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TRANSLOCATION OF CALCIUM IN THE BEAN PLANT [1, 2, 3]

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There are a number of reports in the literature of translocation which indicate that calcium is immobile in the phloem, or relatively so (1, 2, 4, 5, 13). The majority of these are not quantitative studies but merely indicate that calcium translocation compares poorly with that of phosphorus, sulfur, potassium and some other elements. Contrasted with these reports on calcium immobility in the phloem are two which claim that the anesthetizing of leaves with diethyl ether (6) and the application of triiodobenzoic acid (TIBA) (8) cause a reversal of polarity in the phloem and thereby allow calcium to move from leaves. However, neither of these papers established that the calcium transport from the leaf was via the phloem.

The purpose of the present investigation is therefore 2-fold: to examine calcium translocation in more quantitative terms, and to investigate the effects of certain substances or treatments on calcium translocation. In addition we have carefully determined the tissue through which calcium movement occurs.

The quantitative study was made by appraising the mobility of cotyledonary calcium, by observing the partition of the calcium absorbed from the nutrient medium between various plant parts, by observing the

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

subsequent exchange of the calcium between these parts, and finally, by making quantitative measurements of the export of foliarly applied calcium to other parts of the plant.

To accomplish the second part of the study we have investigated the effect on calcium transport of ether and TIBA, substances claimed to reverse polarity, and also the effect of NaCl since in our laboratory we have observed that foliar sprays of high salt content induce movement of calcium from leaves under certain conditions.

Methods

General: Red Kidney bean plants were grown in a Hoagland-type nutrient solution which was changed each 4 days. A ½ strength solution was used during the first 4 days and subsequently a solution of 2.5 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 1.0 $MgSO_4$, 0.25 mM KH_2PO_4, and with Fe and micronutrients. The solutions were aerated and held near pH6. The growth conditions were: temperature 23 ± 1° C, relative humidity 60 ± 5 %, and light at 1000 to 1200 ft-c (fluorescent) on a 12-hour photoperiod. The special conditions of each of a rather large number of experiments are listed separately.

Partition of Available Calcium: As the young plants developed they had a potential source of calcium from the cotyledons and they were given various additional amounts of calcium via the nutrient medium. Plants survived when grown between the nutrient levels of 0.05 and 5.0 mM Ca. Beyond these limits a deficiency or a chlorosis developed which resulted in the death of the plants. $CaCl_2$ or $NaNO_3$ was used to obtain calcium levels different from 2.5 mM without varying the concentration of essential nutrients Plants were grown to an age of 12 days from the straightening of the hypocotyl. Six plants in 6 liters of solution served as a unit, and the composited similar parts of each 6 plants constituted the samples for analysis. Experiments were in duplicate and determinations were by flame spectrophotometry. The data are compiled in figures 1 A and 1 B.

Exchange of Calcium Between Plant Parts: The exchange of calcium was shown by use of the Ca^{45} isotope. All plants received 2.2 μc of Ca^{45} per liter of nutrient solution between the 4th and 8th days from the straightening of the hypocotyl. Immediately after the 8th day 6 plants were harvested for radioactive analysis and 3 for radioautograms to determine the initial distribution of the Ca^{45}. A similar group of plants was transferred to a non-radioactive complete nutrient solution and held for 4 additional days after which 6 plants were harvested for radioactive analysis and 3 for radioautograms. The final distribution of Ca^{45} compared with the initial distribution indicated the amount of exchange between parts. Plants used for radioautograms were quickly dried between blotters in a forced draft oven at 85° C, then exposed to no-screen x-ray film. Repeated tests have shown that no artifacts in calcium distribution occur as the result of the drying procedure unless the leaf is injured by high salt content sprays, TIBA sprays, anesthetization with ether, and presumably other means (see below).

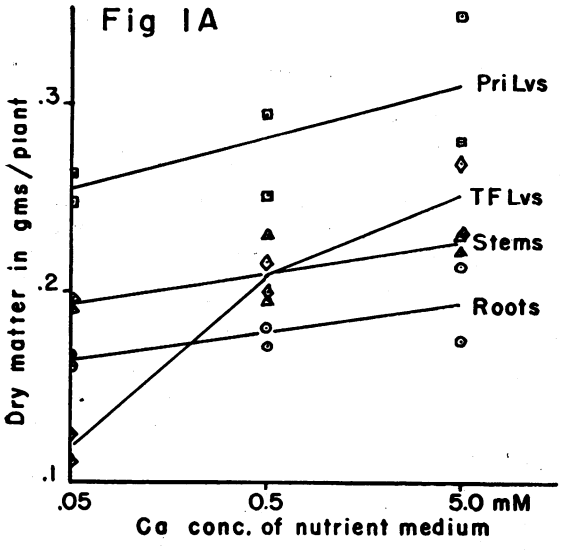
Ca^{45} was determined quantitatively in the calcium oxalate precipitate from the ashed samples; then the total calcium was determined by volumetric analysis. All radioactive analyses were corrected for background, geometry, decay, and self absorption when applicable. Data are represented in figures 2 and 3.

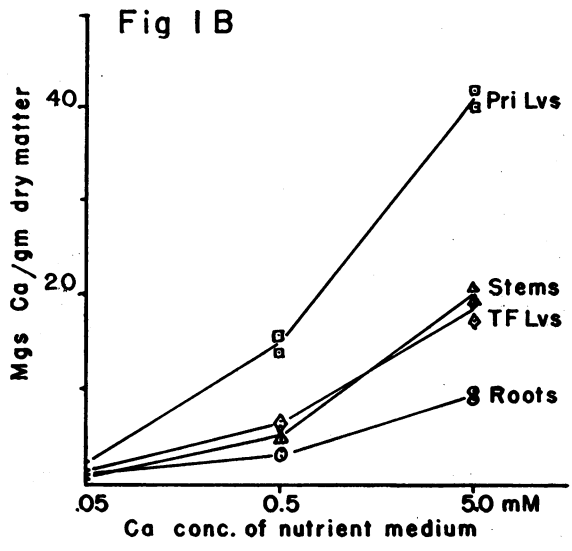
Foliar Export of Calcium: All studies were based on the foliar application of Ca^{45} and its subsequent detection in other parts of the plant by radioactive analysis or radioautography. The tracer applications were all made to the lower surface of the terminal leaflet of the 1st trifoliate leaf. A glass sprayer delivered the solution to an area approximately 1.5 inches in diameter which was centered laterally and located between one fourth to one third of the distance from the base to the tip of the leaflet. The pH of the solutions applied was near 4.0. The migration periods were 24 hours in all cases except those performed to establish the time sequence for the appearance of tracer calcium in the stem.

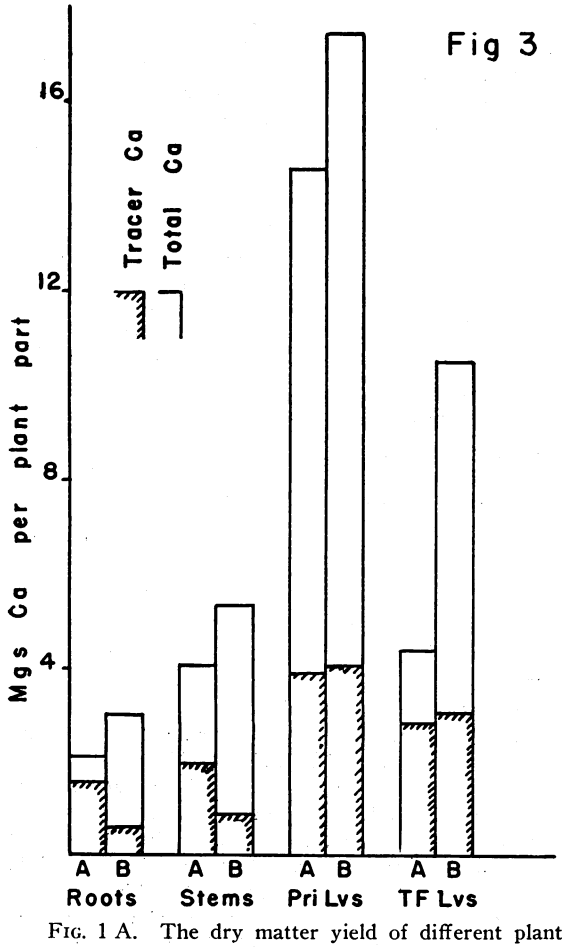
A quantitative evaluation of calcium translocation was made for each of several levels of foliar applied tracer calcium. The foliar spray consisted of 20 μc of Ca^{45} in 50 μl of $CaCl_2$ solution varying in concentration from 0.01 to 1.0 M. Following the 24-hour migration period each plant part was analyzed separately for Ca^{45}. The total Ca^{45} and the percentage of the applied Ca^{45} which was translocated was then calculated. The conventional procedure of steaming the petiole to restrict phloem movement was used to establish the tissue through which movement occurred. The data are compiled in figure 4.

Attempts to Increase Foliar Export: *1. Nutrient treatments*: It was our intent to determine whether or not the foliar export of calcium could be influenced by nutritional conditions. The effects of high and low internal concentrations of certain nutrient elements, and of pH, were investigated. Groups of plants grown under all 8 possible combinations of nutrient conditions resulting from calcium at 0.5 and 5.0 mM, phosphorus at 0.05 and 5.0 mM, and hydrogen ion concentrations at pH 4.0 and 7.0 served as experimental material. A second experiment similar to the above was conducted in which sulfur at 0.01 and 1.0 mM was a variable instead of phosphorus. This made a total of 16 different treatments. Twelve days after the straightening of the hypocotyl a foliar application of approximately 50 μl of 0.2 M $CaCl_2$ containing 16 μc of Ca^{45} was made. Following the 24-hour migration period the plants were dried and radioautograms prepared. The experiment was conducted in duplicate with 6 additional replications at low Ca, low P, and pH 4.0. The results showed no significant differences between treatments so the radioautograms, 38 in all, are not included here.

2. Foliar Treatments: An investigation was made of the effects of NaCl, TIBA and ether treat-







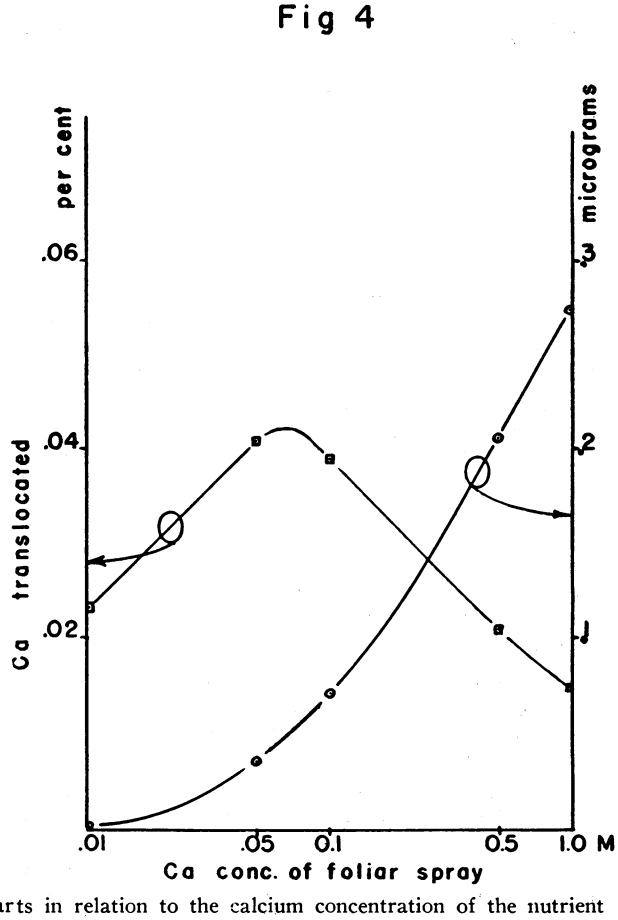


FIG. 1 A. The dry matter yield of different plant parts in relation to the calcium concentration of the nutrient medium. FIG. 1 B. The calcium concentration of different plant parts in relation to the calcium concentration of the nutrient medium. The plant was divided as follows: Primary leaf blades (Pri Lvs), trifoliate leaf blades (T F Lvs), stem including hypocotyl and petioles (Stems), and roots (Roots).

FIG. 3. Histogram showing the distribution of tracer calcium (and total calcium) at 2 intervals following application to the root via the nutrient solution. A. Distribution immediately following an application between the 4th and 8th day from the straightening of the hypocotyl. The nutrient solution contained 2.2 μc of Ca^{45} per liter per plant. B. Distribution at the end of the 12th day. From the 8th to the 12th day the plant was in a normal (nonradioactive) nutrient solution.

FIG. 4. The amount of tracer calcium translocated from a leaf in 24 hours shown in relation to the concentration of the foliar spray applied to the leaf. The foliar spray consisted of 20 μc of Ca^{45} in 50 μl of $CaCl_2$ solution varying in concentration from 0.01 to 1.0 M. The percent of the applied tracer translocated is shown on the left; the total amount translocated on the right.

ments on calcium export from a leaf. The details of each treatment follow:

a. NaCl: A foliar spray of 33 μc of Ca^{45} in 50 μl of 0.03 M $CaCl_2$ and 1.18 M NaCl was applied to the lower surface of the terminal leaflet of the 1st trifoliate leaf. Following the 24-hour migration period plants were either dismembered before radioactive analysis or dried intact and then analyzed. The analyses did not include the treated leaf. The data are compiled in figure 5.

b. TIBA: Seven ml of a TIBA spray at 100 ppm containing 0.05 % Tergitol-7 surfactant was applied to completely wet the aerial parts of each plant. Thirty minutes later a foliar spray consisting of 33 μc of Ca^{45} in 50 μl of 0.015 M $CaCl_2$ was applied. The experiment was otherwise similar to part a. above.

c. Diethyl ether: One minute after a foliar application of 33 μc of Ca^{45} in 50 μl of 0.015 M $CaCl_2$ was made, the treated leaflet was enclosed in a transparent leaf chamber containing liquid ether in a Petri dish. The experiment was otherwise similar to part a. above.

As the ether treatment was the only one which induced an abnormal calcium movement to occur during the migration period, a refined ether treatment procedure was developed in order to determine the tissue through which movement occurred: One minute after a foliar application of 32 μc of Ca^{45} in 50 μl of 0.384 M $CaCl_2$ the treated leaflet was inclosed in a transparent leaf chamber of 2 liters capacity. Air, containing ether vapor, was circulated through the chamber at the rate of 250 ml per minute for 8 minutes. The 2000 ml of air contained the vapor from 1.88 ml of liquid ether. Following an 8-minute ether treatment period a subsequent migration period of either 6, 11, or 22 minutes was allowed during which time the ether-laden air was replaced by moisture-laden air circulated at the rate of 1 liter per minute. Immediately following the migration period, the stem for a distance of 3 inches above and 5 inches below the node of the treated leaf was cut into 1-inch sections, the bark stripped from the wood, and each part analyzed separately for Ca^{45}. The experimental conditions resulted in only a very slight flagging of

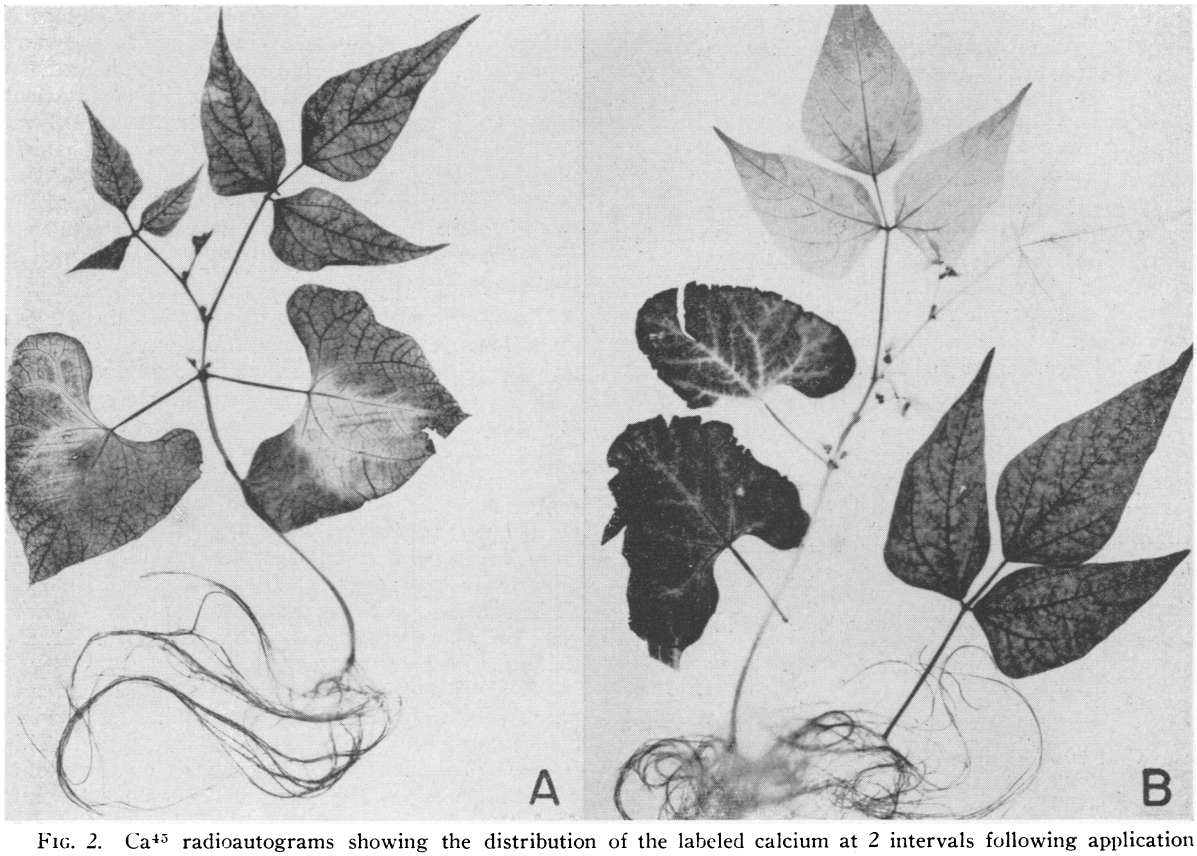


FIG. 2. Ca^{45} radioautograms showing the distribution of the labeled calcium at 2 intervals following application to the root via the nutrient solution. A. Distribution immediately following an application between the 4th and 8th day from the straightening of the hypocotyl. The nutrient solution contained 2.2 μc of Ca^{45} per liter per plant. B. Distribution at the end of the 12th day. From the 8th to the 12th day the plant was in a normal (nonradioactive) nutrient solution. One primary leaf and the 1st trifoliate leaf have been rearranged to fit on the film. The primary leaves of B were injured during the preparation of the radioautograms. Exposure to no-screen x-ray film 60 days.

the ether treated leaf. If the treatment exceeded 8 minutes the leaf collapsed and the results showed that this was invariably followd by a rapid and marked movement of Ca^{45} from the leaf. The data are recorded in table I. Photomicrographs of stripped bark and wood are shown in figure 6. "Bark," as herein used, refers to all tissues exterior to the cambium (7) and "wood" to all tissues interior to the cambium. Under the above growth conditions a well-developed cambium is formed so that stripping is easily accomplished.

Results

Partition of Available Calcium: The relationship between the dry matter yield and the logarithm of the calcium concentration of the nutrient medium was approximately linear for all plant parts. The increment gain in dry matter per unit increase in the logarithm of the nutrient calcium concentration was slight for primary leaves, stems and roots. The high intercept of these curves on the axis representing the lowest calcium concentration which could support growth of the trifoliate leaf system indicated that the early establishment of root, hypocotyl, and primary leaves occurred principally on cotyledonary calcium. For the growth of the trifoliate leaf system an external source of calcium was clearly necessary. This is distinctly indicated by the very meager growth of these parts at the 0.05 M calcium level and the very pronounced gains as the amount of available calcium was increased (fig 1 A).

The primary leaves accumulated and retained a higher concentration of calcium than any other plant part. This was true at each level of calcium nutrition (fig 1 B). The concentration in the stems and trifoliate leaves was intermediate while that of the roots was lowest. These results suggest that the leaves with the longest history of accumulation have the highest calcium concentration since it is not withdrawn in significant amounts for use elsewhere.

Exchange of Calcium Between Plant Parts: Once labeled calcium is deposited in leaves, following a particular absorption period, a significant quantity is not withdrawn to supply leaves formed at a subsequent period. It then follows that the growth of the plant is immediately dependent on the current acquisition of calcium from the growth medium; little, if any, is mobilized from previous depositions. This is evident from a comparison of the Ca^{45} radioautograms in figure 2 A and 2 B.

The same conclusions are supported by the analytical data of figure 3. Here it is shown that the trifoliate leaves which developed during the last 4 days when no tracer calcium was available in the nutrient solution gained only 0.3 mg of labeled calcium from the remainder of the plant while making a gain of 6.2 mg of calcium from the nutrient medium. It is probable that a part of the 0.3 mg gain, if it is significant at all, was from labeled calcium in transit within the plant when the shift to unlabeled calcium was made.

A loss of labeled calcium from root and stem during the later growth period is evident from figure 3. In the root this loss can be explained best by assuming an exchange of the labeled calcium within or upon the root for the unlabeled calcium in the nutrient medium. The loss from the stem, if it is significant at all, must also be explained by this same mechanism since the labeled calcium did not move into the leaves. However, it is only the movement of calcium to the root, bringing it into a position for exchange, and not the exchange itself which would be of significance in the present investigation.

Foliar Export of Calcium: When the amount of radioactive tracer calcium translocated from the leaf was plotted against the molarity of the applied foliar spray, it became evident that the two were related and that calcium translocation was quantitatively measurable. These results are shown in figure 4. The important concept here is that as the concentration of the foliar spray was increased the amount of calcium translocated away from the leaf also increased in a regular manner. The maximum amount of calcium translocated was 0.3 μg in 24 hours. Steaming the petiole of otherwise uninjured leaves completely prevented export of Ca^{45} thereby indicating that movement was via the phloem.

Comparisons can be made between calcium and phosphorus translocation under similar conditions of application when using a 30 mM foliar spray. This is the highest phosphorus concentration available for comparison (9). The approximate values for calcium and phosphorus translocation, the latter applied as $NaH_2P^{32}O_4$, was 0.02 and 15 μg respectively, or a ratio of 1 : 750. The spray concentration resulting in the most efficient translocation of calcium was between 50 and 100 mM (fig 4) whereas for phosphorus it appeared to be between 10 and 30 mM (9). At the most efficient concentration of each approximately

Table I

Distribution of Ca^{45} in the Bark and Wood of the Stem Following Ether Treatment of the Leaflet Receiving the Foliar Spray.*

Migration period following 8 min ether treatment						
Section	6 min		11 min		22 min	
	Bark	Wood	Bark	Wood	Bark	Wood
	counts per minute per section					
3 up	0	16	24	139	116	12,600
2 "	0	83	39	298	4,170	27,450
1 " **	25	102	44	394	4,540	35,750
1 down	180	597	500	1700	9,640	73,250
2 " ***	0	104	0	375	1,848	43,950
3 "	0	0	0	0	53	360
4 "	0	0	0	0	16	83
5 "	0	0	0	0	0	15

* The foliar spray consisted of 32 μc of Ca^{45} in 50 μl of 0.384 M $CaCl_2$ (pH 4).

** Node where tracer entered stem.

*** Primary leaf node in this section.

0.06 μg of Ca and 6 μg of P were translocated—a ratio of 1 : 100. This was approximately 0.04 % and 12 % of the calcium and phosphorus respectively which was applied to the leaves.

Injury to the leaf occurred with foliar sprays of 0.1 M $CaCl_2$ and above. A water-soaked appearance of the treated area characterized the injury and necrotic spots developed as the concentration reached 1.0 M. As will be shown later the magnitude of injury was insufficient to cause artifacts in movement unless undue stress was placed on the water columns within the plant.

Attempts to Increase Foliar Export: *1. Nutrient Treatments.* There was no indication that nutritional conditions involving the level of phosphorus, calcium, or sulfur, or the hydrogen ion concentration within the limits herein used, had any influence on calcium translocation away from leaves. This was investigated rather thoroughly because of an earlier report (1) that calcium became mobile in the phloem under growth conditions of low calcium, low phosphorus, and pH 4.0. This earlier result must now be attributed to some undetected injury to the plant which enabled calcium to enter the xylem and be distributed within the plant during the drying process. In 6 replications of the above conditions no plant showed any marked movement of calcium away from the leaf. It should be stressed that the radioautograms of dried plants all gave evidence of a very small amount of calcium being translocated away from the treated leaf. The amount, however, was consistent with the quantitative figures given in figure 4 for uninjured plants.

2. Foliar treatments: a. The Effects of NaCl: A foliar spray of 0.03 M $CaCl_2$ and 1.18 M NaCl resulted in injury to the leaf at the site of application. The injury was manifested by a water-soaked appearance, a partial collapse of the mesophyll, and an ultimate development of brown spots scattered over the area contacted by the spray. The leaf, however, remained turgid. The data, shown in figure 5, indicate that the injury was not severe enough to cause abnormal calcium movement during the 24-hour migration period. However, it was found that a very marked movement of calcium occurred during the drying process if the plants were left intact.

b. The effects of triiodobenzoic acid: A complete foliar treatment with 100 ppm TIBA had no stimulating effect on calcium movement during a 24-hour migration period. In fact the treatment appears to have hampered normal calcium transport. But, as above, a very pronounced movement occurred during the drying process if the plants were left intact (fig 5). The visual effects of the TIBA on the plants were a twisting of the terminal bud and adjacent tissue and a cupping of the leaves which were still expanding.

c. The effects of ether: Exposing a Ca^{45} treated leaflet to the vapors from liquid ether during the 24-hour migration period resulted in a complete collapse of the leaflet and a very pronounced Ca^{45} movement from the leaf (fig 5). No additional effect was observed if similarly treated plants were dried intact before being analyzed.

When using our refined method of exposing leaves to ether vapor it was found that a treatment period of less than 6 minutes had no effect on Ca^{45} movement and no visible effect on the leaflet. An 8 minute treatment caused about 50 % of the leaves to flag slightly and all treated leaves to lose Ca^{45} to other parts of the plant. Longer treatment caused complete collapse of the leaflet and a near maximal movement. An 8 minute treatment period proved best for

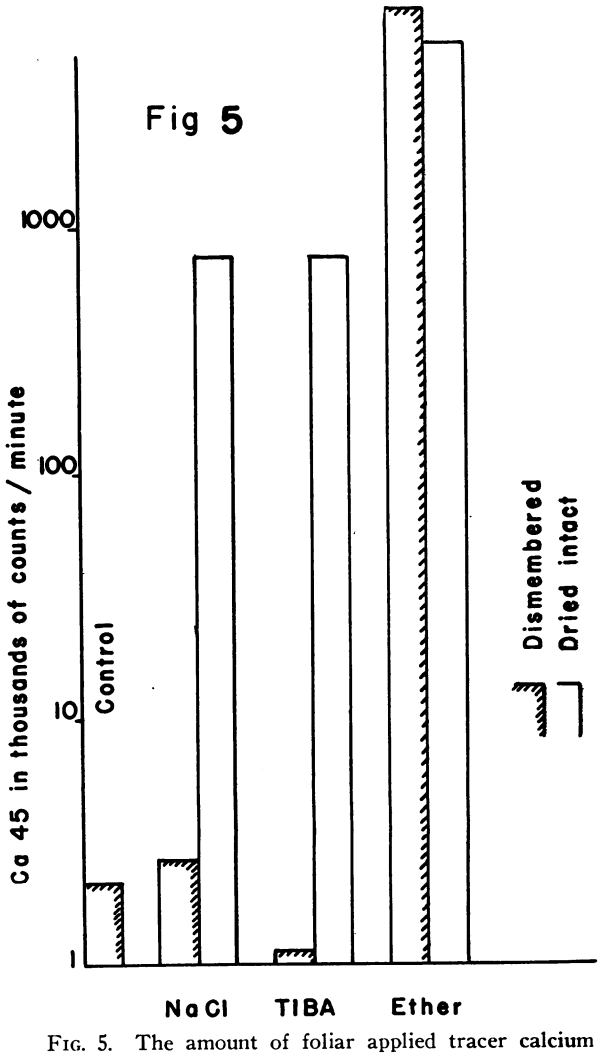


Fig. 5. The amount of foliar applied tracer calcium moving from the treated leaf to all other parts of the plant in 24 hours following several treatments and including 1 normal control. For details of treatment see text. Each member of the pair was treated similarly except that before drying one was dismembered to show the movement which had occurred during the migration period (crosshatched) from the movement which occurred during drying (plain).

the study of tissues involved in transport when the transport was induced by ether. The results are shown in table I.

An analysis of the results shows: (A) That the Ca^{45} moved from the leaf only when the controlling cells comprising the bundle sheath, or the bundle sheath extensions, around the veinlets of the leaf, were injured to the extent that they permitted the leaf to serve as a source of water and minerals to other parts of the plant. (B) That the pathway of movement from the leaf was initially the xylem. The Ca^{45} which subsequently appeared in the phloem both above and below the node of the treated leaf entered this tissue by lateral movement from the xylem. This appears to be true both because the extent of the Ca^{45} is not as great in the phloem as in the xylem and because the actual quantity of Ca^{45} in the phloem is only one-fourth to one-seventh that in the xylem. At the same time, there was little or no evidence that negative tensions developed in the phloem and were responsible for rapid calcium advancement in this tissue. (C) That the Ca^{45} descended in the xylem elements of the leaf traces to the next lower node where anastomosing of bundles occurred before it ascended to leaves higher on the stem. Only a small amount moved on downward toward the base of the stem.

Figure 6 is included to show that in a stripped stem the break between "bark" and "wood" occurs at the cambial region, effectively separating the phloem from the xylem.

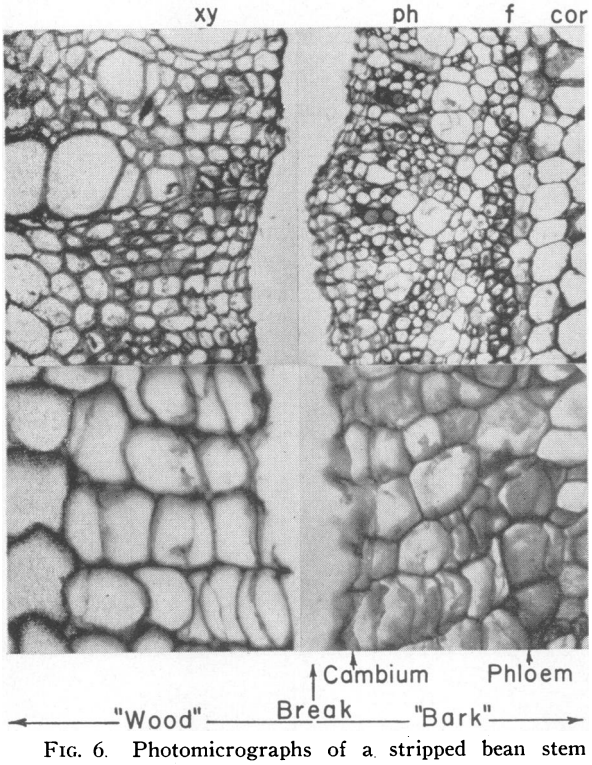


FIG. 6. Photomicrographs of a stripped bean stem showing that the break between "bark" and "wood" occurs at the cambium. Upper pair shows the break over an extended area: xy = xylem, ph = phloem, f = fibers, cor = cortex. Lower pair, at higher magnification, shows details of the break at the cambial region. Sieve plates are visible in some of the sieve elements of the phloem.

DISCUSSION

We have shown that the withdrawal of calcium from the cotyledons during the early growth of the seedling is the most striking incidence of calcium mobilization within the plant. This is in agreement with a recent report of Lausch (10). Since cotyledonary calcium is limited in amount, an external calcium source is required by the time the rapid growth of the trifoliate leaves begins. The cotyledonary calcium incorporated in the older tissues is by then relatively immobile and little is available for subsequent growth.

The calcium from the nutrient medium which enters the leaves via the transpiration stream likewise remains relatively immobile after the initial deposition. This was found to be true under a wide variety of nutrient conditions so that the traditional view that calcium is relatively immobile within the plant can be supported as a generality.

Results obtained with foliar applied Ca^{45} also show that the amount of calcium translocated from leaves is comparatively small although it is definitely measurable with radioisotope techniques. The amount has been found to compare poorly with the amount of phosphorus withdrawn. Under conditions of equal application, i.e. 30 mM, only 1 μg of calcium to approximately 750 μg of phosphorus is exported. At the most efficient concentration for exportation of each the ratio is 1 : 100. Phosphorus, in turn, has been found to be exported in far lesser quantity than sucrose under certain experimental conditions (3), the ratio being in the order of magnitude of 1 μg of phosphorus to 1 mg of sucrose. To show calcium withdrawal from foliage leaves by other means than radioactive tracing would seem very difficult as the amount involved is so small; however, Lausch (10) has recently reexamined this possibility and claims some success.

Were calcium more mobile within the plant it might be anticipated that foliar sprays could be used to alleviate certain calcium insufficiencies which develop during maturation of fruits under some environmental conditions, e.g. black end of tomatoes. This would justify a search for a calcium carrier to render it mobile, or for conditions which would increase its mobility.

To the authors, it does not seem possible to mobilize calcium by reversing polarity in the phloem as has been suggested by Bukovac et al (6) and Kessler and Moscicki (8), for when positive and negative ions enter the stem from a leaf it is known that each may move either upward or downward in the phloem. This would preclude a mechanism of movement based on electrical polarity, and polarity in any other sense

has no precise meaning, unless elaborate systems of carriers and peculiarly charged channels were invoked. Furthermore, in the present study, when calcium was induced to move from the leaf by some form of injury it definitely moved via the xylem. This is consistent with the early work of Yendo (14) and others (see Roach (12)) wherein back transport in the xylem of dyes and other markers was attained following application to cut leaves, etc., and with the work of Pallas and Crafts (11) who observed artifacts in the translocation of C^{14}-labeled 2,4-D when plants were frozen before they were dried. Perhaps this interpretation is also consistent with the results of Bukovac et al and of Kessler and Moscicki, and all are concerned with the movement of fluids in the xylem as a result of stresses which occur or develop in other parts of the plant. Only the mechanism of injury which permitted entry into the xylem differed.

Summary

The instance of greatest calcium mobilization was that from cotyledons during the early growth of root, hypocotyl and primary leaves. The growth of apical meristem and trifoliate leaves was dependent on an external source of calcium since the cotyledonary calcium was not only limited in amount but by then largely immobile.

The order of increasing calcium concentration among plant parts was roots, stems, trifoliate leaves, and primary leaves—indicating that an important factor in calcium accumulation was the total volume of the transpiration stream received. Also important was the meagre loss following the initial deposition, prohibiting reutilization in growth and metabolism elsewhere.

The amount of foliar-applied calcium exported from a leaf was small, approximately only 1/100 that of phosphorus under conditions of maximum percentage translocation. The amount exported from a leaf was related to the amount applied, but not to the nutritional conditions imposed on the plants. Calcium, then, is not immobile in the phloem; it is the volume of flow which is small.

Calcium could not be induced to move from a leaf via the phloem in extra-ordinary quantities, but movement via the xylem occurred following treatment of the leaf with the vapor of diethyl ether. Applying NaCl with the foliar spray, or treating the plant with triiodobenzoic acid induced calcium movement in the xylem during the dehydration of the plant.

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