Cuticular penetration of calcium salts: effects of humidity, anions, and adjuvants

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Accepted 17 January 2001

Summary – Zusammenfassung

Physical laws of cuticular penetration of calcium salts have been studied using astomatous isolated pear (*Pyrus communis* L.) leaf cuticular membranes (CM). Penetration followed first order kinetics and was greatly affected by humidity, hygroscopicity, solubility of salts, and nature of adjuvants. Penetration required dissolution of the salts and this is determined by their point of deliquescence (POD). POD corresponds to the humidity over a saturated salt solution containing undissolved salt. When humidity was above POD the salt residue on the cuticle dissolved, while below a solid residue was formed and penetration ceased. $CaCl_2$ and $Ca(NO_3)_2$ have POD's of 32 and 55%, respectively, while POD's of organic calcium salts (acetate, lactate, propionate) were between 95 and 100%. Furthermore, aqueous solubility of the inorganic calcium salts is one to two orders of magnitude higher than that of the organic salts. Thus, organic calcium salts are not well suited for foliar nutrition since POD's are very high and driving forces of penetration are low due to low solubility even at 100% humidity. $CaCl_2$ and $Ca(NO_3)_2$ penetrate even at low humidity and solubility is much higher. When humidity was above the POD, rate constants of penetration increased with increasing humidity by about a factor of three and maximum rates were measured at 100%. Temperature did not affect rate constants of penetration from which it can be concluded that penetration is most rapid during the night when humidity is high. All salts should be used with an effective wetter as with an alkyl polyglucoside half time of penetration was decreased from 204 to 17 h. All other adjuvants tested (protein hydrolysates, EDTA, gum guar) decreased rate constants of penetration by factors of 3 to 9. This finding is discussed in relation to mixing foliar nutrients with fungizides.

Key words: Bitter pit / calcium salts / cuticular penetration / foliar nutrition / physiological disorders / postharvest diseases

Penetration von Calciumsalzen durch die pflanzliche Kutikula: Einfluss von Luftfeuchte, Anionen und Additiven

Die Gesetze der Penetration von Calciumsalzen wurden unter Verwendung von isolierten Kutikularmembranen (CM), die von Birnenblättern (*Pyrus communis* L.) isoliert wurden, untersucht. Diese CM haben keine Stomata, so dass die Ergebnisse die Eigenschaften der Kutikeln über normalen Epidermiszellen widerspiegeln. Die Penetration der Calciumsalze durch die CM ist ein Prozess erster Ordnung und wird maßgeblich durch die Luftfeuchte, die Hygroskopizität, die Wasserlöslichkeit der Salze und die Anwesenheit von Additiven beeinflusst. Damit Penetration stattfinden kann, müssen die Salze auf der Kutikula in gelöster Form vorliegen, und das wird vom Deliqueszenzpunkt (DQ) der Salze bestimmt. Der DQ entspricht der Luftfeuchte über einer gesättigten Lösung, die noch ungelöstes Salz enthält. Für $CaCl_2$ und $Ca(NO_3)_2$ beträgt der DQ 32% bzw. 55%, während für organische Calciumsalze (Acetat, Laktat, Propionat) DP's zwischen 95 und 100% gemessen wurden. Da die Wasserlöslichkeit der anorganischen Calciumsalze um ein bis zwei Größenordnungen höher ist als die der organischen Calciumsalze, sind die treibenden Kräfte der Penetration bei $CaCl_2$ und $Ca(NO_3)_2$ entsprechend höher. Daher sind die organischen Salze für die Blattdüngung infolge ihrer hohen DQ's and der geringen Löslichkeit wenig geeignet. Die Penetrationsraten von $CaCl_2$ und $Ca(NO_3)_2$ nahmen mit der Luftfeuchte erheblich zu und waren bei 100% immer am höchsten. Die Temperatur hatte keinen signifikanten Einfluss auf die Penetrationsraten, so dass maximale Raten in der Regel nachts, wenn die Luftfeuchte am höchsten ist, zu erwarten sind. Alle Salze sollten zusammen mit einem geeigneten Netzmittel ausgebracht werden. Mit nur 0.2 g l^{-1} eines Alkylpolyglukosids nahm die Halbwertszeit der Penetration von 204 auf 17 h ab. Alle anderen Additive (Proteinhydrolysate, EDTA, Guarmehl) erhöhten die Halbwertszeiten um Faktoren von 3 bis 9. Dieser Befund wird im Hinblick auf die Gepflogenheit, Blattdünger mit Fungiziden zu mischen, diskutiert.

1 Introduction

Calcium ions operate as second messengers in signal transduction and are involved in the regulation of many metabolic processes (*Trewavas* and *Malhó*, 1998). Calcium ions form bridges between pectin chains and play a central role in cell wall stability and in regulating plant senescence and fruit ripening (*Brady*, 1992). In apple fruits calcium deficiency leads to physiological disorders (bitter pit, water core, internal breakdown), to softening and senescence (*Bangerth*, 1979; *Poovaiah*, 1979; *Ferguson* and *Watkins*, 1989). Other crops and organs may also suffer from calcium deficiency symptoms (*Marschner*, 1995), and increased susceptibility to fungal diseases has been reported (*Conway*, 1982; *Conway* and *Sams*, 1983; *Conway* et al. 1987; 1994).

Calcium deficiency cannot always be ameliorated by fertilizer application to the soil, and in these instances aqueous solutions of calcium salts have been sprayed on leaves and fruits or postharvest calcium treatments such as dips or infiltration have been used (*Scott* and *Wills*, 1979; *Sams* and *Conway*, 1987; *Hewett* and *Watkins*, 1991;

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Conway and *Sams*, 1983; *Conway* et al. 1994). Foliar applications have not always resulted in a significant increase in calcium concentrations of tissues and in elimination of deficiency symptoms. Infiltration of apple fruits was generally much more effective because calcium solutions can penetrate through lenticels but surfactants or other adjuvants had to be incorporated into treating solutions (*Lidster* and *Porrit*, 1978; *Lee* and *Dewey*, 1981; *Harker* and *Ferguson*, 1991). This is certainly a useful approach in research, but for commercial applications infiltrating fruits is not universally accepted by consumers and regulation agencies. In the European Community preharvest application is the sole legal method available to the growers and apple orchards are sprayed ten times or more often with calcium salts to combat physiological disorders.

A limited number of studies directly measured penetration of calcium salts across cuticles and it was concluded that cuticles are the major barriers in foliar nutrition (*McFarlane* and *Berry*, 1974; *Schönherr* and *Huber*, 1977; *Chamel*, 1989). *Van Goor* (1973) studied penetration of $Ca(NO_3)_2$ in apple fruits and higher rates of penetration were observed at 50 to 80% than at 100% humidity. 10 to 90% of the applied dose penetrated within 5 days but it is not clear what caused this variability and to which extent lenticels participated in transport. *Glenn* and *Poovaiah* (1985) used isolated apple fruit cuticles and found slower penetration with nitrate and acetate as compared to chloride. *Chamel* (1989) worked with isolated cuticles from "Golden Delicious" fruits but permeability coefficients were about 50 times higher than those reported by *Glenn* and *Poovaiah* (1985). These conflicting results are difficult to reconcile and lenticels and cracks in the cuticle have repeatedly been suggested as selective pathways in the apple fruit skin, especially with "Golden Delicious" fruits around harvest time (*Glenn* and *Poovaiah*, 1985; *Conway* et al.; 1994).

Recently, detailed mechanistic studies with isolated cuticles have become available. *Krüger* (1999) measured diffusion of Ca^{2+} and NO_3^- across astomatous poplar leaf cuticles and he observed equivalent fluxes, that is for each calcium ion two nitrate ions penetrated no matter if deonized water or diluted HCl was used as receiver solution. Rates of diffusion from saturated solutions decreased in the order chloride>nitrate>acetate> propionate by a factor of 6 and permeability coefficients followed the same order but varied only by a factor of 2. From a study of diffusion of calcium chloride across astomatous pear leaf cuticles *Schönherr* (2000) concluded that hydrated ions penetrated the cuticles via aqueous pores. However, penetration of $CaCl_2$ across cuticles was a relatively slow process and in a follow–up study reported here it was investigated which factors affect rates of penetration and if rates could be increased using suitable adjuvants or other means.

2 Materials and methods

2.1 Cuticular membranes

Cuticles can be isolated from apple fruits, but they have stomata and lenticels and may even have cracks. As it was the aim to study cuticular penetration cuticles lacking stomata and lenticels other cuticles had to be used. Astomatous adaxial pear leaf cuticles (*Pyrus communis* L. cv. Conference) had been shown to be a suitable model system for studying salt penetration across cuticles (*Schönherr*, 2000) and they were also used in this study. Fully expanded healthy leaves were isolated from trees growing in our experimental orchard. After punching discs of 20 mm diameter out of the leaves cuticles were isolated enzymatically (*Schönherr* and *Riederer*, 1986), air dried and stored at 5 °C until used. These isolated cuticles will be referred to as cuticular membranes (CM's). Data obtained with this type of CM reflect permeability of CM over "ordinary" epidermal walls comprising more than 95% of leaf surfaces. Permeability of cuticles over guard cells to calcium salts is currently being investigated and will be reported separately.

2.2 Penetration experiments

Rates of cuticular penetration were measured at 20±1 °C using the SOFU procedure (simulation of foliar uptake) as described by *Schönherr* (2000). The method was developed to study the time course of cuticular penetration for each CM separately. Briefly, the CM were mounted between lid and bottom of the desorption chambers using silicon grease (Baysilon, Bayer, Leverkusen, Germany), such that the morphological inner side of the CM faced the receiver chamber. After mounting each CM was tested for leaks and imperfections by applying a 20 µl drop of absolute ethanol to the center of the CM. If defects existed, the ethanol penetrated the CM within 1–3 minutes. This could be detected with the bare eye, as in incident light the area of the CM around the imperfection changed color from whitish to dark. Only CM which passed the test were used for subsequent experiments. The test detects even submicroscopic defects which could not been seen in the microscope at 100 to 200 fold magnification. The test had no influence on membrane permeability (unpublished results). Next, the chambers were filled with 0.5 ml receiver solution and were placed into the wells of a thermostated aluminum block which was rocked at 80–100 rpm for mixing of receiver solutions. After an equilibration period of about 24 h the receiver solutions were withdrawn and a 5 µl drop of donor solution containing $^{45}Ca^{2+}$ was applied to the center of the morphological outer surface of each CM. Evaporation of the water under a stream of ambient air (~50% humidity) took about 40 to 60 min and the receiver chambers were again filled with 0.5 ml receiver solution and returned to the aluminum block. During penetration air of constant humidity was blown over the salt residue on the CM to ensure a constant hydration of the Ca-salt residues. Humidity of the air was adjusted using the dew point method. Receiver solutions were quantitatively withdrawn in 24 h intervals for scintillation counting and were replaced by fresh one. After the last sample had been taken the CM was cut out, residual salt on the CM was dissolved in receiver solution and after adding scintillation cocktail they were counted to determine the amounts of salt left on the surface of the CM. The amount initially applied to the CM (M_0) was obtained by summing amounts penetrated (M_t) and the amount left on the CM at the end of the experiment for each CM individually. Thus, M_t/M_0 is the fraction of salt which penetrated and $1\text{-}M_t/M_0$ is the fraction remaining on the surface of the CM. Radioactivity of samples was determined at constant quench for all samples using a Packard CA 2000 scintillation counter (*Downers Grove, Il. USA*) set at a 2σ error of 3%. Recoveries calculated from the amounts of radioactivity applied (determined by counting five 5 µl droplets of donor solution) and recovered (M_0) ranged from 90 to 100% and data were discarded if recovery was <90%.

Data were plotted as $-\ln(1\text{-}M_t/M_0)$ vs. time (t), the slope of this plot being the first order rate constant (k), and this was performed for each CM separately. Variability among individual CM can be large (*Baur*, 1997) and for this reason 100 CM were used as replicates and arithmetic means with 95% confidence intervals are reported.

2.3 Chemicals

Donor solutions were prepared by dissolving calcium salts (Merck, reagent grade) in deionized water and $^{45}CaCl_2$ (NEN, specific activity 1.07 GBq. mg^{-1}, radiochemical purity 99.9%) was added as a tracer (10 000 cpm μl^{-1}). The total concentration calculated as anhydrous calcium-salts ranged from 1 to 10 g l^{-1} and acetate, chloride, lactate, nitrate, and propionate were used as anions. As receiver medium aqueous citric acid buffer (Merck) at 2 g l^{-1} was used and the pH was adjusted to 4.0 using KOH. Rates of cuticular penetration were not affected by the type of receiver media (deionized water, diluted HCl, citric acid buffer), but as some calcium salts tend to be sorbed to the glass of syringes citric acid buffer was used as receiver medium to avoid this problem. The wetting agent Glucopon 215 CSUP (a $C_{8/10}$-alkyl polyglucoside from Henkel, Düsseldorf, Germany) was added to donor solutions at a concentration of 0.2 g l^{-1}. In one instance the $C_{9/11}$-alkyl polyglucoside (Agrimul PG 2069, Henkel) and $C_{12/16}$-alkyl polyglucoside (Plantacare 1200 UP, Henkel) were tested at the same concentration. Other adjuvants were gum guar (Roth, Karlsruhe, Germany), the protein surfactants V70744, V70745 and 30V-SLL (obtained from Aglukon, Düsseldorf, Germany) and the disodium salt of ethylenediamin tetraacetic acid (Na_2EDTA from Fluka, Neu-Ulm, Germany). Concentrations will be given in the figure legends. Donor and receiver solutions contained 20 mg l^{-1} chloramphenicol (Sigma-Aldrich, Deisenhofen, Germany) to prevent the growth of micro-organisms during experimentation.

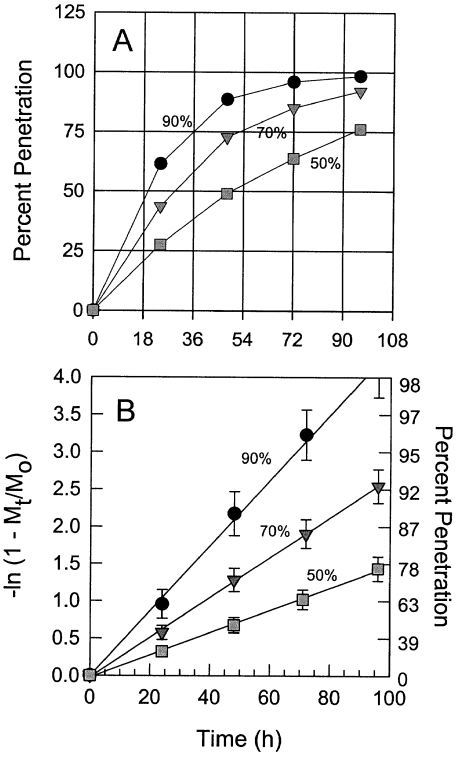

Figure 1: Penetration of $Ca(NO_3)_2$ across pear leaf cuticular membranes at 50, 70, and 90% humidity. Salt concentration was 6 g l^{-1} and 0.2 g l^{-1} Glucopon 215 CSUP was added as wetting agent. Percent penetration was plotted vs. time (A) and in (B) the same data are presented as logarithmic plots. Slopes of the lines in B are the first order rate constants (k).
Abbildung 1: Penetration von $Ca(NO_3)_2$ durch Kutikularmembranen von Birnenblättern bei 50, 70 und 90% Luftfeuchte. Die Salzkonzentration betrug 6 g l^{-1} und 0,2 g l^{-1} Glucopon 215 CSUP diente als Netzmittel. In A wurde die penetrierte Menge (%) gegen die Zeit aufgetragen. Dieselben Daten sind in B logarithmisch aufgetragen. Die Steigungen der Geraden entsprechen den Geschwindigkeitskonstanten 1. Ordnung.

3 Results

Rates of penetration of $Ca(NO_3)_2$ were highest initially, tended to level off with time and increased with increasing humidity (Fig. 1A). Logarithmic plots of these data ($-\ln(1-M_t/M_0)$ *vs.* time (t)) resulted in straight lines (Fig. 1B). Hence, salt penetration can be completely described by a single constant, the rate constant (k) of penetration. Rate constants increased from 15×10^{-3} h^{-1} (50% humidity) to 44×10^{-3} h^{-1} (90% humidity) by a factor of almost 3 showing humidity to be a major factor determining velocity of cuticular penetration of $Ca(NO_3)_2$. Wetting turned out to be another important determinant of calcium salt penetration as slopes measured with $CaCl_2$ in presence of alkyl polyglucosides were 12 times steeper than in absence of wetting agents. All three wetting agents were equally effective as there was no difference in slopes (Fig. 2).

When $CaCl_2$ and lactate were applied in equal amounts (1 g l^{-1}) and pH was adjusted to 4.0 humidity had a much greater effect on rate constants (Fig. 3) than in absence of

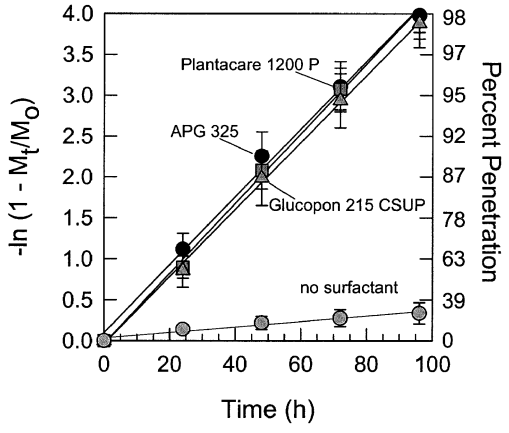

Figure 2: Effects of wetting agents (0.2 g l^{-1}) on rate constants of penetration (slopes) of $CaCl_2$ (6 g l^{-1}) at 90% humidity.
Abbildung 2: Der Einfluss von Netzmitteln (0,2 g l^{-1}) auf die Geschwindigkeitskonstanten (Steigungen) der Penetration von $CaCl_2$ bei 90% Luftfeuchte.

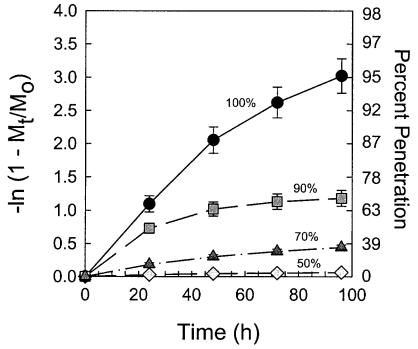

Figure 3: Effects of lactic acid (1 g l^{-1}) buffered at pH 4 in the donor on rate constants of penetration (slopes) of $CaCl_2$ (1 g l^{-1}) at 50, 70, 90, and 100% humidity. Donor solutions contained 0.2 g l^{-1} Glucopon 215 CSUP as wetting agent.
Abbildung 3: Der Einfluss von Milchsäurepuffer (1 g l^{-1}, pH 4.0) im Donator auf die Geschwindigkeitskonstanten der Penetration von $CaCl_2$ (1 g l^{-1}) bei 50, 70, 90 und 100% Luftfeuchte. Donatorlösungen enthielten 0,2 g l^{-1} Glucopon 215 CSUP als Netzmittel.

lactate (Fig. 2). At 100% initial slopes (up to 48 h) were similar as in absence of lactate at 90% humidity (Fig. 2) but they decreased rapidly with decreasing humidity and at 70 and 50% rate constants were extremely small. In this particular instance rate constants decreased with time and this was always observed when salt concentrations were < 2 g l^{-1}. This was attributed to an inhomogeneous distribution of the salt residue (*Schönherr*, 2000) at low coverage. This problem can be avoided by using higher salt concentrations (2 to 10 g l^{-1}) which is also closer to actual practice in foliar nutrition. Using lactic acid buffer it was also tested if velocity of penetration of $CaCl_2$ might depend on pH but rate constants measured at pH 3.0, 3.5, and 4.0 were identical (data not shown).

With salt concentrations of 5 g l^{-1} first order plots (-ln (1-M_t/M_0) *vs.* time (*t*)) were linear with all calcium salts tested and data are summarized in Fig. 4. At 100% humidity rate constants were highest with $CaCl_2$ and $Ca(NO_3)_2$ but they were much smaller with organic calcium salts. The organic salts also exhibited a pronounced dependence on humidity and at 70% rate constants were close to zero, while with the inorganic salts k was still around 25×10^{-3} h^{-1} at 70% and 15×10^{-3} h^{-1} at 50% humidity, respectively. When humidity was 80% or lower the organic calcium salts formed whitish salt residues on the CM.

Protein surfactants have a good ecotoxicological profile and they are used in commercial formulations of calcium salts. As they were expected to increase retention it was tested if rates of penetration of $CaCl_2$ are affected in their presence. This was clearly the case as rate constants were reduced by a factor of about 3 (Fig. 5) with Arlypon V70744 and 707045. With 30V-SLL similar results were obtained (data not shown). Gum guar is a natural product obtained from the guar bean (*Cyamopsis tetragonobulus*) containing as major ingredient a polysaccharide composed of galactose and mannose. It is used as thickener and it was tested if it might affect penetration of $CaCl_2$. Rate constants of penetration of $CaCl_2$ were drastically reduced and reduction was even greater at 70% humidity (a factor of 9) than at 90%

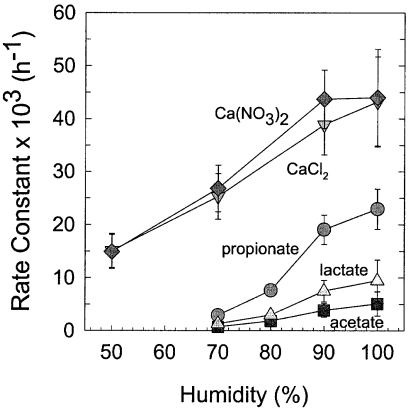

Figure 4: Effects of anions on penetration of calcium salts (5 g l^{-1}). Donor solutions contained 0.2 g l^{-1} Glucopon 215 CSUP as a wetting agent.
Abbildung 4: Der Einfluss von Anionen auf die Penetration von Calciumsalzen (5 g l^{-1}). Donatorlösungen enthielten 0,2 g l^{-1} Glucopon 215 CSUP als Netzmittel.

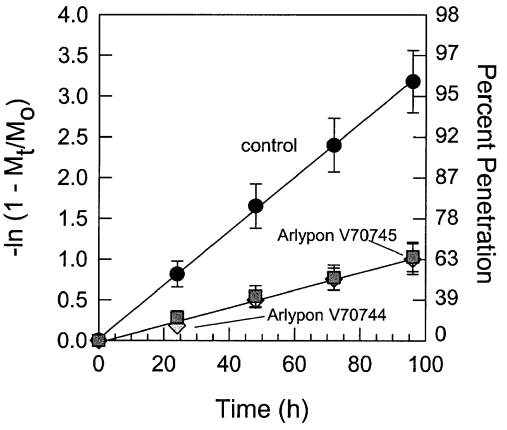

Figure 5: Effects of protein surfactants Arlypon V70744 and V70745 (5 g l^{-1}) on cuticular penetration of $CaCl_2$ (5 g l^{-1}). Donor solutions contained 0.2 g l^{-1} Glucopon 215 CSUP as wetting agent. Humidity was 90%.
Abbildung 5: Der Einfluss der Proteintenside Arlypon V70744 und V70745 (5 g l^{-1}) auf die Penetration von $CaCl_2$ (5 g l^{-1}). Donatorlösungen enthielten 0,2 g l^{-1} Glucopon 215 CSUP als Netzmittel. Die Luftfeuchte betrug 90%.

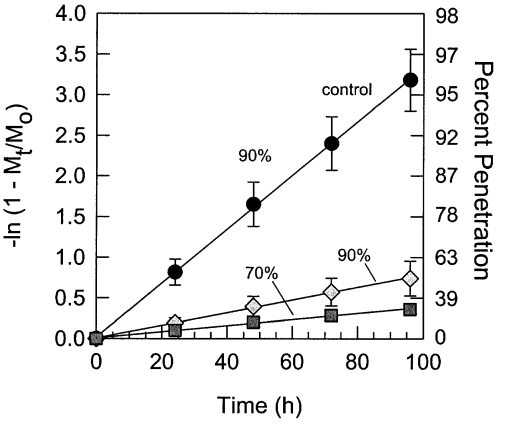

Figure 6: Effect of gum guar (5 g l^{-1}) on rates of penetration of $CaCl_2$ (5 g l^{-1}) at 90 or 70% humidity. Donor solutions contained 0.2 g l^{-1} Glucopon 215 CSUP as wetting agent.
Abbildung 6: Einfluss von Guarmehl (5 g l^{-1}) auf die Penetrationsraten von $CaCl_2$ (5 g l^{-1}) bei 70 bzw. 90% Luftfeuchte. Donatorlösungen enthielten 0,2 g l^{-1} Glucopon 215 CSUP als Netzmittel.

(a factor of 4) (Fig. 6). Mixing $CaCl_2$ with equimolar concentrations of Na_2EDTA also reduced rate constants by a factor of 4 (Fig. 7).

4 Discussion

Following application of donor solutions to the CM the water evaporated under a flow of ambient air having a humidity around 50%. With organic salts their residues looked whitish once they were dry. The residues formed by $CaCl_2$ and $Ca(NO_3)_2$ appeared clear. After receiver had been added the salt residues were subjected to air streams having constant humidity such that the salt residues could sorb moisture depending on humidity and hygroscopicity of the salt (see below). Equilibrium was established quickly as

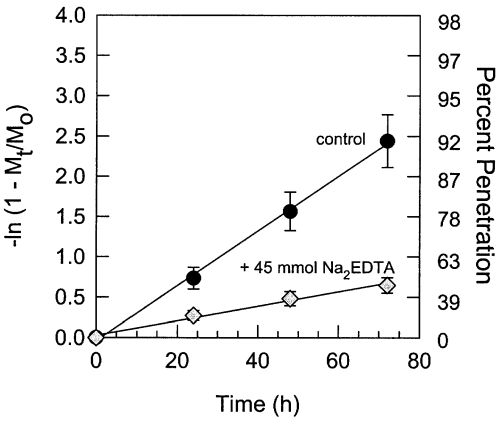

Figure 7: Effect of Na_2-EDTA on rates of penetration of $CaCl_2$. Both compounds were used at 45 mmoles l^{-1}. Donor solutions contained 0.2 g l^{-1} Glucopon 215 CSUP as wetting agent and humidity was 90%.
Abbildung 7: Der Einfluss von Na_2-EDTA auf die Geschwindigkeit der Penetration von $CaCl_2$. Die Konzentration beider Verbindungen betrug 45 mMol l^{-1}. Donatorlösungen enthielten 0,2 g l^{-1} Glucopon 215 CSUP als Netzmittel und die Luftfeuchte war 90%.

logarithmic graphs (for instance Fig. 1B) always intersected the origin. The amount of water taken up by the salt residues is not known, however.

Cuticular penetration of calcium salts was a first order process (Figs. 1, 2, 5, 6, 7), that is, the amount of salt disappeared exponentially with time from the surface of the CM. Rate constants (slopes) were constant and independent of time if salt concentration was 2 g l^{-1} or higher. Decreasing rates of penetration (slopes) seen when percentage penetrated was plotted against time (Fig. 1A) were caused solely by the decreasing driving force, that is by decreasing salt concentration on the CM. From the rate constant k as defined by eq. 1

$$\frac{M_t}{M_0} = 1 - e^{-kt} \qquad (1)$$

the fraction of the salt that penetrated can be calculated for any time interval. For instance, if k is 0.04 h^{-1} and t is 10 h M_t/M_0 is 0.33, that is 33% of the dose applied penetrated in 10 h. Selected percentage figures were added to the right ordinate of each penetration graph but this scale is not linear. From eq. (1) the time required for 50% penetration ($t_{1/2}$) can be calculated by setting $M_t/M_0 = 0.5$. For instance, if k is 0.04 h^{-1} the half time is 17.3 h. Hence, rate constants are very useful as amounts penetrated can be calculated for any time interval and effects of treatments can be conveniently expressed as half times. The slopes seen in Fig. 1B correspond to half times of 47 h (50% humidity), 26 h (70%), and 16 h (90%), respectively. Once it has been established that penetration is a first order process, only one determination at a convenient time interval is needed to calculate k. This is superior to plotting percent penetrated vs. time which invariably results in nonlinear graphs when more than 50% has penetrated (Fig. 1A) and many determinations are necessary to fully describe penetration.

With $CaCl_2$ and pear leaf CM rate constants were found to be independent of salt concentration in the range of 2 to 10 g l^{-1} (*Schönherr*, 2000) and this probably holds for all inorganic calcium salts. Constant k implies, that the absolute amounts which penetrate the CM are proportional to concentration, hence 5 times more salt penetrates per unit time at 10 g l^{-1} compared to 2 g l^{-1}. In Fig. 1A it is seen that rates of penetration (not rate constants!) decreased with time. When the time for 50% penetration is 15 h, only 25% more penetrate during the next half time and in the third one additional salt penetration amounts to only 12.5% of the amount applied. This is caused by the reduction in driving force as seen from the fact that k was constant (Fig. 1B). Rain would wash of most of the deposit if it falls a few hours after spray application but with increasing time the losses decrease in an exponential fashion.

The alkyl polyglucosides proved very effective in increasing rate constants of penetration as in absence of wetting agents half time was 204 h, while in the presence of only 0.2 g l^{-1} wetter half times were only 17 h (Fig. 2). These surfactants penetrate pear leaf cuticles very slowly (unpublished results) and they are not phytotoxic, which is an essential requirement for foliar nutrition. Static surface tension of a $CaCl_2$ solution (10 g l^{-1}) containing 0.2 g^{-1} Glucopon 215CSUP was 30 mN m^{-1} and at 1 g l^{-1} or higher 28.4 mN m^{-1} were measured (unpublished results). These low surface tensions will be reached during droplet drying and since stomatal infiltration has been observed when surface tensions are below 30 mN m^{-1} (*Schönherr* and *Bukovac*, 1972) penetration of stomata and lenticels might occur when stomata are open and lenticels are present. Ethoxylated alcohols are good wetters too, but they should be avoided because many of them are phytotoxic (*Uhlig* and *Wissemeier*, 2000). Furthermore, polyoxyethylene chains strongly bind Ca^2+ ions (*Cross*, 1987) and these ionic surfactant complexes do not penetrate cuticles (*Uhlig* and *Wissemeier*, 2000), which is certainly an undesirable property with regard to foliar nutrition. Many ethoxylated alcohols surfactants increase rates of cuticular penetration of non-ionic organic compounds because they plastisize cuticular waxes (*Schönherr*, 1993; *Schreiber* et al., 1996; *Baur* et al., 1997), but with ions this is not a useful property, since hydrated ions do not enter the lipophilic wax phase. Ions diffuse in aqueous pores and for this reason plastisizers did not increase rates of penetration of ions and salts (*Schönherr*, 2000).

With lactic acid as buffer pH had no effect on rates of penetration of $CaCl_2$ at high humidity, but rate constants of penetration were drastically reduced at low humidity (Fig. 3). This was caused by the formation of calcium lactate which has a much higher point of deliquescense and a much smaller solubility than $CaCl_2$ (see below). Plant cuticles are polyelectrolytes with isoelectric points around 3. Above pH 3 cuticles are negatively charged (*Schönherr* and *Huber*, 1977) and the fixed carboxyl groups bind Ca^{2+} very strongly (*Schönherr* and *Bukovac*, 1973). This, together with the high ionic strengths in the salt residue on the surface of the CM depress ionization of fixed charges. Hence, Donnan-potentials were small or absent as demonstrated by the fact that Ca^{2+} and nitrate ions penetrated in equivalent amounts

and rates of penetration of $CaCl_2$ was proportional to salt concentration over the entire range of concentration (*Krüger*, 1999).

Equivalence of Ca^{2+} and anion fluxes maintains electroneutrality and it implies that large amounts of anions penetrate leaves and fruits. However, not only the physiological effect of the anions should be considered in choosing a calcium salt for foliar nutrition. Water solubility and hygroscopicity are also important (Fig. 4). Penetration requires that the salts are dissolved and at least a saturated liquid phase must exist between cuticle and solid salt residue for penetration to occur. Hydration and dissolution of salts is determined by the point of deliquescence (POD), which refers to the humidity over a saturated salt solution containing solid salt. At higher humidity solids slowly dissolve and if humidity is lower salt crystallizes out (*Kolthoff* et al., 1969). This is the main reason, why humidity and point of deliquescence are major determinants for rates of salt penetration (Figs. 1, 3, and 4). Points of deliquescence do not vary much with temperature and at 20 °C they are 32% for the hexahydrate of $CaCl_2$ and 55% for the tetrahydrate of $Ca(NO_3)_2$ (*Kolthoff* at al., 1969). These are physical laws and do not depend on the type of cuticle used or if stomata are present. Data for the organic calcium salts could not be found in the literature and were measured using a Dostmann P570 hygrometer. With Ca-acetate POD was 100%, with Ca-lactate 97%, and for Ca-propionate 95% were estimated. These salts should penetrate only when humidity is higher than POD. In fact, rate constants for the organic calcium salts at 100% followed the sequence of POD (Fig. 4) and they decreased rapidly with humidity. Nevertheless, at 90 and 80% rate constants were significantly greater than zero and this is likely caused by unstirred layer effects. As aqueous buffer was used as receiver, water penetrated the cuticle and humidity between salt and cuticle was most likely higher than the humidity of the air stream blown over the CM. Apart from this uncertainty it is clear that the organic calcium salts penetrated much more slowly and below 70% humidity rate constants were practically zero. If lactate was added to $CaCl_2$ rate constants of penetration became very sensitive to humidity and decreased rapidly with humidity, because of the high POD of Ca-lactate (Fig. 3). Relatively high rate constants during the first 24 h were mainly caused by penetration during droplet drying. In the field humidity can be as low as 30 to 50% and for this reason organic calcium salts are not useful as foliar nutrients. Rate constants measured with the chloride and nitrate salts were much higher and there was no significant difference between them. Between 100 and 50% humidity rate constants decreased by about a factor of 3 but at 50% rate constant were still around 15×10^{-3} h^{-1} which corresponds to a half time of 46 h.

Additional factors likely influenced velocity of penetration of calcium salts. Salt concentration in the donor solution serves as driving force of penetration and the concentrations of salts in saturated solutions differ greatly (*Lide*, 1991). With $Ca(NO_3)_2$ and $CaCl_2$ solubility is 6 600 and 2790 g salt per kg water, respectively. The respective figures for calcium propionate, acetate and lactate amount to only 490, 374, and 31 g kg^{-1}. These figures are smaller by one to two orders of magnitude and the fact that rate constants observed with the organic salts were much smaller even at 100% humidity (Fig. 3) is at least partly due to their low solubility. Swelling of the CM affects size or number of aqueous pores and depends on humidity (*Schönherr*, 1982). Large salt molecules are discriminated by narrow pores (*Schönherr*, 1982). Unfortunately, the effects of deliquescence, solubility, and pore size on rates of salt penetration cannot be separated and quantified at this time.

The effect of the wetting agents might indicate that salt solutions can reach pore entrances only when surface tension is sufficiently low. In any event, surfactants greatly increased rate constants (Fig. 2) while all other adjuvants tested significantly reduced k (Figs. 5 and 6). The protein surfactants carry negative charges and salt formation is likely. An effect on POD is possible, but most of the reduction in rate constants can probably be attributed to the bulk of the Ca-polypeptides which are excluded from the pores. A similar argument could be applied to the effect of EDTA (Fig. 7). Gum guar is a natural product and a mixture of many compounds. The nature of the interaction with Ca^{2+} ions is not known, but it is clear that penetration of $CaCl_2$ is greatly slowed, even at 90% humidity (Fig. 6). Whatever the precise reasons for the reduction in rate constants might be it is clear, that they should not be used in formulations of calcium salts intended to be used as foliar nutrients. The best results can be expected when aqueous solutions of $CaCl_2$ or $Ca(NO_3)_2$ are sprayed and nothing is added but a small amount of wetting agent such as the Glucopons used here. These salts are cheap, they have a low POD and they penetrate even at low humidity. Spraying should be done during the late afternoon to take advantage of high humidity during the night. Low temperatures are not detrimental, since rate constants of $CaCl_2$ penetration were not significantly affected by temperature in the range of 15 to 30 °C (*Schönherr*, 2000).

Various interactions between Ca^{2+} ions and active ingredients or formulation components of fungicides can be imagined and mixing Ca-salts with fungicides should be recommended only when it has been shown experimentally that rates of penetration of Ca-salts are not adversely affected.

Leaves have stomata at least on one side and fruit cuticles are porous due to lenticels. As indicated above, penetration of liquids into these openings is possible when surface tension is sufficiently low. Stomata may be important beyond this simple effect, as it has been demonstrated that penetration of organic ions (succinic acid-2,2-dimethyl hydrazide) into leaves was much faster when stomata were open even though liquid entry did not occur (*Schönherr* and *Bukovac*, 1978). These authors suggested that selective permeability of cuticles around cuticular ledges of guard cells might have been responsible for this observation. Preferential penetration into guard cells through the cuticle around guard cells was recently demonstrated using the disodium salt of fluoresceine (*Eichert* et al., 1998) and silver nitrate (unpublished results). This implies that rates of penetration of foliar nutrients into stomatous leaf surfaces might be faster than the rates observed in the present study using astomatous cuticles. Since the effect of humidity on deliquescense and swelling of cuticles is a purely physical

phenomenon these laws are expected to apply to stomatous leaf surfaces as well. This is currently under investigation.

Acknowledgments

I extend thanks to *Petra Crohnfeld* and *Karin Lader* for skilful technical assistance, to *Aglukon*, Düsseldorf for samples of protein surfactants and to *Henkel*, Düsseldorf for the alkyl polyglucoside surfactants. *F. Bangerth*, Universität Hohenheim, *B. Sattelmacher*, Universität Kiel, and *L. Schreiber*, Universität Würzburg, read an earlier version of the manuscript. Their valuable comments are gratefully acknowledged. This study was supported by a grant from the *Deutsche Forschungsgemeinschaft*.

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