Cucumber, pea, bean, and corn excised roots were used to assess species differences in metabolic uptake of calcium. It was found that Ca was mostly taken up by these root systems by non-metabolic processes. The apparent free space ranged from 26 to 28 percent of the total root volume and beyond that no large accumulation of Ca was observed. Although a small rate of increase was observed during the 12 hour experimental period, this was attributed to either an increase in the total non-metabolic component or to a manifestation of the small percentage of younger vacuolated segments of the roots. These results pointed out that the physiological maturity of the roots may be the controlling factor in Ca absorption and that mature root cells are relatively impermeable to Ca.

Excised roots and intact plants of six-week-old tomato were compared to evaluate the influence of the top in the uptake of calcium. On a per unit of root basis, the uptake of calcium was 5.5 times
faster for intact plants than for the most rapid sustained phase of absorption for excised roots. Intact plants absorbed calcium almost 39 times faster than the slower rate observed for the excised roots. Several hypotheses were advanced to explain these observations. Mass flow in the transpirational stream or migration along charged surfaces would represent non-metabolic processes. Another possibility is an indirect enhancement of metabolic uptake by transpiration due to a stimulation of bleeding in the root. A third possibility is the operation of a uni-directional transport of ions across the endodermis in intact plants which goes undetected in excised root systems because the stele is opened to the external solution.
NATURE OF CALCIUM UPTAKE BY PLANTS

by

BERNARDO SILVA NORAMBUENA

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1963
APPROVED:

Redacted for privacy

Assistant Professor of Soils

In Charge of Major

Redacted for privacy

Head of Department of Soils

Redacted for privacy

Dean of Graduate School

Date thesis is presented November 6, 1962

Typed by Jolene Wuest
ACKNOWLEDGMENT

I am grateful to Dr. D. P. Moore for his unfailing advice and encouragement, and for his helpful criticism of the manuscript.

The writer also wishes to testify his gratitude to Professors H. B. Cheney, T. L. Jackson, and J. A. Milbrath for their stimulating discussion and useful suggestions.

Thanks are also due to Messrs. B. J. Mason and E. V. Mass for their continuous and helpful assistance throughout this investigation.

Greatly appreciated is the contribution of the Atomic Energy Commission that helped defray part of the expenses associated with the conduct of this thesis problem.

The author would like to express his deep appreciation to the Agency for International Development whose scholarship made it possible for him to undertake graduate study at Oregon State University.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>MATERIAL AND METHODS</td>
<td>10</td>
</tr>
<tr>
<td>Excised Roots</td>
<td>10</td>
</tr>
<tr>
<td>Analytical Procedure for Excised Roots</td>
<td>12</td>
</tr>
<tr>
<td>Intact Plants</td>
<td>12</td>
</tr>
<tr>
<td>Analytical Procedure for Intact Plants</td>
<td>14</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>16</td>
</tr>
<tr>
<td>Excised Roots</td>
<td>18</td>
</tr>
<tr>
<td>Excised Root vs Intact Plant</td>
<td>28</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSION</td>
<td>35</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>37</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>41</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES AND TABLE

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
</tr>
</tbody>
</table>
"There is considerable evidence that the absorption mechanisms of a root system behaves much like the absorption mechanisms of individual cells"


INTRODUCTION

Several workers in dealing with calcium absorption have concluded that much of it occurs in plants as a non-metabolic process. The majority of studies have been conducted with tissue segments or excised roots. The observations resulting from their experiments have been assumed to hold also for intact plants, without regard to the complication that the transpiration stream and the presence of the shoot may introduce.

Of the voluminous literature on ion absorption and translocation, little actually deals specifically with calcium uptake. Other ions are known to be absorbed metabolically by plants while the available information on calcium would seem to indicate that this ion may behave differently. In addition, species differences are known to occur in the uptake of many ions and data on this point is sorely lacking for calcium uptake. In this study, cucumber, corn, pea, and bean excised roots were used to assess any species differences which may occur in metabolic calcium uptake. These species represent a broad range of calcium requirements when these plants
are grown under normal conditions.

Opinions with regard to the role of the transpiration process on ion uptake have been strongly divergent. Whereas, some like Hylmo (24) contend that it plays a direct effect on ion uptake, there are others, like Brouwer (4, 5, 6) who take an opposite view of the problem, stating that transpiration plays an indirect influence. On the other hand, Russel and Shorrocks (36), state that transpiration influences the removal of ions already accumulated, thus influencing the bleeding. In a later paper, Pettersson (35), took transpiration process as having dual influence, acting on ion transport by mass flow and at the same time acting as a bleeding-stimulant as well. In an attempt to evaluate the effect of transpiration and the influence of the top on Ca uptake, excised roots and intact plants of tomato were compared in this study.
LITERATURE REVIEW

The entrance of ions into plant roots has been considered to consist of different components, which are divided into active and passive ones. Accumulation and bleeding are considered to be active components supposedly requiring energy from metabolic processes. Ion uptake components dependent on strictly physical processes, such as diffusion, adsorption and mass flow, are classed among the passive ones. The size and the importance of these different components, however, is a subject of strong controversies among the investigators.

Although, no proposed mechanism for ion uptake is entirely accepted, there are a few processes that are thought to occur in plants:

1) A passive, rapidly completed, initial phase, consisting of
   a) diffusion into the free space (AFS) of the root.
   b) Adsorption to electrically charged points in the plasma surface of the root cells. The considerable differences in the adsorption of cations and anions is in agreement with the plasma colloids being predominantly negative charged.

2) A slower accumulation in the root cells. This process proceeds at a constant rate, provided that the ion concentration
is kept practically constant in the surrounding media and
that the plant roots maintain a constant metabolic activity.

3) A transport of ions which is dependent on metabolic activi-
ties in the roots principally due to root pressure, a pheno-
menon known as "bleeding".

Chasson (12) found that when DNP was added with radioactive
calcium, the uptake of the latter by potato slices was increased over
that of the control. The respiration and accumulation in the vacuole
was increased. This effect was found to be null below a critical
concentration of DNP, and have a different behaviour when applied
over a maximum critical concentration. For instance, at a con-
centration of 5.9 × 10^{-5} M, DNP increased the Ca absorption by
potato slices and greatly inhibited the uptake of radioactive Rb by the
same tissue. When carrot slices were substituted for potato, there
was a depressing effect for both Ca and Rb, indicating that the stimu-
lation of Ca uptake appeared to be specific with respect to the ion
and the tissue. By measuring the uptake of Ca at reduced metabolic
level (low temperature and by lowering the pH from 6 to 4), they
found that DNP increased the uptake into the cell and that at the same
time, metabolic accumulation was inhibited.

Moore, et al. (33) working with excised barley roots at pH 5,
found no influences whatsoever of DNP upon the radioactive and inert
uptake of Ca. This indicates that the isotopic exchange and the Ca
*DNP, as used hereafter in this thesis, refers to dinitrophenol.*
uptake were both largely non-metabolic in nature, under the conditions of these experiments; K, which has been shown to be absorbed by plants metabolically, was completely inhibited by DNP.

It is interesting, however, to look at the differences found by Chasson and Moore, et al. While the former used potato slices and carrot slices, the latter used excised barley roots, and while Chasson took his experiment up to 24 hours, the latter investigators carried it out for only a 3 hour period. It may be that the differences found are due to the different tissues involved and to the different experimental periods used. The former worker found that the stimulation of DNP on Ca uptake usually became apparent following a lag period of four to six hours after the experiment started and that if DNP was introduced two hours after the experiment began, the same lag period was observed.

Chasson and Levitt(11) found that the absorption of Ca appeared to follow a typical two-phase time course. A rapid initial rise was followed by a small linear increase with time. This linear increase (steady state) suggests, they said, a metabolic absorption of Ca. However, this explanation was discounted by Moore, et al. (33) on the grounds that Ca\(^{45}\) exchanged for initially present inert Ca in the tissue. The small slope of their time curve was explained as an increased non-metabolic absorption as a result of the growth that could have occurred in that period of time in the plant tissue.
Florell (15, 16) observed that the amount of mitochondria was closely related to the calcium concentration in the nutrient solution. He found, for example, that differences in Ca concentration between $10^{-7}$ M and $10^{-4}$ M resulted in an increase of about 60% in dry weight of mitochondria in contrast to the 30% increase observed with root length (fresh and dry weight). This effect, though, was observed not only for Ca, but for Mn as well.

Burstrom (8) demonstrated that Ca increased the uptake of nitrate and showed that it was primarily an increase in the absorption, rather than an increase in the assimilation.

On the basis of this investigation and on the findings previously noted, Florell concluded that if Ca could influence the formation of mitochondria this might in turn explain the increased nitrate uptake. This suggests that Ca may have its action beyond the surface region, that is, in the cytoplasm itself. In this way, with an increased amount of mitochondria in the root there were more sites of absorption and more respiratory activity available for the ions and their rate of absorption was thus promoted. This is in contrast to the view of Moore, et al. who felt that the rapid isotopic equilibration of Ca$^{45}$ with inert Ca in the root indicates that the calcium is located primarily on the cell surfaces.

Handley and Overstreet (20) working with Zea mays found that Ca exerts a stimulating effect upon the respiration of the tip sections,
indicating that more than the cell wall was involved in the uptake of Ca by this tissue. By preventing development of the cell during the experiment and thereby avoiding a possible side effect on the non-metabolic uptake, they found that Ca had a physical effect on the protoplasm and that the older sections (the more vacuolated cells) absorbed Ca apparently by metabolic processes. Their experiments were restricted to the first 12 mm of the root tip.

Kramer (29) in experiments with radioactive tracers indicated that ions enter roots and move to the tops most rapidly in the same region through which most of the water enters. He pointed out that an active transport plays an important part in salt movement into roots, especially in young roots (less vacuolated cells). He also considers that passive movement by diffusion and mass flow occurs, especially in older roots and rapidly transpiring plants. Thus, he presents the situation differently from the conclusion of Handley and Overstreet mentioned earlier. It should be recognized that the latter investigators carried out their experiments with excised roots in absence of the transpiration process, a factor which had been considered by Kramer to take a major role in ion absorption.

It is known that ion absorption, whether actively accumulated or moving through free space, is not restricted to the root system but includes movement to the shoot. According to Fried and Shapiro (17) the ions moving to the tops are not necessarily those ions
accumulated by the roots, and the ions may move directly to the aerial organs chiefly in the xylem. However, they say, there are doubts as to whether a prior active step is a necessary part of this transport, yet they go along with the idea that an active step is an essential component of ion transport to tops, and they related this active step to a carrier mechanism.

Hylmo (24) in an extensive paper concluded that the increased Ca and Cl transport to the shoots associated with increased transpiration was dependent on mass flow of ions in the transpiration stream. Brouwer (4, 5, 6) contended also that ions were moving by passive means through the shoots as a result of a change in water and ion conductivity that the increased transpiration brought about.

Van den Honert, et al. (22) could not find any relationships at all between water and ion transport in their material. Biddulph, et al. (3) working with radioactive Ca on beans showed that the entry into specific stem sections consisted of two phases: a reversible exchange and an irreversible accumulation phase. The former process was completed in three hours and constituted less than 10% of the total calcium. The Ca on exchange sites could in turn, be exchanged up to the stem; the loss curve for the Ca$^{45}$ tracer fell through three half values in six hours. They also concluded that Ca was mostly in the calcium oxalate crystal system, the low solubility being the driving force for accumulation. The order of increasing
calcium concentration was found to be roots, stems, trifoliate leaves and primary leaves, indicating that an important factor was the total volume of the transpiration stream received.
MATERIAL AND METHODS

Excised Roots

Alaska pea (Pisum sativum), KA3 corn (Zea mays), Ohio Mr 17 cucumber (Cucurbita pepo), and Top Crop bean (Phaseolis vulgaris) varieties were used in these experiments. To ensure reproducibility of the experiments, plants were grown under carefully standardized conditions. Approximately 500 grams of pea, corn, and bean seeds, and about 100 grams of cucumber seed were soaked for 24 hours in 2 liters of distilled water with continuous aeration. The germinating seeds were then washed several times with distilled water and spread uniformly on cheese cloth placed over stainless steel racks. The racks were placed in plastic rat cages of 10 liters capacity containing 3 liters of the prepared nutrient solution found to be suitable for the growth of the plant material. The level of the nutrient solution was adjusted to within 1/2 inch of the germinating seeds. To secure uniform growth of the seedlings over the entire tray, forced aeration of the culture solution was necessary. A moist double layer of cheese cloth was placed over the seeds to maintain a water saturated air environment. The entire assembly was then placed in the dark and maintained at a constant temperature of 22°C for 8 days.

The culture solution used was CaSO₄·2H₂O at a concentration
of 0.1 meq of Ca per liter, which was renewed three days after setting out the trays. At this time the roots were well established in the nutrient solution so the upper cheese cloth was removed. At the end of the eight days the plants were harvested.

The roots were cut off just below the rack with a razor blade and cut into about 3 cm lengths. These excised roots were washed in distilled water and wrapped in moist cheese cloth for centrifugation to remove the excess water adhering to them. The centrifugal force and the time used were 65 x g for 5 minutes. Roots with swollen root tips were discarded. Approximately 60 grams per tray were obtained by this culture technique. After centrifugation, representative samples of roots were weighed out and placed in bottles of the desired salt solution, which were kept at constant temperature (22°C). In all experiments, 1 gram of roots per liter of solution was used. The solution used throughout was CaSO₄ · 2H₂O at a concentration of 5 meq of Ca per liter. The pH of 5 was rigorously controlled by adding H₂SO₄ or Ca(OH)₂ during the experimental period. The amount of base or acid added was negligible compared to the actual CaSO₄ concentration. The solutions were aerated continuously during the experiments by bubbling compressed air through the system.
Analytical Procedure for Excised Roots

At the conclusion of an experiment, the roots were separated on a Nylon mesh filter and washed for 10 seconds with running distilled water. The washed roots were dried in an oven and then digested with nitric and perchloric acid (19). The digest was filtered, diluted to volume and an aliquot of the unknown was passed through a column of resin, as described by Carlson, et al. (10). This step was taken to get rid of the interfering ions, such as Mn, Al, and Fe, which interfere with the end point of the titration. NaCyDTA (sodium cyclo hexane di-amine tetra acetic acid) with Calcein as indicator was used to titrate the samples.

The data are expressed as net uptake of Ca, given in meq per kilogram of roots, as a result of the different time periods of absorption.

Intact Plants

Tomato plants (Lycopersicum esculentum), OSU 465, of the crop of 1960, were germinated, transplanted and allowed to grow in plastic buckets of 10 liters volume, which were placed in a controlled environment chamber.

The growth chamber used had the following characteristics:
Day length period of 15 hours.
Temperature during the day of 22°C ± 1°C, and 20°C ± 1°C at night.
Relative humidity of 50% ± 1%.
The light was provided by incandescent bulbs and fluorescent lamps, giving off approximately 2,000 foot candles at the top of the plants.

The nutrient solution employed was 1/8 Hoagland solution (21), with the exception that the Ca salt was added at a rate of 0.10 mM of Ca per liter and 0.5% iron tartrate solution at a rate of 1 cc per liter was used as a source of iron. Care was taken to detect any deficiency symptoms. Phosphorus and iron in its respective salts were added periodically for plants showing slight deficiency symptoms of these nutrients. The whole nutrient solution was renewed each week, and the growth of the tomato plants was vigorous, yet in a low salt condition. A week before the experiment started, the tomato plants were placed under continuous light to reduce the diurnal variation, a phenomenon which according to Gunar, et al. (18), is an important factor to consider in ion uptake by intact plants. The plants growing under natural conditions show diurnal variations which added to their inherent genetic variability may lead to erroneous interpretation of results.

Three days before the experiment began, the plants were transferred to distilled water to allow the roots to become depleted of ions.

At the time of the experiment, enough plants to assure
sufficient root material were excised and subjected to the same experimental treatment followed with the younger excised roots as described above. With the rest of the material, 5 plants for each treatment with 2 replications were selected on the basis of uniformity. The plants were placed in plastic buckets of 10 liter volume with a solution made of 5 meq of Ca per liter, supplied as CaSO$_4$·2H$_2$O labeled with about 30 μc of Ca$^{45}$. The pH of 5 was maintained throughout the experimental period by adding acid or base. The solutions were aerated during the experiment.

**Analytical Procedure for Intact Plants**

At the end of the experimental period, a careful separation of roots and tops was made. The roots were blotted to remove excess moisture adhering to them. Tops and roots were then placed in the oven to be dried. After drying, tops were ground and a representative sample of 1 gram was digested by the nitric-perchloric acid method. The whole root material was, at the same time, digested following the same digestive procedure as with the excised roots. Aliquots of the digested solution were then titrated with NaCyDTA to determine the total calcium content in tops and roots. In addition, 25 mls of the original digested solution was neutralized, diluted to 50 mls, from which 2 mls were taken and placed in aluminum
planchets and dried. The samples were then counted twice using a thin window flow counter to detect the beta radiation. Duplicate planchets were prepared for each sample. Standards were made which had the same mass as the unknowns. These standards consisted of aliquots taken from the original labeled solution to determine the amount of counts per milliequivalent of Ca.

Variations in count due to differences in mass and geometry were negligible.

The data are expressed as meq of Ca in tops on a kilogram of root basis as it was related to time of absorption.
RESULTS AND DISCUSSION

The carrier hypothesis used to describe the process of ion accumulation by plant cells is based on the assumption that the entrance of ions into living cells is accompanied by a binding or adsorption by some protoplasmic constituent, i.e., to a "carrier molecule". According to Epstein and Hagen (14) this process can be pictured as follows: "at the outer surface of a membrane which is impermeable to the free ions, the ions combine with metabolically produced binding compounds or carriers. They traverse the membrane in this form. Upon reaching the inner surface, the carrier is chemically altered by metabolic processes so that the ions are set free". These assumptions can be expressed in the following equation:

\[
\begin{align*}
1) \quad & R + M \xrightarrow{k_1} MR \\
& \xrightarrow{k_3} \text{outside} \xrightarrow{k_2} \text{inside} \\
2) \quad & MR \xrightarrow{k_4} R' + M
\end{align*}
\]

where \( R \) and \( R' \) represent different chemical states of the metabolically produced carrier, \( M \) the ion, \( MR \) the unstable carrier-ion complex, and \( k \) the rate constant for each reaction indicated. The over-all reaction is essentially irreversible.

Jacobson, et al. (25) suggested that these binding compounds form complexes with cations with the liberation of hydrogen ion, and similar compounds combine with anions with the liberation of
hydroxyl or bicarbonate ions. In the case of cations the chemical reaction taking place would be

3) \( \text{HR} + \text{M}^+ \rightleftharpoons \text{MR} + \text{H}^+ \), and for anions

4) \( \text{R'O'H} + \text{A}^- \rightleftharpoons \text{R'A} + \text{OH}^- \),

where HR and R'O'H represent the metabolically produced cationic and anionic binding substances.

Fried and Shapiro (17), following Epstein and Hagen suggestions, stated that if Equation (1) and (2) or a variation of these equations describes ion accumulation, mathematical expressions of these equations could be used to describe the process. A distinct advantage of this approach is that the equation can be quantitatively tested, provided steady-state conditions are established. If it is assumed that \( k_3 \) is much larger than \( k_1 \) or \( k_2 \) and that \( k_4 \) is essentially zero, one form of the kinetic expression for ion accumulation is \( v = MRk_3t \). In this expression \( v \) is the amount of an ion actively accumulated, \( M \) is the concentration of ion in the external solution, \( R \) is the carrier concentration, \( t \) is time, and \( k_3 \) is a constant.

Where the concentration of \( M \) in solution is maintained essentially constant and the plant root keeps a constant carrier concentration, then a plot of uptake versus time should give a straight line (steady-state). The assumption that \( k_4 \) is essentially zero leads to the conclusion that active absorption is an irreversible process. Active transport as compared to passive or exchange adsorption has been
shown to be linear with time.

**Excised Roots**

Figures 1, 2, 3 and 4 show the net uptake of Ca, expressed as milliequivalents of Ca per kilogram of root (fresh weight) of cucumber, pea, bean, and corn excised roots, as a function of time. In all these experiments the time periods used were: 0, 1/2, 1, 2, 3, 6, 9 and 12 hours. The first treatment (0 time) was used to determine the initial calcium content of the roots. The initial value was subtracted from the Ca content of the roots for the treatments to arrive at the net uptake for the various time periods. These curves show the rapid initial equilibration, characteristic of apparent free space (AFS), followed by a much slower process. The value for the 30 minutes uptake period was used to assess the AFS. While this is somewhat arbitrary, it was felt that the initial equilibration was complete in this time period. Kylin and Hylmo (30) working with labelled sulphate and wheat roots have reported the passive uptake to be finished after 15 minutes. Butler (9) working with radiosulphate and wheat roots reported 30 minutes, and Epstein (13) obtained an equilibration period of 1 hour from sulphate measurements on barley roots. However, in Epstein experiments the AFS equilibration was approximately 96% complete in 30 minutes. Hope and Stevens (23) reported equilibration of the AFS within 10 minutes.
FIGURE 1

The uptake of calcium by excised cucumber roots as a function of time
FIGURE 2

The uptake of calcium by excised pea roots as a function of time

Net Ca uptake in meq Ca/kg roots (fresh weight)

Time in Hours
FIGURE 3

The uptake of calcium by excised bean roots as a function of time.
FIGURE 4

The uptake of calcium by excised corn roots as a function of time.
The values of AFS are calculated according to the equation given by Pettersson (35), which is:

$$\frac{\mu\text{mol initial uptake/ g fresh weight}}{\text{ambient concentration (mM)}} \times 100.$$  

The error in estimating the AFS in this way is very small because of the extremely slow steady-state uptake. Even for corn, where the metabolic absorption rate may be significant, the error is probably less than 1/10 of the AFS value. The AFS values found for cucumber, pea, bean, and corn excised roots were 26, 28, 27 and 28% respectively. Of particular interest is the fact that all of the plants tested showed an essentially constant AFS value. These results are in agreement with the ones found by Jacobson, et al. (26) who gave values of this magnitude for several cations and with Butler (9) and Epstein (13) who found AFS values ranging from 24 to 33%. Kylin and Hylmo (30), Hope and Stevens (23), and Levitt (31) found values close to 10%.

The net uptake and the respective rates of uptake for the initial phase (AFS), for the period from 30 minutes to 3 hours, and from 3 hours up to 12 hours are presented in Table 1.

Cucumber excised roots (Figure 1 and Table 1) showed a net uptake of 1.39 meq Ca per kilogram of roots (fresh weight) during the 12 hour experimental period. Almost 95% of the total uptake was already in the plant root at 30 minutes occupying the so-called outer
TABLE I

Net uptakes and rates of uptake of calcium for excised roots systems.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>NET UPTAKE 0-12 hours (meq Ca/kg)</th>
<th>RATE OF UPTAKE FOR AFS (meq Ca/Kg/hour)</th>
<th>RATE OF UPTAKE 30 min to 3 hours (meq Ca/Kg/hour)</th>
<th>RATE OF UPTAKE 3 hours to 12 hours (meq Ca/Kg/hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cucumber</td>
<td>1.39</td>
<td>2.64</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Pea</td>
<td>1.78</td>
<td>2.90</td>
<td>0.010</td>
<td>0.022</td>
</tr>
<tr>
<td>Bean</td>
<td>1.58</td>
<td>2.76</td>
<td>0.040</td>
<td>0.001</td>
</tr>
<tr>
<td>Corn</td>
<td>2.65</td>
<td>2.86</td>
<td>0.310</td>
<td>0.075</td>
</tr>
</tbody>
</table>
or free space. With pea excised roots, 80% of the total net uptake should be credited to AFS. (Figure 2). Bean excised root (Figure 3) in turn, showed that almost 90% of the total net uptake was already in the plant root at 30 minutes. These results indicate that the total uptake is largely non-metabolic in these systems and that no appreciable accumulation of Ca was obtained. These results are in agreement with the ones found by Moore, et al. (33) working with excised barley roots. However, they are in disagreement with Chasson (12) who found Ca being accumulated in significant amounts by potato tissue slices. The results of Chasson may be open to argument since he determined uptake using Ca$^{45}$ and appreciable isotopic exchange may have occurred (33).

Figure 4 shows the Ca uptake by corn excised roots. It appears here that the time curve can be divided easily in three sections. The rapid initial uptake of Ca completed at 30 minutes was responsible for 54% of the total net uptake during the 12 hour period. Following this, the period between 30 minutes and 3 hours is an indication that Ca was being accumulated by these roots. This part of the time curve is in agreement with the results of Handley and Overstreet (20) who found Ca was accumulated by younger sections of corn roots. It is interesting to note that after the uptake due to AFS was deducted, this period of accumulation was responsible for 87% of the remaining uptake. After 3 hours the time curve has clearly leveled off to
become almost parallel to the time axis, indicating that a very slow rate of increase was occurring similar to the other species.

There are two possibilities by which the small positive slope of Ca uptake can be explained.

1) Since growth may have occurred during the 12 hour experimental period, an increase in the total non-metabolic uptake component could have also occurred. The positive slope of the time curves would then indicate an increase of this component, rather than showing a metabolic accumulation. This point was supported by Moore, et al. (33) who found that Ca was mostly taken up by non-metabolic processes in working with excised barley roots.

2) The continued uptake of Ca might be a reflection of the accumulatory power of the younger segments of the root system. This may be especially true for the part of the time curve between 30 minutes and 3 hours for corn excised roots. This is supported by the results of Handley and Overstreet (20) who found a rapid metabolic absorption of Ca by the young portion of excised corn roots. The net metabolic absorption for the whole root system might, therefore, be a reflection of the active uptake of a small portion of the roots. These considerations would lead to the conclusion that the mature root cells are relatively impermeable to Ca. This point is in agreement with the results found by Florel (15, 16) and Burstrom (8) who found that Ca was accumulated by the mitochondria of the
younger root segments which are known to be actively metabolizing as compared with the older sections or more vacuolated cells of the root system.

At any rate comparing the two components, namely AFS and accumulation, the AFS was the larger of the two even if all of the uptake after 30 minutes was assumed to be metabolic accumulation. The conditions for these experiments were purposely chosen to enhance metabolic Ca absorption. It should be recalled that these roots were grown in a dilute solution so that the experimental material would be in a "low salt" condition. The initial Ca contents of these roots were low and it is unlikely that the cells were already saturated with Ca. The initial contents of Ca were 14.51, 15.80, 26.32, and 25.75 meq per kilogram of roots for cucumber, pea, bean and corn respectively. It is interesting to note that corn actually had a higher initial content than did cucumber and pea, yet showed considerable more evidence for a metabolic component. Ions such as Cs, Rb, Na, K, Li and Mg, which are known to be absorbed metabolically, are readily taken up by systems such as the ones used here (27, 28, 34). Their rates of absorption are many times larger than those observed for Ca on a whole root basis. It would appear that Ca absorption is at least quantitatively different from the other cations. The results of Handley and Overstreet (20) suggest that the physiological maturity of the cells may be the controlling factor in
Ca absorption.

Excised Root vs Intact Plant

Figures 5 and 6 record the calcium uptake by 6 weeks old excised tomato roots and radiocalcium uptake by intact tomato plants of the same age. Just as with the younger materials, tomato plants were reared in dilute solutions, with the difference that five days before the experiment started, the plants were placed in distilled water in order to further increase the "low salt" condition. Hylmo (24) showed that plants growing in a suboptimal calcium condition, took up considerable more calcium than those which had been well supplied with Ca.

The time curve for the excised roots can be separated into three portions (Figure 5). First, there is the characteristic rapid initial equilibration in the first 30 minutes. A larger AFS (35%) than with the younger excised roots was observed which may be due either to a species difference or to a greater proportion of mature cells as a consequence of a longer growing period (6 weeks as compared with 8 days). The segment between 30 minutes and 3 hours shows a rate of increase of about 0.17 meq per kilogram per hour. This is about 7 times faster than the 0.024 meq/hr observed for the 3 to 48 hour period.

Figure 6 shows the uptake of Ca by the intact tomato plants.
FIGURE 5

The uptake of calcium by excised tomato roots as a function of time
The uptake of calcium by intact tomato plants as a function of time

Regression equation:
\[ \hat{y}_x = \hat{y} + b(x - \bar{x}) \]
\[ = 18.57 + 0.93(x - 25) \]

Correlation coefficient:
\[ r = 0.97 \]
This figure records a marked relationship between uptake and time of absorption. The regression line and the correlation coefficient were calculated, assuming a straight line relationship. The high correlation coefficient indicates the closeness with which the experimental points approached the regression line. A rate of uptake of 0.93 meq of Ca per hour on a kilogram of root basis (fresh weight) was observed. Comparison with the absorption by excised tomato roots at the faster rate of uptake (between 30 minutes and 3 hours) indicates that intact plants absorbed Ca about 5.5 times faster than the excised roots. When compared on the basis of the 3 to 48 hour period, the intact plants absorbed Ca almost 39 times faster. These are minimum values since the absorbed Ca retained by the roots of the intact plants is not included here. The results in Figure 6 refer only to the Ca actually reaching the top during the experimental period. Isotopic exchange (33) in the root made it impossible to evaluate the amount of Ca taken up and retained by the roots during the experiment.

The most obvious difference between the excised root and intact plant systems is the presence of an active transpiration process in the latter case. But whether this transpiration effect is direct or indirect is open to argument. According to Hylmo (24) there is no need for assuming a chain of absorption, secretion or "leakage", and absorption by a neighbouring cell in order to account for absorption and translocation of the ions through the roots and on to the shoot.
He said that ions are drawn through the root by mass flow in the transpiration stream, passively going through without intervention of the active absorbing mechanism. Brouwer (4, 5, 6) concluded that salt uptake is an active process, and that at increasing suction tension in the vessels, there occurs an increase in conductivity for water and ions. Van den Honert, et al. (22) concluded from experiments with corn that there was no direct influence whatsoever of transpiration in ion absorption. It is possible that stimulation of metabolic uptake by bleeding and removal of ions to the tops brought about by transpiration may occur.

Whatever the influence of transpiration may be, the question still remains as to how the ions reached the xylem vessels. Presumably, the ascending sap in the transpiration stream passes through the xylem bundles. In the experiments with excised roots apparently most of the Ca was in the AFS of the roots, and beyond that no big observable accumulation was seen. However, by excising the roots, obviously the plants were devoid of the transpiration process. In addition by cutting them into pieces the normal structure of the root system is altered by opening the xylem bundles to the external solution. Therefore, any unidirectional active transport across the endodermis to the xylem would "leak" out the cut endodermis and thus go undetected. According to the views of Hylmo (24) and Kramer (29), the free space extends radially through the roots to the water
conducting elements of the xylem making it possible for the external solution to move by mass flow through free space of the surrounding cells until it reaches the xylem and is carried up to the shoot by the transpiration stream, thus denying a possible role of endodermal cells in ion accumulation. Sandstrom (37) showed that in roots without epidermis the ions moved passively with the water and passed into the xylem vessels in the same concentration as in the external solution. Selective absorption seemed to have disappeared, and the cells surrounding the xylem pumped the solution into the vessels without any selective action. Lundegardh (32) is of the opinion that the active cells are situated contiguous to xylem vessels, and that ions on their way to the xylem have to pass through the vacuoles. Broyer (7) demonstrated, on the other hand, with radioactive bromide ions that the salt may be moved to the xylem without entering the vacuoles. Butler (9), van den Honert (22), Arisz (1) and Brouwer (4, 5, 6) believe that most of the ions reach the conducting system via a metabolic pathway.

In a recent paper Bernstein and Nieman (2) proposed an alternative hypothesis to the mass flow one. They said that if free space extends radially into the root as far as some limiting layer, such as the endodermis, an accumulation of solutes in this free space is effected as water is selectively absorbed by cortical and endodermal cells under the influence of transpiration and that this fact may
account for increased salt uptake by plants under conditions of high transpiration.

Moore, et al. (33) in their study of Ca uptake on barley excised roots, advanced the hypothesis that ions may move from the external solution along surfaces into the xylem without ever being absorbed actively. There may be fixed negative charges in the cell wall as well as in the plasmalemma, with Ca migrating along these charged surfaces. And they furthermore stressed the point that if we associated these fixed charges with phosphatides and pectic substances, this type of movement could be rather specific for certain ions, Ca being one of the most important ones.

If the endodermal cells are acting and influencing the transport of ions there is no way of comparing excised roots versus intact plants, for in the latter the stelar region is not readily accessible to the external solution. If a barrier is located at the endodermal cells, any major movement of ions by means of mass flow would be precluded.
SUMMARY AND CONCLUSIONS

Most of the Ca, under the working conditions, appeared to be absorbed by excised plant roots by purely physical means. A slow rate of accumulation was also observed which varied according to the plant species used. However, when working with different plant species, differences in physiological maturity of the root cells in a given species may be important. As Kramer (29) pointed out, the absorption mechanism of a root system behaves much like the absorption mechanisms of the individual cells. The results reported here would seem to indicate that as cells mature they become impermeable to Ca. If this is true, the presence of an active or a passive Ca uptake would be a matter of the physiological stage of the cells which we are dealing with.

The presence of the top definitely exerts a strong influence on calcium absorption by plants. Three possibilities exist to explain the differences in uptake between excised root and intact plants observed in this study.

1) Ca moves by mass flow along with the water through channels in the cell wall. Migration of ions along charged surfaces (33) is a special case of mass flow inasmuch as the energy required to move the ions is supplied by the moving transpirational stream. These represent strictly passive processes.
2) Movement of ions to the top could stimulate metabolic transport by increasing the gradient across the root system. This stimulation or "bleeding" would represent an indirect effect of transpiration on the metabolic accumulation process.

3) The endodermal cells may actually be responsible for the transport of ions which ultimately are translocated to the top. The uptake by excised roots would therefore represent only the accumulation of ions by the individual cells of the root. Because of the cut ends of excised roots, uni-directional transport of calcium to the stele would merely "leak" out the cut ends. This type of transport would be classed as metabolic.

The diversity of opinion whether an active or passive process is of a greater importance need not be contradictory to each other. Instead, the environmental conditions under which plants are growing, species differences, and physiological stage of the cells in a given root system might be factors in determining the predominance of one process over another.
BIBLIOGRAPHY


APPENDIX
# APPENDIX

**Excised Root Systems**

1. - Cucumber

<table>
<thead>
<tr>
<th>Time</th>
<th>0 time</th>
<th>14.51 milliequivalent of Ca per kilogram of fresh roots.</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 minutes</td>
<td>15.83</td>
<td></td>
</tr>
<tr>
<td>1 hour</td>
<td>15.88</td>
<td></td>
</tr>
<tr>
<td>2 &quot;</td>
<td>15.83</td>
<td></td>
</tr>
<tr>
<td>3 &quot;</td>
<td>15.83</td>
<td></td>
</tr>
<tr>
<td>6 &quot;</td>
<td>15.88</td>
<td></td>
</tr>
<tr>
<td>9 &quot;</td>
<td>15.88</td>
<td></td>
</tr>
<tr>
<td>12 &quot;</td>
<td>15.90</td>
<td></td>
</tr>
</tbody>
</table>

2. - Pea

<table>
<thead>
<tr>
<th>Time</th>
<th>0 time</th>
<th>15.80 milliequivalent of Ca per kilogram of fresh roots.</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 minutes</td>
<td>17.25</td>
<td></td>
</tr>
<tr>
<td>1 hour</td>
<td>17.31</td>
<td></td>
</tr>
<tr>
<td>2 &quot;</td>
<td>17.28</td>
<td></td>
</tr>
<tr>
<td>3 &quot;</td>
<td>17.31</td>
<td></td>
</tr>
<tr>
<td>6 &quot;</td>
<td>17.39</td>
<td></td>
</tr>
<tr>
<td>9 &quot;</td>
<td>17.35</td>
<td></td>
</tr>
<tr>
<td>12 &quot;</td>
<td>17.58</td>
<td></td>
</tr>
</tbody>
</table>

3. - Bean

<table>
<thead>
<tr>
<th>Time</th>
<th>0 time</th>
<th>26.32 milliequivalent of Ca per kilogram of fresh roots.</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 minutes</td>
<td>27.70</td>
<td></td>
</tr>
<tr>
<td>1 hour</td>
<td>27.88</td>
<td></td>
</tr>
<tr>
<td>2 &quot;</td>
<td>27.83</td>
<td></td>
</tr>
<tr>
<td>3 &quot;</td>
<td>27.83</td>
<td></td>
</tr>
<tr>
<td>6 &quot;</td>
<td>27.88</td>
<td></td>
</tr>
<tr>
<td>9 &quot;</td>
<td>27.88</td>
<td></td>
</tr>
<tr>
<td>12 &quot;</td>
<td>27.90</td>
<td></td>
</tr>
</tbody>
</table>
4. - Corn

<table>
<thead>
<tr>
<th>Time</th>
<th>Ca Milliequivalents/kg Fresh Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 time</td>
<td>25.75</td>
</tr>
<tr>
<td>30 min</td>
<td>27.18</td>
</tr>
<tr>
<td>1 hr</td>
<td>27.50</td>
</tr>
<tr>
<td>2 &quot;</td>
<td>28.00</td>
</tr>
<tr>
<td>3 &quot;</td>
<td>28.25</td>
</tr>
<tr>
<td>6 &quot;</td>
<td>28.30</td>
</tr>
<tr>
<td>9 &quot;</td>
<td>28.35</td>
</tr>
<tr>
<td>12 &quot;</td>
<td>28.40</td>
</tr>
</tbody>
</table>

5. - Tomato Excised Roots

<table>
<thead>
<tr>
<th>Time</th>
<th>Ca Milliequivalents/kg Fresh Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 time</td>
<td>12.62</td>
</tr>
<tr>
<td>30 min</td>
<td>14.41</td>
</tr>
<tr>
<td>1 hr</td>
<td>14.40</td>
</tr>
<tr>
<td>2 &quot;</td>
<td>14.68</td>
</tr>
<tr>
<td>3 &quot;</td>
<td>14.91</td>
</tr>
<tr>
<td>6 &quot;</td>
<td>15.04</td>
</tr>
<tr>
<td>9 &quot;</td>
<td>15.17</td>
</tr>
<tr>
<td>12 &quot;</td>
<td>15.52</td>
</tr>
<tr>
<td>24 &quot;</td>
<td>15.56</td>
</tr>
<tr>
<td>36 &quot;</td>
<td>15.94</td>
</tr>
<tr>
<td>48 &quot;</td>
<td>16.00</td>
</tr>
</tbody>
</table>

6. - Tomato Intact Plants

<table>
<thead>
<tr>
<th>Time</th>
<th>Ca Milliequivalents/kg Fresh Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 time</td>
<td>It was not calculated due to isotopic exchange.</td>
</tr>
<tr>
<td>10 hrs</td>
<td>7.55</td>
</tr>
<tr>
<td>20 &quot;</td>
<td>9.75</td>
</tr>
<tr>
<td>30 &quot;</td>
<td>22.74</td>
</tr>
<tr>
<td>40 &quot;</td>
<td>34.23</td>
</tr>
</tbody>
</table>