Foliar Nutriton Using Inorganic Salts: Laws of Cuticular Penetration

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Abstract

Laws of cuticular penetration have been elucidated using calcium and potassium salts. These salts have hydration shells and they penetrate cuticles by diffusing in aqueous pores of molecular dimensions. Cations and anions penetrate in equivalent amounts, because electrical neutrality must be maintained. Penetration is a first order process and salts deposited on the surface of the cuticles disappeared exponentially with time. Velocity of penetration can be best quantified using first order rate constants or half times of penetration. Rates of penetration were greatly affected by humidity over cuticles, and hygroscopicity of salts. Penetration requires dissolution of the salt. This is determined by the point of deliquescence (POD) of the salt and humidity over the salt residue. POD is defined as that humidity over a saturated solution containing solid salt. When humidity is above POD the salt residue on the cuticle dissolves, while below a solid residue is formed and penetration ceases. Hence, salts suitable for foliar nutrition should have a low POD. The following salts meet this criterion: $CaCl_2$ (33%), $MgCl_2$ (33%) K_2CO_3 (44%), $Ca(NO_3)_2$ (56%) and $Mg(NO_3)_2$ (56%). Salts having POD's above 90% (for instance K_2HPO_4, KH_2PO_4, KNO_3, Ca-acetate, Ca-lactate and Ca-propionate) are not suitable for foliar nutrition, as they penetrate only at a humidity close to 100%. When humidity is above POD rate constants of penetration increased with increasing humidity by about a factor of three and maximum rates were measured at 90 to 100%. This is attributed to swelling of cuticles. The driving force for cuticular penetration is the concentration difference across the CM. Ion concentrations in the apoplast are in the millimolar range while water solubility of inorganic salts can be very high. Fortunately, with the exception of $MgCl_2$ all salts mentioned above having a low POD's also have high aqueous solubility ranging from 1.25 to 6.60 kg per kg water which shows that driving forces are very large following spray application and evaporation of excess water. Plasticiser and temperature did not affect rates of penetration and spraying should be done in the evening to take advantage of high humidity during the night. The effect of wetting agents and other adjuvants on rates of penetration is discussed.

INTRODUCTION

All primary above ground organs of plants are covered by a cuticle, which protects cells and tissues against excessive loss of water and apoplastic solutes (leaching). Permeability of cuticles to water, ions and polar solutes were found to be extremely small (Yamada et al., 1964; McFarlane and Berry, 1974; Schönherr, 1982; Tyree et al., 1992; Krüger, 1999). From these findings one would not expect leaves and fruits to take up large amounts of foliar nutrients. However, foliar nutrition has been practised for decades and often successfully (Hanway, 1988; Weinbaum, 1988; Marschner, 1995). In an attempt to reconcile these contrasting findings we have investigated cuticular penetration of ionic compounds across isolated cuticles and into detached leaves. From these and other studies the laws of cuticular penetration of ionic compounds evolved and they will be presented below.

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IONIC COMPOUNDS ARE HYDRATED AND ARE CONFINED TO AN AQUEOUS ENVIRONMENT

Inorganic foliar nutrients are strong electrolytes. In water the ions are surrounded by water molecules, which form a hydration shell. The exact number of water molecules is difficult to measure and varies depending on type of ion and method of measurement (Cussler, 1984). Hydration is responsible for the fact that strong electrolytes are highly soluble in water but are practically insoluble in organic solvents. The free energy of interaction between an ion and a water molecule (charge-dipole-interaction) is about 167 kJ mol^{-1}. This is 20 times more than the dipole-dipole-interactions involved in hydrogen bonding (Stein, 1967). Due to this huge amount of energy involved in charge-dipole interactions all ions and salts are hydrated under physiological conditions and they are confined to an aqueous environment. It follows that for ion and salt penetration across lipid membranes aqueous pores are required. In cell membranes these pores are formed by proteins embedded within the phospholipid double layer (Taiz and Zeiger, 2000).

Cuticles are solid-state lipid membranes and the transport-limiting layer at the outer surface of the cuticle is composed of cutin and associated waxes (Schönherr and Baur, 1996). Waxes embedded in the cutin are responsible for barrier properties of cuticles (Riederer and Schreiber, 1995) and diffusion of non-electrolytes takes place in an amorphous methylene group environment (Baur et al., 1997; Buchholz and Schönherr, 2000). This extremely lipophilic environment is not accessible to ions. For penetration of ions alternative parallel pathways are required.

LATERAL ASYMMETRY OF LEAF SURFACES, LOCALISATION AND SIZE OF AQUEOUS PORES

Most mechanistic studies of cuticular penetration have been carried out using astomatous cuticular membranes (CM) isolated enzymatically from fruits or adaxial leaf surfaces. CM from 5 plant species investigated so far were permeable to $CaCl_2$ which demonstrates the presence of aqueous pores in these isolated astomatous CM (Schönherr, 2000). However, most fruit and leaf surfaces have stomata and various types of trichomes. Cuticles also cover these structures, but there is ample evidence showing that permeability of cuticles over stomata and trichomes differs from that over ordinary epidermal cells (Strugger, 1939; Butterfass, 1956; Maercker, 1965; Franke, 1967; Schönherr and Bukovac, 1970).

The radius of aqueous pores in *Citrus aurantium* cuticles was 0.45 nm, which is about the size of a glucose molecule (Schönherr, 1976). This estimate is the average pore size and smaller as well as larger pores probably exist, but pore size distribution has not been studied. Average pore size did not depend on pH but the number of pores increased with pH from about 5×10^{10} (pH 3) to 16×10^{10} (pH 9) per cm^2 of membrane. Due to technical problems determinations were made using wax free cuticles and it might be argued that during incrustation of cuticles with wax aqueous pores are plugged up. However, this is not a very likely scenario, since aqueous pores in cuticles arise due to hydration of polar groups of cutin (-COOH, -OH and ester groups) and it is improbable that highly lipophilic wax monomers should enter that aqueous phase. This thermodynamic argument is supported by the fact, that extracting waxes from pear leaf CM increased rates of penetration of $CaCl_2$ only by factors of two (at 90% humidity) to three (50% humidity) (Schönherr. 2000). Possibly, embedded waxes increase rigidity of cuticles, which interferes with swelling. Swelling of CM depends on pH and humidity of the surrounding air (Schönherr, 1982; Chamel et al., 1991) and is a major determinant of rates of penetration of salts across CM (Schönherr, 2000).

THE VELOCITY OF CUTICULAR PENETRATION OF IONS DEPENDS ON HUMIDITY OF THE AIR

In foliar nutrition aqueous salt solutions are sprayed on leaves. After evaporation of excess water a hydrated salt residue is formed on the cuticle. Penetration of ions across the cuticle proceeds from this residue and this situation was simulated in the SOFU

(simulation of foliar uptake) experiments to be discussed. Isolated astomatous CM were inserted in a penetration apparatus, a 5 µl droplet of salt solution was positioned on the outer surface of the CM and after evaporation of water (40 to 60 min) the receiver compartment was filled with a citric acid buffer of pH 4.0. This receiver was withdrawn quantitatively at certain time intervals and replaced by fresh one. Since the salt solutions were doped with either $^{45}Ca^{2+}$ (with calcium salts) or ^{86}Rb (potassium salts) the salt fluxes could be monitored using a scintillation counter. During penetration a stream of air of constant humidity was blown over the salt residue on the CM (Schönherr, 2000).

A typical result obtained with $Ca(NO_3)_2$ is shown in Fig. 1. Rates of penetration of $Ca(NO_3)_2$ were highest initially and levelled off with time. At 90% humidity it took about 18 h until 50% of the applied dose had penetrated the CM. Penetration results in a decrease in amount of salt on the cuticle and in a decrease in salt concentration. This can be tested by plotting the natural logarithm of the fraction of salt (F_t) left on the CM at time *t vs*. time. These plots are linear showing salt penetration to be a first order process (Fig. 1B), *i.e*. the amount of salt disappeared exponentially with time. Only dissolved salt can penetrate and linearity implies that the salt concentration also decreased exponentially with time and all salt was dissolved throughout the experiment at each humidity level tested. If initially a saturated salt solution containing undissolved crystals had been present on the CM the plots would not have been linear throughout. However, this was the case with all salts tested, provided salt concentration in the donor was >2 g l^{-1} (Schönherr, 2000, 2001; Schönherr and Luber, 2002). The slopes of the plots in Fig. 1B are the first order rate constants (k) of penetration. From the rate constant the half time of penetration $t_{1/2}$ can be calculated ($t_{1/2}$ =-ln 0.5/k = 0.693/k). Half times decreased from 46.2 h (50% humidity) to 15.8 h (90% humidity). This is a factor of 2.9 showing humidity to be a major factor in cuticular penetration of calcium nitrate and all other inorganic salts (Schönherr 2000, 2001; Schönherr and Luber, 2002).

The two calcium salts penetrated at the same velocity and with increasing humidity half times decreased from about 45 to 15 h (Fig. 2A). Both salts are fairly hygroscopic, as POD's amount to 32 % ($CaCl_2$) and 55 % ($Ca(NO_3)_2$), respectively (Table 1). At 50% humidity calcium nitrate should have crystallised out since POD is 55%. However, this did not occur, as the salt residue was shiny rather than white. Apparently, humidity directly over the salt residue was higher than 50% due to unstirred layers. Hence, with these two salts humidity was higher than their POD's, the salts were always dissolved and the effect of humidity on half times can be attributed to swelling of the CM. The situation is different with organic calcium salts, which penetrated more slowly even at 100% humidity. Half times with calcium propionate, lactate and acetate were 30, 73 and 136 h, respectively. At 70% humidity half times were 239 h (propionate), 533 h (lactate) and 962 h (acetate). This can be attributed to their very high POD values ranging from 95% to 100 % (Table 1). When measuring penetration at a humidity level of 90% and lower white salt residues were clearly visible. Some penetration was detectable even at 70% humidity, again possibly due to unstirred layer effects. Apart from this it is clear that these organic calcium salts are not very suitable for foliar nutrition.

Potassium carbonate has a POD of 44% and half times decreased with increasing humidity (Fig. 2B) up to 90% and again this can be attributed to swelling. At higher humidity half times decreased and measurements were difficult because the salt residue took up moisture resulting in run-off problems. POD of KCl is 86% and relatively short half times can be rationalised only by invoking an unstirred layer effect resulting in higher humidity directly over the salt residue. Absolute values of half times of the two potassium salts were similar and at 90% humidity they were as high as those measured with calcium chloride and calcium nitrate. Monopotassium phosphate and potassium nitrate have rather high POD's of 97% and 95%, respectively (Table 1). At 100% humidity half times were 16 h (KNO_3) and 27 h (KH_2PO_4) but at 90% humidity half times were 141 h (KNO_3) and 115 h (KH_2PO_4) respectively. Comparable results have been obtained with *Citrus aurantium* CM (Schönherr and Luber, 2002). Hence their usefulness as foliar fertilisers is rather limited. From the four potassium salts tested K_2CO_3 has the

most desirable properties, as it has the lowest POD, the highest solubility and the highest K content (Table 1).

CATIONS AND ANIONS PENETRATE CUTICLES IN EQUIVALENT AMOUNTS

From the above examples it is evident, that humidity over the salt residue affects velocity of penetration by two independent mechanisms, *viz* (1) swelling of the cuticle and (2) dissolution of salt as related to POD. Both are purely physical properties. These laws can be expected to hold for all types of cuticles (including leaf surfaces having stomata and trichomes) and salts. Even though only a small number of calcium and potassium salts have been studied, it is obvious that a salt should have low POD and a high aqueous solubility. In foliar nutrition electrical neutrality must be maintained, that is cations and anions penetrate in equivalent amounts and this has been demonstrate using calcium nitrate, were each calcium ion was accompanied by two nitrate ions (Krüger, 1999). Depending on nutritional requirements either the chloride or the nitrate can be chosen, but the POD is usually lower for the chloride (Table 1). The very high POD of KNO_3 renders it a poor nitrogen fertiliser. The calcium, magnesium and maganese salts are superior, also ammonium nitrate. Unfortunately, there is no good phosphorus fertiliser. POD values in Table 1 were determined at 20 to 23 °C but POD generally does not depend much on temperature (Kolthoff at al., 1969) and repeated measurements showed deviations not lager than ±2%. In Table 1 molecular weights are also given, even though available data do not show a clear dependence of rates of penetration on molar mass of the salts. This may be due to uncertainties related to the number of water molecules accompanying the salts on their journey through the cuticles. Since aqueous pores in cuticles are very narrow smaller ions should penetrate faster.

THE ROLE OF WETTING AGENTS, ACCELERATORS AND OTHER ADJUVANTS

All data discussed were generated using the alkyl-polyglucoside Glucopon 215 CSUP as wetting agent. This is very important, because half time of penetration of $CaCl_2$ without wetting agent was 204 h, while 0.2 g l^{-1} decreased half time to 17 h. Higher concentrations were not better and varying the length of the alkyl chain had no effect either (Schönherr, 2001). It appears that the wetter is needed to make to pore entrances accessible to the hydrated ions. Ethoxylated surfactants are good wetters as well, but they should be avoided, because many of them are phytotoxic (Uhlig and Wissemeier, 2000) and polyoxyethylene chains strongly bind Ca^{2+} and other divalent cations (Cross, 1987). These large surfactant complexes do not penetrate the cuticles and this is the reason, why surfactant phytotoxicity can be eliminated by the addition of calcium salts to spray solutions (Uhlig and Wissemeier, 2000). However, as these calcium ions are not available to the plant ethoxylated surfactants should not be used in foliar nutrition.

Calcium ions interact with many other compounds. For instance gum guar at 0.5% increased half times of penetration of $CaCl_2$ by up to 9 fold. Protein surfactants also increased half times of penetration by a factor of three and mixing $CaCl_2$ with equimolar amounts of Na_2EDTA increased half times by a factor of four. These examples demonstrate that one should be cautious, when mixing salts with adjuvants that interact with divalent cations. Foliar nutrients are often mixed with fungicides and interactions with active ingredients and formulants might slow rates of penetration of foliar nutrients. This needs to be investigated.

Rates of penetration of non-electrolytes can be greatly increased by adding certain adjuvants called accelerators (Schönherr, 1993; Schreiber, 1995; Schreiber et al. 1996; Schönherr and Baur, 1996; Schönherr et al. 2001). Accelerators are plasticiser, which increase fluidity of cutin and amorphous waxes, which in turn increases rates of diffusion of molecules which can access these phases (Buchholz and Schönherr, 2000). Since ions are confined to a aqueous pores, accelerators have no influence on velocity of ion penetration (Schönherr, 2000). Thus, apart from using an effective wetting agent, there is

no way to increase rates of cuticular penetration of foliar nutrients by including adjuvants. Even increasing temperature from 15 to 30 °C did not increase rates of penetration of calcium and potassium salts (Schönherr, 2000; Schönherr and Luber, 2002).

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Table

Table 1. Physical properties of selected inorganic salts for foliar nutrition.

Salt	POD[a] %	Solubility[b] g/kg H_2O	Nutrient content %	Molecular weight g/mol
$CaCl_2$ x 6 H_2O	33	2790	18.3 (27.2)	219
$MgCl_2$ x 6 H_2O	33	1670	12.0	203
K_2CO_3 x 2 H_2O	44	1469	44.9	174
$Ca(NO_3)_2$ x 4 H_2O	56	6600	10.3	236
$Mg(NO_3)_2$ x 6 H_2O	56	1250	9.5	256
NH_4NO_3	63	1183	35.0 (N)	80
KCl	86	344	52.1	75
K_2HPO_4	92	167	44.9	174
KH_2PO_4	95[a]	33	28.8	136
KNO_3	95	133	38.7	101
Ca-propionate x H_2O	95[a]	490	19.6	204
Ca-lactate x 5 H_2O	97[a]	31	13.0	308
Ca-acetate	100[a]	374	25.4	158
$FeCl_3$ x 6 H_2O	44	919	20.7	270
$Fe(NO_3)_3$ x 9 H_2O	54	1500	13.8	404
$Mn(NO_3)_2$ x 4 H_2O	42	426	21.9	251
$MnCl_2$ x 4 H_2O	60	1510	30.5	180
$ZnNO_3$ x 6 H_2O	42	1843	22.0	297

[a] own determinations, [b]Lide (1991).

Figures

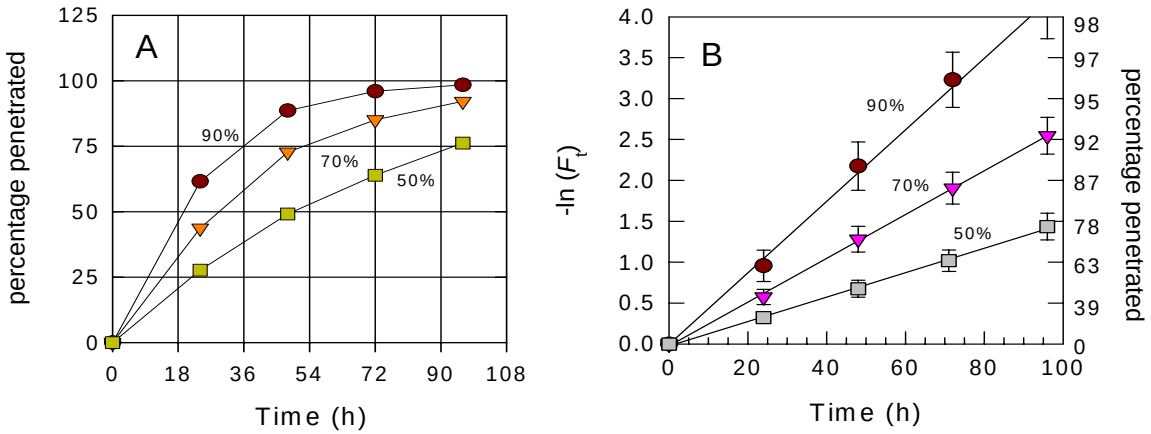

Fig. 1. Penetration of calcium nitrate across pear (*Pyrus communis*) leaf cuticular membrane at 50, 70 and 90% humidity. Salt concentration was 6 g l^{-1} and 0.2 g l^{-1} Glucopon 215 CSUP was added as wetting agent. In A percentage penetrated was plotted vs. time and the same data are presented in B as first order plots. Data were taken from Schönherr, 2001.

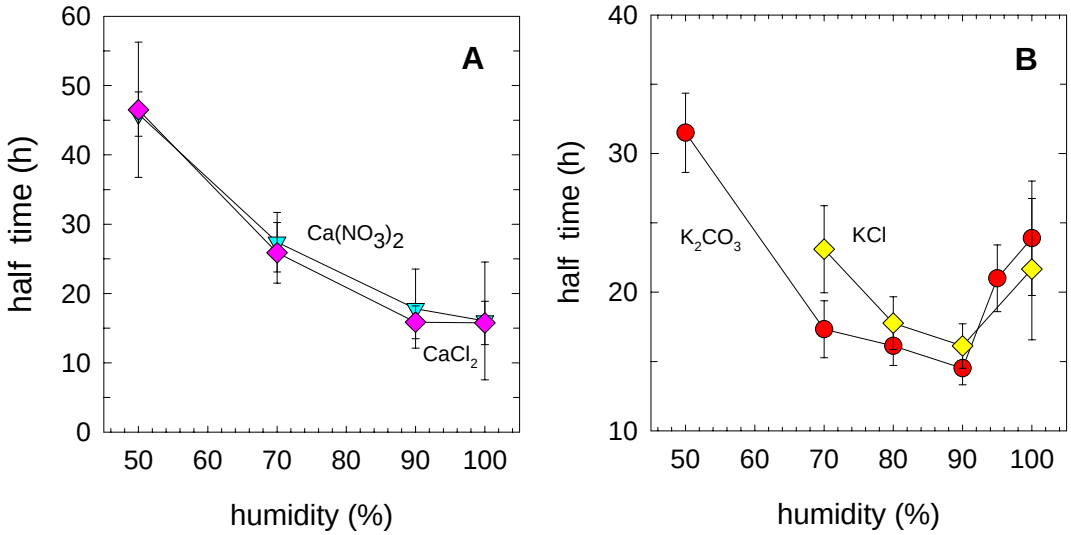

Fig. 2. The effect of humidity at 20 °C on velocity of penetration across pear leaf CM of calcium (A) and potassium salts (B), respectively. Donor concentrations were 5 g l^{-1} with each salt and 0.2 g l^{-1} Glucopon 215 CSUP was added as wetting agent. Data taken from Schönherr, 2001 and Schönherr and Luber, 2002).