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# Cuticular Permeability to Calcium Compounds in 'Golden Delicious' Apple Fruit

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***Abstract.*** **Isolated cuticles from 'Golden Delicious' apples (*Malus domestica* Borkh.) were mounted in a flow-through diffusion cell. Donor solutions containing 0.5M Ca chloride, Ca acetate, Ca nitrate, and two commercial formulations of Ca were allowed to flow over the outer surface of the cuticle. Calcium that permeated the cuticle was collected and analyzed by atomic absorption spectrophotometry. The effect of temperature and pH on Ca penetration also was investigated. Calcium chloride permeated the cuticle significantly faster than other organic or inorganic forms of Ca tested. The Ca penetration rate decreased with decreasing temperature as predicted by the temperature coefficient for diffusion of strong electrolytes. The decrease in the rate of diffusion at low temperatures seemed to be due to the increase in viscosity of the solution. Uptake of $CaCl_2$ tended to be higher at pH 3 than at pH 11. The pCa was also higher at pH 3 than at pH 11. Solutions of $CaCl_2$ dried significantly slower than other forms of Ca tested. Cuticular Ca movement and the potential advantages of the system for screening cuticular permeability of various forms of Ca were investigated.**

Pre- and postharvest Ca treatments have been shown to reduce the incidence of bitter pit, scald, internal breakdown, and other Ca related disorders (1, 6, 7, 17, 25). Calcium treatments also have been shown to maintain fruit firmness and prolong storage life (1, 12, 13, 14, 16). Since postharvest treatments are not effective in controlling disorders that occur on the tree and may cause unacceptable levels of fruit damage, preharvest Ca treatments have gained a wide use (4, 19). Nevertheless, a major drawback of preharvest Ca treatments is that many spray applications, each of a considerable cost to the grower, are needed to attain significant increases in fruit Ca levels (3, 15, 26). Fewer preharvest sprays might be needed if the factors and conditions that influence Ca uptake through the cuticle were understood and exploited. The formulation of a compound, its concentration, pH, and rate of drying are some of the factors reported to be involved in spray uptake (21).

The major barrier to spray penetration is the plant cuticle. Spray solutions penetrate the cuticle by diffusion and by mass flow through stomata, lenticels, and other surface breaks (8, 23). A direct approach to study the effects of various factors on cuticular penetration is by the use of isolated cuticles (10, 21). Such a system allows an investigation of the influence of a single factor on cuticular permeability independent of complications introduced by other factors. Much of the work using isolated cuticles has been carried out using labelled isotopes. A study done by McFarlane and Berry (9), however, utilized isolated cuticles mounted in a flow-through diffusion cell to measure the penetration rates of various nonlabelled inorganic cations. Utilizing a similar system, investigations were conducted to study the effect of various Ca compounds and commercial formulations of Ca, as well as the effect of temperature, viscosity, pCa, and pH on the rate of Ca diffusion through isolated apple fruit cuticles.

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## Materials and Methods

Golden Delicious apples were harvested from the horticulture orchards located in Pullman on 15 Aug. and 15 Sept. 1982. Cylinders of tissue were taken from the equatorial region of the fruit using a No. 13 (1.8 cm) cork borer from which a thin disk containing the cuticle was sliced. The tissue adhering to the cuticle was removed enzymatically as described by Orgell (11).

A flow-through diffusion cell constructed of plexiglas was composed of 2 rectangular halves 30 cm × 7 cm × 1.3 cm. Each half had 2 rows of 16 holes (0.635 cm in diameter) on the inner face precisely matching those of the opposite half (Fig. 1). Two wax gaskets with holes matching those of the diffusion cell were made to seal around the periphery of the cuticles. Supply and exit tube attachments were fixed permanently into the channel ports. Screws were used to bind the cell halves together and create a water-tight seal around each cuticle.

Diffusion of Ca through the cuticle is reported to be a slow process (9). Subsequently, any leak in the cuticle through which mass flow could occur would alter the measurements of the true

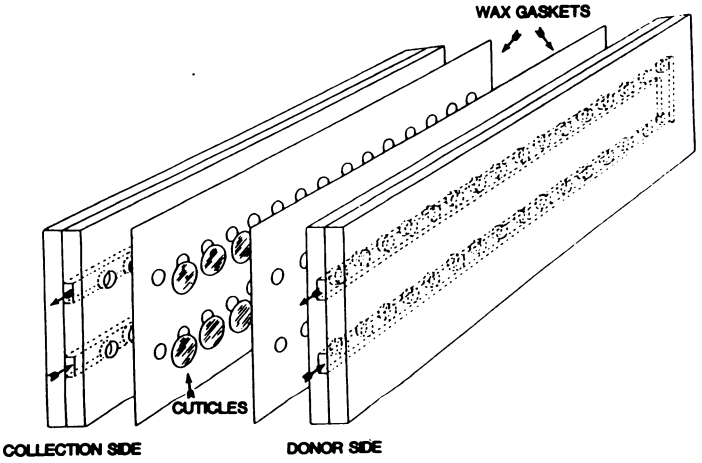


Fig. 1. Diagramatic view of flow-through diffusion cell showing donor and collection ports and the arrangement of cuticles. Inlet and outlet arrows indicate the direction of flow of donor and collection solutions. Channel tube attachments and binding screws are not illustrated.

rate of diffusion drastically. Aided by a light microscope, 32 cuticles were examined carefully and then positioned in the diffusion cell such that no lenticels or other probable sites of mass flow were exposed. After loading the diffusion cell, the cuticles were tested rigorously for submicroscopic leaks by using distilled water as the collection medium and 0.5% blue dextran (2,000,000 MW) as the donor solution. The solutions were allowed to remain stagnant within the cell for 24 hr after which the collection solution was analyzed for blue dextran using a Gilford spectrophotometer (model 2600). Any detection of the dye in the collection medium was considered unacceptable, and the cuticles were discarded.

A more sensitive method as described earlier (9) consisted of using $CaCl_2$ as the donor solution and NaCl as the collection solution. A positive pressure was created in the collection channel by elevating its exit tube 10 cm above the exit tube of the donor channel. This pressure inhibited mass flow of $CaCl_2$ solution to the collection solution. Once equilibrium was reached, the Ca concentration of the collection solution was measured using an atomic absorption spectrophotometer (Instrumentation Laboratory, Model 151). A positive pressure then was created in the donor channel by elevating its exit tube 10 cm above the exit tube of the collection channel, thus favoring mass flow of the $CaCl_2$ donor solution toward the collection solution. Once equilibrium was reached, the Ca concentration of the collection solution again was measured. Significant differences at the 5% level in the Ca concentration of the collection solution under the different pressures were attributed to holes allowing mass flow of solution, and, subsequently, the cuticles were discarded.

Once the cuticles in the diffusion cell were determined to be free of holes allowing mass flow, 0.5 M donor solutions of the cation to be measured were allowed to flow over the outer surface of the cuticle. A collection medium of NaCl was allowed to flow over the inner surface of the cuticle. The concentration of the collection solution was adjusted to that of the donor solution using the Chardakov method (22). The pH of both the donor and collection solutions was adjusted to pH 7 unless otherwise indicated. Equal flow rates were maintained in both channels of the cell using an ISCO peristaltic pump (model WIX). Five to 10 samples (5 ml) per treatment were collected in glass culture tubes, using an ISCO fraction collector (model 328). Between each treatment, the diffusion cell was purged with 0.4 M nitric acid and distilled water. The permeability of chlorides of K, Mg, and Ca was studied 1st to determine whether the system was sensitive enough to detect differences in the permeability of cations having different ionic radii. Subsequently, the permeability of 3 Ca compounds ($CaCl_2$, Ca acetate, and Ca nitrate) and 2 commercial Ca formulations (formulation no. 1 and formulation no. 2) having surfactant properties were studied. All treatments using formulation No. 2 were carried out at pH 2 rather than pH 7, since precipitation occurred at higher pH levels. The Ca concentration of both the collection and donor solutions was measured using an atomic absorption spectrophotometer. The permeability coefficients for each treatment were calculated as reported earlier (9).

To study the effect of temperature and pH, 32 cuticles from fruit harvested on 15 Sept. were mounted in the diffusion cell and tested for leaks. The movement of 0.5 M $CaCl_2$ through the cuticle at temperatures of 24°, 12°, and 1°C was studied in temperature controlled rooms. Calcium chloride solutions of pH 3 and pH 11 were used to determine the effect of pH on the rate of Ca penetration. The viscosity of dilute (0.034 M) and concentrated (0.5 M) Ca solutions was measured using an Ostwald viscosimeter. The viscosity of $CaCl_2$ solutions at various temperatures also was measured. The pCa of $CaCl_2$ solutions at pH 3, pH 7, and pH 11, as well as the pCa of various 0.5 M Ca compounds was determined using an Orion Ca ion electrode (model 93-20). The drying rate of five 2 ml samples of 0.5 M solutions of each of the Ca compounds was determined by calculating the percentage of the initial water content the compounds retained after an 8-week drying period in 1.3 cm × 10.0 cm glass culture tubes.

## Results

A steady state usually was attained within 3 hr for each cation tested (Fig. 2). Potassium was found to move nearly 10 times faster than Ca through the cuticle. The penetration of Mg was slightly faster than that of Ca. The permeability coefficients obtained for Ca and Mg were precisely the same values as reported by McFarlane and Berry (9) in their work with astomatous apricot (*Prunus armeniaca* L.) leaf cuticles. Due to the small variability in the readings within different runs, it became apparent that the cuticular permeability was not significantly altered by 0.5 M salt solutions and, furthermore, that the system was sensitive enough to measure differences in the penetration rates of various cations.

The penetration rates for different Ca compounds and formulations using the same set of cuticles resulted in detectable differences. Calcium chloride was found to have the highest penetration rate of all the forms of Ca tested (Table 1). Formulation no. 2 had a Ca penetration rate of 50% of the rate observed for $CaCl_2$. The remaining 3 forms of Ca tested (Ca

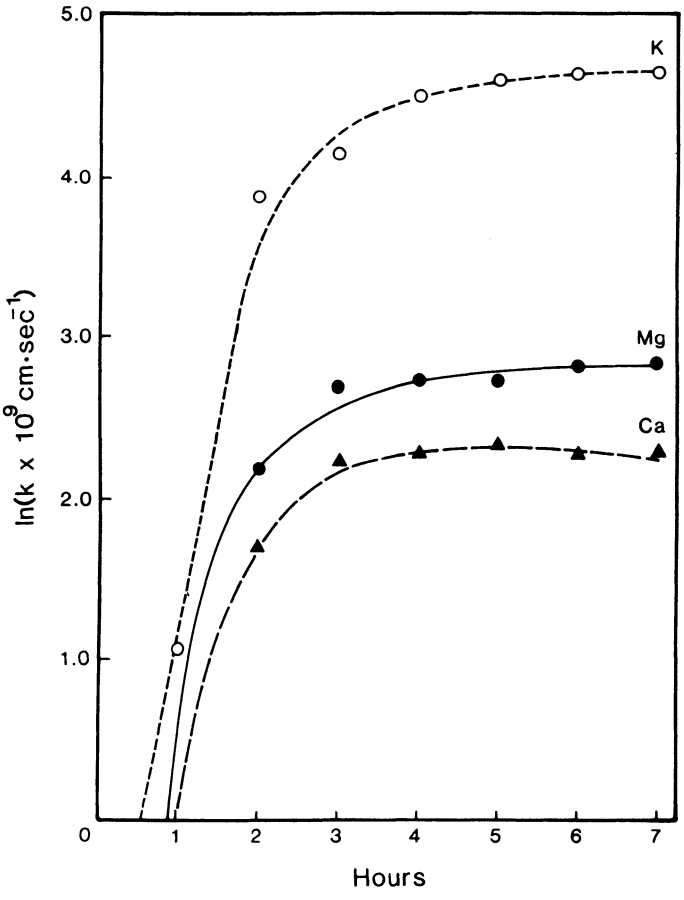


Fig. 2. Time course study of permeability coefficients for $CaCl_2$, $MaCl_2$, and KCl using isolated apple fruit cuticles.

Table 1. Permeability coefficients (k) of isolated apple cuticles, viscosity (η) of 0.5 M and 0.034 M solutions, Ca ion concentration (pCa) and percentage of moisture retained for different Ca salts and formulations.

| Test solution | Permeability coefficient (k) (X $10^{-8}$ cm·sec$^{-1}$) | η (Centipoises) 0.5 M | η (Centipoises) 0.034 M | Ca conc (pCa) | Moisture[z] retention (%) |
|---|---|---|---|---|---|
| Calcium chloride | 1.05 a[y] | 1.02 a | 0.90 a | 0.30 a | 9.2 a |
| Calcium acetate | 0.78 b | 1.23 b | 0.92 b | 0.52 b | 0.1 b |
| Calcium nitrate | 0.88 b | 0.99 c | 0.91 a | 2.50 c | 8.3 c |
| Formulation no. 1[x] | 0.89 b | 1.11 d | 0.91 a | - - - | 8.4 c |
| Formulation no. 2[w] | 0.53 c | 1.06 e | 0.90 a | 2.80 d | 6.7 d |
| Distilled water | - - - | 0.89 f | 0.89 c | - - - | - - - |

[z]Percentage of moisture $\frac{\text{final wt} - \text{dry wt}}{\text{initial wt} - \text{dry wt}} \times 100$ was calculated after 8 weeks of drying of 2 ml of solution in a 1.3 cm × 10.0 cm glass culture tube. Dry weights were obtained by oven drying the samples for 6 hr at 100°C.
[y]Means followed by a common letter are not significantly different at 5% level.
[x]Formulation no. 1: This Ca from Stoller Chemical Company.
[w]Formulation no. 2: Link Ca of Wilbur-Ellis Company.

acetate, Ca nitrate, and formulation no. 1) had similar Ca penetration rates (Table 1). Temperature was found to have a significant effect on the rate of Ca penetration (Table 2). The penetration rate of Ca at 24°C was significantly higher than that at 12° or 1° (Table 2).

The viscosity of $CaCl_2$ solution was found to be inversely proportional to the solution temperature (Table 2). Calcium nitrate was found to be the least viscous followed by $CaCl_2$, formulation no. 2, formulation no. 1, and Ca acetate, respectively (Table 1). When the viscosity of dilute solutions (0.034 M) of the various compounds was measured, only Ca acetate was found to be significantly more viscous than the remaining Ca compounds tested.

The pH of the $CaCl_2$ solution tended to affect Ca penetration (Table 3). Greater Ca uptake was observed at pH 3 than at pH 11. Calcium chloride molecules dissociated more under neutral or acid conditions than basic conditions. The pCa of the various Ca compounds revealed that $CaCl_2$ had the greatest Ca ion concentration among the various Ca compounds tested. The pCa of Ca nitrate and formulation no. 2 were very low, in comparison to $CaCl_2$. The pCa of formulation no. 1 could not be measured due to interfering substances causing excessive drift in the electrode reading. The percentage of initial moisture content retained by each Ca compound after an 8-week drying period demonstrated that $CaCl_2$ dried slower (Table 3) than other Ca compounds. The viscosity of each solution appeared to correlate with the observed percent moisture retention.

Table 2. The effect of temperature on permeability coefficients of isolated apple cuticles and on viscosity of a 0.5 M $CaCl_2$ solution.

| Temperature | Permeability coefficient (k) (X $10^{-8}$cm·sec$^{-1}$) | $Q^z_{10}$ | Flow rate (sec.)[y] | $V_{10}$[x] |
|---|---|---|---|---|
| $t_1$ 24°C | 1.06 a[w] | | 73.0 a | |
| | | 1.44 | | 1.39 |
| $t_2$ 12°C | 0.69 b | | 108.4 b | |
| | | 1.38 | | 1.37 |
| $t_3$ 1°C | 0.48 b | | 153.2 c | |

[z]$Q_{10}$ was calculated using formula $\log Q_{10} = \frac{10}{C_2 - t_1} \log \frac{k_2}{k_1}$ where t = temperature and k = rate.
[y]Flow rates were obtained from viscosimeter readings.
[x]$V_{10}$ is a viscosity coefficient calculated from formula $\log V_{10} = \frac{10}{t_2 - t_1} \log \frac{r_1}{r_2}$ where t = temperature and r = rate.
[w]Means followed by a common letter are not significantly different at 5% level.

Table 3. The effects of pH on calcium ion concentration and permeability of 0.5 M $CaCl_2$ through isolated apple cuticles.

| pH | Permeability coefficient (k) (X $10^{-8}$ cm·sec$^{-1}$) | Ca ion concn (pCa) |
|---|---|---|
| 3 | 2.20 a[z] | 0.31 a |
| 7 | - - - | 0.31 a |
| 11 | 1.56 a | 0.36 b |

[z]Means followed by a common letter are not significantly different at 5% level.

## Discussion

The factors involved in the movement of Ca through the cuticle are complex. The hydrated ionic radius is reported to be inversely proportional to the penetration rate of an ion (9). This study showed that K traversed the cuticle at a higher rate than Mg or Ca, both of which have larger hydrated ionic radii. The penetration rate of a single cation associated with different anions also could vary. Efforts to understand the factors involved in cuticular Ca movement often are focused on the chemical interactions of the ions with the cuticle. The cuticle is composed of an outer waxy epicuticular layer which forms the major barrier to spray penetration (24). Since nonpolar compounds are known to dissolve in the epicuticular layer of the cuticles, various organic Ca compounds have been used to enhance Ca movement. Riley and Kolattukudy (20) synthesized and studied the effects of 3 organic Ca compounds having lipophilic anions. Their results, after dip treatments with various Ca compounds, showed $CaCl_2$ to be more effective than any of the organic Ca compounds in maintaining fruit firmness. Similarly, in this study, $CaCl_2$ was found to permeate the cuticle at a faster rate than the organic Ca compounds tested. Because of the seemingly poor correlation between the lipophilic nature of an anion associated with a Ca compound and the rate of Ca penetration (20), other factors that may influence this process were investigated.

The pH of a solution has been reported to affect cuticular

permeability. McFarlane and Berry (9) reported a 5-fold increase in the rate of K movement through the cuticle under basic vs. acidic conditions. Our results indicated that $CaCl_2$ tended to permeate the cuticle faster under acidic than basic conditions. It was noted further that the pCa of $CaCl_2$ was higher at pH 3 than at pH 11. These results suggest that pCa is a factor of importance in Ca movement through the cuticle.

Temperature is another important factor in Ca penetration. Temperature is known to affect the rate of diffusion. It has been suggested that changes in the viscosity of a solution with temperature may largely account for the observed changes in diffusion rates (5). A comparison of the $Q_{10}$ vs. viscosity coefficient (Table 2) shows that the decrease in the rate of diffusion can be attributed largely to the increased viscosity at lower temperatures. It would seem that viscosity is a major factor influencing the movement of Ca. A measure of the viscosity of the different Ca compounds (0.5 M) indicated that there are significant differences in their viscosity. When viscosity and pCa parameters were considered together with regard to Ca movement, a possible correlation was observed. Low viscosity and low pCa (high Ca ion concentration) were both associated with increased rates of Ca uptake. When 2 Ca compounds differed in only 1 of these 2 factors, the differing factor seemed to determine the relative rate of Ca penetration. For instance, the viscosities of $CaCl_2$ and formulation no. 2 were similar, but the pCa for $CaCl_2$ was much lower than for formulation no. 2. The pCa of $CaCl_2$ and Ca acetate were similar; however, $CaCl_2$ had a lower viscosity. In both instances, $CaCl_2$ showed increased Ca penetration.

An additional factor of major importance in field applications is the drying rate of Ca sprays. Once a compound has dried on the cuticle surface, little additional penetration can occur. Van Goor (27) noted that the relative humidity affected the penetration of $^{45}Ca$ into apple fruit by affecting the drying time (18, 27). Calcium choride not only dried slower than the other Ca compounds studied, but seemed less viscous which would enhance Ca movement further.

The chemical properties of a Ca compound (2), its viscosity, pCa, and drying rate are all important in Ca penetration of the cuticle. The results of this study implicate $CaCl_2$ as the most effective form of Ca in cuticular penetration. An understanding of the factors involved in Ca penetration would be beneficial in screening compounds for field use and in maximizing their effectiveness. Field studies need to be performed to establish a correlation between in vitro and in vivo conditions. Nevertheless, the results do agree with other in vivo studies comparing $CaCl_2$ to other forms of Ca (3, 20).

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