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Studies on Water Transport through the Sweet Cherry Fruit Surface. 7. Fe^{3+} and Al^{3+} Reduce Conductance for Water Uptake

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The effects of the chloride salts LiCl, $CaCl_2$, $MgCl_2$, $AlCl_3$, $EuCl_3$, and $FeCl_3$ and the iron salts $FeCl_2$, $FeCl_3$, $Fe(NO_3)_3$, $FeSO_4$, and $Fe_2(SO_4)_3$ on water conductance of exocarp segments (ES) and rates of water uptake into detached sweet cherry fruit (*Prunus avium* L. cv. Adriana, Early Rivers, Namare, Namosa, and Sam) were studied. ES were excised from the cheek of mature fruit and mounted in stainless steel diffusion cell; water penetration was monitored gravimetrically from donor solutions containing the above mineral salts into a PEG 6000 (osmolality = 1.14 osM, pH 4.8, 25 °C) receiver solution. Conductance of ES was calculated from the amount of water taken up per unit of surface area and time by dividing by the gradient in water activity across ES. LiCl, $CaCl_2$, $MgCl_2$, $FeCl_2$, and $FeSO_4$ had no significant effect on conductance, but $AlCl_3$, $FeCl_3$, $Fe(NO_3)_3$, and $Fe_2(SO_4)_3$ significantly reduced conductance compared to water only as a donor. Also, $EuCl_3$ lowered conductance; however, this effect was not always significant. Effects of salts on water conductance of ES and rates of water uptake into detached fruit were closely related ($R^2 = 0.97$***). Upon application of an $FeCl_3$-containing donor conductance decreased instantaneously. $FeCl_3$ concentrations of $<6.6 \times 10^{-4}$ M had no effect on conductance, but concentrations at or above this threshold decreased conductance. $FeCl_3$ lowered water conductance at a receiver pH of 4.8, but not at pH $\leq$2.6. The effect of $FeCl_3$ on conductance was largest in cv. Namare and smallest in cv. Adriana. There was no significant effect of $FeCl_3$ on conductance for transpiration. Formation of aluminum and iron oxides and hydroxides in the exocarp as a result of a pH gradient between donor and receiver solution is discussed as the potential mechanism for Fe^{3+} and Al^{3+} reducing conductance for water uptake.

KEYWORDS: ***Prunus avium*; water penetration; water conductance; exocarp; cuticle; fruit cracking; cation**

INTRODUCTION

Rain-induced fruit cracking is a limitation in sweet cherry production worldwide (*1*, *2*). Earlier studies have indicated that fruit cracking may be lowered by foliar sprays of $CaCl_2$, $Ca(OH)_2$, or $Cu(OH)_2$ before or during rainfall (*3*−*5*) or by incubating fruit in solutions of mineral salts in standardized laboratory assays (*1*, *6*−*8*). Using the latter system, Bullock (*6*) observed decreased cracking of mature cv. Bing sweet cherry fruit when immersed in solutions containing Al^{3+}, Cu^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+}, Th^{4+}, or U^{2+} ions (concentration range = 0.001−0.01 M) as compared to deionized water. Anions had no or only small effects. Also, Bangerth (*7*) reported reduced cracking in the presence of Al^{3+} (−78%), Fe^{3+} (−69%), Ca^{2+} (−65%), Sr^{2+} (−62%), Ba^{2+} (−18%), and Mg^{2+} (−15%), but K^+ (−5%) had only little effect (all at concentrations of 0.1 M). The mechanistic basis of reduced cracking is not clear. Potential explanations include modified mechanical properties of the fruit and/or altered water uptake. For example, Ca^{2+} is generally believed to reduce fruit cracking by improving cross-linking of cell wall components (*7*, *9*, *10*), and Fe^{3+} and Al^{3+} may have similar effects (*7*). Indeed, postharvest applications of $CaCl_2$ increased fruit firmness of sweet cherry fruit (*11*). Alternatively, cations may directly affect water uptake, thereby reducing fruit cracking. To our knowledge, effects of cations on water uptake through the sweet cherry fruit surface have not been studied in detail.

The rate of water uptake (F in kg s^{-1}) across the fruit surface equals the product of the surface area (A in m^2) and the flux of water (J in kg m^{-2} s^{-1}). J, in turn, corresponds to the product of the conductance ($g_{tot.uptake}$ in m s^{-1}) of the cuticular membrane (CM) for water uptake, the density (ρ in kg m^{-3}) of water, and the gradient in water potential ($\Delta\Psi$ in MPa) across the fruit surface. Multiplying $\Delta\Psi$ by the partial molar volume of water ($\bar{V}_w$ in m^3 mol^{-1}) and dividing by the universal gas constant (R in m^3 MPa mol^{-1} K^{-1}) and the absolute temperature (T in K) converts the driving force from a gradient in water potential to a gradient in water activity (eq 1; *12*, *13*).

$$F = AJ = Ag_{tot.uptake}\rho(\bar{V}_w/RT)\Delta\Psi \quad (1)$$

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The F in eq 1 is a composite quantity that depends on $g_{tot.uptake}$, A, and $\Delta\Psi$. In contrast, $g_{tot.uptake}$ is characteristic for a particular surface, that is, the CM of the exocarp, because it is independent of A and $\Delta\Psi$. Thus, $g_{tot.uptake}$ is useful in characterizing and comparing transport across treatments or cultivars (*14*).

Water uptake into sweet cherry fruit may be low because A, $g_{tot.uptake}$, or $\Delta\Psi$ is low (eq 1). However, it is unlikely that A would be affected by treatment with mineral salts. Therefore, a reduction in water uptake may be related to effects on $g_{tot.uptake}$ and/or $\Delta\Psi$.

The objective of our study was to investigate the effects of selected mineral salts on water conductance of the sweet cherry fruit exocarp. We focused on cations because earlier studies found them to be more effective than anions in reducing fruit cracking (*6, 7*). Because the A and Ψ values of sweet cherry fruit are inconvenient to determine on a routine basis, a standardized laboratory-based water uptake assay was employed that allowed us to monitor water uptake through ES under defined conditions (*15*). In this system, A and $\Delta\Psi$ are known and $g_{tot.uptake}$ can be calculated.

MATERIALS AND METHODS

Plant Material. Mature sweet cherry fruit (*Prunus avium* L. cv. Sam and Early Rivers, both grafted on *P. avium* cv. Alkavo rootstocks) was obtained from commercial orchards near Eisleben, Germany (latitude 51° 31′ N and longitude 11° 44′ E). Cv. Adriana sweet cherry (grafted on cv. F12/1 rootstocks) was sampled from an experimental orchard of the Horticultural Research Center LVG Erfurt (latitude 50° 58′ N and longitude 11° 01′ E), and cv. Namare and Namosa (both grafted on cv. Alkavo rootstocks) were obtained from an orchard of the Bundessortenamt, Prüfstelle Marquardt, Marquardt (latitude 52° 31′ N and longitude 12° 51′ E). Fruit samples were selected for uniformity of development and freedom from defects by visual inspection, transferred to the laboratory, and stored at 1.4 ± 0.6 °C, 89.0 ± 9.0% relative humidity, and CO_2 and O_2 concentrations of 18.1 ± 0.3 and 17.1 ± 0.1%, respectively. Fruit samples were removed from storage at weekly intervals and held at 1 ± 1 °C and 90 ± 5% relative humidity until initiation of the experiment. Previous studies established that fruit can be stored for up to 71 days after harvest with no significant effect on conductance for water uptake (*15*).

Water Uptake: ES. Conductance of ES was determined gravimetrically as previously described (*15*). Briefly, ES were excised from the cheek of the fruit using a razor blade, adhering mesocarp tissue was carefully removed, and ES were mounted in stainless steel diffusion cells using a high-vacuum grease (Hochvakuumfett schwer; Wacker-Chemie, München, Germany). Diffusion cells were filled with a receiver solution containing polyethylene glycol (PEG 6000; Merck Eurolab GmbH, Darmstadt, Germany) via a sampling port in the bottom. Unless specified otherwise, nonbuffered PEG-receiver solution (lot 1, pH 4.8; lot 2, pH 5.0) was used at an osmolality of 1.14 osM (by vapor pressure osmometry; model 5520; Wescor Inc., Logan, UT). Diffusion cells were tape sealed [Tesa Film; tesa-Werke, Offenburg, Germany; see Figure 1A in Beyer and Knoche (*15*)], and experiments were initiated by weighing diffusion cells and subsequently applying ~0.15 mL of donor solution to the ES that completely covered the exposed surface area of the segment (A = 38.5 mm^2). Diffusion cells were incubated at 25 ± 1 °C. At predetermined time intervals donor solutions were removed completely by blotting with soft tissue paper, diffusion cells reweighed on a microbalance (BP 211 D, Sartorius AG, Göttingen, Germany) for determining water uptake, and, subsequently, fresh donor solutions reapplied. To prevent changes in osmolality due to evaporation of water from donor solutions that contained salts, initial experiments were carried out using tape-sealed donor solutions on diffusion cells. However, data from later experiments established that effects of $FeCl_3$ on water conductance were independent of $FeCl_3$ concentration above 6.6×10^{-4} M and, hence, would not be affected by the ~15% decrease in donor volume that would occur during a regular sampling interval at ambient temperature and humidity as a result of evaporation of the donor (and water uptake). Hence, subsequent experiments were carried out without the tape seal.

Conductance of ES for water uptake ($g_{tot.uptake}$ in m s^{-1}) was calculated from the flow rate (F in kg s^{-1}) across the exposed segment area (A in m^2) and the driving force for penetration according to eq 1. F was obtained from the slope of a linear regression line fitted through a plot of mass of diffusion cells versus time (only observations with $R^2 > 0.50$, grand mean $R^2 = 0.90$, $n = 312$). The driving force equaled the difference in water potential across the ES ($\Delta\Psi$ in MPa). Because the pressure exerted by the 0.15 mL of donor solution on the ES and the gravitational potential across the ES were negligible (*15*), $\Delta\Psi$ equaled the difference in osmotic pressure between receiver (Π_{rec}) and donor solutions (Π_{don}, eq 2):

$$\Delta\Psi = \Psi_{don} - \Psi_{rec} = \Pi_{rec} - \Pi_{don} = RT(\Phi_{rec}C_{rec} - \Phi_{don}C_{don}) \quad (2)$$

In eq 2, Φ_{rec} and Φ_{don} represent the osmotic coefficients that depend on the solute(s) in donor and receiver solution and their respective concentrations. Multiplying C_{rec} and C_{don} by the respective Φ corrects for nonideal behavior of solutes. For a nonbuffered receiver solution containing PEG 6000 at 522.7 g/kg of water, $\Phi_{rec}C_{rec}$ was 1.14 osM, which gives a Π_{rec} at 25 °C of 2.83 MPa (eq 2). For buffered PEG-receiver solutions and for all donor solutions containing solutes, osmolalities were determined directly by vapor pressure osmometry. Π_{rec}, Π_{don}, and $\Delta\Psi$ were calculated from eq 2. Inserting $\Delta\Psi$ in eq 1 and rearranging yielded $g_{tot.uptake}$.

Experiments. The effect of selected chloride salts (LiCl, $CaCl_2$, $MgCl_2$, $AlCl_3$, $EuCl_3$, and $FeCl_3$; all at 66 mM, $n \geq 17$ ES) and iron salts [$FeCl_2$, $FeCl_3$, $Fe(NO_3)_3$, $FeSO_4$, and $Fe_2(SO_4)_3$; all at 66 mM except for $Fe_2(SO_4)_3$ at 33 mM, $n \geq 15$ ES] on water conductance of ES (cv. Sam) was established by performing sequential treatments on an individual ES basis. During the initial period of the experiment (phase I from 0 to 4 h), water served as a donor. At 4 h, water was replaced by solutions containing salts, and water uptake was monitored up to 8 h (phase II). Osmolality values of donor solutions containing salts ($\Phi_{don}C_{don}$; as determined by vapor pressure osmometry) were as follows: LiCl, 0.12 osM; $CaCl_2$ and $MgCl_2$, 0.18 osM; $AlCl_3$, 0.17 osM; $EuCl_3$, 0.21 osM; and $FeCl_3$, 0.19 osM (in the comparison of selected chloride salts) and $FeCl_2$, 0.15 osM; $FeCl_3$, 0.19 osM; $FeSO_4$ and $Fe_2(SO_4)_3$, 0.08 osM; and $Fe(NO_3)_3$, 0.20 osM (in the comparison of selected iron salts). Deionized water served as control. Water conductance was calculated as described above. The effect of salts on water conductance was indexed by the ratio ($g^{II/I}_{tot.uptake}$) of conductance after replacing the water donor by a salt solution (phase II; $g^{II}_{tot.uptake}$) to the initial conductance established from water as a donor (phase I; $g^{I}_{tot.uptake}$).

The effect of $FeCl_3$ concentration on water conductance of ES (cv. Sam) was established in the range from 6.6×10^{-6} to 6.6×10^{-2} M. Donor solution containing only water served as control (n = 8 ES).

Because donor pH values differed among the different salts tested and among concentrations of $FeCl_3$, resulting in potential confounding between solute and donor pH, the effect of donor pH was investigated using citric acid/NaOH buffer only at pH 2.2, 3.5, 5.0, and 6.5 (100 mM buffer) and pH 8.0 and 9.5 (50 mM buffer; all n = 8 ES). In a further experiment, the effects of $CaCl_2$ and $EuCl_3$ (both at 25 mM) on water conductance were studied at a donor pH of 2.0 (pH adjusted using HCl; equivalent to the pH of 25 mM $FeCl_3$) and at a donor pH of 5.3 ($CaCl_2$) or 4.8 ($EuCl_3$). $FeCl_3$ (pH 2.0 at 25 mM) and deionized water served as controls (n >14 ES).

To establish the dependence of effects of $FeCl_3$ on receiver pH, two experiments were conducted. In cv. Early Rivers, receiver pH of the PEG solution was adjusted to pH 2.6 using citric acid (100 mM)/NaOH buffer, and the donor contained 31 mM $FeCl_3$ (osmolality = 0.1 osM). For control treatments without and with $FeCl_3$ in the donor, nonbuffered PEG-receiver solutions (pH 4.8, osmolality = 1.14 osM) were used. In cv. Sam, a complete two-factorial experiment was carried out. Here, the receiver pH of the PEG solution was adjusted to pH 2.0 using HCl. PEG solution without HCl served as control (pH 5.0). Donor solutions were as described for cv. Early Rivers.

Reversibility of the effect of $FeCl_3$ (31 mM, osmolality = 0.1 osM) on water conductance was investigated in cv. Early Rivers (n = 10

Table 1. Sequence of Treatments for Studying Reversibility of the Effect of $FeCl_3$ on Water Conductance of Exocarp Segments Excised from Cv. Early Rivers Sweet Cherry Fruit

		time interval		
treatment	compartment	phase I, 0–1.5 h	phase II, 1.5–3 h	phase III, 3–4.5 h
treatment 1	donor	H_2O	H_2O	buffer,[a] pH 2.6
	receiver	PEG, pH 4.8	PEG, pH 4.8	PEG, pH4.8
treatment 2	donor	H_2O	$FeCl_3$[b]	buffer,[a] pH 2.6
	receiver	PEG, pH 4.8	PEG, pH 4.8	PEG + buffer,[a] pH 2.6
treatment 3	donor	H_2O	$FeCl_3$[b]	H_2O
	receiver	PEG, pH 4.8	PEG, pH 4.8	PEG, pH 4.8

[a] 100 mM citric acid buffer at pH 2.6 adjusted using NaOH. [b] Osmolality of $FeCl_3$ = 0.10 osM.

ES). The treatments performed in sequence on an individual segment basis are summarized in **Table 1**.

The effect of sweet cherry cultivar on water conductance in the absence or presence of 31 mM $FeCl_3$ (osmolality = 0.1 osM) was studied using ES excised from cv. Adriana, Early Rivers, Namare, and Namosa fruit (n = 10−45 ES). Stomatal density was quantified by fluorescence microscopy on the same batch of fruit using the procedure by Knoche et al. (*16*).

Water Uptake: Detached Fruit. The effect of LiCl, $CaCl_2$, $MgCl_2$, $AlCl_3$, $EuCl_3$, and $FeCl_3$ (all at 10 mM, n = 10 fruit, osmolality ≤ 0.05 osM) and the iron salts $FeCl_2$, $FeCl_3$, $Fe(NO_3)_3$, $FeSO_4$, and $Fe_2(SO_4)_3$ (all at 10 mM, n = 10 fruit; osmolality ≤ 0.05 osM) on water uptake by whole fruit was quantified gravimetrically. Because pedicel/fruit juncture and pedicel end serve as sites of preferential water uptake into detached fruit (*17*), the pedicel was removed by careful pulling. The resulting hole above the stony endocarp was sealed using silicone (Dow Corning 3140 RTV coating; Dow Corning Corp., Midland, MI) such that the area covered by the silicone corresponded approximately to the initial area covered by the receptacle. This procedure ensured that water uptake into detached fruit was restricted to penetration through the exocarp (*17*). Following curing of the sealant overnight, fruit were weighed, incubated at 25 °C in deionized water for 0.75 h (phase I; one fruit per vessel), blotted dry, and weighed again to determine water uptake from water as a donor. Subsequently, fruit were transferred to one of the above salt solutions, incubated for another 0.75 h (phase II), and reweighed to quantify water uptake from the salt solution. Following the experiment, fruit samples were carefully inspected for visible cracks. Data analysis was restricted to fruit that remained without visible cracks. Rates of water uptake during phase I (F^{I} in kg s^{-1}) and phase II (F^{II} in kg s^{-1}) were calculated by dividing the increase in fruit mass by the duration of the incubation period. The effect of salts on water uptake was indexed by the ratio of the rate of uptake ($F^{II/I}$) determined from a salt solution (phase II; F^{II}) to the one established initially from water as a donor (phase I; F^{I}).

Transpiration: ES. Effects of $FeCl_3$ on conductance for transpiration were quantified following pretreatment of cv. Sam fruit by incubating whole fruit in 31 mM $FeCl_3$ (osmolality = 0.1 osM) for 1 h at 25 °C. Fruit incubated in water (1 h, 25 °C) served as control. ES were excised from pretreated fruit and mounted in diffusion cells. During the initial period of the experiment (0−3 h), conductance for transpiration was determined by monitoring mass loss from diffusion cells. Briefly, diffusion cells were incubated upside down above dry silica (at 25 °C) as described previously except that PEG solution (1.14 osM) was used as the donor [see Figure 1A in Knoche et al. (*14*)]. Subsequently (3−6 h), conductance for water uptake from water as a donor was established on the same ES using the procedure described above (n = 10 segments). Conductance for transpiration ($g_{tot.transp}$) was calculated according to eq 3 (*18*)

$$F = AJ = Ag_{tot.transp}\rho(a_w - a_{wv}) \quad (3)$$

where a_w and a_{wv} (both dimensionless) represented the activity of water in the donor and of water vapor in the receiver, respectively (*18*). The a_{wv} above dry silica is practically zero (*19*), and the a_w is related to the osmotic pressure of the PEG-donor solution according to eq 4 (*15*). For $1.0 > a_w > 0.95$, the term $\ln a_w$ approximately equals $a_w - 1$ (*20*):

$$\frac{RT}{-\bar{V}_w}\ln a_w \approx \frac{RT}{-\bar{V}_w}(a_w - 1) = \Pi_{don} \quad (4)$$

At Π_{don} = 2.83 MPa, a_w was calculated at 0.9797. Inserting a_w in eq 3 and rearranging yielded $g_{tot.transp}$ (in m s^{-1}), which may be compared to $g_{tot.uptake}$ calculated from eq 1 in the same units.

Hydrodynamic Radii of Cations. Radii of cations including their hydration shells (r in m; subsequently referred to as hydrodynamic radii) were calculated from their respective diffusion coefficients in water (D in m^2 s^{-1}; *21*) using the Stokes−Einstein equation (*22*):

$$D = kT/6\pi\eta r \quad (5)$$

In this equation k equals the Boltzmann constant ($k = 1.381 \times 10^{-23}$ J K^{-1}), T the absolute temperature (in K), and η the viscosity of water ($\eta = 0.8909 \times 10^{-3}$ kg s m^{-1} at 25 °C). Solving eq 5 for r yields the hydrodynamic radius of the respective cation in water.

Statistics and Data Presentation. Because conductance for water uptake and transpiration through ES had a log-normal distribution (*14*, *15*), conductance data were log-transformed prior to analysis of variance (ANOVA) and multiple comparisons of treatment means. Unless individual observations or log-transformed data are shown, conductance data in the tables and figures represent the back-transformed arithmetic mean of the log-transformed data. ANOVA, multiple comparisons of means, and regression analysis were carried out using the Statistical Analysis System software package (version 6.12; SAS Institute Inc., Cary, NC). Significance of coefficients of determination (R^2) at p = 0.05, 0.01, or 0.001 is indicated by *, **, or ***, respectively.

RESULTS

Conductance for water uptake through ES was not significantly affected by LiCl, $CaCl_2$, or $MgCl_2$, but $AlCl_3$, $EuCl_3$, and $FeCl_3$ significantly decreased conductance as compared to water only as a donor (**Table 2**). Qualitatively similar data were obtained in an earlier experiment except for $EuCl_3$, which had no significant effect on water conductance (M. Beyer, unpublished data). Among the iron salts tested, the largest decrease in water conductance was obtained with $FeCl_3$, followed by $Fe(NO_3)_3$ and $Fe_2(SO_4)_3$. $FeSO_4$ and $FeCl_2$ had only little effect. Effects of salts on rates of water uptake into detached sweet cherry fruit were closely related to their effects on water conductance of ES, suggesting that conductance must have decreased also on a whole fruit basis (**Tables 2** and **3**). The linear regression equation describing the relationship between salt effects on rates of water uptake ($F^{II/I}$) into detached fruit and on water conductance ($g^{II/I}_{tot.uptake}$) of ES was $F^{II/I}$ (ratio) = 0.14 (± 0.11) + 0.80 (± 0.15) * $g^{II/I}_{tot.uptake}$ (ratio), R^2 = 0.72***. The y-axis intercept of this regression line was not significantly different from zero (p = 0.21). When the regression line was forced through the origin, the slope was 1.00 (± 0.05) (R^2 = 0.97***).

Analysis of penetration time courses revealed that uptake from water as a donor increased linearly with time up to 8 h, and during this time period conductance remained essentially constant (**Figure 1**). When the water donor was replaced at 4 h by a donor solution containing $FeCl_3$, rates of water uptake decreased instantaneously (phase II; **Figure 1A**). Because the osmolality of the receiver solution remained essentially constant over the course of a typical experiment (M. Beyer and M. Hinz, unpublished data), the driving force for water uptake was constant and, hence, $FeCl_3$ must have lowered conductance (**Figure 1B**). In contrast, there was no significant effect of $CaCl_2$ on the amount of water taken up or on conductance (**Figure 1**).

Table 2. Effect of Selected Chloride Salts on Relative Conductance for Water Uptake ($g^{II/I}_{tot.uptake}$) through Excised Exocarp Segments (ES) and on Relative Rate of Water Uptake ($F^{II/I}$) through the Exocarp of Detached Cv. Sam Sweet Cherry Fruit[a]

donor phase II[b]	diffusion coefficient of cation in H_2O[c] ($\times 10^{-10}$ m^2 s^{-1})	hydrodynamic radius of cation (nm)	ES		detached fruit	
			donor pH	$g^{II/I}_{tot.uptake}$ (ratio)[d]	donor pH	$F^{II/I}$ (ratio)[d]
H_2O			5.4	0.97 a	5.3	0.86 b
LiCl	10.30	0.24	5.8	1.01 a	5.4	1.05 a
$CaCl_2$	7.92	0.31	5.5	0.76 ab	5.3	0.80 b
$MgCl_2$	7.06	0.35	5.6	0.84 ab	5.3	1.01 ab
$AlCl_3$	5.41	0.45	3.6	0.59 b	4.0	0.54 c
$EuCl_3$	6.02	0.41	4.8	0.58 bc	4.9	0.58 c
$FeCl_3$	6.04	0.41	1.7	0.27 c	2.3	0.49 c

[a] Effects of salts were determined by performing sequential treatments on an individual ES or fruit basis, respectively. $g^{II/I}_{tot.uptake}$ and $F^{II/I}$ were calculated by dividing the conductance or rate of water uptake established using a salt solution as a donor (phase II; $g^{II}_{tot.uptake}$ or F^{II}) by the initial conductance or rate of water uptake established from water as a donor (phase I; $g^{I}_{tot.uptake}$ or F^{I}). Hydrodynamic radii of cations were calculated from diffusion coefficients in water at 25 °C using the Stokes–Einstein equation (eq 5). [b] Salt concentrations were 66 and 10 mM for ES and whole fruit, respectively. [c] Data from Lide and Kehiaian (*21*) for 25 °C. For comparison, the diffusion coefficient of $H_2{}^{18}O$ in H_2O is 22.75×10^{-10} m^2 s^{-1} (*23*). [d] Means within columns followed by the same letter are not significantly different (Tukey's studentized range test, $p = 0.05$). Grand means for $g^{I}_{tot.uptake}$ and F^{I} were $0.44(\pm 0.04) \times 10^{-7}$ m s^{-1} ($n = 169$) and $2.33(\pm 0.09)$ mg·h^{-1} ($n = 82$), respectively.

Table 3. Effect of Selected Iron Salts on Relative Conductance for Water Uptake ($g^{II/I}_{tot.uptake}$) through Excised Exocarp Segments (ES) and on Relative Rate of Water Uptake ($F^{II/I}$) through the Exocarp of Detached Cv. Sam Sweet Cherry Fruit[a]

donor phase II[b]	ES		detached fruit	
	donor pH	$g^{II/I}_{tot.uptake}$ (ratio)[c]	donor pH	$F^{II/I}$ (ratio)[c]
H_2O	5.6	0.92 a	5.5	0.82 a
$FeCl_2$	3.9	0.66 ab	4.2	0.68 b
$FeCl_3$	2.0	0.32 b	2.6	0.47 c
$Fe(NO_3)_3$	1.7	0.54 b	2.6	0.42 c
$FeSO_4$	3.7	0.61 ab	4.0	0.69 ab
$Fe_2(SO_4)_3$	1.9	0.60 b	2.4	0.41 c

[a] Effects of salts were determined by performing sequential treatments on an individual ES or fruit basis, respectively. $g^{II/I}_{tot.uptake}$ and $F^{II/I}$ were calculated by dividing the conductance or rate of water uptake established using a salt solution as a donor (phase II; $g^{II}_{tot.uptake}$or F^{II}) by the initial conductance or rate of water uptake established from water as a donor (phase I; $g^{I}_{tot.uptake}$ or F^{I}). [b] Salt concentrations were 66 and 10 mM for ES and whole fruit, respectively, except for $Fe_2(SO_4)_3$ at 33 mM for ES. [c] Means within columns followed by the same letter not significantly different (Tukey's studentized range test, $p = 0.05$). Grand means for $g^{I}_{tot.uptake}$ and F^{I} were $0.45(\pm 0.05) \times 10^{-7}$ m s^{-1} ($n = 106$) and $2.76(\pm 0.12)$ mg·h^{-1} ($n = 60$), respectively

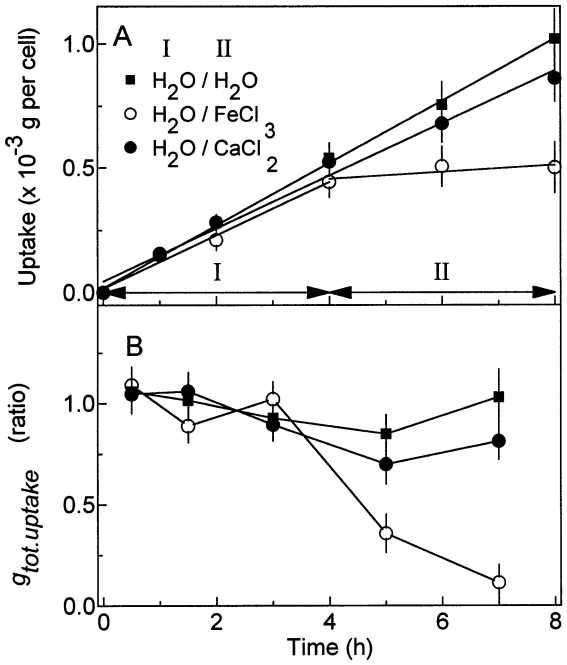

Figure 1. Effect of $CaCl_2$ or $FeCl_3$ on the time course of (A) water uptake and (B) relative conductance for water uptake ($g_{tot.uptake}$) through exocarp segments (ES) excised from cv. Sam sweet cherry fruit. Arrows indicate duration of phase I (0–4 h, water as a donor) and phase II of the experiment (4–8 h, water donor replaced by solutions of $CaCl_2$ or $FeCl_3$). ES having deionized water during phase I and II as a donor served as control. The relative $g_{tot.uptake}$ was calculated by dividing the $g_{tot.uptake}$ in a given time interval by the average $g_{tot.uptake}$ during phase I.

Water conductance in the presence of $FeCl_3$ as a donor was positively related to conductance of the same segments from water as a donor ($R^2 = 0.77$***; **Figure 2**). Also, calculating the decrease in conductance ($\Delta g_{tot.uptake}$) caused by $FeCl_3$ by subtracting the conductance from $FeCl_3$ as a donor (phase II; $g^{II}_{tot.uptake}$) from that established initially using water as a donor (phase I; $g^{I}_{tot.uptake}$) revealed a significant positive relation with $g^{I}_{tot.uptake}$ (**Figure 2**, inset; $R^2 = 0.93$***). In contrast, there was no significant relationship between the ratio of conductances in the presence and absence of $FeCl_3$ ($g^{II/I}_{tot.uptake}$) and $g^{I}_{tot.uptake}$ ($R^2 = 0.01$; data not shown). The effect of $FeCl_3$ on water conductance depended on the concentration of $FeCl_3$ applied (**Figure 3**). At or above 6.6×10^{-4} M $FeCl_3$, water conductance was significantly reduced with no difference among concentrations, but below this concentration, $FeCl_3$ was not effective (**Figure 3**).

It may be argued that the effect of salts on water conductance was confounded by a change in donor pH, because $g^{II/I}_{tot.uptake}$ or $g^{II}_{tot.uptake}$ and the pH of the donor solution were positively and significantly related (**Figure 4**). Linear regression equations for these relationships were $g^{II/I}_{tot.uptake}$ (ratio) $= 0.10$ ($\pm$ 0.02) $\times$ pH $+ 0.30$ ($\pm$ 0.07), $R^2 = 0.14$*** and log $g^{II}_{tot.uptake}$ (m s^{-1}) $= 0.06$ ($\pm$ 0.01) $\times$ pH $- 7.83$ ($\pm$ 0.06), $R^2 = 0.08$***, respectively. The pH of a citric acid buffer donor solution, however, had no significant effect on conductance for water uptake in the range from pH 2.2 to pH 9.5, suggesting that donor pH alone was not the critical factor [log $g_{tot.uptake}$ (m s^{-1}) $= -0.02$ ($\pm$ 0.01) $\times$ pH $- 6.98$ ($\pm$ 0.09); $R^2 = 0.03$]. Also, solutions of $CaCl_2$ or $EuCl_3$ at a pH equivalent to a solution of $FeCl_3$ (pH 2.0 at 25 mM) remained without significant effect on water conductance, but $FeCl_3$ decreased conductance (**Table 4**).

Water conductance in the presence of $FeCl_3$ was markedly affected by receiver pH (for cv. Early Rivers, see **Figure 5**). At a receiver pH of 4.8, $FeCl_3$ decreased water uptake as compared to the control, but $FeCl_3$ had no significant effect at the receiver pH of 2.6 (**Figure 5A**, data for cv. Early Rivers).

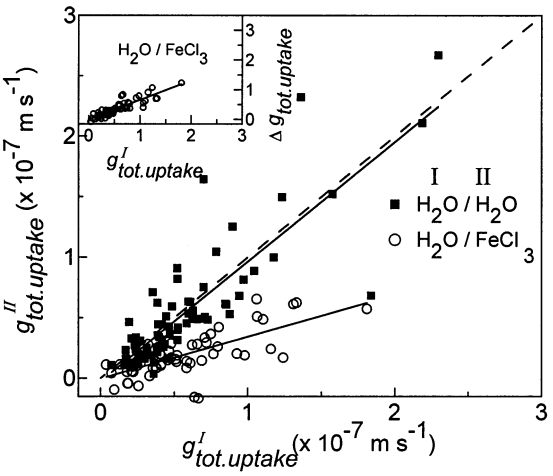

Figure 2. Relationship between conductance for water uptake through exocarp segments (ES) of cv. Sam sweet cherry fruit during phase I ($g^{I}_{tot.uptake}$) and phase II ($g^{II}_{tot.uptake}$) of an experiment: (- - -) 1:1-relationship; (—) regression lines. (Inset) Decrease in water conductance following replacement of water donor by $FeCl_3$ in phase II ($\Delta g_{tot.uptake}$) vs $g^{I}_{tot.uptake}$. $\Delta g_{tot.uptake}$ was calculated by subtracting the $g^{II}_{tot.uptake}$ established using an $FeCl_3$ solution as a donor from the $g^{I}_{tot.uptake}$determined using water was a donor. Data points represent individual ES.

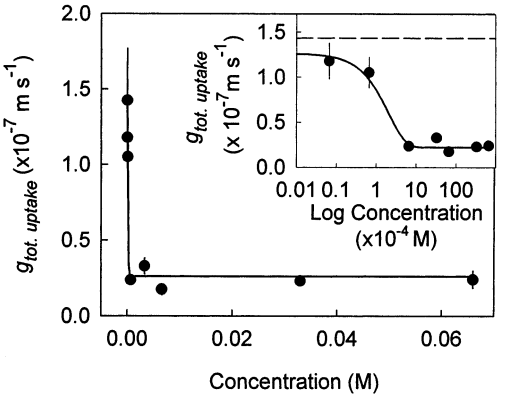

Figure 3. Effect of concentration of $FeCl_3$ in the donor on conductance for water uptake ($g_{tot.uptake}$) through exocarp segments of cv. Sam sweet cherry fruit. (Inset) $g_{tot.uptake}$ versus logarithm of the $FeCl_3$ concentration: (- - -) water conductance in the absence of $FeCl_3$.

Qualitatively similar data were obtained in the factorial experiment using cv. Sam fruit (**Table 5**), where $FeCl_3$ decreased conductance at pH 5.0 but not at pH 2.0. Conductance in the absence of $FeCl_3$ was higher at a receiver pH of 5.0 than at a pH of 2.0.

In cv. Early Rivers, the effect of $FeCl_3$ on the rate of water uptake and water conductance was partly reversed when the receiver pH was decreased from pH 4.8 to 2.6 and the donor was replaced by buffer at pH 2.6 (**Figure 5B**,**C**). However, replacing the $FeCl_3$-containing donor by deionized water and maintaining the receiver at pH 4.8 did not reverse the effect of $FeCl_3$ on the rate of water uptake and conductance (**Figure 5B**,**C**).

The decrease in water conductance in the presence of $FeCl_3$ was not limited to cv. Sam fruit but also occurred in other cultivars (**Table 6**). The largest reduction in water conductance ($\Delta g_{tot.uptake}$) was observed with cv. Namare, which also had the highest conductance in the absence of $FeCl_3$. The smallest effect of $FeCl_3$ was obtained in cv. Adriana, which had the lowest conductance when only water served as a donor. Across

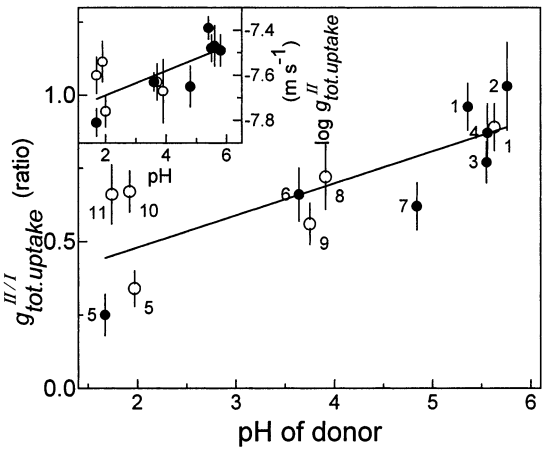

Figure 4. Relationship between relative conductance for water uptake ($g^{II/I}_{tot.uptake}$) through exocarp segments of cv. Sam sweet cherry fruit in the presence of selected mineral salts and pH of the respective salt solutions. $g^{II/I}_{tot.uptake}$ was calculated by dividing the conductance established using a salt solution as a donor (phase II; $g^{II}_{tot.uptake}$) by the initial conductance established using water as a donor (phase I; $g^{I}_{tot.uptake}$). Data taken from **Table 2** (solid symbols: 1, H_2O; 2, LiCl; 3, $CaCl_2$; 4, $MgCl_2$; 5, $FeCl_3$; 6, $AlCl_3$; 7, $EuCl_3$) and **Table 3** [open symbols: 1, H_2O; 5, $FeCl_3$; 8, $FeCl_2$; 9, $FeSO_4$; 10, $Fe_2(SO_4)_3$; 11, $Fe(NO_3)_3$]. (Inset) Log $g^{II}_{tot.uptake}$ vs pH of the respective salt solutions.

Table 4. Effect of $FeCl_3$, $CaCl_2$, $EuCl_3$, and pH on Relative Conductance for Water Uptake ($g^{II/I}_{tot.uptake}$) through Excised Exocarp Segments (ES) from Cv. Sam Sweet Cherry Fruit[a]

donor phase II[b]	donor pH	$g^{II/I}_{tot.uptake}$ (ratio)[c]
H_2O	5.6	1.00 a
$FeCl_3$	2.0	0.55 b
$CaCl_2$	5.3	1.04 a
$CaCl_2$	2.0[d]	0.90 ab
$EuCl_3$	4.8	0.84 ab
$EuCl_3$	2.0[d]	0.83 ab

[a] Effects of salts were determined by performing sequential treatments on an individual ES basis. $g^{II/I}_{tot.uptake}$ was calculated by dividing the conductance for water uptake established using a salt solution as a donor (phase II; $g^{II}_{tot.uptake}$) by the initial conductance established from water as a donor (phase I; $g^{I}_{tot.uptake}$). [b] Salt concentrations = 25 mM. [c] Means followed by the same letter not significantly different (Tukey's studentized range test, $p = 0.05$). Grand mean for $g^{I}_{tot.uptake} = 0.47(\pm 0.07) \times 10^{-7}$ m s^{-1} ($n = 106$). [d] pH of solutions adjusted using HCl.

Table 5. Effect of $FeCl_3$ and Receiver pH on Water Conductance ($g_{tot.uptake}$) of Exocarp Segments Excised from Cv. Sam Sweet Cherry Fruit[a]

	$g_{tot.uptake}$[b] ($\times 10^{-7}$ m s^{-1})		
receiver pH	H_2O	$FeCl_3$	mean$_{donor}$[c]
2.0	0.33 b	0.37 b	0.35
5.0	0.79 a	0.19 c	0.39
mean$_{pH}$[c]	0.51	0.26	

[a] Conductance data represent means of log-transformed data following back-transformation. [b] Data log-transformed prior to ANOVA. Mean separation by Tukey's studentized range test at $p = 0.05$. [c] Main effects donor (H_2O vs $FeCl_3$) and receiver pH (pH 2.0 vs 5.0) significant at $p = 0.0001$ and $p = 0.3788$, respectively, and the interaction donor × pH at $p = 0.0001$.

cultivars, water conductance in the absence and presence of $FeCl_3$ and the decrease in conductance caused by $FeCl_3$ were significantly and positively related to stomatal density (**Table 7**).

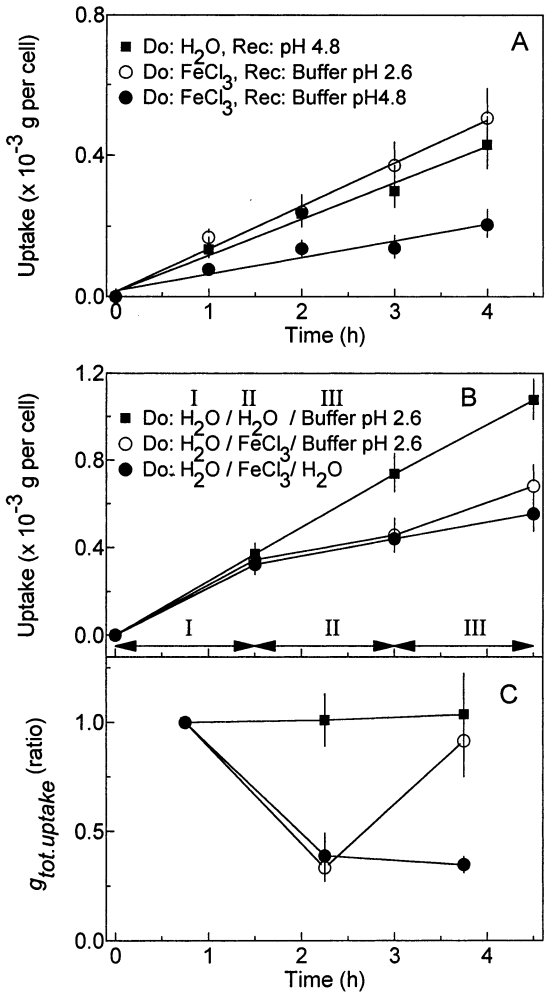

Figure 5. (A). Effect of the receiver pH on water uptake through exocarp segments (ES) of cv. Early Rivers sweet cherry fruit in the presence and absence of $FeCl_3$. The receiver pH was adjusted using citric acid/NaOH buffer. (B, C) Effect of donor and receiver pH in the absence and presence of $FeCl_3$ on water uptake (B) and on relative conductance for water uptake ($g_{tot.uptake}$; C) through ES of cv. Early Rivers sweet cherry fruit. Arrows indicate duration of phases I–III of the experiment. Relative $g_{tot.uptake}$ values were calculated by dividing the $g_{tot.uptake}$ during phases I–III by the average initial $g_{tot.uptake}$ during phase I. For further details see the text.

Table 6. Stomatal Density (d_{sto}) and Water Conductance of Exocarp Segments Excised from Fruit of Selected Sweet Cherry Cultivars before (Phase I; $g^{I}_{tot.uptake}$) and after the Water Donor Was Replaced by a Donor Containing $FeCl_3$ (Phase II; $g^{II}_{tot.uptake}$)[a]

cultivar	d_{sto} (mm^{-2})	$g^{I}_{tot.uptake}$ [b] ($\times 10^{-7}$ m s^{-1}), H_2O as donor	$g^{II}_{tot.uptake}$ [b] ($\times 10^{-7}$ m s^{-1}), $FeCl_3$ as donor	$\Delta g_{tot.uptake}$ [b] ($\times 10^{-7}$ m s^{-1})
Adriana	0.04 ± 0.01 a	0.29 a	0.08 a	0.21 a
Early Rivers	0.77 ± 0.06 b	0.73 b	0.27 b	0.61 b
Namosa	2.79 ± 0.16 c	2.08 c	0.47 bc	1.64 cd
Namare	1.47 ± 0.09 d	2.87 c	0.79 c	2.33 d
Sam	1.06 ± 0.10 e	0.95 b	0.22 b	0.84 bc

[a] Decrease in conductance caused by $FeCl_3$ ($^{\Delta}g_{tot.uptake}$) was calculated by subtracting $g^{II}_{tot.uptake}$ from $g^{I}_{tot.uptake}$. Data for d_{sto} represent means ± SE, those for $g_{tot.uptake}$ means of log-transformed data following back-transformation. [b] Data log-transformed prior to ANOVA. Mean separation within columns by Tukey's studentized range test ($p = 0.05$).

The effect of $FeCl_3$ on conductance was limited to water uptake; transpiration was not affected (**Table 8**). Interestingly, conductance for water uptake in the absence of $FeCl_3$ was ~40-fold larger than conductance for transpiration. This factor reduced to an 11-fold difference in the presence of $FeCl_3$.

Table 7. Regression Equations for the Relationship between Conductance for Water Uptake before (Phase I; $g^{I}_{tot.uptake}$) and after Application of Donor Solution Containing $FeCl_3$ (Phase II; $g^{II}_{tot.uptake}$), the Decrease in Water Conductance Due to $FeCl_3$ ($\Delta g_{tot.uptake}$), and Stomatal Density (d_{sto}) of Exocarp Segments (ES) of Cv. Adriana, Early Rivers, Namare, Namosa, and Sam Sweet Cherry Fruit[a]

dependent variable	donor	no. of observations (n)	regression parameters $a \pm$ SE	regression parameters $b \pm$ SE	coefficient of determination (R^2)[b]
$g^{I}_{tot.uptake}$	H_2O	120	0.80 ± 0.11	0.48 ± 0.14	0.30***
$g^{II}_{tot.uptake}$	$FeCl_3$	69	0.15 ± 0.04	0.21 ± 0.05	0.18***
$\Delta g_{tot.uptake}$		69	0.56 ± 0.13	0.45 ± 0.17	0.22***

[a] $\Delta g_{tot.uptake}$ was calculated by subtracting $g^{II}_{tot.uptake}$ from $g^{I}_{tot.uptake}$. Regression models were $Y = aX + b$, where Y and X represented the respective $g_{tot.uptake}$ ($\times 10^{-7}$ m s^{-1}) and d_{sto} (mm^{-2}). Regression analysis was carried out using conductance data on individual ES and an average d_{sto} determined on fruit from the same sample. [b] ***, significant at $p = 0.001$.

Table 8. Effect of Preincubating Sweet Cherry Fruit for 1 h in Water or in a Solution of $FeCl_3$ on Conductance of Exocarp Segments for Transpiration ($g_{tot.transp}$) and Water Uptake ($g_{tot.uptake}$)[a]

pretreatment	conductance[b] ($\times 10^{-7}$ m s^{-1}) $g_{tot.transp}$	conductance[b] ($\times 10^{-7}$ m s^{-1}) $g_{tot.uptake}$	$mean_{process}$[c]
H_2O	0.029 a	1.072 b	0.174
$FeCl_3$	0.030 a	0.324 c	0.098
$mean_{pretreatment}$[c]	0.030	0.589	

[a] Data represent means of log-transformed conductance data following back-transformation. [b] Data log-transformed prior to ANOVA. Mean separation by Tukey's studentized range test. [c] Main effects process (transpiration vs uptake) and pretreatment (H_2O vs $FeCl_3$) significant at $p < 0.0004$ and $p < 0.0001$, respectively, and the interaction process × pretreatment at $p < 0.0002$.

DISCUSSION

Conductance estimates for water uptake in our paper are apparent conductances calculated from the initial gradient in osmotic potential between donor and receiver, implying no penetration of solutes. Although this assumption is likely to hold for the large PEG 6000 molecule in the receiver (*24*), it may not apply to donor solutions containing mineral salts (*25*). To our knowledge, there are no published data for conductance of the sweet cherry fruit exocarp to these salts and, hence, driving forces and conductances for water uptake cannot be corrected. However, several arguments suggest that the potential error resulting from taking the initial gradient in osmotic potential as the driving force is small. First, penetration time courses in the absence and presence of salts in the donor were linear, indicating that the driving force must have remained constant. Second, we did not detect a significant change in osmolality of the receiver solution during a typical uptake experiment in the presence of a donor containing 66 mM $FeCl_3$ (M. Beyer, unpublished data). Third, sampling intervals were short and donor solutions were always replaced by fresh solutions. Fourth, even if salt penetration occurred, the resulting change in driving force and, hence, conductance would be small relative to the marked reduction of conductance observed in the presence of some salts. Last, effects of salts on rates of water uptake into detached fruit paralleled their effects on water conductance of ES. Because the osmotic pressure of salt solutions was low (Π_{don}

= 0.12 MPa) compared to the water potential of sweet cherry fruit [e.g., $\Psi = -1.42$ MPa (*26*)], any decrease in the rate of water uptake in excess of ~8.7% relative to the water control must be attributed to decreased water conductance of the exocarp.

The absence of a significant effect of $CaCl_2$ on water conductance of ES may be unexpected considering that $CaCl_2$ is often applied in sweet cherry orchards to reduce fruit cracking (e.g., see ref *27*). However, decreased fruit cracking by $CaCl_2$ may be accomplished by improved mechanical properties of the fruit and, particularly, the cell wall (*7*, *9*, *11*). Alternatively, $CaCl_2$ may decrease water uptake by reducing the driving force for transport. For example, Lang and Flore (*27*) used overhead sprinklers to apply up to 1% (w/w) $CaCl_2$ (anhydrous product) in sweet cherry orchards during rainfall. Assuming complete wetting of the fruit surface and no dilution of $CaCl_2$ by rain as first approximations, the driving force and, hence, the rate of water uptake into fruit would be reduced by ~39% at a fruit water potential of −1.42 MPa (*26*). This effect of $CaCl_2$ is nonspecific and would also be accomplished by application of any other osmoticum at equal osmolality. The salt concentrations used in our experiments, however, were too low to significantly reduce driving forces and, hence, water uptake by this mechanism. Thus, other processes must have been involved.

Effects of salts on water conductance were limited to $AlCl_3$, the ferric salts $FeCl_3$, $Fe(NO_3)_3$, and $Fe_2(SO_4)_3$, and, in some experiments, $EuCl_3$, whereas salts of other cations were not or, in the case of the ferrous salts, less effective. Because donor pH (**Table 4**), osmolality (relationship with $g^{II/I}_{tot.uptake}$ not significant, $R^2 = 0.17$), or concentration of the Cl^- ion (**Table 3**) in donor solutions may be excluded as factors, effects on conductance must have been specific for the Al^{3+}, Fe^{3+}, and, possibly, Eu^{3+} ions. Theoretically, two mechanisms may be involved.

Sorption of Cations to the CM. Cations sorb to negative charges in the CM, particularly at pH values above its isolectric point (IEP; *28*−*30*). These charges result from carboxylic groups of pectic materials, of proteins embedded in the cutin matrix, or of nonesterified fatty acids of the cutin polymer and from hydroxyl groups of phenols associated with the CM (*29*). The IEP of plant CM is around pH 3 (*29*, *31*). In our system, the pH of the nonbuffered PEG-receiver solution (pH 4.8 or 5.0 depending on lot) and the pH of donor solutions containing LiCl, $CaCl_2$, $MgCl_2$, $FeCl_2$, $FeSO_4$, and $EuCl_3$ were above the IEP (**Tables 2** and **3**). Only solutions of $FeCl_3$, $Fe(NO_3)_3$, $Fe_2(SO_4)_3$, or $AlCl_3$ had a donor pH lower than or close to the IEP (**Tables 2** and **3**). However, because the rate-limiting barrier is thought to be located toward the morphological outer side of the CM (*32*), a major portion of the CM would be above the IEP also in the presence of the latter salts. Therefore, all cations were likely to sorb to the CM in our system. Schönherr (*30*) reported increased CM permeability in the presence of monovalent alkali metal ions that may be related to swelling of the CM resulting from sorption. However, a swelling effect would not account for the decrease in conductance as observed in our study. A decreased conductance may be accomplished by sorption of divalent and trivalent cations that could improve cross-linking and reduce chain mobility in the CM polymer. For example, Schreiber and Schönherr (*33*) observed decreased thermal expansion of the CM in the presence of Ca^{2+}. This effect was attributed to a "physical form of cross-linking" resulting from binding of Ca^{2+} (*33*), and one would expect the trivalent ions Al^{3+}, Eu^{3+}, and Fe^{3+} to exert similar effects (*7*). However, if improved cross-linking was the mechanism in decreasing water conductance, the effects of these cations should increase in the same order as the pH of their solutions, and, hence, the number of charges in the CM and the amount of cation sorbed would increase. But, this was not the case (**Table 2**). Also, the divalent ions Ca^{2+} and Mg^{2+} should have some effects based on this hypothesis. An alternative potential explanation for sorption of trivalent cations decreasing the water conductance of the CM is based on the assumption of a "porous" CM having polar pathways with charged walls (*28*, *30*, *34*−*39*). It should be pointed out that these pathways do not necessarily represent holes in the CM but may arise from polar domains of orientated polar functional groups that under certain conditions hydrate and form an aqueous continuum, that is, a polar pathway (*37*), through the CM. Such domains would allow for localized binding of polar substances in a lipoidal CM (*28*, *40*). Provided that this model of the CM applies to sweet cherry fruit, a specific effect of the trivalent cations would be accounted for by their similar hydrodynamic radii (**Table 2**) that may be particularly effective in lowering the free path for water transport, thereby reducing conductance. Schönherr (*30*) estimated the equivalent diameter (d) of polar pores at $2r = d = 0.92$ nm (dewaxed *Citrus aurantium* L. CM), which is in close agreement with the estimate derived by Luque et al. (*41*). Al^{3+}, Eu^{3+}, and Fe^{3+} ions would "fit" these structures (**Table 2**) and, hence, sorption could indeed lower the free volume of these pathways. However, this hypothesis does not explain why $FeCl_3$, despite the low pH, was markedly more effective in reducing conductance than $AlCl_3$ or $EuCl_3$. Also, the Ca^{2+} or Mg^{2+} ions that are smaller than the Al^{3+}, Eu^{3+}, and Fe^{3+} ions should at least have some effect (**Table 2**). Furthermore, water permeability of track-etched synthetic membranes that contain negative charges (carboxylate) was only little affected (<30% decrease) by divalent cations (*42*). Thus, the sorption hypothesis is unlikely to account for the effects of Al^{3+} and, in particular, Fe^{3+} on water conductance.

Formation of Iron(III) and Aluminum(III) Oxides and Hydroxides. Aqueous solutions of ferric salts often are strongly acidic, colorless at a pH close to zero, but yellow in color at pH >0 (*43*), as was the case in our study. As the pH is increased to pH 2−3, condensation occurs, resulting in the formation of colloidal gels and, finally, red-brown precipitates that comprise complex hydrated, amorphous iron(III) oxides [most likely FeO(OH); *43*]. This reaction may be reversed when the pH is decreased. Similarly, $AlCl_3$ forms the hydrated cation $[Al(H_2O)_6]^{3+}$ in water that is hydrolyzed to $[Al(H_2O)_5(OH)]^{2+}$, $[Al(H_2O)_4(OH)_2]^{2+}$, etc., yielding a solution of low pH (*43*). As the pH is increased, aggregation occurs and hydrated oxides precipitate (*43*). On the basis of these arguments, it may be hypothesized that formation of insoluble precipitating oxides and hydroxides of Fe^{3+} and Al^{3+} as a result of the pH gradient across the ES decreased the free volume within the polymer available for water penetration, thereby reducing conductance. This hypothesis would account for (1) the effect of $AlCl_3$, $FeCl_3$, $Fe(NO_3)_3$, and $Fe_2(SO_4)_3$ on water conductance (**Tables 2** and **3**), (2) the pH dependence of the effect of $FeCl_3$ (**Figure 5A**; **Table 5**), and (3) the partial reversibility of the effect of $FeCl_3$ as the receiver pH is decreased (**Figure 5C**). Furthermore, the effects of $AlCl_3$, $FeCl_3$, $Fe(NO_3)_3$, and $Fe_2(SO_4)_3$ on water uptake by detached fruit (**Table 3**) are also explained by the above hypothesis. The pH of the plant's apoplast is ~5.5 (*44*) and averaged pH 4.3 for cell sap extracted from sweet cherry fruit mesocarp (M. Beyer, unpublished data). Thus, when fruit is incubated in donor solutions containing the above salts, a pH gradient from the acidic donor into the fruit's apoplast is established that would result in precipitation of oxides and

hydroxides of Fe^{3+}. Also, no precipitation occurred when solutions of the ferrous salts $FeCl_2$ and $FeSO_4$ were titrated to the pH of the PEG-receiver solution (M. Hinz, unpublished data), as would be expected on the basis of their markedly smaller effect on conductance and water uptake. A potential effect of $EuCl_3$, however, cannot be explained on these grounds, because solutions were stable over the relevant pH range.

It may be speculated that this mechanism for reducing CM conductance by Fe^{3+} and Al^{3+} is active in all penetration pathways including the hypothetical high-flux polar pathways discussed above that (1) are accessible to water and the Fe^{3+} or Al^{3+} ions and (2) maintain the pH gradient necessary for precipitation. This hypothesis is supported by the following arguments. First, conductance for water uptake in the absence of $FeCl_3$ and the decrease in water uptake following application of $FeCl_3$ were linearly and positively related (**Figure 2**). Second, water conductance and the effect of $FeCl_3$ thereon increased as stomatal density increased (**Table 7**), and guard cells were shown to have a greater frequency of polar pathways (*35*, *37*, *45*). Third, $FeCl_3$ affected only water uptake, but not transpiration (**Table 8**). This would be expected if polar pathways were involved only in water uptake (*15*) and the presence of these pathways was limited to fully hydrated CM (*46*). Clearly, these arguments are speculative and further studies are necessary to prove the existence of high flux pathways and potential effects of $FeCl_3$ thereon.

The data presented in this paper provide conclusive evidence for decreased conductance for water uptake through the sweet cherry fruit exocarp by Al^{3+} and Fe^{3+}. The most likely explanation for this effect is the formation of precipitates in the presence of a pH gradient across the CM. These precipitates may lower conductance for water uptake by decreasing the free volume of the polymer and possibly of any high-flux pathway for polar solutes associated with the CM of sweet cherry fruit. From a practical point of view, salts of Fe^{3+} would be more acceptable than those of Al^{3+}, because aluminum has been implicated as a neurotoxic agent (*47*). Provided that a salt can be identified that has no characteristics that limit use in spray application, for example, acidity and toxicity, the effect and mechanism described herein may form a basis for developing cultural treatments in sweet cherry orchards that reduce water uptake and, ultimately, fruit cracking.

ABBREVIATIONS USED

CM, cuticular membrane(s); d_{sto}, stomatal density; ES, exocarp segment(s); F, rate of water uptake into detached fruit; $g_{tot.transp}$, conductance for transpiration through ES; $g_{tot.uptake}$, conductance for water uptake through ES; IEP, isoelectric point of CM; PEG, polyethylene glycol.

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Registry No. (supplied by the author): $AlCl_3$, 7446-70-0; $CaCl_2$, 7774-34-7; $EuCl_3$, 13759-92-7; $FeCl_2$, 13478-10-9; $FeCl_3$, 7705-08-0; $Fe(NO_3)_3$, 7782-61-8; $FeSO_4$, 7782-63-0; $Fe_2(SO_4)_3$, 10028-22-5; LiCl, 7447-41-8; $MgCl_2$, 7791-18-6.

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