A Mechanism for the Leaching of Calcium from Foliage

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Summary. Young bean plants (Phaseolus vulgaris) containing root-absorbed 45Ca and 86Rb were leached to determine the pathway and mechanism of cation loss by leaching. Calcium is leached from the exchangeable calcium fraction within the plant by a process of ion exchange and diffusion involving exchange sites both within the leaf and on the leaf surface. Leaching of cations is primarily a passive process, although some metabolites may be deposited upon leaf surfaces by active processes. The exchange and diffusion explanation is compatible with current theories of ion uptake and translocation and explains the results of numerous experiments on leaching reported in the literature.

Inorganic and organic metabolites are leached from foliage, stems, flowers, and fruits by aqueous solutions including rain, dew, and mist (1,14,15). Metabolites which are leached include all of the inorganic minerals found in plants, large quantities of carbohydrates, 21 amino acids, and at least 15 organic acids (10).

Tukey et al. (15) have shown that as cations are leached from the foliage, they are replaced by translocation from the roots and other plant parts. In fact, leaching of the foliage accelerated the rate of root uptake and translocation of 45Ca into the stems and foliage of bean plants (8). These results indicate a close relationship between translocation and leaching of calcium, as the following experiments verify.

Materials and Methods

Young bean seedlings (Phaseolus vulgaris L.) in the primary leaf stage were grown in the greenhouse in aerated nutrient solution cultures to which either 45Ca or 86Rb was added. Subsequent to or during the uptake of the radioisotopes, the stems and foliage were leached either by exposure to an atomized mist of distilled water or immersion in aerated aqueous solutions for periods of up to 24 hours (15). The leachates were collected and fractionated on Amberlite IR-120 cation exchange resin which adsorbed the leached cations, including the radioactive materials. The leached constituents were removed from the resin by elution with 2N HCl and analyzed for radioactivity. Tissue samples from the leached plants were collected, and ashed at 500°C for 12 hours. The residue was taken up in 0.1 N HNO3, and a 1-ml aliquot was analyzed for radioactivity with a Geiger-Müller detector.

In another series of experiments the specific activity of 45Ca (cpm 45Ca/mg Ca) was determined in the foliar leachate and in 3 calcium fractions of bean shoot tissue. Bean plants were placed in nutrient solution containing 25 μC of 45Ca/liter of solution Simultaneously, the plants were leached by the atomized mist spray and the radioactive leachate was collected (leachate fractions). The leached plants were then placed in a hydraulic press and the cell sap was expressed and collected (cell sap fractions). The pressed tissue was homogenized in 1 N ammonium acetate (pH 7), heated to 70°C to precipitate the homogenate, and filtered to separate the exchangeable fractions from the residue or nonexchangeable fractions. Although some exchange did occur between the cell sap and the cell wall during pressing, it had no significant effect upon the results.

The plant tissue fractions and the foliar leachate were dried, ashed, and taken up in 0.1 N HNO3. Manganese, iron, aluminum, and phosphate were removed by precipitation and centrifugation (11). Calcium, including 45Ca in the supernatant fraction was precipitated and centrifuged into planchets as calcium oxalate (3), which was then analyzed for radioactivity. The weight of the calcium oxalate precipitate was determined and used for calculating the amount of total calcium and for correcting for self-absorption in the determination of 45Ca. The specific activity of each fraction was calculated by dividing the cpm of 45Ca in each sample by the mg of total calcium.

Results

Energy Relations. Arens (1) used the term kutikularie exkretion to describe leaching, suggesting...
that there was an active mechanism involved, whereas others (13) maintained that leaching was primarily a passive process such as diffusion. If the leaching process is an active one, presumably energy is required to maintain it, and by manipulating the energy level within a leached plant, some insight might be gained as to the mechanism of loss.

Accordingly, bean plants containing root-absorbed $^{45}$Ca and $^{86}$Rb were transferred to nonradioactive nutrient solution. Half of the plants were lightly sprayed, twice within a 12-hr period, with an atomized mist of $10^{-4}$ M 2,4-dinitrophenol (DNP). The spray solution contained a wetting agent (Tween 20) and was adjusted to pH 6.0 with NaOH. The DNP solution caused no apparent injury to the leaves when applied in this fashion, but resulted in a subsequent decreased rate of growth. Losses by leaching with a distilled water mist from the DNP-treated plants were compared with nontreated plants over a 4-day period following treatment. Loss of $^{45}$Ca averaged 0.8 % per day from DNP-treated plants and 1.0 % per day from untreated plants. Loss of $^{86}$Rb averaged 3.8 % per day from both sets of plants.

In a second experiment, the reserve energy of bean plants was depleted by placing them in continuous darkness for up to 5 days prior to and during leaching. Losses of $^{45}$Ca and $^{86}$Rb (root-absorbed immediately prior to treatment) from these plants were compared with plants grown in natural light. The results show again very little difference between the 2 sets of plants. Loss of $^{45}$Ca averaged 1.2 to 1.6 % per day and loss of $^{86}$Rb averaged 3.75 % per day.

These results indicate that the energy level had little influence on the leaching, thus supporting the proposal that leaching is primarily a passive process. However, metabolites may be deposited upon leaf surfaces by active processes such as guttation from hydathodes and trichomes, later to be washed away by rain and dew. This, too, is properly called leaching (14).

Source of Leached Calcium. Bean plants, growing in complete nutrient solution, were divided into 8 groups of 16 plants each. The first group of 16 was placed in a solution containing $^{45}$Ca for 24 hours and then returned to the complete nutrient solution in which they had been growing. A second group of 16 plants was similarly treated the second day, and so on until all 8 groups had been treated with $^{45}$Ca on successive days. All plants from all treatments were then leached simultaneously on the eighth day. The last group was thus being leached during the 24-hour period during which it was absorbing $^{45}$Ca from the radioactive solution.

During the 8-day period, the plants increased in dry weight due to the full expansion of the primary leaves. Uptake of $^{45}$Ca from the nutrient solution increased with each succeeding day. Thus, uptake on the eighth day was almost double the uptake on the first day. Because of these differences in uptake, losses of $^{45}$Ca by leaching were expressed as a percentage of the $^{45}$Ca in the plants.

The results presented in figure 1 indicate that the most recently absorbed $^{45}$Ca was the most easily leached. Only 0.5 % of the $^{45}$Ca was leached from the first group of plants which had been treated on the first day, 7 days prior to leaching. The losses increased with each succeeding day to a maximum loss of 4.8 % of the $^{45}$Ca which was root-absorbed on the same day that the plants were leached.

These results again suggest that the calcium that is being translocated within the plant is the major source of the calcium that is leached. It might be expected that a major portion of the recently absorbed $^{45}$Ca would be in the translocation stream of the plant, whereas a major portion of the previously absorbed calcium would be incorporated in relatively unleachable forms in the leaf.

One way of directly determining the source of leached calcium is to determine the specific activity of $^{45}$Ca (ratio of $^{45}$Ca to total calcium) in various calcium fractions within the plant and in the foliar leachate. In such an experiment, the specific activity of $^{45}$Ca in various calcium fractions in the plant will increase with time as the $^{45}$Ca is absorbed by the roots. Those fractions most closely related to absorption and translocation will increase rapidly, whereas those fractions remote from the translocation process will increase slowly. The fraction within the

![Figure 1](https://example.com/image1.png)
Fig. 2. Specific activity of $^{45}$Ca (cpm $^{45}$Ca/mg calcium) in the foliar leachate and in the cell sap fraction, the exchangeable fraction, and the residue fraction of bean plants (Phaseolus vulgaris L.) during 24 hours of leaching with a distilled water mist.

This hypothesis explains the results presented in this paper and is compatible with the results of other leaching experiments. Although developed for calcium, it is applicable to other cations such as potassium, rubidium, and strontium. For example, it explains the characteristic alkaline nature of plant leachates noted by Arens (1) and others. Water on the leaf surface dissolves CO$_2$ from the air to form carbonic acid. The carbonic acid dissociates and the released hydrogen exchanges with cations on the cuticle exchange sites to form alkaline carbonates, which either remain in the leaching solution, or are precipitated onto the leaf surface. In this manner, leaves may become even encrusted with whitish accumulations of carbonates, as reported by Morgan (9) on the leaves of chrysanthemum growing in the greenhouse. This substantiates the conclusion of Greendale and Nye (5) that bicarbonate was the primary anion in leachates from tropical trees.

The exchange and diffusion hypothesis explains why the volume of the leaching solution had little influence upon the leaching of $^{45}$Ca (7, 9). On the basis of the exchange hypothesis, the volume of leaching solution need only be sufficient to wet the leaf surface, and additional volume would increase the exchange only slightly, perhaps not enough to be detected. This also substantiates reports that dew and light rain of long duration are more effective in leaching metabolites than are heavy rains of short duration (14).

Changes in temperature, light (15), and energy levels in the plant have little influence upon the leaching of cations such as $^{45}$Ca, $^{40}$K, $^{22}$Na, $^{24}$Mg, $^{88}$Rb, $^{89}$Sr, and $^{65}$Zn. This would be expected in that foliar leaching is a physical process of cation exchange and diffusion, which would be affected by such factors to such a small degree as to make detection difficult.

Matute leaves are more susceptible to the leaching of organic and inorganic metabolites than are young leaves (1, 13, 14, 15). Calcium is easily leached from the exchangeable calcium pool in mature tissues. However, young, vigorously growing tissues accumu-
late calcium within cells and cell walls, from where it is not readily leached, thus reducing the amount of exchangeable calcium available for leaching. Thus, in effect, foliar leaching successfully competes with cellular metabolism and vice versa, for exchangeable cations and for other metabolites which are being exported, even to the extreme of killing plants by starvation during prolonged periods of rain.

**Literature Cited**