TRANSPIRATION STREAM & ASCENSION OF CALCIUM [1, 2]

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INTRODUCTION

Since Cline's report (5) of a very slow equilibration in the leaves of tritiated water absorbed by the roots of bean plants, no resolution has been made between his expressed alternative views that this was due A, to failure of tissue water to exchange with the incoming tritium-labeled water, or B, to a large isotope effect. During the preparation of the present manuscript Vartapetyan and Kursanov (14), using H_2O^{18}-enriched water in the nutrient solution, have also reported a slow equilibration of this tracer in the leaves (& roots) of the bean plant. In addition, they grew sunflower plants from seed with H_2O^{18}-enriched water and observed almost complete equilibration in all parts of the plant and thereby showed that the failure to equilibrate was due to failure of mixing, and that there remained the possibility of only an insignificant isotope effect. Even this was explainable by unlabeled water brought into the experiment in the seed, or to the formation of metabolic water in which there was a slight isotope effect.

Our report confirms the slow equilibration in leaves of the labeled water delivered in the transpiration stream, and in addition shows the progress of the labeled water through the stem and into the leaves in a way which discloses the path and manner of movement of the transpiration stream. The Ca^{45} isotope was used with the tracer water in order to compare the ascent of a mineral marker. Calcium was selected as it is not subject to a significant redistribution from leaves and could be expected to reside in the leaves into which it was delivered (4). The degree of dependence between the movement of a mineral component and the movement of the transpiration stream has never been satisfactorily investigated. The traditional concept is that mineral nutrients move upward in the xylem vessels in conjunction with the transpiration stream; once started the water and minerals move together and, with the exception of those salts removed by absorption along the way, reach their final destination in the leaves (6). The data obtained in the present study indicate that neither the water nor the calcium ascends exclusively in the vessels, but the stem as a whole functions, to some extent, as a pathway for ascent. As a result, a substantial portion of the ascending calcium moves by exchange on biocolloids rather than by mass movement in the vessels.

METHODS

Bean plants (*Phaseolus vulgaris* L. var. Red Kidney) were grown in a half-strength Hoagland solution, with micronutrients and aeration, to a stage where the third trifoliate leaf was about half expanded. The growth conditions were: temperature 23± 1° C, light 1,000 to 1,200 ft-c (fluorescent) on a 12-hour photoperiod, relative humidity 60 ± 5%. Two series of four plants each were then placed in a treatment chamber maintained under conditions similar to those above and wherein the water in the nutrient solution was labeled with THO and the calcium with Ca^{45}. In the first series tritium, as THO, was present at 0.0207 μc/ml and Ca^{45} at 0.167 μc/ml; in the second series THO was present at 0.0196 μc/ml and Ca^{45} 0.0167 μc/ml. The treatment chamber was designed to exhaust the air flowing over the leaves in order to remove any THO vapor transpired.

Plants were removed from the tracer solutions in the treatment chamber at 0.75-, 1.5-, 3-, and 6-hour intervals in the first series and 6-, 24-, 48-, and 72-hour intervals in the second. As each plant was removed, the hypocotyl and root were cut from the stem at the cotyledonary node (which was 2± ⅛ in above the root) and discarded. The top was divided into the following sections: primary leaves, first, second, and third trifoliate leaves, five basal stem sections of 1-inch length, each divided into bark and wood, two sections above these (undivided), and top (the remainder). Bark, as herein referred to, is the tissue exterior to the cambium, and wood, or xylem cylinder, that interior to it (4). The petioles of the leaves were analyzed separately in the second series (6–72 hr). Each section was analyzed for tritiated water (2) and Ca^{45} (4). Total calcium analyses were made on separate comparable plants by a chemical method (1).

The original experiment was designed to include a second part using the growth of bean plants from seed in tritiated water as an additional test for an isotope effect. The results of the first experiment, however, settled this matter against a measurable isotope effect, so that the latter experiment was unnecessary.

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[2] The radioisotopes were acquired from the U.S. A.E.C., Oak Ridge, Tenn.

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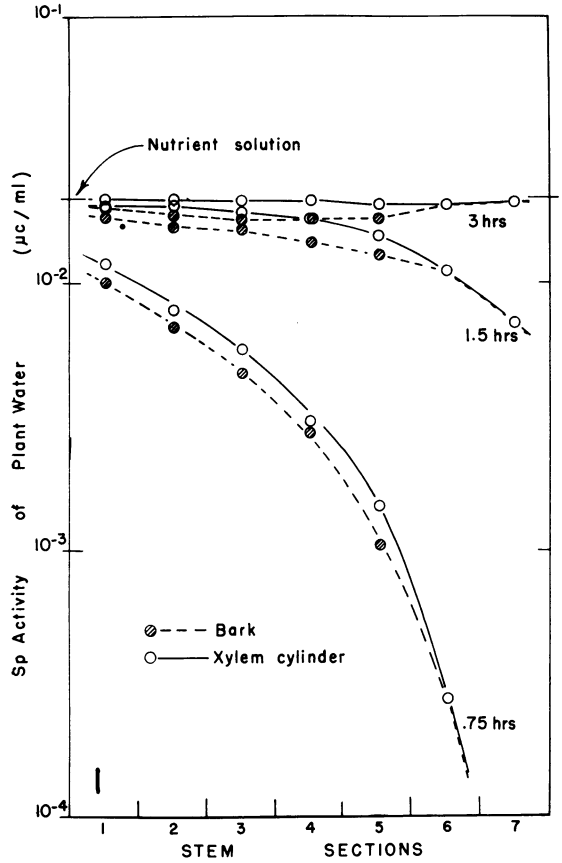
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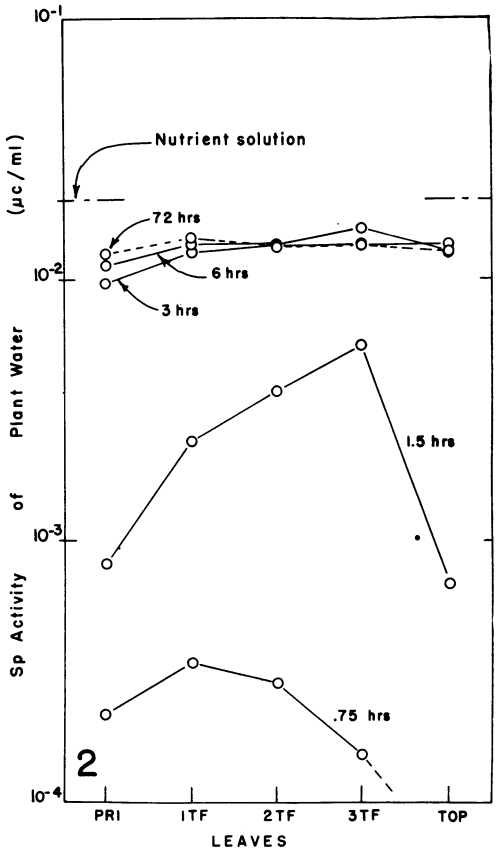
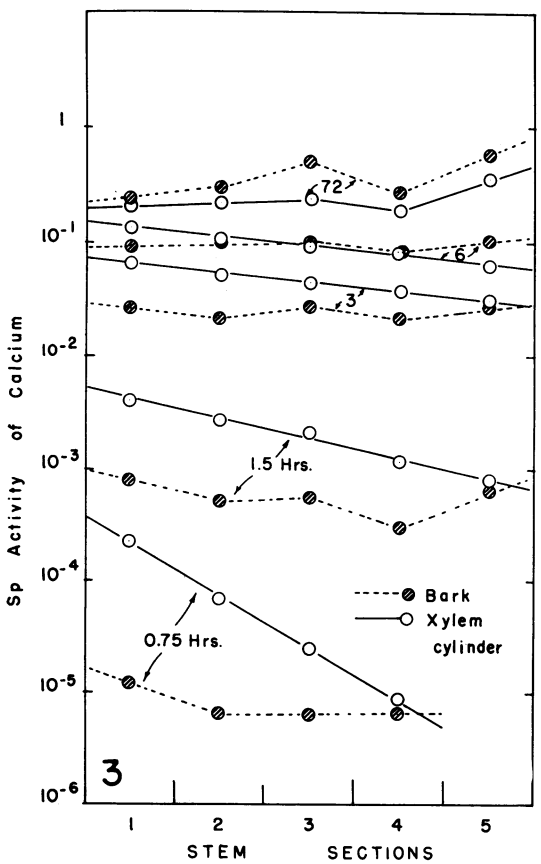
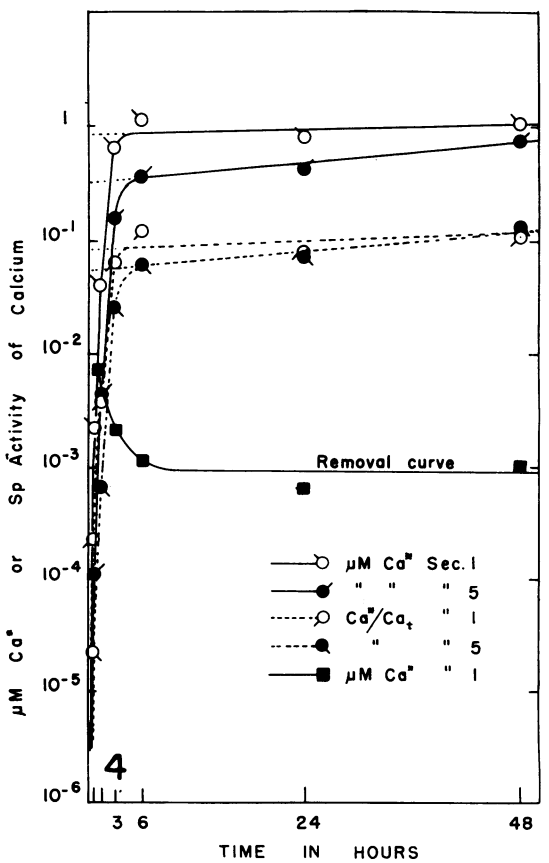
3 hrs
1.5 hrs
.75 hrs
Bark
Xylem cylinder
Sp Activity of Plant Water (μc/ml)
STEM SECTIONS
1


Nutrient solution
72 hrs
6 hrs
3 hrs
1.5 hrs
.75 hrs
Sp Activity of Plant Water (μc/ml)
PRI 1TF 2TF 3TF TOP
LEAVES
2


72
6
3
1.5 Hrs.
0.75 Hrs.
Bark
Xylem cylinder
Sp Activity of Calcium
STEM SECTIONS
3


Removal curve
μM Ca" Sec. 1
" " " 5
Ca"/Ca$_t$ " 1
" " 5
μM Ca" " 1
μM Ca" or Sp Activity of Calcium
TIME IN HOURS
4

The results presented herein are from single plants, each a member of a sequence of treatments. How well these results represent the progress of the two substances through the plant can be judged best by their consistency with each other rather than their conformity, as individuals, to an average, for there was no replication in that sense.

Results & Discussion

THO: Xylem Cylinder. A comparison of the specific activity of the water recovered from each plant section with the specific activity of the water in the nutrient solution was used to indicate the extent to which the labeled water from the nutrient solution had replaced the indigenous water or that initially present in the tissues. The data for individual stem sections at each time interval are shown in figure 1. These data show the measurable front of the tracer water to be near the top of the sixth section after 45 minutes. This is 8 inches from the root, and assuming, from an extrapolation, a starting point 1½ inches below this, at zero time, the measurable average rate of ascension of the tracer water in the xylem cylinder was approximately 12 inches per hour (30.5 cm/hr). The tracer water was not confined to movement in the vessels, for 50 % of the indigenous water in the central cylinder of the first stem section above the cotyledonary node was replaced by tracer water in 45 minutes. At 1½ hours (45 min later) the 50 % replacement value was 4½ inches higher up the stem. Thus, it appeared that the zone of 50 % replacement progressed up the xylem cylinder at the rate of about six inches per hour. Similarly, it can be shown that the zone of complete replacement progressed up the xylem cylinder at about three inches per hour. All water in the two lowest sections of the xylem cylinder and 98 % or more in the third and fourth sections was replaced within 3 hours. Therefore, it was concluded that while the xylem vessels may have served as the channel of least resistance for the flow of the transpiration stream, all of the xylem cylinder served to some extent for the ascent of water cf., (12).

Failure of the transpiration stream to remain confined to the xylem vessels was also shown by the distribution pattern of the tracer in the stem sections. A logarithmic plot of the specific activity of the tracer water in each stem section against the distance from the root resulted in a series of non-linear curves, figure 1. The nature of the curves and the manner in which they approached zero slope resembled the theoretical curves obtained by Horwitz (8) for the flow of a tracer through tubes in which a reversible loss through the lateral walls occurred. If the loss were irreversible, with no return movement, the curves were linear. Conformity of the present data with the theoretical curves for reversible loss corroborated the former evidence for the involvement of the entire xylem cylinder in the ascent of water.

That part of the transpiration stream moving in the vessels had little continuous molecular identity, since individual molecules exchanged out and into the stream at random. Because of the exchange, only the entity *water* could display the maximum theoretical rate of ascension in the vessels. The rate displayed by individual tracer molecules would, on the average, fall far short of this value. This can be shown as follows:

By dividing the maximum volume per hour of tracer water which had moved past the cotyledonary node by the observed rate of ascension (obtained above), the cross-sectional area of xylem cylinder necessary to carry the stream was obtained. The cross-sectional area required, for the indicated rate of ascension, was almost exactly twice the actual cross-sectional area of the xylem cylinder. This indicated the observed rate of ascension of THO, taken as an average value through 9½ inches of stem, was, at best, only half the maximum rate. The front of the THO tracer was obviously more or less continu-

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Fig. 1. The specific activity of the water recovered from each section of bean stem at 0.75, 1.5, and 3 hours, after placing the plants into a nutrient medium containing THO. At 1.5 hours the specific activity in the lower part of the xylem cylinder had approached that in the nutrient medium, indicating an almost complete replacement of water. At 3 hours replacement was complete throughout. In the bark the maximum replacement was 87 %, even after 72 hours.

Fig. 2. The specific activity of the water recovered from bean leaves at 0.75, 1.5, 3, 6, and 72 hours after the plants had been placed into a nutrient medium containing THO. PRI: primary leaves, 1 TF, 2 TF, and 3 TF: first (oldest), second and third trifoliate leaves, respectively. Top: the stem and young leaves above the third trifoliate leaf. The indigenous water in the leaves was not more than 65 % replaced in 72 hours.

Fig. 3. The specific activity of the calcium from each section of bean stem at 0.75, 1.5, 3, 6, and 72 hours after placing the plants into a nutrient medium containing Ca^{45}. The specific activity in the bark exceeded that in the xylem cylinder after 6 hours, indicating a more rapid rate of replacement of indigenous calcium in this tissue.

Fig. 4. The specific activity of the calcium and the μmoles of tracer calcium in sections one and five of the xylem cylinder at various time intervals after placing the plants into the tracer solution. The uptake curves show two phases, an exchange phase and an irreversible accumulation phase. A removal curve is shown for section one of the xylem cylinder. These plants had received Ca^{45} for 1 hour only, and by subsequent exchange the tracer had fallen through almost three half values in 6 hours. The curves are characteristic of those depicting cation exchange in a variety of tissues.

ously dissipated into extra-vessel tissue as it ascended.

Further investigation into the rate of ascension of the THO tracer showed that the highest values occurred in the plants with the longest hypocotyl and basal internodes. It was in the leafy portion of the stem, and particularly in the immature portion, where the rate of ascension fell off most rapidly. The measurable ascension rate was then related to the growth form.

THO: Bark. The bark, or tissue exterior to the cambium, which contained the phloem, reached equilibration values of 86 to 93 % (avg. 88 %) in 3 hours (fig 1). At 72 hours the values were 83 to 96 % (avg. 86 %). There are two possible reasons for the failure of the bark to equilibrate: either the bark retained water which did not mix with currently incoming water, or the phloem was replenished with water which had not completely equilibrated. There are two possible sources of unequilibrated water: the first from the leaf blades (see below), and the second from the roots. Biddulph and Cory (2) have shown a downward movement of tritiated water from leaves to roots via the phloem, and Vartapetyan and Kursanov (14) have shown the roots of intact bean plants to retain unlabeled water in amounts of 47 to 59 % after having been immersed in a labeled solution (H_2O^{18}) for 24 hours. Our own results show roots of intact bean plants to be 77 % equilibrated after 24 hours' exposure in the labeled solution. A portion of the root system was then not involved in the uptake and transport of transpirational water. This portion, wherever it may have been, was not excluded as a possible source of unequilibrated water to the bark.

The exchange of all but 13 % of the water in the tissues exterior to the cambium each 3 hours showed that a part of the transpiration stream ascended in this area.

THO: Leaves. The data showing the rapidity and degree of equilibration of tracer water in the leaves and stem tip are shown in figure 2. The lower, older leaves, though they had the opportunity of acquiring tracer water ahead of the upper leaves, reached their maximum equilibration value more slowly. The maximum value, approximately 65 % of complete equilibration, was reached by all but the oldest leaves in 3 hours. These data show that the water currently lost by transpiration moved quite directly from vessels in the leaf veins to the stomata with comparatively little mixing in the mesophyll. Cline (5) and recently Vartapetyan and Kursanov (14) have both reported low equilibration rates for leaves. In Cline's report, as in ours, the leaves reached approximately 65 % of equilibration quickly and maintained this value to 72 hours—the terminal period for both experiments.

An analysis of the petioles for the 6- to 72-hour plants showed them to be from 95 to 100 % equilibrated, indicating either the passage, or very improbably, the retention of some unequilibrated water.

The retention in the leaf blades of 35 % of their indigenous water through three daily transpiration cycles suggests that in some particular part of the leaf a significant part of the water is held in a structured form. Szent-Györgyi (13) has suggested some possible roles of structured water in energy transfer systems, which would suggest an investigation of this unexchangeable water.

Ca: Xylem Cylinder. The distribution of the calcium in the various stem sections is shown in figure 3. In this figure the logarithm of the specific activity [Ca*/Ca (total)] was plotted against the distance along the stem. In contrast to the nonlinear relationship between the logarithm of the THO concentration (sp. act.) and distance along the stem, indicating reversible loss of THO from the vessels, the curves for the Ca^{45} tracer were almost linear. A linear relationship has been shown to result from an irreversible loss of tracer from the vessels (8), or from the movement of a tracer by an exchange process (2). Removal of tracer calcium from the vessels with a subsequent movement by exchange of all or a fraction of that removed would still produce a linear pattern of distribution. Movement by exchange was then definitely indicated.

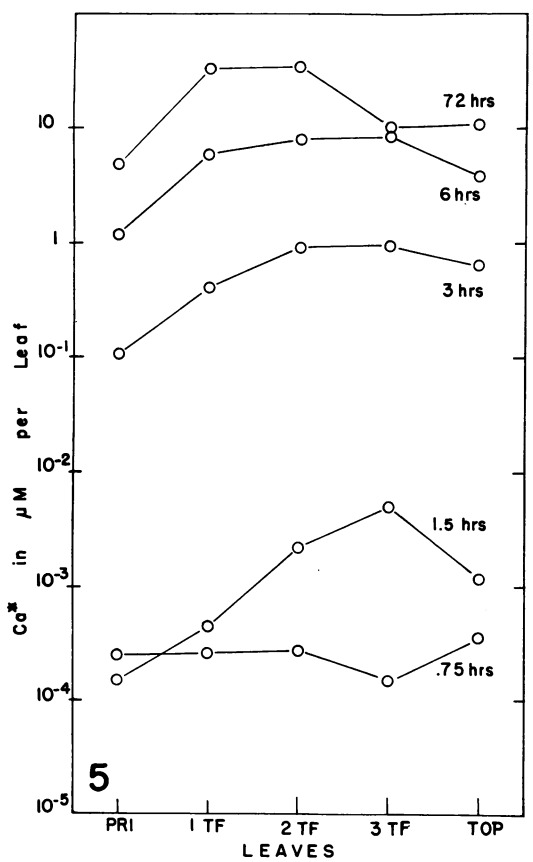
When the specific activity of the calcium in individual sections was plotted against the absorption time, the curves that resulted were identical in nature to those obtained for cation uptake in a variety of tissues (10). Specifically, the curves showed both an exchange phase and an irreversible accumulation phase (fig 4). The exchange phase reached completion in the xylem cylinder of the stem base in approximately three hours. An increase in the specific activity of the calcium in these sections showed that the exchange was accomplished by the replacement of exchangeable indigenous calcium by tracer calcium. The approximate amount of tracer calcium present on the exchange sites in sections one and five was 0.85 and 0.32 μmoles respectively, which was 8.5 and 5.5 %, respectively, of the total calcium present in those sections.

By the same process of exchange as that shown above, the exchangeable fraction of the tracer calcium could, in turn, be replaced with nontracer calcium. This was experimentally accomplished as follows: After exposure of a group of plants to tracer calcium for 1 hour, they were moved back into a nontracer solution and analyzed at intervals. The spaced intervals for analysis allowed the transpiration stream to act for varying periods and to effect the exchange in a natural way, thereby obviating the necessity of removing sections and washing out the exchangeable tracer. The removal curve of figure 4 shows the time course of the removal, by upward exchange, of the aliquot of tracer calcium, the tracer now giving way to the nontracer calcium ascending the stem. The amount of tracer fell through almost three half values (to 14 % instead of 12.5 %) in 6 hours. A further significant reduction of the 14 % could not be obtained in 72 hours, indicating that this amount

had been irreversibly accumulated and quite permanently immobilized.

Much of the indigenous calcium in the xylem cylinder remained in an unexchangeable condition, for when the exchange phase was complete, only 6 % to 9 % of that present had been replaced by tracer calcium. This increased to only 11 % to 12 % in 72 hours. This slow removal together with the retention of 14 % of the upward-moving aliquot shown above, clearly indicated the presence of a relatively permanent deposition system for a portion of the calcium which ascended the stem. The calcium oxalate crystal system, rather than metabolic accumulation in the traditional sense, appeared to be the trapping mechanism for most of this calcium. Detailed data on the Ca* oxalate system are to be presented elsewhere.

Ca: BARK. The distribution curves for Ca* in the bark sections are shown in figure 3. Initially (for the 0.75-hour period), the Ca* was low because of the time required for ascent in and removal from the xylem. At 3 hours the concentration (Ca*/g fr wt) in the bark equaled that in the adjacent xylem cylinder. At 6 hours the total amount in adjacent sections was equal and shortly thereafter the specific activities in the bark surpassed those in the xylem cylinder. Finally, at 72 hours, 50 % to 60 % of the indigenous calcium in the upper bark sections had been replaced with tracer calcium. The rapid turnover of calcium in the upper bark indicated that it, too, could serve as a channel for the ascent of calcium. This substantiates some of the contentions of Curtis (7), namely, that minerals could ascend in the bark near the stem tip.



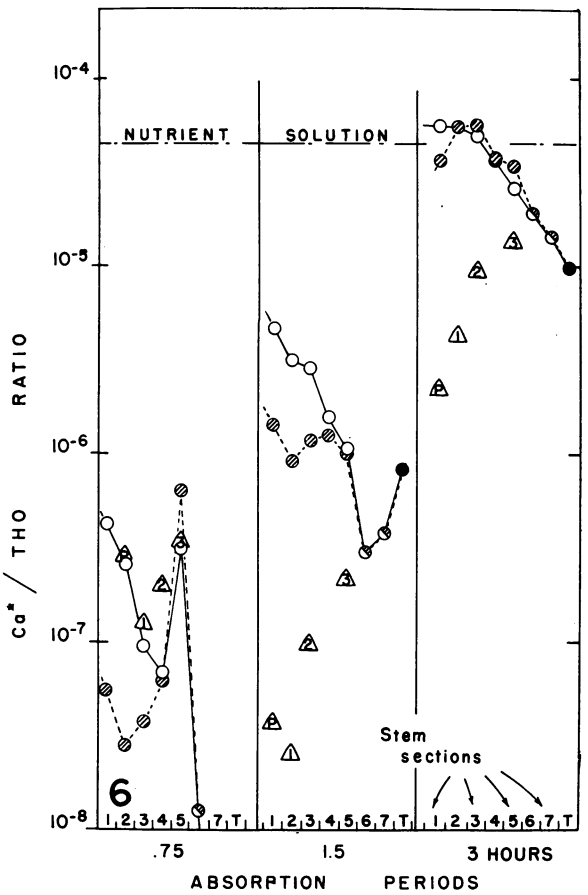


FIG. 5. The amount of tracer calcium in various bean leaves at 0.75, 1.5, 3, 6, and 72 hours after the plants had been placed into a nutrient medium containing Ca^{45}. PRI: primary leaves. 1 TF, 2 TF, 3 TF: first (lowermost), second, and third trifoliate leaves, respectively. Top: the stem and young leaves above the third trifoliate leaf.

FIG. 6. The Ca*: THO ratios for various parts of bean plants at 0.75, 1.5, and 3 hours after the plants had been placed in a nutrient medium containing Ca^{45} and THO at the ratio shown. Open circles: xylem cylinder sections. Filled circles: bark sections. Triangles with numbers: leaves; P, 1, 2, and 3 are primary and first, second, and third trifoliate leaves, respectively. The position of each leaf on the stem can be determined by relating its triangle to the stem section immediately below it.

Ca: LEAVES. The leaves did not receive tracer calcium in proportion to either size or weight during the first 6 hours; instead, position was the determining factor (fig 5). The third trifoliate leaf received the most Ca* while progressively less moved into the second, first, and the combined two primaries. This was true for periods up to and including 48 hours. As the periods lengthened, the Ca* content of the first and second trifoliate leaves approached that of the third. At 72 hours they were higher, but the 72-hour value for the third trifoliate leaf seemed low, for both the 24- and 48-hour values exceeded it. Expressed on a fresh weight basis, the ranking was not altered, but the differences merely accentuated, for the average fresh weights of the leaves were: 4.3, 3.0, 2.5, 1.9, and 1.4 g for the combined primaries, the first, second, and third trifoliate leaves and the top (0- to 6-hr averages only), respectively.

Ca*/THO RATIOS: The ratios Ca*/THO in the various leaves and stem sections for the intervals of 45 minutes, 1½ hours, and 3 hours are shown in figure 6. Similarities between the ratios for the leaves and the sections of xylem cylinder from which they diverged were found for only the 45-minute period. This indicated that the first influx of tracers into both stems and leaves was largely confined to a movement in the xylem vessels. Subsequent movement into leaves must continue via the xylem vessels as no extensive extra-vascular tissue extends through the petiole.

At 1½ hours the Ca*/THO ratio for the first (lowermost) section of xylem cylinder was higher by a factor of ten than for the fifth section. This was because the THO was approaching its equilibrium value in all sections, while the Ca* was just entering into the stem base in significant quantity.

The Ca*/THO ratios for the leaves were much lower than those for the stem sections. Among leaves the lowest ratio, that for the primary leaf, was smaller by a factor of ten than the highest ratio, that for the third trifoliate leaf. In order for a more dilute transpiration stream to enter the lower leaves than moved into the upper ones, the removal of some Ca* from the leaf traces leading to the lower leaves would be required. The leaf traces in the bean plant traverse a full node after diverging from the vascular cylinder. It was during the passage through this internode that the Ca* content of the transpiration stream was reduced. A significant and possibly a major portion of that which left the xylem was found in the adjacent phloem. The transfer occurred predominantly at the node of divergence of the leaf, and some Ca* extended downward in the phloem traces as though it had moved in the assimilate stream. This mineral extracting mechanism near the pulvinus of mature leaves augments exchange toward the apex by returning to the stem a portion of the minerals carried in the leaf traces. Figure 7 shows Ca^{45} and Sr^{89} radioautograms of the bark wherein the lateral transfer at the nodes is evident. A similar transfer has been reported earlier for P^{32} and S^{35} (3). The details of the transfer mechanism will be reported at a later time.

At the 3-hour period the Ca*/THO ratios for the stem sections had approached their original ratio in the nutrient medium. This was because the THO values had been at equilibrium for approximately 1½ hours while the Ca* was just completing its exchange up the stem. Following this period, increases in the ratio were slower and were due to augmentation of Ca* to that fraction which was irreversibly accumulated.

It is now quite clear that Ca*/THO ratios should not be interpreted as representing solution concentrations. Neither should the value, mineral absorbed/water transpired, be used to convey the impression that the two move only *en masse* and as a solution corresponding to this particular concentration, cf., Hylmo (9), and Russell and Shorrocks (11). Our data show the xylem cylinder of the plant stem fundamentally to operate as an exchange column through which narrow vessels with porous walls permit solvent movement at somewhat less resistance than is encountered in the ground tissue as a whole.

A translocation mechanism involving an exchange process explains the observed unequal rates of ascent of Ca* and THO, and their different distribution characteristics in different plant parts, more successfully than a mechanism based on mass flow. With mass flow there is no flexibility or opportunity for control over delivery save that inherent in transpirational loss from individual tissues or organs. On the other hand, ascent by exchange would be dependent upon both the nature of the ion and of the exchange sites, the current loading of the sites, and the rapidity of removal from the exchange system. Removal at the stem apex, and other metabolically active places, would be into new space created by either growth or utilization thus bringing control of ascent more nearly under the influence of tissue metabolism at each receiving site than could be so with a mass flow controlled only by transpiration. Such an exchange system, composed of a variety of biocolloids over which movement is actuated by water of the transpiration stream, offers the flexibility and the control mechanisms necessary for explaining most of the observed characteristics of mineral ascent.

SUMMARY

The degree of dependence between water movement and calcium movement was determined by the sequence and pattern of entrance of THO and Ca^{45}, used simultaneously, into various tissues and organs of the bean plant. The root-water reached only 77 % of equilibration with nutrient water in 24 hours while equilibration in the xylem cylinder of the stem was complete to the third trifoliate leaf in 3 hours. The bark and leaves reached 87 % and 65 %, respectively, of equilibration in 3 hours and neither exceeded this value in 72 hours.

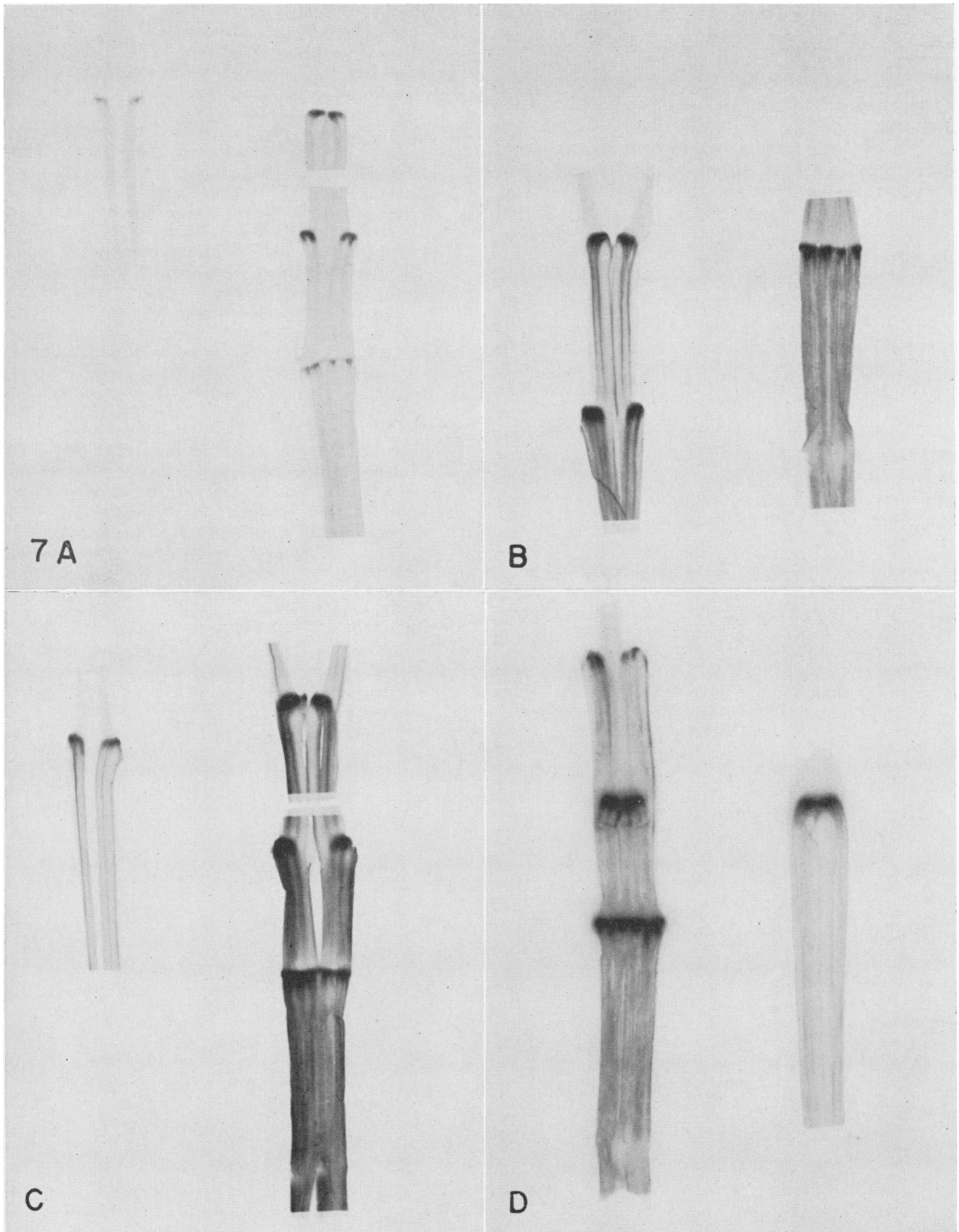


FIG. 7. Ca^{45} radioautograms of frozen-dried bark of bean stems. The plants remained in a half-strength Hoagland solution containing Ca^{45} for A, 0.75 hours; B, 1.5 hours; and C, 3 hours, respectively, prior to stripping the bark from the xylem cylinder. The tissues were frozen in isopentane chilled in liquid N_2. The base of the section of bark on the left joins the top of the section on the right to show the primary, first, second, and third (except B) trifoliate leaf nodes, respectively. A distinct transfer of Ca^{45} from the xylem component of the leaf traces to the phloem is evident at each node. D is a similar radioautogram of Sr^{89} corresponding to B, except the position of bark sections is reversed.

The time course curve for Ca^{45} entry into specific stem sections showed that entrance consisted of two phases: a reversible exchange phase and an irreversible accumulation phase. The exchange phase was complete in 3 hours and the exchangeable fraction constituted less than 10 % of the total calcium. The Ca^{45} on exchange sites could, in turn, he exchanged up the stem, the loss curve for the tracer falling through three half values in 6 hours. The non-exchangeable Ca^{45} was mostly in the calcium oxalate crystal system, the low solubility constituting the driving force for accumulation.

The ratio of Ca*/THO in leaves increased stepwise from the primary leaves (lowest values) to the third trifoliate leaves (highest values). Radioautograms of bark tissues showed a transfer of Ca* from the xylem of leaf traces to the adjacent phloem sufficient to account for the low Ca*/THO ratios in older leaves.

The data showed the xylem cylinder of the bean stem fundamentally to operate as an exchange column for calcium. *En masse* flow in the vessels was inadequate for explaining the rapid deep-seated exchanges observed for this tracer.

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