
THE TRANSPIRATION STREAM AND UPWARD TRANSLOCATION OF MINERAL IONS^{1, 2}

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ABSTRACT

Ca⁴⁵ and P³² were supplied simultaneously to young *Phaseolus vulgaris* L. seedlings grown in solution. After short periods of uptake, the Ca⁴⁵/P³² ratios in opposite leaves and opposite leaflets were very similar. This relationship also held when Ca⁴⁵ and Cs¹³⁷ were paired. Ca⁴⁵/P³² ratios in lateral halves of the same leaf were in good agreement, also. However, the basal halves of leaves always had higher Ca⁴⁵/P³² ratios than terminal halves. There was a negative acropetal gradient of Ca⁴⁵/P³² ratio in the stem, but the ratio in the trifoliolate leaves was higher than in the primary leaves. When roots were removed prior to supplying the radioisotopes, the Ca⁴⁵/P³² ratios in the foliage and stems were lower than in plants with roots intact. The data obtained support the *en masse* flow concept of ion movement through the stem.

The usual mechanism proposed for the upward translocation of mineral ions in plants is visualized as a bulk transport of these ions in the transpiration stream. This concept has led to considerable investigation attempting to ascertain the precise dependency of salt uptake and distribution on the movement of water through the plant. Several investigators have shown that an increase in transpiration rate is accompanied by an increase in salt uptake and movement to the shoots (Hylmo, 1953, 1955, 1958; Brouwer, 1954, 1956; Kylin and Hylmo, 1957; Smith, 1957). However, the mechanism whereby transpiration influences the overall

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movement of salts from the outer surface of the roots to the shoots is not agreed upon. There are, basically, two opposing views at present. The point of conflict lies in explaining the transfer of salts from the root surface, across the cortex, to the stele of the root. One view, the "passive transfer" hypothesis, visualizes the movement of ions as a mass flow through the apparent free space of the root. The opposing view, the "active transfer" hypothesis, visualizes the movement of ions across the root as an active process dependent upon the release of energy through metabolic processes. The pros and cons of these hypotheses have been reviewed by Russell and Barber (1960).

In spite of differences of opinion as to how ions reach the xylem of the root, it is generally agreed that the subsequent movement of ions to the shoots occurs as a flow *en masse* in the transpiration stream. If this is so, it follows that the ratio of ions accumulating in similar leaves or leaflets after transpiration periods of short duration should be relatively uniform, assuming that the pathways traversed by these ions to the respective leaves are also relatively uniform. For test areas at similar levels of insertion on the plant, such as opposite leaves or opposite leaflets, this assumption seems reasonable, and any significant disagreement among ratios at these comparable test sites should, therefore, indicate that a mechanism of upward solute movement visualized as a flow *en masse* in the transpiration stream does not adequately describe this process. The investigation reported here tests this mass flow hypothesis. Ca^{45} and P^{32} (or Ca^{45} and Cs^{137}) were supplied simultaneously to plants for short periods. It was assumed that after short term transpiration periods, the relative uptake and distribution of two concurrently supplied radioisotopes could be ascertained and be accurately interpreted as material which entered the plant only during these limited time periods.

MATERIALS AND METHODS

Asgrow's Black Valentine bean (*Phaseolus vulgaris* L.) was used as the experimental plant in all investigations described herein. Seedlings were raised in a modified Hoagland solution (Meyer et al., 1955) in a controlled environment room operating on a 14-hr photoperiod. The temperature was maintained at 76 ± 1 C during the light period and 72 ± 1 C during the dark period. Relative humidity was 60 to 65 per cent during the light period and 90 to 100 per cent during the dark period. The light intensity was approximately 1000 ft-c at the base of the plants. All experiments were conducted in this room, also.

Radioisotopes were supplied in pairs to the plants as constituents of an aerated solution of the following composition: 10^{-3} M KNO_3 , 10^{-3} M MgSO_4 , 3×10^{-4} M $\text{Ca}(\text{NO}_3)_2$, and 2×10^{-4} M KH_2PO_4 . After an absorption period of about 2 hr, when a suitable amount of activity could be detected in the plant parts designated for analysis, the plants were harvested and prepared for assay. Plant parts were sliced into small pieces, dried overnight at 50 C, and wet ashed at a temperature of 85 C in a mixture of three parts concentrated nitric acid and one part 60 per cent perchloric acid. The digestion solutions were refluxed until clear, and then evaporated to dryness, resulting in the deposition of a clear precipitate in the bottom of each beaker. The precipitate was dissolved in 1 ml of 0.1 N HCl, transferred to a glass planchet, and dried under 300-w flood lamps mounted 14 inches directly overhead. This procedure ensured the deposition of clear, uniform samples, which was essential for valid self-absorption corrections.

Samples were counted with an ultra-thin end window gas flow detector. A sufficient number of counts was totalized in all cases to insure less than 1 per cent error at the 95 per cent confidence level. Counts were corrected for background and coincidence losses in all cases and, in the case of Ca^{45} , for self-absorption as well. The counts for Ca^{45} were separated from those of P^{32} or Cs^{137} by the technique of differential absorption (Comar, 1955). Samples were counted first in the normal manner and then recounted with an aluminum absorber (42.2 mg/cm²)

inserted between the geiger tube and the sample. The partial counts for the respective radioisotopes were then calculated by substitution of the total counts (C_0 and C_t) in the following set of simultaneous equations:

$$(1) \quad \frac{B}{A} = \frac{F_a - (C_t/C_0)}{(C_t/C_0) - F_b}$$

$$(2) \quad A + B = C_0$$

- where: A = partial activity of P^{32} (or Cs^{137}), in counts per minute
 B = partial activity of Ca^{45} , in counts per minute
 C_0 = total counts per minute with no absorber
 C_t = total counts per minute with absorber
 F_a = fraction of P^{32} (or Cs^{137}) counted with absorber
 F_b = fraction of Ca^{45} counted with absorber.

F_a and F_b were determined empirically for each experiment by counting standards of the respective radioisotopes with each group of samples counted.

EXPERIMENTS AND RESULTS

Experiment I.—Four plants in which the third trifoliate leaf had just begun to expand were placed in radioactive solution for 2.5 hr. The Ca^{45}/P^{32} ratio of the experimental solution was 4.4. The root systems were removed from two of the plants, and two of the plants were left intact. The Ca^{45}/P^{32} ratios in the leaves and stem sections after 2.5 hr of absorption are shown in figure 1. It is apparent that there was a considerable degree of correlation of Ca^{45}/P^{32} ratios between similar leaves and leaflets of the intact plants (fig. 1, plants 1 and 2). The ratios in the primary leaves were nearly identical, and the ratios in opposite leaflets of trifoliate leaves, though not as close, nonetheless showed reasonable conformity. These results, then, accord with at least one of the requirements of the *en masse*

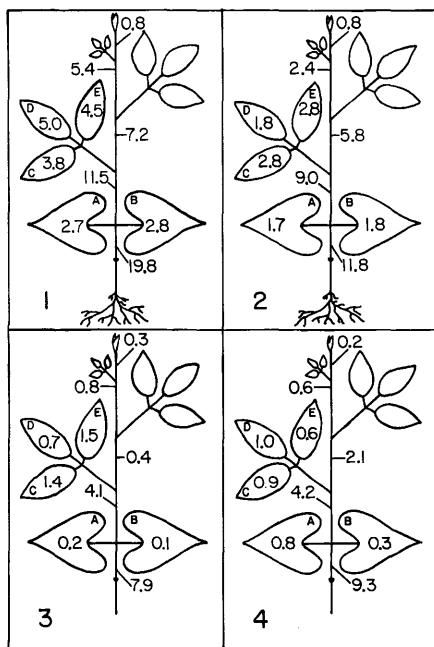


FIGURE 1. Ca^{45}/P^{32} ratios in various regions of bean plants after a short transpiration period.

flow hypothesis, namely that the ratio of ions accumulating at comparable test sites should be similar.

The ratio of $\text{Ca}^{45}/\text{P}^{32}$ in the stem decreased at successively higher internodes. In spite of this negative acropetal gradient, however, the ratios in the trifoliolate leaves were consistently higher than those in the primary leaves. The ratio in the trifoliolate leaflets was 1.6 times that of the primary leaves for both plants 1 and 2. Thus, in spite of different absolute values for the two plants, the general pattern was much the same.

The general picture for the root-excised plants (fig. 1, 3 and 4) was quite similar to that of the intact plants, the only exception being that the ratios in the former are weighted in favor of P^{32} , indicating that the entry of P^{32} was greatly increased relative to Ca^{45} . It appears that the roots exhibited a high degree of discrimination between P^{32} and Ca^{45} .

Experiment II.—In this experiment it was desired to determine to what extent foliage above or below a particular leaf (the first trifoliolate) influenced the distribution of the radioisotopes to that leaf, and to investigate the effect of the leaves on

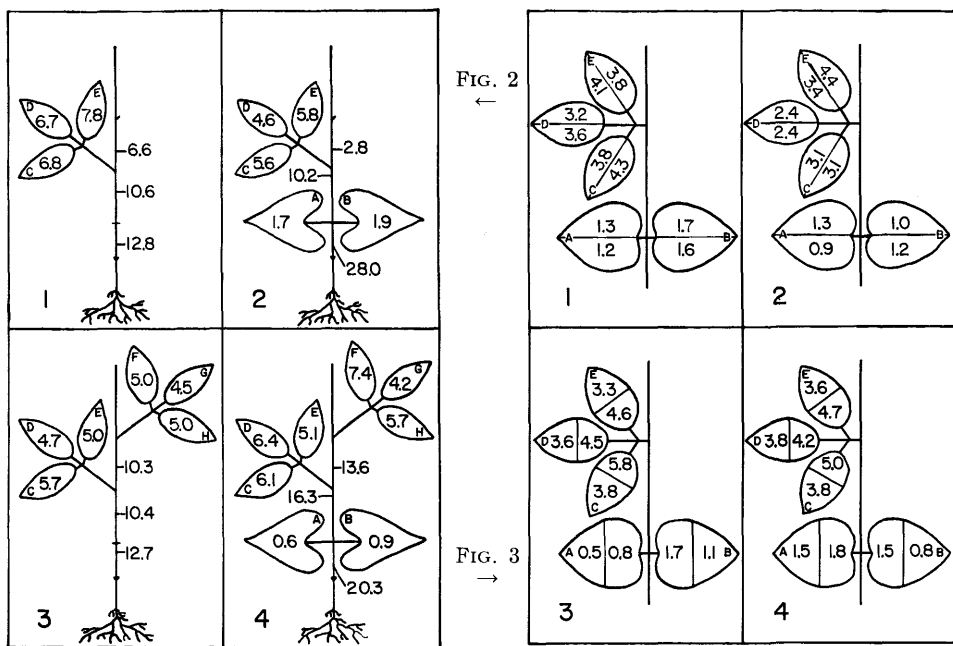


FIGURE 2. $\text{Ca}^{45}/\text{P}^{32}$ ratios in various regions of bean plants, pruned as shown, after a short transpiration period.

FIGURE 3. $\text{Ca}^{45}/\text{P}^{32}$ ratios in designated half-leaves of bean plants after a short transpiration period. Only test leaves are shown in the figure.

the gradient in the stem. Plants similar to those used in Experiment I were pruned in various ways and placed in experimental solution with a $\text{Ca}^{45}/\text{P}^{32}$ ratio of 5.4 for two hours. The results of this experiment are shown in figure 2. The plants were pruned as illustrated.

In general, the $\text{Ca}^{45}/\text{P}^{32}$ gradients in the stem were qualitatively similar, but important quantitative differences were revealed. The primary leaves appeared to be a sump for P^{32} , thus leading to a very high ratio of $\text{Ca}^{45}/\text{P}^{32}$ in the internode just below (compare plants 2 and 4 versus 1 and 3 in fig. 2). The ratios in the leaflets of the first trifoliolate were not influenced to any great extent by the presence or absence of other leaves on the plant.

Experiment III.—Since there were a few observations of disagreement in ratios between similar test areas in previous experiments, it was decided to investigate differences within the leaves or leaflets themselves. Four plants similar to those used in previous experiments were placed in experimental solution with a $\text{Ca}^{45}/\text{P}^{32}$ ratio of 3.9 for 2 hr. When harvesting these plants, the leaves from two of them (fig. 3, plants 1 and 2) were sliced to give two lateral halves, the midrib not being included in either sample. In the other pair of plants (fig. 3, 3 and 4), the leaves were divided into a basal half and a terminal half.

It has been shown that particular areas of plants, and even halves of leaves, are supplied by specific root areas, and little or no translocation occurs to the unsupplied area (Ririe and Toth, 1952; Rinne and Langston, 1960). This being the case, it was thought that if a root which might supply one-half of a leaf or leaflet were broken in handling the plants, there may be a greater mass flow of solution in through the cut root, and, accordingly, the longitudinal halves of a given leaf or leaflet may be expected to show greater disparities in the $\text{Ca}^{45}/\text{P}^{32}$ ratios than the transverse halves. There was, however, considerable agreement between lateral halves of the same leaf. On the other hand, in the second pair of plants (fig. 3, 3 and 4), the basal half had a consistently higher ratio of $\text{Ca}^{45}/\text{P}^{32}$ than the terminal half of each leaf. The gradient of decreasing ratios in the leaf

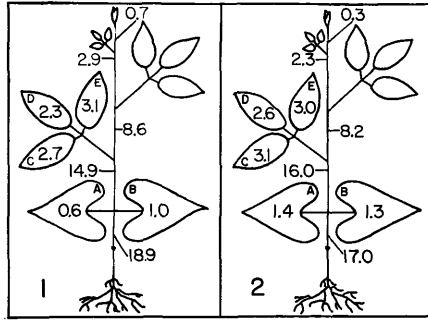


FIGURE 4. $\text{Ca}^{45}/\text{Cs}^{137}$ ratios in various regions of bean plants after a short transpiration period.

follows, therefore, that in the stem. From the results of this experiment, it seems probable that broken roots did not contribute to disparities in ratios between similar leaves or leaflets.

Experiment IV.—Whereas the previous experiments were concerned with the ratio of a cation and an anion, this experiment was concerned with the ratio of two cations. Two intact plants, similar to those used in the first experiment, were placed in experimental solution having a $\text{Ca}^{45}/\text{Cs}^{137}$ ratio of 1.7 for 2 hr. The results, shown in figure 4, were very similar to those of Experiment I. There was a decreasing acropetal gradient of the ratio in the stems, and the trifoliolate leaves had higher values than the primary leaves. Thus, as regards uptake and distribution relative to Ca^{45} , it appears that Cs^{137} resembled P^{32} . Even though there is a possibility that there were volatile losses of Cs^{137} during sample preparation, it is not likely that the qualitative patterns of distribution were affected much. The literature at present seems inconsistent with regards to the care necessary to avoid such losses (Bange and Overstreet, 1960; Cline and Hungate, 1960).

DISCUSSION

In general, the results obtained support the *en masse* flow concept of ion movement through the stem. Ratios of two mineral ions, whether two cations or one cation and one anion, are quite similar in comparable leaves or leaflets after short

periods of uptake. It is important to note, however, that even though most of the data obtained support the view that ions move *en masse* in the xylem, the complications arising from differential absorption and differential metabolic accumulation in transit must be underscored, and hence no one-to-one correspondence in water-ion transport rates should be expected.

The mass flow of ions in the transpiration stream has been seriously questioned in recent years (Biddulph et al., 1961; Bell and Biddulph, 1963). Using tritiated water and Ca^{45} , Biddulph et al. (1961) obtained data which they felt was not compatible with a mass flow explanation of calcium movement in the transpiration stream. They concluded that the xylem cylinder of the bean stem operates as a chromatographic column, and calcium movement up the stem occurs as exchange along the walls of the conducting channels. Bell and Biddulph (1963), again studying Ca^{45} movement in bean plants, obtained results which they felt were consistent with exchange movement and "wholly incompatible with mass flow." A serious difficulty of the chromatographic hypothesis, however, in the present study, is the positive $\text{Ca}^{45}/\text{P}^{32}$ and $\text{Ca}^{45}/\text{Cs}^{137}$ gradient in the leaves in contrast to the negative acropetal gradient in the stems. If the calcium is visualized as chromatographing through the stem, then this pattern is not easily explained. Therefore, it seems that complete dismissal of the mass flow theory of ion movement in the transpiration stream appears highly improbable.

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