solute. It is important, therefore, to verify that water flow was not a factor in bringing about the pH effects discussed, but that they indeed reflected changes in the centripetal passage of isotope. Such verification is afforded by the close agreement between the xylem sap data and those for delivery of radiophosphorus to the plant top (Fig. 4b). Delivery to the top was not affected by the acid solutions, but was diminished by increases in pH to 7.0 and above.

DISCUSSION

Time-Course. The current results represent the first records of their kind of centripetal passage of ions across intact roots, so similar data are not available for comparison. However, the time-course of centripetal passage has been plotted in exudation studies with detopped roots. Pettersson (19) recorded an initial equilibration period similar to that in Figure 2a for sulfur in sunflower roots. This was followed by a steady state condition (as in the current data), or a decline in passage, depending on when the isotope was introduced following detopping. Läuchli *et al.* (16) also noted a steady state condition for chloride in exudate from corn roots. Other exudate experiments, however, showed little similarity to current data for intact plants (Broyer for bromide (3); Hodges *et al.* for chloride (12); Lopushinsky for phosphorus (17); Vaadia for calcium, potassium, magnesium (21)).

The time-course of centripetal passage has also been followed by measuring delivery of test ion to the plant top. Hanson and Biddulph (11) found an accelerated increase in potassium in the tops of intact bean plants over the 1st hr,

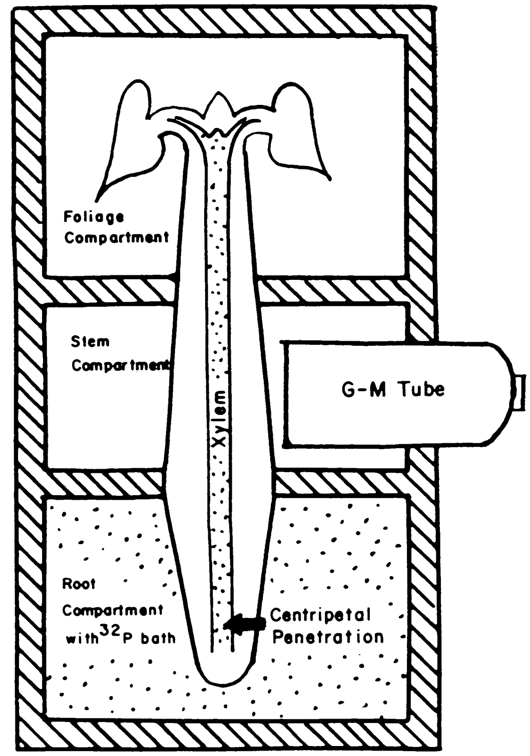


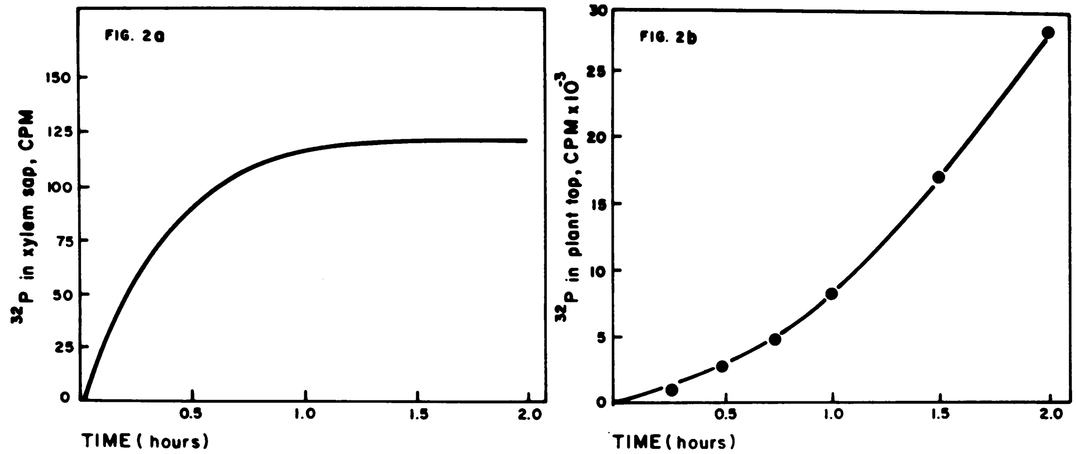
FIG. 1. The environment control system for measuring centripetal passage of radiophosphorus across intact bean roots by noting the level of isotope in the xylem sap. Controls for the foliage compartment were: 26 ± 1 C; relative humidity 50 ± 2%; light 1500 ft-c at plant height. The stem compartment was at 0.0 ± 0.2 C. The root compartment was at 24 ± 0.5 C, with continuous aeration, and 0.1 mM $KH_2{}^{32}PO_4$ plus 0.3 mM $Ca(NO_3)_2$.

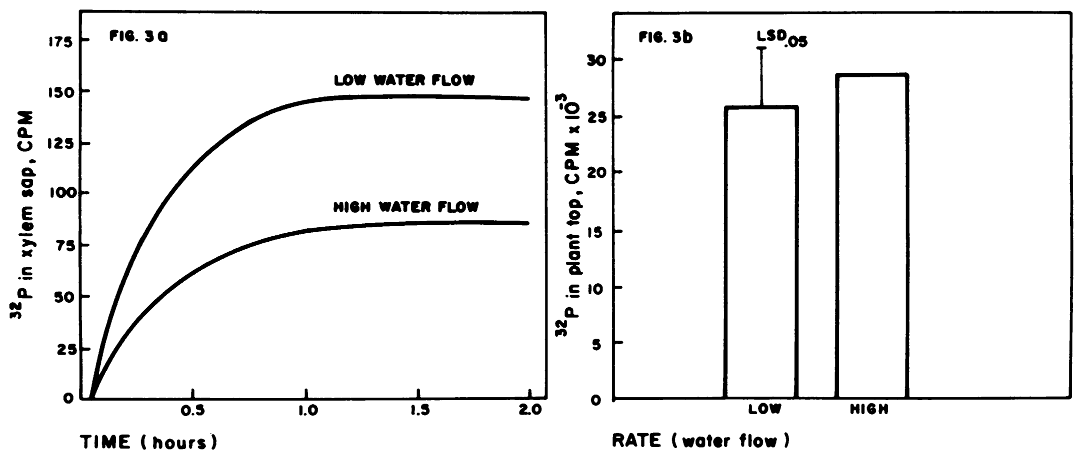
followed by a linear increase. Crossett (4) showed a similar pattern for phosphorus in barley plants, except that initial equilibration occurred over a 5-hr period. These results on the whole agree with findings in Figure 2b.

The time-course pattern for centripetal passage of phosphorus may provide a clue to the mechanics of passage. The smooth and gradual rise during the initial equilibration period suggests that passage did not occur as an abrupt front. Length of the equilibration period (about 1 hr) also argues against an abrupt front. Rather, the results favor Crossett's interpretation of a phosphorus "pool" through which passage must occur (4). The specific radioactivity of the pool and of the ion complement leaving the pool would initially increase with time as the stable phosphorus was replaced by radioactive phosphorus. This change would be reflected in the xylem sap in the pattern described in Figure 2a.

Rate of Water Flow. The relationship of transpiration-induced water flow to plant nutrition has been given extensive review by Russell and Barber (20), Brouwer (2), Kramer (13), and others. The view is commonly held that the bulk of ion passage to the xylem involves metabolic processes and is affected little if at all by normal changes in mass flow of water through the system. Some water-dependent passive flux may occur with the transpiration stream, but this fraction is usually minor. The current data which showed little effect of water flow on passage of radiophosphorus to the xylem are in general agreement with this scheme.

Water did, however, exert a profound influence on the composition of the xylem stream by affecting the ratio of solvent to radiophosphorus. Such dilution by water may serve to ex-





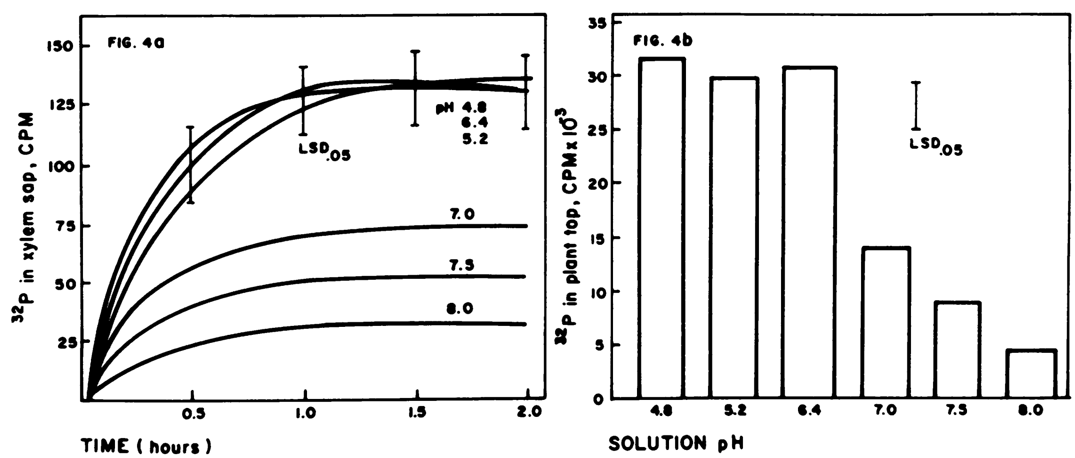


FIG. 2. Radiophosphorus in the xylem sap (a), and isotope delivered to the plant tops (b), as functions of time after addition of radiophosphorus to the root medium of intact bean plants.

FIG. 3. Effects of "high" and "low" rates of water flow through intact bean plants on (a) radiophosphorus in the xylem sap over a 2-hr period following addition of the isotope to the root medium, and (b) total amounts of radiophosphorus delivered to the plant tops during this period.

FIG. 4. The effect of pH of the radiophosphorus solution on (a) radiophosphorus in the xylem sap following its addition to the root medium of intact bean plants, and (b) total amounts of isotope delivered to the plant tops during the test period.

plain past contradictions in results comparing exudate concentration with ambient solution concentration. Most reports represented conditions in which mass flow of water through the roots was eliminated by detopping, and further movement restricted to osmotic phenomena. Exudate concentration under these circumstances invariably exceeded source solution concentration. Distinctly different results were obtained in the fewer instances where mass flow of water was induced by pressure or tension devices. A dilution of the exudate occurred under these circumstances so that exudate concentration was less than that of the source solution (7, 19). This situation may hold as well for the xylem stream of intact rapidly transpiring plants (5, 18).

Ambient pH. The effect of pH on centripetal passage of radiophosphorus (Fig. 4a) is similar to that demonstrated for uptake of phosphorus in excised barley roots (9, 10). This comparison is of particular interest as it demonstrates a similarity of response between the over-all process of centripetal passage and a component step of that process. It may thereby provide a clue as to the rate-limiting step in passage to the xylem.

To explain, the studies with excised barley roots dealt with ion passage into individual cells. Centripetal passage to the xylem, on the other hand, is considerably more complex. It involves a sequence of steps of which uptake by the cell is only the first. Other steps include centripetal transport of ions via the symplast, and finally, copious efflux of ions from the symplast into the xylem lumen (1, 15, 16). Each of these steps could conceivably limit phosphorus transport to the xylem. Yet, the similarity of response of such transport to that of cell uptake, as described above, suggests cell uptake as the rate-limiting step. Whatever the influences of other events involved in passage of radiophosphorus to the xylem, they apparently were not of sufficient magnitude to mask or seriously distort the impression made during initial movement of ion across the cell membrane to the symplast.

Similar deductions were expressed by Läuchli *et al.* (15) based on findings that chloride passage across corn roots mirrored cell uptake of this element with respect to kinetics, interaction with bromide, response to the phosphorous uncoupler, carbonylcyanide *m*-chlorophenylhydrazone, and the inhibitor, oligomycin. These workers suggested initial movement across the plasmalemmas of cortical cells as the rate-limiting step to centripetal passage.

Further evidence may be cited to show that ion movement to the xylem reflects directly the conditioning influence of the initial cell uptake step. Franklin (8) found that potassium, magnesium, and ammonia enhanced phosphorus uptake in cells of excised root material. Earlier work in this series (7) showed a similar effect of these ions on centripetal passage of phosphorus.

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