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Rates of Cuticular Penetration of Chelated Fe^{III}: Role of Humidity, Concentration, Adjuvants, Temperature, and Type of Chelate

JÖRG SCHÖNHERR, VICTORIA FERNÁNDEZ, AND LUKAS SCHREIBER*

Institute of Vegetable and Fruit Science, University of Hannover, Am Steinberg 3, 31157 Sarstedt, Germany, and Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1, 53115 Bonn, Germany

Time courses of cuticular penetration of $FeCl_3$ and Fe^{III} complexes of citric acid, EDTA, EDDHA (Sequestrene 138Fe), imidodisuccinic acid (IDHA), and ligninsulfonic acid (Natrel) were studied using astomatous cuticular membranes (CMs) isolated from *Populus x canescens* leaves. At 100% relative humidity, the Fe^{III} chelates disappeared exponentially with time from the surface of the CMs; that is, penetration was a first-order process that can be described using rate constants or half-times of penetration ($t_{1/2}$). Half-times ranged from 20 to 30 h. At 90% humidity, penetration rates were insignificant with the exception of Natrel, for which $t_{1/2}$ amounted to 58 h. Rate constants were independent of temperature (15, 25, and 35 °C). Permeability decreased with increasing Fe chelate concentration (IDHA and EDTA). At 100% humidity, half-times measured with FeIDHA were 11 h (2 mmol L^{-1}), 17 h (10 mmol L^{-1}) and 36 h (20 mmol L^{-1}), respectively. In the presence of FeEDTA, penetration of $CaCl_2$ was slowed greatly. Half-times for penetration of $CaCl_2$, which were 1.9 h in the absence of FeEDTA, rose to 3.12 h in the presence of an equimolar concentration of EDTA and 13.3 h when the FeEDTA concentration was doubled. Hence, Fe chelates reduced permeability of CMs to $CaCl_2$ and to the Fe chelates themselves. It is suggested that Fe chelates reduced the size of aqueous pores. This view is supported by the fact that rate constants for calcium salts were about 5 times higher than for Fe chelates with the same molecular weights. Adding Tween 20 (5 g L^{-1}) as a humectant did not increase permeability to FeIDHA at 90% humidity and below, while addition of glycine betaine did. Penetration of $FeCl_3$ applied at 5 g L^{-1} (pH 1.5) was not a first order process as rate constants decreased rapidly with time. Only 2% of the dose penetrated during the first 2 h and less than that in the subsequent 8 h. Recovery was only 70%. This was attributed to the formation of insoluble Fe hydroxide precipitates on CMs. These results explain why in the past foliar application of Fe compounds had limited success. Inorganic Fe salts are instable and phytotoxic because of low pH, while Fe chelates penetrate slowly and 100% humidity is required for significant penetration rates. Concentrations as low as reasonably possible should be used. These physical facts are expected to apply to stomatous leaf surfaces as well, but absolute rates probably depend on leaf age and plant species. High humidity in stagnant air layers may favor penetration rates across stomatous leaf surfaces when humidity in bulk air is below 100%.

KEYWORDS: Iron chlorosis; foliar nutrition; EDTA; EDDHA; IDHA; Natrel; Sequestrene 138Fe

INTRODUCTION

Many economically important plant species suffer from iron deficiency when grown in calcareous soils. Citrus, grapevines, kiwifruit, peach, and pear are examples of susceptible crops whose leaves may turn yellow because chlorophyll synthesis is impaired. Iron chlorosis can cause considerable economic losses because of reduced yields and poor fruit quality (*1*).

In calcareous soils with high pH values, uptake of Fe into roots is limited because Fe occurs as Fe^{III} hydroxides, carbonates, and phosphates. Solubility of these compounds is extremely low, and they are not readily available for root uptake (*2*). However, other factors such as UV light, pH, and organic acids in the apoplast and activity of the plasma membrane-bound ferric-chelate reductase affect availability of Fe and may also be involved in iron chlorosis (*3*−*8*).

Various remedies have been tried to cure iron chlorosis. Soil application of Fe^{III} chelates is the dominating correction practice (*9*−*13*). Solid Fe sulfate implanted into the stems of pear and

* To whom correspondence should be addressed. Telephone: ++49 +228 73 4687. Fax: ++49 +228 73 6811. E-mail: lukas.schreiber@uni-bonn.de.

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peach trees has been found to increase Fe and chlorophyll concentrations in leaves (*14*). Foliar applications of a large variety of iron-containing compounds have also been tried, usually with limited success (*15*−*20*). Foliar application of Fe has been studied since the 1930s (*21*), and numerous reports have been published. In the majority of cases, inorganic Fe^{II} and Fe^{III} compounds have been tested and regreening of yellow leaves and increases in chlorophyll and/or Fe concentrations in leaves have been used as criteria for assessing treatment success. This literature has been reviewed recently (*17*, *22*). In a few studies, penetration was examined directly by using radio-labeled ^{59}Fe. Basiouny and Biggs (*23*) investigated penetration into *Citrus aurantium* leaves of a number of synthetic Fe^{III} chelates, $FeCl_3$, Fe^{III} citrate, tartrate phosphate, Ferbam, and Fe^{II} ammonium sulfate. Very slow penetration was observed with all compounds, and penetration of chelates was faster than that of inorganic Fe. Furthermore, translocation of chelates was superior compared to inorganic Fe. High relative humidity (100%) increased rates of penetration into citrus leaves. Poor penetration of $FeCl_3$ was likely due to the formation of hydroxide polymers, because $FeCl_3$ is stable only below pH 1. Eddings and Brown (*18*) studied penetration of $^{59}FeCl_3$ at pH 2 into sorghum, tomato, and bean leaves. They included the anionic surfactant Vatsol OT (0.1%) and observed a contribution of stomatal penetration. The anionic surfactant probably formed Fe^{III} salts of high molecular weight, and formation of Fe hydroxides may have been involved. Kannan (*24*) studied penetration of $FeSO_4$ and FeEDDHA through astomatous *Euonymus* cuticles and observed higher rates with $FeSO_4$. No information as to pH, type, and concentration of surfactant was given, and oxidation of Fe^{II} with subsequent formation of Fe hydroxide polymers is likely to have occurred. Chamel (*25*) investigated penetration of radio-labeled $FeSO_4$ (at pH 3.9, 4.3, and 4.7) and $Fe(NO_3)_3$ (at pH 3.1) into maize leaves at 48−60% humidity. All solutions contained 0.02% Tween 20 as a wetting agent and 0, 0.5, or 1% DMSO as an adjuvant. Very slow penetration was observed, and DMSO increased rates of penetration of $FeSO_4$ but not of $FeCl_3$. Once again, oxidation of Fe^{II} and formation of hydroxide polymers must have occurred, but this was not considered. At the end of the experiments, leaf surfaces were washed with Na_2EDTA, but effectiveness of this procedure for completely removing Fe polymers is doubtful. Neumann and Prinz (*19*) and Levy and Horesh (*20*) observed stomatal infiltration of citrus leaves when dipped into $FeSO_4$ solutions containing Silwet L-77. The effect decreased with time, probably because this surfactant is not stable under highly acid or alkaline conditions (*26*).

These examples demonstrate that penetration of Fe salts and chelates is slow and very poorly understood. This is due in part to the empirical nature of these experiments but also to the poor understanding of the laws of cuticular penetration at the time. During the past decade, cuticular penetration has been studied systematically and quantitative laws have evolved. 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 (*27*). Neutral noncharged molecules cross cuticles by dissolving and diffusing in lipophilic domains composed of cutin and amorphous cuticular waxes. This route is called the lipophilic pathway (*28*−*30*).

Ionic species are not lipid-soluble, because they are hydrated at physiological temperatures (*31*). Hence, ions are excluded from the lipophilic pathway, and they can cross lipid membranes (including cuticles) only if aqueous pores traverse them. Such pores arise by hydration of polar functional groups in the membrane (*29*, *32*−*34*), and penetration of ionic species across lipid membranes is direct evidence for the existence of aqueous pores. All astomatous cuticles studied so far exhibited polar pores, albeit to different degrees (*29*). Aqueous pores in astomatous cuticular membranes (CMs) isolated from gray poplar (*Populus x canescens*) leaves are size-selective, and permeability decreased by a factor of up to 13 when molecular weight of ions increased from 100 to 500 g mol^{-1} (*33*).

Plant leaves have stomata at least on one side, and higher water and solute permeability of cuticles over and in the vicinity of guard cells has been reported (*35*−*42*). These observations have been interpreted as evidence for the presence of aqueous pores in the cuticle over guard cells and trichomes. Because ions are confined to the aqueous pathway, their rates of diffusion depend on swelling of cuticles, which in turn depends on the relative air humidity (*32*, *34*, *43*, *44*). Permeability of poplar cuticles to ionic glyphosate salts increased by factors of 5−10 when humidity was increased from 70 to 100% (*43*). High humidity increased herbicidal efficacy of glyphosate (*45*, *46*).

With this background, the important variables involved in cuticular penetration of Fe compounds can be identified and meaningful mechanistic investigations can now be performed. Rates of penetration must be measured rather than amounts penetrated in an arbitrary time interval, as was usually done in the past. Humidity, adjuvants, temperature, and concentration are likely important variables, and their effects on rates of penetration of Fe compounds should be studied. Because cuticular penetration is a purely physical process, these measurements can be conducted using a model cuticle, which has aqueous pores of known properties. Penetration of calcium, potassium, and glyphosate salts as well as proline and glycine betaine across gray poplar CMs has been studied extensively. These data can serve as a basis for comparing penetration rates observed with Fe compounds. The role of stomata and variability in rates of penetration among plant species must be studied using leaves. Such studies are underway and will be reported separately.

MATERIALS AND METHODS

Plant Material. Experiments were conducted using astomatous adaxial CMs from leaves of gray poplar [*Populus x canescens* (Aiton) Sm.]. Adaxial cuticles from leaves just fully expanded were isolated enzymatically (*47*), dried, and stored at 8 °C until used. Similar CMs have previously been employed to characterize permeability of cuticles to various ionic compounds (*33*, *43*, *47*).

Chemicals. Donor solutions were prepared by dissolving calcium chloride, calcium pantothenate, or Fe compounds in deionized water. Molecular weights and other relevant properties of test compounds are given in **Table 1**. $^{45}CaCl_2$ (NEN, Boston, MA; specific activity of 44.7 GBq $mmol^{-1}$, radiochemical purity of 99.9%) was added as tracer (approximately 100 Bq μL^{-1}) to the calcium salts. Fe compounds were spiked with $^{59}FeCl_3$ at a concentration of approximately 500 Bq μL^{-1}, which corresponds to a concentration of ^{59}Fe of 2.55 μmol L^{-1} (Perkin−Elmer, Boston, MA; specific activity of 3500 MBq mg^{-1}; radiochemical purity of 99%). The concentrations of calcium salts in the donor solutions were 10 or 20 mmol L^{-1}, while the concentrations of Fe^{III} contained in the test compounds ranged from 0.2 to 20 mmol L^{-1}. The C8/10-polyglycoside Glucopon 215 CSUP (Fluka, Neu-Ulm, Germany) was added to all donor solutions at a concentration of 0.25 g L^{-1}. This is a nonionic surfactant, which does not interact with Ca^{2+} or Fe^{3+} ions. Addition of an effective surfactant is absolutely necessary to establish good contact between aqueous solutions and aqueous pores in cuticles (*29*, *34*, *43*). Solutions were freshly prepared for each experiment unless mentioned otherwise. As $^{59}FeCl_3$ stock was dissolved in 0.5 mol L^{-1} HCl, an equivalent amount of KOH was added to the donor solutions to neutralize the acid and maintain the pH at the desired level (**Table 1**).

Table 1. Properties of Calcium Salts and Fe^{III} Compounds Used in the Experiments

compound	chemical name of chelator	Fe weight fraction	molecular weight (g mol^{-1})	pH[a]	source
FeEDTA	ethylenediaminetetraacetic acid	0.12–0.14	367	4.9	Fluka
FeIDHA	imidodisuccinic acid	0.09	393	5.1	Agrex
sequestrene 138Fe (FeEDDHA)	ethylenediamine *N,N*′-di(*o*-hydroxyphenylacetic) acid	0.06	368	8.3	Syngenta
natrel	ligninsulfonic acid	0.11	507[b]	3.4 adj. to 5.0	Agrex
ferric citrate	citric acid	0.18–0.20	263	adj. to 5.0	Fluka
ammonium ferric citrate	citric acid	0.20–0.23	254[b]	7.0	Fluka
ferric chloride		0.35	162	1.5–2.7[c]	Fluka
calcium chloride			111	6.5	Fluka
Ca-pantothenate			477	7.2	Fluka

[a] pH value of a 0.02 M aqueous solution. [b] Average molecular weight calculated from Fe weight fraction and atomic weight of Fe. [c] Depending on the concentration.

Preparation of ^{59}FeIDHA. Because rates of penetration were studied by monitoring radioactivity of the receiver solutions, it is essential that equilibration between nonradioactive Fe^{3+} in Fe chelates with $^{59}Fe^{3+}$ added as $^{59}FeCl_3$ takes place rapidly such that ^{59}Fe chelates are uniformly labeled. This was tested by comparing penetration rates measured with ^{59}FeIDHA obtained by mixing nonradioactive FeIDHA with $^{59}FeCl_3$ with those observed when ^{59}FeIDHA was prepared by reacting Na_4IDHA with $^{59}FeCl_3$. The procedure used by Shenker et al. (*48*) was adopted. At room temperature, $^{59}FeCl_3$ was added dropwise to Na_4IDHA under vigorous stirring. The reactants had a concentration of 2 mmol L^{-1}, and they were mixed at a molar ratio of 1:1. After the mixture was stirred for 2 h at room temperature, the pH of the mixture was adjusted to 5.0 with KOH. After dilution with deionized water, the final concentration was 1 mmol L^{-1}. Recovery of radioactivity was 100%, and a visible precipitate did not form.

Penetration Experiments. Rates of cuticular penetration were measured using SOFU (simulation of foliar uptake) as described in detail elsewhere (*29*, *34*, *43*). The method allows studying the time course of cuticular penetration for each individual CM (*49*). CMs were mounted between the ring-shaped lid and bottom of the desorption chamber using silicon grease (Baysilon, Bayer, Leverkusen, Germany). After mounting, each CM was tested for leaks and imperfections as described previously (*29*, *33*). The chambers were filled with 0.6 mL receiver solution consisting of 2 g L^{-1} citric acid buffer adjusted with KOH to pH 6.0. They were placed into the wells of a thermostated aluminum block, which was rocked at 75 rpm for mixing of receiver solutions. After an equilibration period of about 24 h, the receiver solutions were withdrawn and a 5 μL droplet of donor solution was applied to the center of each CM. The water evaporated within 60 min when a stream of ambient air was blown over the droplets. At this time, the donor compartment was sealed with clear adhesive tape (Tesafilm, Beiersdorf, Hamburg, Germany) to ensure 100% humidity above the salt residue. The chambers were then filled again with receiver solution and returned to the aluminum block. The TESA method was used in all experiments with constant humidity of 100%. When the effect of humidity over the salts or chelates was investigated (**Figures 7−10**), air of constant humidity was blown over their residues on the CMs to ensure a constant level of hydration of the residues. The air flow rate was 2.5 mL s^{-1}. With an air volume of 0.25 mL over the CMs, this equated to 10 exchanges of air per second. Humidity of the air was adjusted by using the dew point method (KF-24 Kältefalle, Heinz Walz GmbH, Effeltrich, Germany).

Receiver solutions were quantitatively withdrawn for scintillation counting at intervals ranging from 2 to 24 h and were replaced by fresh ones. After the last sample had been taken, each CM was washed 3 times with 0.35 mL of deionized water to determine the amount of salt that had not penetrated. The wash water was collected in a scintillation vial; scintillation cocktail was added; and the combined washes were assayed for radioactivity. In this way, one and the same set of CMs could be used for more than one experiment (paired comparisons). The amount applied (M_0) was obtained by summing up the amounts penetrated and the amount left on the CM for each CM individually. The amount applied in the 5 μL droplet was determined independently from 5 replicates. Average recovery (radioactivity recovered/radioactivity applied) ranged from 95 to 100%. Radioactivity of samples was determined at a constant quench using a Wallac 1409 liquid scintillation counter (Wallac, Turku, Finland) set to a 2σ error of 3%.

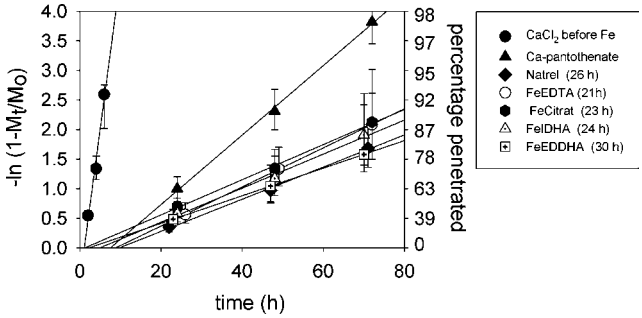

Figure 1. First-order plots of penetration of selected calcium salts and Fe^{III} chelates at 20 mmol L^{-1} across poplar CMs at 20 °C and 100% relative humidity.

Data were plotted as $-\ln(1 - M_t/M_0)$ versus time (t), where M_t is the amount of ^{45}Ca or ^{59}Fe recovered from the desorption medium at time t. When the natural logarithm of the fraction left on the CM is plotted against time, the slope of the line is the first-order rate constant of penetration (k). Variability among individual CMs can be large, and for this reason, 40 CMs were used as replicates. Geometric means and 95% confidence intervals were calculated from log-transformed data and are shown in the figures.

RESULTS

Permeability at 100% Humidity over the Donor Residues. When penetration of Fe chelates was measured at 100% humidity plots of $-\ln(1 - M_t/M_0)$ versus time resulted in straight lines (**Figure 1**). This shows that the fraction of chelates left on the surface of the CMs (M_t/M_0) decreased exponentially with time. The slopes of the lines (rate constants k in h^{-1}) were steeper with the Ca salts than with the Fe chelates. The same set of CMs was used for all compounds, and experiments were started with $CaCl_2$ for which k was 0.51 h^{-1}. In the next experiment, Ca pantothenate was used and the slope was 0.058 h^{-1}. Mean rate constants for the four Fe chelates were around 0.033 h^{-1}, which amounts to a half time ($\ln 0.5/k$) of about 21 h. The differences among the chelates were not significant as the confidence intervals overlap (**Figure 1**). Before changing to a new test compound, the surfaces of the CMs were washed with deionized water and the combined radioactivity of the wash fluid and the receiver samples was always between 95 and 100% of the radioactivity applied. These excellent recoveries are evidence that Fe chelates did not form insoluble residues on the CMs nor were significant amounts sorbed in the CMs.

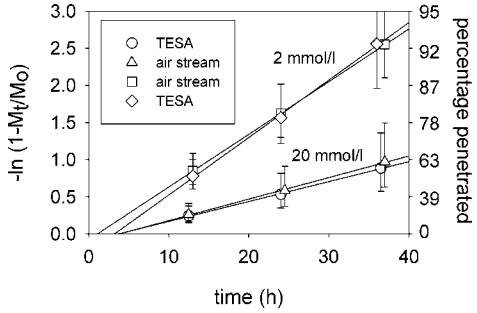

Figure 2. First-order plots of penetration of Fe^{III}IDHA chelates at 2 and 20 mmol l-1 across poplar CMs at 20 °C and 100% humidity.

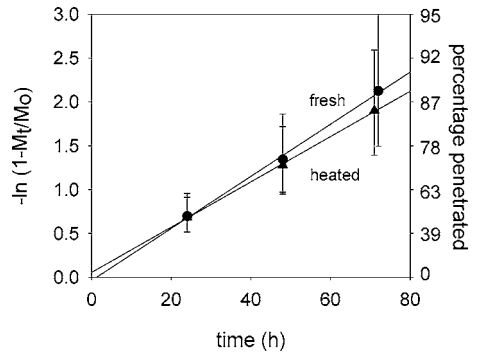

Figure 3. First-order plots of penetration of Fe^{III}-citrate at 20 mmol L^{-1} across poplar CMs at 20 °C and 100% humidity. Data for the graph marked "heated" were obtained with a donor solution that had been heated to 80 °C for 12 h and cooled to 20 °C prior to application.

At the beginning of each experiment, compounds dissolved in water precipitated on the CMs when the water of the donor evaporated, but for penetration to occur, compounds must be in solution. All test compounds are hydrophilic ionic solutes with high solubility in water, and dissolution at 100% humidity (which has the same chemical potential as liquid water) can be taken for granted. Two methods for obtaining 100% humidity were tested. When the donor was dry, the donor chambers were sealed with sticky transparent TESA film and citric acid buffer was added to the receiver chamber. As water penetrated across the CMs, humidity over the residues increased and eventually reached 100%. The second method consisted of directing an air stream of 100% humidity onto the residue during the entire experiment (see the Materials and Methods). Linear penetration graphs were obtained with FeIDHA at 2 and 20 $mmol^{-1}$, and the slopes obtained with the two methods were identical (**Figure 2**). Interestingly, the rate constant at 2 mmol L^{-1} was 0.074 h^{-1}, while at 20 mmol L^{-1}, k was only 0.028 h^{-1}. The x intercepts did not differ significantly either, which shows that in the TESA method 100% humidity was reached quickly (**Figure 2**).

Next, it was tested if the measured flow rates of ^{59}Fe did indeed represent penetration of chelated Fe. To test whether isotopic exchange was rate-limiting, penetration was studied using Fe citrate freshly prepared by mixing nonradioactive Fe citrate with $^{59}FeCl_3$. The penetration graph was linear (**Figure 3**, fresh), and the slope was 0.028 h^{-1}. While this experiment was conducted, the remainder of the donor solution contained in an airtight vial was heated in the dark to 80 °C for 12 h followed by cooling to room temperature. With the same set of CMs, the experiment was repeated using the donor that had been heat-treated. The rate constant was slightly smaller (0.026 h^{-1}) (**Figure 3**, heated). A similar experiment was conducted with FeEDTA, and the rate constants obtained were 0.037 and 0.030 h^{-1} for the "fresh" and "heated" treatments, respectively (data not shown). The differences in rate constants obtained with cold- and heat-treated donor solutions were not significant with either compound. Rate constants measured with a donor obtained by mixing nonradioactive FeIDHA with $^{59}FeCl_3$ (1 mmol L^{-1}) amounted to 0.058 h^{-1}, while k was 0.064 h^{-1} with a donor prepared by mixing Na_4 IDHA with $^{59}FeCl_3$. The concentrations of the chelates were 1 mmol L^{-1} in each case, and the same set of CMs was used. Because recovery was 95−100% in every case, it is concluded that insoluble Fe hydroxides were not formed during donor preparation and experimentation. These three sets of results obtained with 3 different chelates indicate that isotope exchange was rapid and mixing nonradioactive Fe chelates with $^{59}FeCl_3$ is a valid procedure for studying cuticular penetration of chelated Fe^{III}. This method was used in experiments shown in **Figures 1** and **2** and in all subsequent experiments.

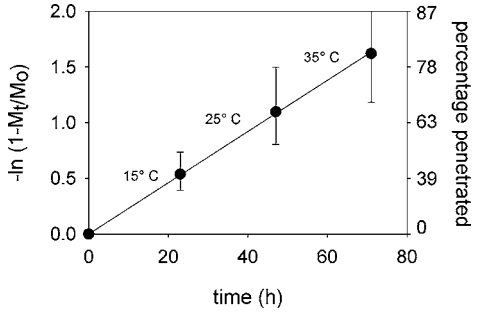

Figure 4. First-order plots of penetration of Fe^{III}IDHA at 10 mmol L^{-1} across poplar CMs at 100% humidity. The experiment was started at 15 °C, and temperature was raised in 24 h intervals.

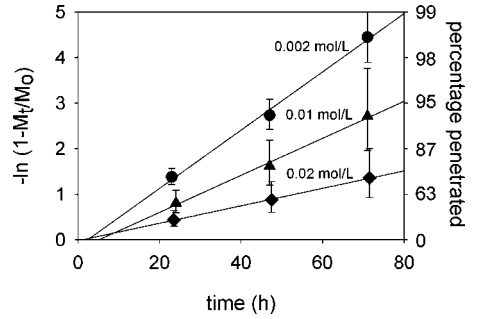

Figure 5. First-order plots of penetration of Fe^{III}IDHA chelates at 2, 10, and 20 mmol L^{-1} across poplar CMs at 20 °C and 100% humidity.

The effect of temperature on penetration of Fe chelates was studied using FeIDHA. The experiment was started at 15 °C, and temperature was raised to 25 and 35 °C at intervals of 24 h. It took less than 0.5 h for the penetration chambers, CMs, and solutions to reach the new higher temperature. The slope of the penetration graphs was not affected by temperature (**Figure 4**). The average rate constant was 0.023 h^{-1}.

Rate constants of penetration, that is, permeability of CMs, decreased with an increasing concentration of FeIDHA (**Figure 5**). At 2, 10, and 20 mmol L^{-1}, rate constants amounted to 0.064, 0.041, and 0.019 h^{-1}, respectively. That is, an increase in concentration by a factor of 10 resulted in a decrease in the rate constant by a factor of 3.4. Penetration of $^{45}CaCl_2$ was also affected in the presence of FeEDTA (**Figure 6**). In the absence of FeEDTA (marked no FeEDTA), the rate constant for $CaCl_2$ penetration was 0.37 h^{-1}. Adding an equimolar concentration of FeEDTA (0.01 mol L^{-1} FeEDTA) reduced the rate constant to 0.22 h^{-1}; with twice this concentration of FeEDTA (0.02 mol L^{-1} FeEDTA), rate constants were only 0.052 h^{-1}.

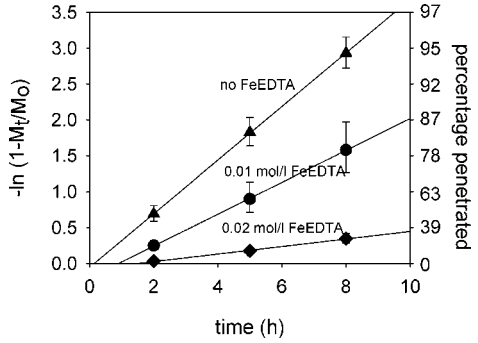

Figure 6. First-order plots of penetration of $CaCl_2$ at 10 mmol L^{-1} in the absence or presence of Fe^{III}EDTA at 10 and 20 mmol L^{-1} across poplar CMs at 20 °C and 100% humidity.

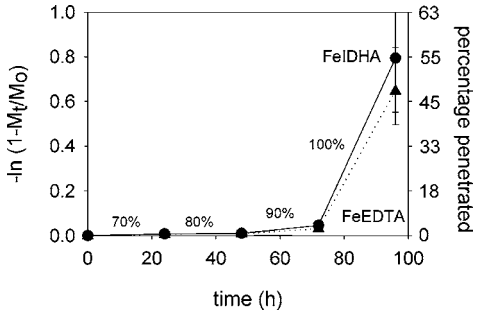

Figure 7. First-order plots of penetration of Fe^{III}IDHA (●) and Fe^{III}EDTA (▲) at 20 mmol L^{-1} across poplar CMs at 20 °C and 100% humidity. The humidity of the air blown over the chelate on the CMs was 70% during the first interval and was increased every 24 h as shown in the graph.

Table 2. Rate Constants of Fe Salts Measured at 2 Different Humidities

compound	rate constant[a] at 100% relative humidity (h^{-1})	rate constant at 90% relative humidity (% of 100% relative humidity value)
$Fe^{III}NH_4$-citrate	0.025	1.6
dito	0.027	4.1
dito	0.020	2.3
Fe^{III}-citrate	0.058	4.6

[a] Measured at 1 g L^{-1}.

Effect of Humidity. The effect of humidity over the donor residue was studied using the same set of CMs for all humidities. Experiments were started at 70% humidity, and after 24 h, receiver samples were taken and the humidity was raised to the next higher level. Rates of penetration were greatly affected by humidity. At 70, 80, and 90% humidity over the residue of FeIDHA, penetration was so slow that it was difficult to measure. Only when humidity was raised to 100% did constants rise to 0.024 h^{-1} (FeEDTA) and 0.031 h^{-1} (FeIDHA) (**Figure 7**). These values are similar to those measured with constant 100% humidity (**Figure 1**). Comparable results were obtained with Fe citrate and $FeNH_4$ citrate (**Table 2**). Rate constants measured at 90% humidity amounted to only 1.6−4.6% of those obtained at 100% humidity.

These data indicate problems with dissolution of chelates at humidity below 100%. However, adding 5 g L^{-1} of Tween 20 as a humectant had no effect at all even though a shiny and apparently liquid residue was observed on the CMs throughout the experiment (**Figure 8**).

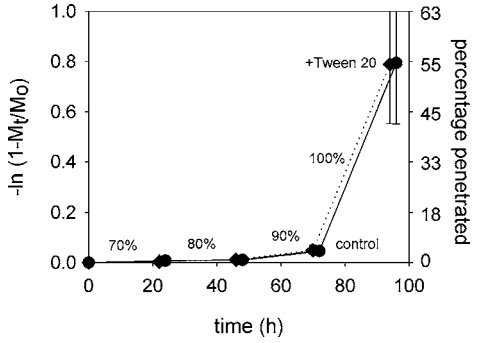

Figure 8. First-order plots of penetration of Fe^{III}IDHA at 20 mmol L^{-1} across poplar CMs at 20 °C. The humidity of the air blown over the chelate on the CMs was 70% during the first interval and was increased every 24 h as shown in the graph. In the treatment marked "+Tween 20" (◆), the donor contained 5 g L^{-1} Tween 20.

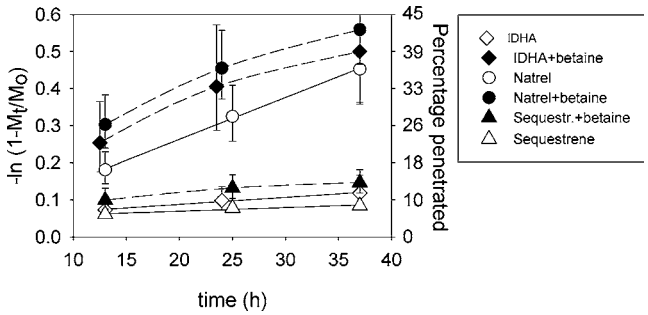

Figure 9. First-order plots of penetration of Fe^{III} chelates at 5 mmol L^{-1} across poplar CMs at 20 °C and 90% humidity. Closed symbols indicate where glycine betaine (5 g L^{-1}) was added to the donor solutions.

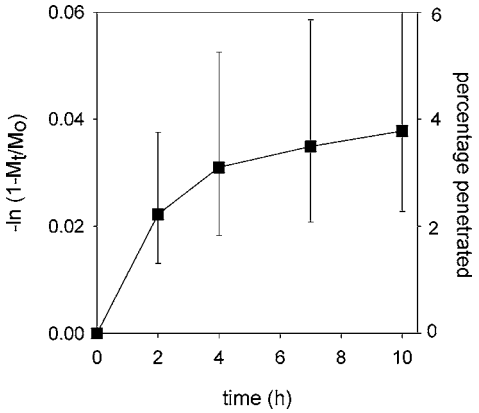

Figure 10. First-order plots of penetration of $FeCl_3$ at 5 g L^{-1} across poplar CMs at 20 °C and 90% humidity. Geometric means and 95% confidence intervals obtained with 15 CMs are shown.

Penetration graphs obtained for FeIDHA and Natrel at 90% humidity were linear (open symbols in **Figure 9**), but Natrel produced much steeper slopes (0.011 h^{-1}) than FeIDHA (0.002 h^{-1}). With Sequestrene 138, rate constants were only 8×10^{-4} h^{-1} and most of the FeEDDHA penetrated during droplet drying. Adding glycine betaine at 5 g L^{-1} to FeIDHA or Natrel increased rates of penetration at 90% humidity, while the effect with Sequestrene 138 Fe was negligible (closed symbols in **Figure 9**). The effect was greatest with FeIDHA and smaller with Natrel, and the effects decreased with time.

Penetration graphs with $FeCl_3$ at 90% humidity were not linear (**Figure 10**). About 2% of the dose penetrated during the first 2 h and another 2% over the following 8 h. Even though the donor solution had an initial pH of 1.5 (5 g L^{-1} $FeCl_3$ × 6

H_2O), recovery was only 70% when the CMs were thoroughly washed with water at the end of the experiment. A similar experiment was conducted with a new set of CMs, where the donor concentration was 1 g L^{-1}, resulting in a pH of 2.7. At 90% humidity, only 5.5% of the applied dose penetrated during the first 3 h, after which the humidity was raised to 100% and the experiment was continued for another 69 h. Total penetration in 72 h was 10% of the dose, that is, an additional 4.5% of the dose penetrated in 69 h at 100% humidity. Extensive washing of the residues on the CMs with 0.1 mol L^{-1} HCl yielded a total recovery of only 40%. This indicates that an Fe residue insoluble in 0.1 N HCl must have formed, which amounted to 60% of the dose.

DISCUSSION

Scope and Validation of Procedures. We have studied cuticular penetration of chelated Fe^{III} compounds, using CMs of astomatous poplar leaves as a model system. Penetration across the cuticle into the apoplast is the first and limiting step in foliar nutrition. However, with Fe, it is likely that not all Fe located in the apoplast is available to cells and physiologically active. Cuticular penetration is a purely physical process, and our discussion will focus on physicochemical aspects of cuticular penetration. The absolute rates of penetration are expected to depend on the presence of stomata and to vary greatly among plant species (*50*). However, the physical laws of penetration will also apply to stomatous surfaces and cuticles from different plant species. The presence of aqueous pores in cuticles is a prerequisite for penetration of hydrated ionic compounds (*29*). The number of pores and their size determine absolute values of permeability and penetration rates (*33*).

Penetration of chelated Fe^{III} followed first-order kinetics, as was observed with other ions such as calcium, potassium, and glyphosate salts (*29*, *34*, *43*, *44*, *51*). This indicates that all of these compounds use the same aqueous pathways in cuticles. Recoveries ranged from 95 to 100% of the applied dose. Hence, insoluble residues did not form on the CMs at 70−100% humidity. Time-independent rate constants indicate that photodecomposition of Fe^{III} chelates (*52*, *53*) did not occur. However, there was little chance for this to happen because experiments were conducted in the dark and donor solutions were subjected to dim light from fluorescent tubes for only 1 h during droplet drying. Results with $FeCl_3$ gave nonlinear kinetics and very low recovery, even though initial pH values were only 1.5 or 2.7, respectively. The poor recovery was caused by the instability of inorganic Fe compounds leading to the formation of insoluble Fe hydroxides (*16*, *17*, *22*). Because $FeCl_3$ was used, oxidation was not involved but hydroxides formed, which were neither soluble in water nor in 0.1 mol L^{-1} HCl, and penetration ceased after a few hours. Clearly, foliar nutrition with inorganic Fe salts is not a real option because, with most plants, phytotoxicity will be a problem and only a few percent of the dose applied will actually penetrate even at 100% humidity. This conclusion is based on penetration of poplar CMs, but this in agreement with numerous failures to obtain regreening with inorganic Fe salts (*22*). That is, chelated Fe^{III} compounds are the only realistic alternative for foliar Fe nutrition.

100% Humidity Is Required for Cuticular Penetration of Fe Chelates. Penetration of calcium salts and Fe chelates was a first-order process; that is, the fraction of the salts or chelates remaining on the surface decreased exponentially with time, and the slopes of the graphs represent the first-order rate constants (k). Such a process may also be described by the half-time of penetration ($t_{1/2}$), as with $M_t/M_0 = 0.5$, the half-time $t_{1/2}$ is equal to 0.693/k. The highest rate constants occurred when humidity over the Fe residues on the CMs was 100%. Two mechanisms are responsible for the humidity effects on rates of cuticular penetration. Solids do not penetrate, and ionic compounds must be dissolved. This is determined by the point of deliquescence (POD) (*54*). Apparently, PODs of most chelates were above 90% and close to 100% (**Figures 7**−**9**), with the possible exception of Natrel, which penetrated at significant rates even at 90% (**Figure 9**). Rates of all other chelates at 90% were only 1.6−4.6% of those observed at 100% (**Table 2**). FeIDHA, Sequestrene 138 Fe, and Natrel are technical products, and the contributions of impurities to their PODs are not known. Foliar penetration of chelates included in this study requires very high humidity and can be expected to be highest when dew occurs during the night. Stagnant air layers over stomatous leaf surfaces at low wind velocities will favor rates of penetration when humidity in bulk air is 100% or lower.

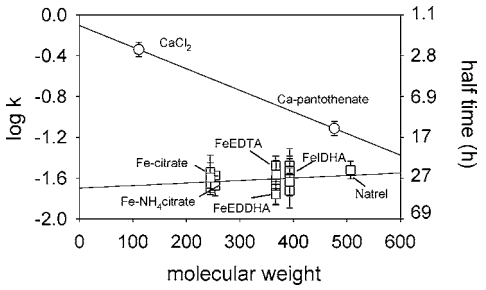

Figure 11. Rate constants of penetration of Ca salts and Fe^{III} chelates (20 mmol L^{-1}) as affected by anhydrous molecular weight of the test compounds. Geometric means of experiments with 40 CMs at 20 °C and 100% humidity are shown. Each symbol refers to one experiment.

Temperature Had No Effect on Rates of Penetration. In the range of 15−35 °C, rate constants of penetration did not depend on temperature (**Figure 4**). The same had been observed previously for calcium and potassium salts (*29*, *34*), and it has practical implications. During periods of nocturnal dew formation, temperatures are usually lower than during the day, but this will not adversely affect rates of penetration of Fe^{III} chelates. It can be concluded that foliar penetration of chelated Fe will preferentially happen during the night. Because Fe^{III} chelates are UV-labile (*52*, *53*), foliar applications during the late afternoon are to be recommended.

Molecular Weight Affected Rates of Penetration of Calcium Salts but Had No Effect on Penetration of Fe Chelates. Poplar CMs are size-selective (*33*). At 100% humidity, rate constants of penetration of calcium salts decreased by factors of 7−13 (depending on which year's sample of poplar CMs was used) when molecular weight increased from 100 to 500 g mol^{-1}. In the present study, rate constants for $CaCl_2$ were higher by a factor of 8.8 than those for Ca pantothenate (**Figure 1**), whose molecular weight is 4.3 times higher than that of $CaCl_2$ (**Table 1**). This agrees with the results of the previous study. Rate constants measured with Fe^{III} chelates were lower than those for Ca pantothenate (**Figure 1**), even though most of them had lower molecular weights (**Table 1**). In fact, there was no correlation between rate constants and molecular weights of Fe chelates (**Figure 11**), and all rate constants were below that of Ca pantothenate. This is an unexpected result that differs from the findings obtained with Ca and glyphosate salts (*33*, *51*).

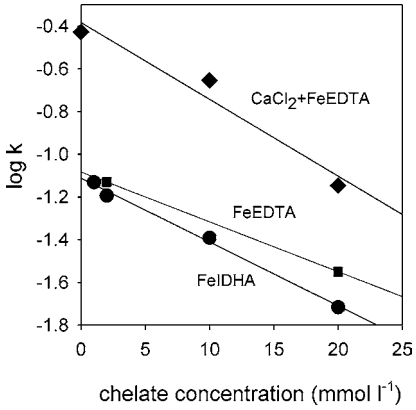

Figure 12. First-order plots of penetration of $CaCl_2$ in the presence of FeEDTA (◆), Fe^{III}IDHA (●), and FeEDTA (■) across poplar CMs at 20 °C and 100% humidity as affected by the chelate concentration.

Permeability of CMs Decreased with Increasing Concentration of Fe Chelate. Rate constants decreased with increasing concentrations of FeIDHA (**Figure 5**), and in the presence of FeEDTA, rate constants of penetration of $CaCl_2$ decreased with an increasing FeEDTA concentration (**Figure 6**). These data are summarized in **Figure 12**, which shows that log *k* of Fe chelates and $CaCl_2$ decreased with an increasing concentration of FeIDHA or FeEDTA. The slopes of the plots are similar. Rate constants of penetration of FeEDTA also decreased with its concentration (**Figure 12**). These results (**Figures 11** and **12**) are very surprising but appear to be related: (1) Rate constants of penetration of Fe chelates were much lower than would be predicted from their molecular weights. (2) Rate constants of penetration of Fe chelates were independent of molecular weight in the range of 254−507 g mol^{-1}. There is some uncertainty as to the molecular weight of Natrel, which was calculated from the atomic weight of Fe and its weight fraction (55.8 g mol^{-1}/0.11 = 507 g mol^{-1}). Presumably, ligninsulfonic acid molecules vary in molecular weight, and the rate constant is an average. (3) Rate constants for $CaCl_2$ (that is, permeability of CMs) were greatly reduced in the presence of FeEDTA (**Figure 12**). Permeability (*k*) of pear leaf CMs for CaEDTA was lower by a factor of 4 than that for $CaCl_2$ (*34*), and this was attributed to the higher molecular weight of the chelate. There is a chance that after mixing $CaCl_2$ with FeEDTA a fraction of the Ca ions might have been complexed by EDTA. However, at pH 5, stability constants for CaEDTA and Fe^{III}EDTA are 7.3 and 14.2, respectively (*54*), and this renders it unlikely that CaEDTA diffused in significant amounts when FeEDTA and $CaCl_2$ were mixed. Also, the formation of CaEDTA could not account for the fact that doubling the concentration of FeEDTA decreased $CaCl_2$ rate constants from 0.22 to 0.052 h^{-1} (**Figure 6**). This low rate constant of 0.052 h^{-1} for $CaCl_2$ in the presence of 20 mmol L^{-1} FeEDTA is only about twice as high as that measured for FeEDTA (**Figure 1**), and this suggests that FeEDTA and FeIDHA reduced the size of the aqueous pores in poplar cuticles. The data indicate deswelling of CMs in the presence of Fe chelates. CMs are weakly acidic ion-exchange membranes with a high selectivity for Ca^{2+} over monovalent ions (*55*). Swelling and water permeability of CMs in the Ca^{2+} form is much lower than in the Na^+ form (*56*). Water permeability of cherry fruit CM in the Fe^{3+} form was even lower than in the Ca^{2+} form. This might explain the reduction Ca permeability in the presence of FeEDTA (**Figure 12**), but it is difficult to see why it should reduce size selectivity of poplar CMs for Fe chelates (**Figure 11**).

Practical Implications. Nevertheless, our findings are of practical importance. Rapid foliar penetration of Fe chelates is desirable because they are subject to photodegradation. Rather than using very high concentrations that penetrate slowly and might cause phytotoxicity, low concentrations are to be preferred. This can be realized by combining high-volume sprays and low chelate concentrations. At a rate of 500 L ha^{-1}, a concentration of 2 mmol L^{-1} FeIDHA would amount to a dose of 620 g of technical FeIDAH, and because it contains 9% Fe, a dose of 55.8 g ha^{-1} Fe^{III} could be delivered with a single spray. For a micronutrient, this represents a significant amount.

The high PODs of the Fe chelates represent a severe limitation to foliar nutrition. Unfortunately, adding Tween 20 as a humectant did not increase rates of penetration at any level of humidity (**Figure 8**). Tween 20 is a nonionic surfactant with a molecular weight of 1260 g mol^{-1}. It has 20 ethyleneoxide units, which can sorb large amounts of water (*57*). The residues on the CMs appeared liquid, yet rate constants were not increased. This is another observation than cannot be explained at this time. On the other hand, glycine betaine, which has a POD of 50% (unpublished), increased rate constants with IDHA and Natrel. Glycine betaine is a small zwitterionic molecule (molecular weight 117 g mol^{-1}) that penetrates at rates comparable to $CaCl_2$ (unpublished results). This rapid penetration was responsible for the decrease in rate constants with time (closed symbols in **Figure 9**). Possibly, the presence of these charged molecules in the pore fluid increased permeability by increasing water content and pore size. This hypothesis needs testing. If it can be verified, looking for hygroscopic ionic humectants might produce candidates that increase rates of penetration of Fe chelates at humidity below 100%.

ABBREVIATIONS USED

CM, cuticular membrane; SOFU, simulation of foliar uptake.

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