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Studies on Water Transport through the Sweet Cherry Fruit Surface. 11. $FeCl_3$ Decreases Water Permeability of Polar Pathways

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The effect of $FeCl_3$ (10 mM) on osmotic water uptake into detached sweet cherry fruit (*Prunus avium* L.) and on the ^3H_2O permeability (P_d) of excised exocarp segments (ES) or enzymatically isolated cuticular membranes (CM) was investigated. ES or CM were mounted in an infinite dose diffusion system, where diffusion is monitored from a dilute donor solution through an interfacing ES or CM into a receiver solution under quasi steady-state conditions. In the absence of $FeCl_3$, ^3H_2O diffusion through stomatous ES was linear over time, indicating that P_d was constant. Adding $FeCl_3$ to the donor decreased P_d by about 60%. P_d remained at a decreased level when replacing the $FeCl_3$ donor again by deionized water. The decrease in P_d was positively and linearly related to the stomatal density of the ES. There was no effect of $FeCl_3$ on the P_d of astomatous sweet cherry fruit ES or CM regardless of the presence of wax (epicuticular or cuticular). $FeCl_3$ decreased P_d when added to the donor (−63%) or receiver (−16%), but there was no effect when it was added to donor and receiver solutions simultaneously. The decrease in P_d depended on the pH of the receiver and the presence of citrate buffer. There was no effect of $FeCl_3$ with citrate buffer as a receiver regardless of pH (range 2.0−6.0). When using nonbuffered receiver solutions with pH adjusted to pH 2.0, 3.0, 4.5, or 6.0, $FeCl_3$ markedly decreased ^3H_2O diffusion at pH ≥ 3 but had no effect at pH 2.0. $FeCl_3$ increased the energy of activation (E_a) for ^3H_2O diffusion (range 15−45 °C) through stomatous ES but had no significant effect in astomatous CM. The increase in E_a by $FeCl_3$ was positively related to stomatal density. $FeCl_3$ decreased the P_d for 2-(1-naphthyl)[1-^{14}C]acetic acid (NAA) and 2,4-dichloro[U-^{14}C]-phenoxyacetic acid (2,4-D) in stomatous ES. The magnitude of the effect depended on the degree of dissociation and was larger for the dissociated acids (pH 6.2) than for the nondissociated acids (pH 2.2). Incubating whole fruit in isotonic solutions of selected osmotica resulted in significant water uptake that was inversely related to the molecular weight of the osmotica and was consistently lower for fruit treated with $FeCl_3$. The $FeCl_3$ induced decrease in water fluxes was larger for osmotica having a low molecular weight than for those with a higher molecular weight. Our data indicate that $FeCl_3$ decreased the permeability of the stomatous sweet cherry exocarp to water and other polar substances by pH-dependent formation of precipitates that decrease transport along polar pathways. Decreasing the permeability of polar pathways by a precipitation reaction is a useful target in developing strategies against rain-induced fruit cracking.

KEYWORDS: Fruit cracking; cuticle; ferric salts; *Prunus avium* L.; stomata

INTRODUCTION

Sweet cherry fruit cracking is a severe limitation in crop production worldwide. Water uptake through the fruit surface is considered to be an important factor in cracking, and various strategies to reduce fruit cracking have been developed including use of rain shelters, antitranspirants, or plant growth regulators (for recent reviews see refs *1* and *2*). Earlier studies from our laboratory established that water uptake and fruit cracking were markedly reduced by several cations of mineral salts including Fe^{3+} (*3*, *4*). Interestingly, the decrease in water uptake depended on the oxidation state and the anion. For Fe-salts, effects on water uptake were limited to the Fe^{3+}-salts $FeCl_3$, $Fe_2(SO_4)_3$, $Fe(NO_3)_3$, and the organic Fe^{3+}-glucoheptonate, but Fe^{3+}-citrate and the Fe^{2+}-salts $FeCl_2$ and $FeSO_4$ had little or no effect (*3*, Weichert, unpublished data). The mechanism of the Fe^{3+}-reduction in water uptake through the sweet cherry exocarp is not entirely clear. Osmotic effects that decrease the driving force for water uptake can be excluded as a factor. Such effects would

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be nonspecific and accomplished by essentially all substances in solution provided that their concentration was sufficiently high. This, however, was not the case, and therefore, decreased permeability must have accounted for the decreased uptake and cracking (*3*, *4*). The use of $FeCl_3$ under orchard conditions is prohibitive for several reasons, including the corrosive nature of the strongly acidic spray solution and the discoloration of fruit (*4*). Nevertheless, a better understanding of the mechanism of the Fe-dependent decrease in water permeability may be helpful in identifying alternative compounds having the same mode of action but avoiding the above shortcomings of $FeCl_3$.

Water transport through the sweet cherry surface occurs by diffusion and by viscous flow along polar pathways across the exocarp (*5*, *6*). Polar pathways represent polar domains in the lipohilic cuticular membrane (CM) that result from orientation of polar functional groups of CM constituents. Upon hydration, they form an aqueous continuum across the CM. This continuum is porous in nature and allows for rapid transport of polar substances including water. It therefore may be hypothesized that the Fe^{3+}-salts cited above decreased water uptake and fruit cracking by decreasing the permeability of the polar pathways in the sweet cherry fruit exocarp.

The purpose of our study was to test this hypothesis and establish the mechanistic basis of the effect of $FeCl_3$ on water transport. Where possible, we employed an infinite dose diffusion system for studying water transport through excised exocarp segments. This technique offers a high degree of control and unique opportunities for manipulating experimental conditions beyond those possible using whole fruit.

MATERIALS AND METHODS

Plant Material. Sweet cherry fruit [*Prunus avium* L., Adriana, Hedelfinger, Sam, all grafted on Alkavo (*Prunus avium*) rootstocks] were collected at commercial harvest time. Tomato (*Lycopersicon esculentum* Mill.) and bell pepper fruit (*Capsicum annuum* L. *var. annuum Grossum Group*) were purchased locally, and names of cultivars were unknown. All fruit were selected for uniformity of maturity and size and visually inspected for freedom from defects. Exocarp segments (ES) consisting of cuticular membrane (CM), epidermis, and some layers of mesocarp tissue were excised from the cheek of sweet cherry fruit unless otherwise specified or from the equatorial region of tomato and pepper fruit using a corc borer (15.3 or 17.4 mm diameter). The sweet cherry ES used in diffusion experiments were blotted with soft tissue paper, mounted in diffusion cells, or stored in deionized water containing 15 mM NaN_3 at 5 °C until use. The study of the effect of selected osmotica on water uptake was conducted using detached fruit of Sam sweet cherries held in storage for up to 34 days. Using stored fruit reduced day-to-day variability in fruit water potential caused by growing conditions in the orchard. Storing conditions were 0.4 ± 0.1 °C, 72.2 ± 0.4% relative humidity, 17.5 ± 0.1% O_2, and 18.0 ± 0.4% CO_2.

Isolation of CM. CM were isolated enzymatically from ES using pectinase (90 mL L^{-1} Panzym Extra, Novo Nordisk Ferment Ltd., Dittingen, Switzerland) and cellulase (5 g L^{-1}; Sigma Chemical Co., St. Louis, MO) prepared in 15 mM NaN_3 and 50 mM sodium citrate buffer at pH 4.0 (*7*, *8*). Solutions were replaced repeatedly until CM separated from the tissue. Subsequently, CM were rinsed in deionized water to remove adhering cellular debris. Sweet cherry fruit CM (Adriana) were stored in deionized water at 5 °C; tomato and pepper CM were air-dried on Teflon sheets and stored at ambient temperature until use. Dewaxed CM (DCM) were prepared by batch extracting CM with 10 consecutive changes of CH_3Cl_3/MeOH (1:1 by volume) at 35 °C. ES excised from sweet cherry fruit subjected to cellulose acetate stripping (*9*) served as a source of CM without epicuticular wax (ECW; CM-ECW).

Diffusion Experiments. *General Procedure for Diffusion Studies Using ^3H_2O.* Diffusion of ^3H_2O (specific activity 0.2 GBq mL^{-1}; Amersham Corp., Arlington Heights, IL) was studied using the infinite dose system (*10*). In this system, diffusion is monitored under quasi steady-state conditions from a donor through an interfacing ES or CM into a receiver. CM or ES were mounted in plexiglass holders using silicone rubber (Dow Corning 3140 RTV Coating; Dow Corning Corp., Midland, MI). The cross-sectional area of CM or ES exposed in the holder ranged from 3.1 to 78.5 mm^2 (equivalent to a 2−10 mm diameter of the orifice). When using the small orifice size and CM or ES isolated from Adriana sweet cherries, astomatous membranes could be selected by light microscopy at 100× magnification (model BX-60; Olympus Optical Co. Europa GmbH, Hamburg, Germany), since this cultivar has a low number of stomata (*11*). ES from sweet cherries and CM from tomato and pepper fruits were mounted in normal orientation such that the morphological outer side faced the donor. For sweet cherry CM, CM-ECW, and DCM, orientation was random, since it was difficult to distinguish outer and inner sides. However, preliminary experiments established that there was no significant difference in ^3H_2O permeability (P_d) between sweet cherry fruit CM mounted in normal vs reversed orientation (P_d: 0.70 ± 0.09 × 10^{-8} m s^{-1} vs 0.82 ± 0.08 × 10^{-8} m s^{-1} for normal vs reversed orientation, respectively). In these experiments, CM orientation was identified by exposing one side of the CM for 2 min to a 0.1% methylene blue solution (Peter Baur, personal communication). Methylene blue only stains the cell wall side of the CM (*12*). All CM and ES were inspected for microscopic cracks at 100×, and those with cracks were discarded. Holders with crack-free ES or CM were mounted between two glass half-cells of diffusion units using silicone grease (Baysilone-Paste hochviskos; GE Bayer Silicones, Leverkusen, Germany). Diffusion units were placed in a thermostated water bath (usually at 25 °C) positioned on a multistirring unit. Donor solutions were prepared at specific activities ranging from 2.6 × 10^4 to 2.3 × 10^6 dpm mL^{-1}. Deionized water was used as the receiver unless specified otherwise. Diffusion experiments were initiated by adding 5 mL of donor and receiver solution to the donor and receiver cells of the diffusion apparatus, respectively. The time course of diffusion was followed by repeated sampling of the receiver. Aliquots (1 mL) were removed from the receiver, radioassayed by liquid scintillation spectrometry (scintillation cocktail Ultima Gold XR; Perkin-Elmer Life and Analytical Sciences, Boston, MA; counter: LS 6500; Beckman Instruments Inc., Fullerton, CA), and replaced by fresh receiver solution. Flow rates were calculated by fitting a linear regression line through a plot of cumulative ^3H_2O penetration vs time. Coefficients of determination were better than 0.98. The slope of this regression line equaled the flow rate (F, dpm h^{-1}). F is related to the permeability in self-diffusion (P_d; m s^{-1}) by eq 1, where A (m^2)

$$F = AJ = AP_d\Delta C \quad (1)$$

represents the cross-sectional area of the ES or CM exposed in the holder and ΔC (dpm m^{-3}) is the driving force for transport, i.e., the gradient in radioactivity between donor and receiver. Since the concentration of radioactivity in the receiver remains negligibly low, ΔC corresponds to the concentration of radioactivity in the donor.

The effect of $FeCl_3$ on P_d was established using sequential treatments performed on an individual CM or ES basis. Briefly, during the first phase of the experiment, ^3H_2O permeability was established in the absence of $FeCl_3$ (P_d^I). In phase II, the ^3H_2O donor solution was replaced by 10 mM $FeCl_3$ containing ^3H_2O and the P_d in the presence of $FeCl_3$ was established (P_d^{II}). In some experiments, pretreatments of ES were performed. Here, phase I (in the absence of $FeCl_3$) was followed by phase II, where CM or ES were pretreated by exposing the morphological outer side to 10 mM $FeCl_3$ that did not contain ^3H_2O. Unless specified otherwise, the Fe-effect was induced against deionized water as a receiver. Phase II was terminated after a minimum period of 26 h, which was sufficient to reach steady-state penetration (for example, see **Figure 1**). Holders with CM or ES were removed from diffusion units, carefully rinsed with deionized water to remove adhering $FeCl_3$, and subsequently remounted in a clean set of diffusion units. This procedure was followed to prevent carry over of $FeCl_3$ into the subsequent phase III. The diffusion experiment was continued by refilling donor cells with 5 mL of ^3H_2O donor solution (no $FeCl_3$) and

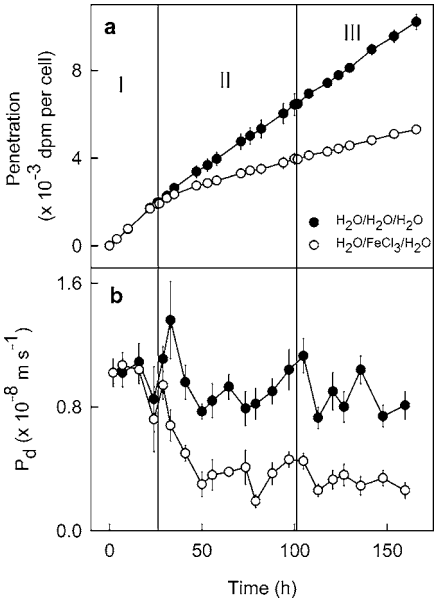

Figure 1. Time course of ^3H_2O penetration through excised exocarp segments (ES) of Sam sweet cherry fruit in the absence and presence of 10 mM $FeCl_3$: (**a**) cumulative penetration; (**b**) permeability (P_d) as a function of time. During phase I of the experiment, deionized water served as a donor. For phase II, the water donor was replaced by 10 mM $FeCl_3$. In phase III, the $FeCl_3$ donor was replaced again by a water donor. For control, water only was used throughout the experiment. Data represent means ± standard errors of means.

receiver cells with deionized water only. The P_d after pretreatment (P_d^{III}) was determined as described above. The effect of $FeCl_3$ on ^3H_2O permeability was indexed by the ratio of permeability in the presence and absence of $FeCl_3$ (P_d^{II}/P_d^{I}) or the ratio of permeability after and before pretreatment with $FeCl_3$ (P_d^{III}/P_d^{I}).

Time Course. The effect of 10 mM $FeCl_3$ on the time course of ^3H_2O diffusion was studied in a three phase experiment. During the first phase, the permeability of Sam sweet cherry fruit ES to ^3H_2O (P_d^{I}) was determined in the absence of $FeCl_3$. In phase II the ^3H_2O donor was replaced by a donor that contained ^3H_2O and 10 mM $FeCl_3$ and the P_d^{II} was determined. For the subsequent phase III, the donor solution was replaced again by a ^3H_2O donor (no $FeCl_3$) to establish P_d^{III} and any reversibility of the $FeCl_3$ effect on ^3H_2O permeability. Diffusion units with a ^3H_2O donor solution throughout the experiment served as control. The experiment was carried out using eight single ES replicates.

Stomatal Density. To establish a relationship between stomatal density (d_{sto}) and ^3H_2O permeability in the absence and presence of 10 mM $FeCl_3$, ES excised from the stem cavity, cheek, and stylar scar regions of Sam sweet cherries were used. These regions were selected to maximize the range in stomatal density (range 0−3.5 mm^{-2}; *11*). Stomatal density was determined at 100× magnification for every ES. The permeability of ES for ^3H_2O was established in the absence (phase I; P_d^{I}) and presence of 10 mM $FeCl_3$ (phase II; P_d^{II}). The reduction in P_d caused by $FeCl_3$ (ΔP_d) was calculated by difference ($\Delta P_d = P_d^{I} - P_d^{II}$) on an individual ES basis. The experiment was carried out using 16 ES.

Effect of Wax. Potential interactions between the effect of $FeCl_3$ on ^3H_2O permeability and epicuticular (ECW) or cuticular wax were studied using astomatous CM, CM-ECW, and DCM of Adriana sweet cherries. To broaden the data base, CM and DCM obtained from tomato and pepper fruit were also included in the comparison. ^3H_2O permeability was determined in the absence (phase I, P_d^{I}) and presence of 10 mM $FeCl_3$ (phase II, P_d^{II}). The experiment was carried out with a minimum of six replications.

Position of $FeCl_3$ and Direction of ^3H_2O Diffusion. To establish whether the Fe-effect on water transport depended on the position of $FeCl_3$ (donor vs receiver) and the direction of ^3H_2O transport, a two factorial experiment was conducted. ES excised from Sam sweet cherry fruit were mounted in diffusion units such that the outer surface faced the donor. In phase I, ^3H_2O permeability (P_d^{I}) in the absence of $FeCl_3$ was determined as described above. At the end of phase I, donor and receiver solutions were removed. In phase II, ES were pretreated with 10 mM $FeCl_3$ by filling the donor cell, the receiver cell, or the donor and receiver cell with 5 mL of $FeCl_3$ (phase II). Except for the latter treatment, the other cell contained deionized water. For phase III, holders were remounted in a clean set of diffusion cells such that the morphological outer side of the ES faced the donor ("normal" orientation) or the receiver compartment ("reversed" orientation). ^3H_2O diffusion from the donor into the receiver compartment was monitored, and the P_d^{III} was calculated. The effect of $FeCl_3$ was indexed by the ratio P_d^{III}/P_d^{I}. The minimum number of single ES observations was eight.

Effect of pH and Citrate Buffer. The pH dependence of ^3H_2O diffusion and the effect of $FeCl_3$ thereon were studied in two sets of experiments. First, the effect of pH on ^3H_2O diffusion in the absence of $FeCl_3$ was investigated using astomatous Adriana CM. Donor and receiver solutions were buffered using 10 mM citrate at pH values ranging from 2.0 to 6.0. Sequential treatments were performed by stepwise increasing pH. The P_d for ^3H_2O was determined from equilibrium flow rates at any one pH as described above. Second, the effect of receiver pH on the $FeCl_3$ induced decrease in water transport was investigated using stomatous Sam ES. The experiment comprised four phases of differing combinations of donor and/or receiver solutions. During phase I, the permeability of ^3H_2O diffusion (P_d^{I}) from an aqueous donor into an aqueous receiver solution was established in the absence of $FeCl_3$. In phase II, the Fe-effect was induced against buffered or nonbuffered receiver solutions of different pH values. During the initial 8 h of this phase II, the receiver side of the ES was equilibrated at pH 2.0, 3.0, 4.5, or 6.0 using either 10 mM citrate buffer or a nonbuffered aqueous solution with pH adjusted (deionized water plus HCl or NaOH). The donor was deionized water only (no pH adjustment). After 8 h, the water donor was replaced by 10 mM $FeCl_3$ (pH 2.4); the receiver remained citrate buffer or nonbuffered water at the respective pH. After 33 h, holders were remounted in a clean set of diffusion units. For the subsequent phase III, a ^3H_2O containing donor (no $FeCl_3$) was added to donor cells, deionized water was added to receiver cells, and the P_d^{III} for ^3H_2O was quantified as described above. Those diffusion units, where the Fe-effect was induced against water at pH ≥ 3 were subjected to a fourth phase (IV), where the water donor and water receiver were replaced by 10 mM citrate buffer at pH 2.4. The change in P_d with time was followed. The minimum number of replications was eight.

Effect of Temperature. The purpose of these experiments was to determine whether the temperature dependence of P_d for ^3H_2O was affected by $FeCl_3$. Astomatous CM from Adriana and stomatous ES from Hedelfinger and Sam sweet cherries were equilibrated at 25 °C with 10 mM $FeCl_3$ in the donor against deionized water in the receiver. Thereafter, temperature was decreased to 15 °C and then increased in 10 °C intervals up to 45 °C. The P_d was determined at any one temperature from steady-state flow rates. The temperature dependence of ^3H_2O diffusion was analyzed by calculating the energy of activation (E_a) from the slope of an Arrhenius plot of the natural logarithm of P_d (P_d in m s^{-1}) vs the inverse of temperature (T in K) on an individual ES basis. The number of replications ranged from 6 to 26.

Effect of $FeCl_3$ on Diffusion of NAA and 2,4-D. To establish whether the $FeCl_3$ reduction of diffusion was limited to ^3H_2O, we studied diffusion of selected plant growth regulators through ES excised from Sam sweet cherry fruit. The molecular probes used were 2-(1-naphthyl)-[1-^{14}C]acetic acid (NAA; specific activity 2.3 Gbq mmol^{-1}, 98.7% radiochemical purity by TLC; Amersham Corp., Arlington Heights, IL) and 2,4-dichloro[U-^{14}C]phenoxyacetic acid (2,4-D; specific activity 0.5 GBq mmol^{-1}, 95.8% radiochemical purity by HPLC; Sigma-Aldrich, Saint Louis, MO). These compounds represent weak organic acids with a pK_a of 4.2 (NAA) and 2.6 (2,4-D). The pH values of donor solutions were adjusted to pH 2.2 and pH 6.2, which is two pH units below and above the pK_a of NAA. Thus, NAA and 2,4-D were predominately in the nondissociated, more lipophilic form at pH 2.2 and in the dissociated, more polar form at pH 6.2. Preliminary experiments were conducted to identify a buffer that did not interfere

with the effect of $FeCl_3$. Glycine (10 mM) and piperazine-dihydrochloride hydrate (10 mM) proved suitable for buffering donor and receiver solutions at pH 2.2 and pH 6.2, respectively, since ratios of ^3H_2O permeabilities (P_d^{III}/P_d^{I}) after (P_d^{III}) and before pretreatment (P_d^{I}) of ES with $FeCl_3$ were similar [P_d^{III}/P_d^{I}: 0.48 ± 0.04 and 0.32 ± 0.04 in glycine buffer (pH 2.2) and in piperazine-dihydrochloride hydrate buffer (pH 6.2), respectively]. Donor solutions were prepared at total NAA and 2,4-D concentrations of 10 and 15 μM, respectively. For NAA, the concentration of radioactivity in the donor was 0.7×10^5 and 7.4×10^5 dpm mL^{-1} at pH 2.2 and 6.2, respectively. For 2,4-D, the corresponding data were 0.5×10^5 and 4.7×10^5 dpm mL^{-1} at pH 2.2 and 6.2, respectively. Buffer solutions containing no NAA or no 2,4-D served as receiver. To prevent microbial growth, NaN_3 was added to donor and receiver solutions at a final concentration of 1 mM. Diffusion experiments were conducted in three phases. During phase I, P_d^{I} was established for NAA and 2,4-D at pH 2.2 and at pH 6.2. Thereafter, phase II was initiated where ES were pretreated with 10 mM $FeCl_3$ as the donor in the absence of radiolabel against glycine or piperazine-dihydrochloride hydrate buffer in the receiver. To standardize the pH-gradient between the 10 mM $FeCl_3$ donor (pH 2.4) and the receiver solutions, the pH values of the glycine and piperazine-dihydrochloride hydrate buffers were adjusted to pH 4.2. In phase III, the $FeCl_3$ containing donor was replaced again by donor solutions containing NAA and 2,4-D in glycine and piperazine buffer at pH 2.2 and 6.2, respectively, and the receiver solutions were replaced by the respective buffer solutions at pH 2.2 and 6.2. Steady-state flow was re-established, and the P_d^{III} was calculated as described above. The effect of $FeCl_3$ on diffusion of NAA and 2,4-D was indexed by the ratio P_d^{III}/P_d^{I}. The number of replications was eight.

Effect of Osmotica on Whole Fruit Water Uptake. The effect of the molecular weight (MW) of selected osmotica on water uptake into detached Sam sweet cherry fruit was determined gravimetrically by incubating fruit in isotonic solutions of osmotica of differing molecular weight as described previously (*6*). Water transport was restricted to the exocarp by removing pedicels and sealing the resulting hole above the stony endocarp using silicone rubber (Dow Corning 3140 RTV coating; Dow Corning Corp.; *13*). Following curing under ambient conditions overnight, fruit was preincubated in 10 mM $FeCl_3$ (45 min) to induce the Fe-effect on water uptake. Fruit preincubated in deionized water (22 min) served as control. The durations of the preincubation periods were selected such that water uptake during preincubation and, hence, the fruit's water potential were identical for control and $FeCl_3$ treated fruit. The fruit water potential (Ψ_{fruit}) was determined by incubating a subsample of fruit in a series of polyethylene glycol 6000 solutions (PEG 6000, mean MW 6000; Merck Eurolab GmbH, Darmstadt, Germany) of differing osmotic potential as determined by water vapor pressure osmometry (model 5520; Wescor Inc., Logan, UT). The highest PEG 6000 concentration had an osmotic potential (Ψ_Π) lower than the Ψ_{fruit}, and hence, incubation of fruit in this solution resulted in negative rates of water uptake (F). The Ψ_{fruit}, determined by fitting a linear regression line through a plot of F vs Ψ_Π of the PEG solutions, was −2.7 MPa. Subsequently, isotonic solutions of selected osmotica of differing molecular weights were prepared at $\Psi_\Pi = -2.7$ MPa. Hydrodynamic radii (r in m) of the osmotica were taken from Weichert and Knoche (*6*). The osmotica, their respective molecular weights, and their hydrodynamic radii (r) were as follows: NaCl, MW 58, r_{Na^+} 0.18×10^{-9} m; glycerol, MW 92, r 0.23×10^{-9} m; mannitol, MW 182, r 0.36×10^{-9} m; sucrose, MW 342, r 0.47×10^{-9} m; and PEG 6000, MW 6000, r 2.3×10^{-9} m. Water (H_2O, MW 18, r 0.11×10^{-9} m) was included as control. The change in fruit mass upon incubation in solutions of these osmotica was determined gravimetrically during two 45 min time intervals at 22 °C (*6*). Thereafter, fruit were inspected for macroscopic cracks. Observations on fruit that cracked in the course of an experiment were excluded from data analysis. Rates of water uptake (F in g h^{-1}) were determined on an individual fruit basis from the slope of a linear regression line fitted through a plot of cumulative water uptake vs time. The flux J (kg m^{-2} s^{-1}) was calculated by dividing F by the fruit surface area (A in m^2). A was estimated from fruit mass, assuming a density of sweet cherry fruit of 1000 kg m^{-3} and a spherical fruit shape as first approximations. The minimum number of single fruit replications was nine per osmoticum.

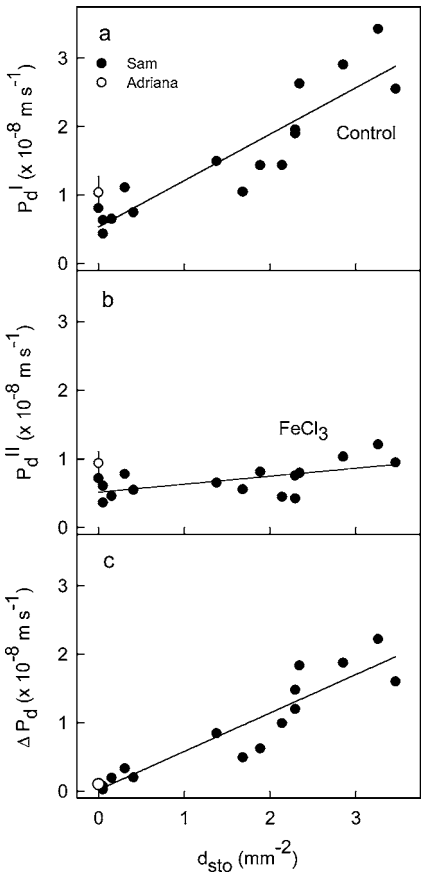

Figure 2. Relationship between ^3H_2O permeability (P_d) of exocarp segments (ES) and stomatal density (d_{sto}) in the absence (P_d^{I}; **a**) and presence of $FeCl_3$ (P_d^{II}; **b**). (**c**) Decrease in P_d (ΔP_d) caused by $FeCl_3$ as affected by d_{sto}. The decrease in P_d caused by $FeCl_3$ was calculated by difference ($\Delta P_d = P_d^{I} - P_d^{II}$). The regression equations were as follows: P_d^{I} ($\times 10^{-8}$ m s^{-1}) = 0.53 (±0.16) + 0.68 (±0.08)[d_{sto} (mm^{-2})], R^2 = 0.83, $P < 0.0001$; P_d^{II} ($\times 10^{-8}$ m s^{-1}) = 0.52 (±0.08) + 0.12 (±0.04)[d_{sto} (mm^{-2})], R^2 = 0.37, $P < 0.013$; ΔP_d ($\times 10^{-8}$ m s^{-1}) = 0.02 (±0.12) + 0.56 (±0.06)[d_{sto} (mm^{-2})], R^2 = 0.86, $P < 0.0001$. Data for Sam represent individual stomatous ES, but those for astomatous ES from Adriana represent means ± standard errors of means.

Data Analysis and Presentation. Data were subjected to analysis of variance (ANOVA). ANOVA (Proc Anova, Proc Glm), multiple comparisons of means, and regression analysis (Proc Reg) were carried out using the Statistical Analysis System software package (version 9.1; SAS Institute Inc., Cary, NC). Unless specified otherwise, regression analysis was performed using individual observations. Data in figures are presented as means ± SE of means except for **Figures 2** and **4c**, where individual observations are shown.

RESULTS

Diffusion of ^3H_2O in the absence of $FeCl_3$ increased linearly with time, indicating a constant P_d (**Figure 1a**). When $FeCl_3$ was added to donor solutions, flow rates decreased by about 60% within 26 h, and they remained constant thereafter. Replacing the $FeCl_3$ containing donor by water did not reverse the decrease in flow rates. Since the driving force for ^3H_2O diffusion remained approximately constant throughout the experiment, the decrease in flow rates must be related to decreased P_d caused by $FeCl_3$ (**Figure 1b**).

Increasing the stomatal density of Sam ES increased the P_d of ^3H_2O in the absence of $FeCl_3$ (**Figure 2a**). When $FeCl_3$ was added to donor solutions, P_d and its dependence on d_{sto} decreased

Table 1. Effect of $FeCl_3$ (10 mM) on the ^3H_2O Permeability of Astomatous Cuticular Membranes (CM), Dewaxed CM (DCM), and CM with Epicuticular Wax (ECW) Removed (CM-ECW) of Adriana Sweet Cherry Fruit[a]

	$P_d \pm$ SE ($\times 10^{-8}$ m s^{-1})		ratio
membrane	P_d^I control	P_d^{II} $FeCl_3$	P_d^{II}/P_d^I
CM	1.5 ± 0.2 b	1.5 ± 0.2 b	1.01 ± 0.02 a
CM-ECW	2.3 ± 0.3 b	2.5 ± 0.3 b	1.06 ± 0.02 a
DCM	11.7 ± 0.9 a	12.8 ± 1.0 a	1.10 ± 0.01 a

[a] During the first phase of the experiment, permeability was established in the absence of $FeCl_3$ (P_d^I); thereafter, it was established in the presence of 10 mM $FeCl_3$ (P_d^{II}). The effect of $FeCl_3$ on P_d was indexed by the ratio of P_d^{II}/P_d^I. Data represent means ± standard errors of means (SE). Mean separation within columns by Tukey's studentized range test, $P < 0.05$.

Table 2. Effect of the Position of $FeCl_3$ (10 mM; Donor vs Receiver) and the Direction of ^3H_2O Transport Relative to the Orientation of Exocarp Segments (ES; Normal vs Reversed) of Sam Sweet Cherry Fruit on ^3H_2O Permeability (P_d)[a]

	$P_d^{III}/P_d^I \pm$ SE		
donor/receiver in phase II	CM side to donor (normal)	cell wall side to donor (reversed)	$mean_{treatment}$
H_2O/H_2O	0.93 ± 0.06 a	1.06 ± 0.03 a	1.02
$FeCl_3/H_2O$	0.36 ± 0.04 b	0.39 ± 0.02 c	0.37
$H_2O/FeCl_3$	0.90 ± 0.04 a	0.73 ± 0.04 b	0.84
$FeCl_3/FeCl_3$	1.03 ± 0.03 a	1.00 ± 0.07 a	1.02
$mean_{orientation}$	0.78	0.80	

[a] During phase I of the experiment, the P_d^I in the absence of $FeCl_3$ was established. In phase II, $FeCl_3$ was added to the donor ("$FeCl_3/H_2O$"), to the receiver ("$H_2O/FeCl_3$") or to both donor and receiver cells ("$FeCl_3/FeCl_3$"). For phase III, holders with ES were remounted in diffusion cells with the CM side oriented to the donor ("normal") or to the receiver ("reversed") and the P_d^{III} in the absence of $FeCl_3$ was re-established. The effect of $FeCl_3$ was indexed by the ratio of P_d^{III}/P_d^I. Data represent means ± standard errors of means (SE). Mean separation within columns by Tukeys studentized range test, $P < 0.05$.

(**Figure 2b**). The reduction in P_d caused by $FeCl_3$ was positively related to d_{sto} (**Figure 2c**). The *y*-axis intercepts of the regression lines in **Figures 2a** and **b** represent the respective P_d values for a hypothetical astomatous Sam ES. These intercepts did not differ in the absence and presence of $FeCl_3$ [0.53 (± 0.16) × 10^{-8} vs 0.52 (± 0.08) × 10^{-8} m s^{-1} for P_d^I vs P_d^{II}, respectively], indicating that there would be no effect of $FeCl_3$ on P_d in astomatous ES. This was indeed observed for astomatous Adriana ES, where P_d^I, P_d^{II}, and ΔP_d did not differ from the relationship predicted using stomatous Sam ES (**Figure 2**).

Also, there was no effect of $FeCl_3$ in astomatous CM from Adriana sweet cherries in the presence or absence of epicuticular or epicuticular and cuticular wax (**Table 1**). To broaden the data base for astomatous CM, additional experiments were carried out using astomatous CM and DCM from tomato and pepper fruit. Again, there was no effect of $FeCl_3$ in CM or DCM from tomato (P_d^{II}/P_d^I: 0.92 ± 0.09 vs 0.91 ± 0.06 for CM vs DCM, respectively) or pepper fruit (P_d^{II}/P_d^I: 1.03 ± 0.01 vs 0.99 ± 0.01 for CM vs DCM, respectively).

The effect of $FeCl_3$ as indexed by the P_d^{III}/P_d^I ratio depended on the side of $FeCl_3$ addition (**Table 2**). Pretreating ES with $FeCl_3$ in the donor was more effective in reducing ^3H_2O permeability than pretreatment with $FeCl_3$ in the receiver cell. Changing the orientation of ES following the pretreatment with $FeCl_3$ generally had little effect on the P_d. There was no effect on ^3H_2O permeability when ES were pretreated with $FeCl_3$ in both donor and receiver cells simultaneously.

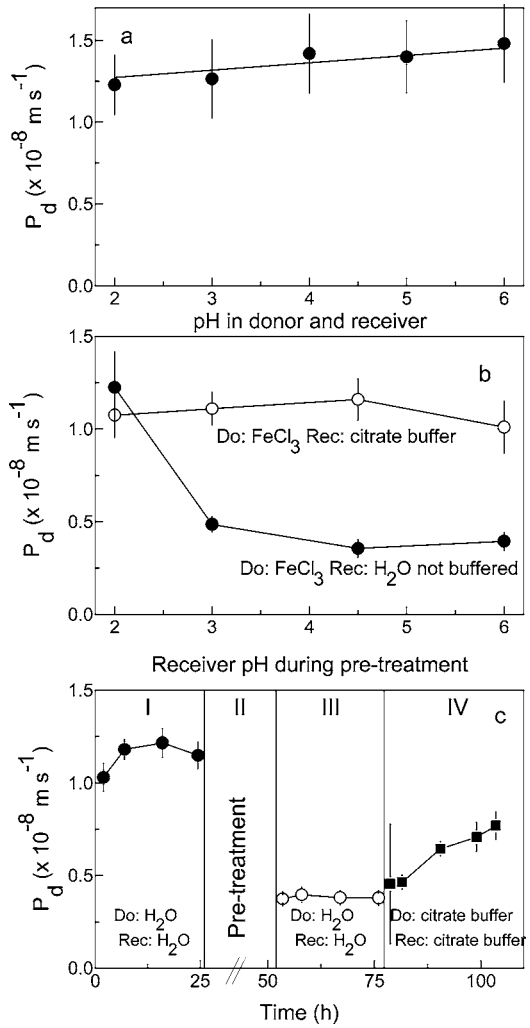

Figure 3. Effect of pH and $FeCl_3$ (10 mM) on ^3H_2O permeability (P_d). (**a**) Effect of donor and receiver pH on P_d of isolated astomatous Adriana cuticular membranes (CM) in the absence of $FeCl_3$. The regression equation was as follows: P_d ($\times 10^{-8}$ m s^{-1}) = 0.06 (±0.01)pH + 1.10 (±0.06), $R^2 = 0.89$, $P < 0.017$. (**b**) Effect of receiver pH during the pretreatment with $FeCl_3$ on the subsequent permeability of the sweet cherry exocarp to water. Stomatous exocarp segments (ES; Sam) were pretreated using 10 mM $FeCl_3$ as a donor against citrate buffer or deionized water with pH values adjusted to those indicated on the *x*-axis as receiver. (**c**) Effect of citrate buffer on the time course of the change in P_d of ES of Sam sweet cherry fruit following preincubation in 10 mM $FeCl_3$. Phase I refers to the P_d from water as donor and receiver (Do: H_2O, Rec: H_2O), phase II refers to the pretreatment phase where the Fe effect on water uptake was induced against a water receiver of pH ≥ 3 (Do: 10 mM $FeCl_3$, Rec: H_2O at pH ≥ 3), and phase III and phase IV refer to the P_d after pretreatment with $FeCl_3$ (phase III Do: H_2O, Rec: H_2O; phase IV Do and Rec: 10 mM citrate buffer at pH 2.4). For details see Materials and Methods.

The pH of the donor and receiver had no effect on P_d in the absence of $FeCl_3$ (**Figure 3a**). Also, pretreating stomatous ES with $FeCl_3$ had no effect on P_d when using citrate buffer as a receiver during pretreatment. However, when deionized water was used as a receiver during pretreatment, P_d depended on receiver pH. The P_d decreased at pH 3.0, 4.5, and 6.0, but there was no effect at pH 2.0 (**Figure 3b**). The decrease in P_d at pH ≥ 3.0 was partially reversed again when the water donor and receiver were replaced by citrate buffer (**Figure 3c**).

The P_d for ^3H_2O diffusion across stomatous ES was positively related to temperature and consistently lower in the presence

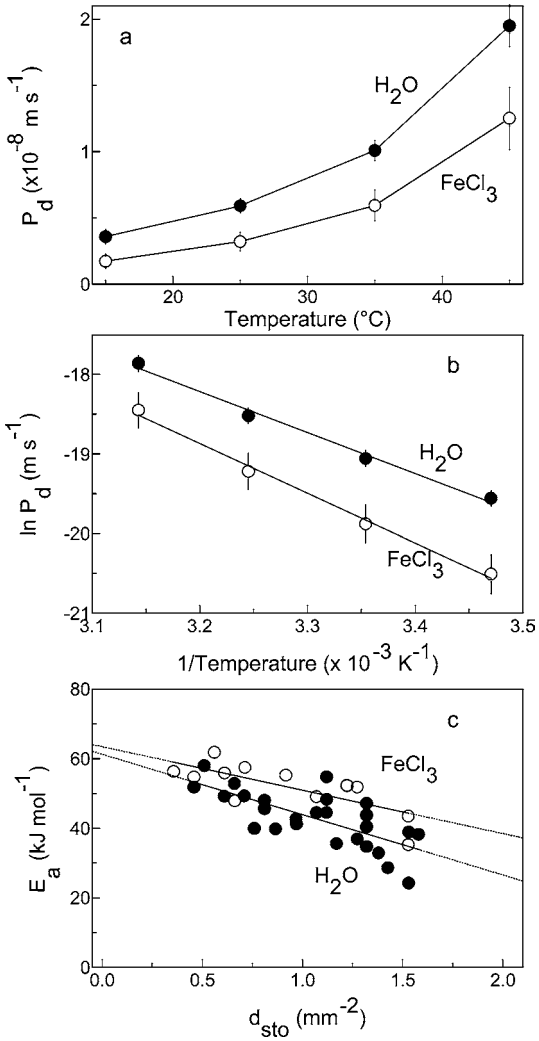

Figure 4. (**a**) Effect of temperature in the presence and absence of $FeCl_3$ (10 mM) on the ^3H_2O permeability (P_d) of excised sweet cherry fruit exocarp segments (ES, Sam). (**b**) Arrhenius plot of the data depicted in part a. (**c**) Energy of activation (E_a) for P_d as a function of stomatal density (d_{sto}.). Regression equations were as follows: in the absence of $FeCl_3$, E_a (kJ mol^{-1}) = –17.3 (±3.5)[d_{sto} (mm^{-2})] + 61.2 (±3.9), $R^2 = 0.51$, $P < 0.0001$; in presence of $FeCl_3$, E_a (kJ mol^{-1}) = –12.5 (±3.5)[d_{sto} (mm^{-2})] + 63.5 (±3.5), $R^2 = 0.54$, $P < 0.004$.

than in the absence of $FeCl_3$ (**Figure 4a**). Arrhenius plots were linear between 15 and 45 °C (**Figure 4b**). $FeCl_3$ significantly increased the energy of activation for ^3H_2O diffusion across stomatous ES on average by about 11.6 kJ mol^{-1} (**Table 3**). A quantitatively similar increase in E_a by $FeCl_3$ was obtained after pretreating stomatous ES with $FeCl_3$ (8.1 kJ mol^{-1}; Weichert, unpublished data). For stomatous ES, E_a was linearly and negatively related to stomatal density in both the absence and presence of $FeCl_3$ (**Figure 4c**). The slope of the regression lines fitted through plots of E_a vs d_{sto} may be interpreted as the decrease in E_a per stoma, and the *y*-axis intercepts may be interpreted as the E_a for diffusion across a hypothetical astomatous ES ($d_{sto} = 0$). $FeCl_3$ decreased the E_a per stoma by about 28%, as indicated by a less negative slope of the regression line (−17.3 ± 3.5 and −12.5 ± 3.5 kJ mol^{-1} in the absence and presence of $FeCl_3$, respectively; data for $d_{sto} = 1$ mm^{-2}). The *y*-axis intercepts, however, predicted no effect of $FeCl_3$ on the E_a of a hypothecial astomatous ES (61.2 ± 3.9 vs 63.5 ± 3.5 kJ mol^{-1} in the absence and presence of $FeCl_3$, respectively), which was indeed observed in astomatous Adriana CM (E_a = 69.3 ± 3.6 and 62.7 ± 5.7 kJ mol^{-1} in the absence and presence of $FeCl_3$, respectively; $P < 0.3475$).

Table 3. Effect of $FeCl_3$ (10 mM) on the Energy of Activation (E_a) of the ^3H_2O Permeability (P_d) of Stomatous Exocarp Segments (ES) from Hedelfinger and Sam Sweet Cherries[a]

cultivar	d_{sto} ± SE (mm^{-2})	E_a ± SE (kJ mol^{-1}) control	E_a ± SE (kJ mol^{-1}) $FeCl_3$	E_a ± SE (kJ mol^{-1}) mean$_{cultivar}$	ΔE_a (kJ mol^{-1})
Hedelfinger	0.74 ± 0.06	39.4 ± 2.4 a	55.6 ± 3.3 b	47.5 a	16.2
Sam	1.07 ± 0.06	44.4 ± 1.6 a	51.4 ± 1.9 b	45.8 a	7.0
mean$_{treatment}$		41.9 b	53.5 a		11.6

[a] The change in E_a (ΔE_a) was calculated by difference from the E_a in the presence of $FeCl_3$ (P_d^{II}) minus that in the absence of $FeCl_3$ (P_d^{I}). Mean comparisons were performed using the least-square difference (LSmeans) at $P < 0.05$. Analysis of variance was performed using d_{sto} as a covarible. Therefore, the mean E_a (±SE) specified represents the mean adjusted for the different d_{sto} values of Hedelfinger and Sam ES.

Table 4. Effect of $FeCl_3$ (10 mM) on the NAA and 2,4-D Permeability (P_d) of Stomatous Exocarp Segments (ES) Excised from Sam Sweet Cherry Fruit[a]

substance	pH	percentage nondissociated (% of total)	P_d ± SE (×10^{-8} m s^{-1}) P_d^{I}	P_d ± SE (×10^{-8} m s^{-1}) P_d^{III}	ratio P_d^{III}/P_d^{I}
NAA	2.2	0.9	7.7 ± 1.8 a	6.4 ± 1.4 a	0.83 ± 0.02 a
	6.2	98.9	0.9 ± 0.1 b	0.4 ± 0.1 b	0.43 ± 0.03 b
2,4-D	2.2	26.6	5.6 ± 1.1 a	4.3 ± 0.8 a	0.78 ± 0.03 a
	6.2	100.0	1.0 ± 0.1 b	0.5 ± 0.0 b	0.47 ± 0.01 b

[a] During the first phase of the experiment, permeability was established in the absence of $FeCl_3$ (P_d^{I}). In phase II, ES were pretreated with $FeCl_3$ in the donor by replacing the NAA or 2,4-D donor by 10 mM $FeCl_3$. Following pretreatment, the $FeCl_3$ donor was replaced again by the NAA or 2,4-D donor and permeability was re-established (P_d^{III}). The effect of $FeCl_3$ on P_d was indexed by the ratio of P_d^{III}/P_d^{I}. Data represent means ± standard Errors of means (SE). Mean separation within columns and PGRs by Tukeys studentized range test, $P < 0.05$.

$FeCl_3$ reduced the P_d for NAA and 2,4-D (**Table 4**). The decrease in P_d depended on the degree of dissociation of the acids and was about 2-fold higher for the predominantly dissociated than the nondissociated species. In the absence of $FeCl_3$, the P_d values for the nondissociated NAA and 2,4-D were about 8.7- and 5.6-fold higher than those of the dissociated form, which were of similar magnitude as the P_d for ^3H_2O.

Incubating Sam sweet cherry fruit in isotonic solutions of selected osmotica resulted in significant water uptake that depended on the molecular weight of the osmoticum and preincubation in $FeCl_3$. Water fluxes were inversely related to molecular weight and consistently lower for fruit pretreated with $FeCl_3$. There was no water uptake for the largest osmoticum PEG 6000 regardless of treatment with $FeCl_3$ (**Figure 5**a). Calculating the decrease in flux (ΔJ) caused by $FeCl_3$ revealed that ΔJ on an absolute scale was also inversely related to the molecular weight and, hence, molecular radii of the osmotica (**Figure 5b**). On a relative scale, however, the $FeCl_3$ induced decrease was largest in an isotonic solution of sucrose that has an hydrodynamic radius of 0.47 nm (**Figure 5b**, inset).

DISCUSSION

In our discussion we focus on the mechanism and site of action of the $FeCl_3$ induced decrease in the permeability of the sweet cherry exocarp.

Mechanism of Action. Beyer et al. (*3*) hypothesized that $FeCl_3$ decreased water transport by a precipitation reaction in the sweet cherry exocarp. Aqueous (donor) solutions of $FeCl_3$

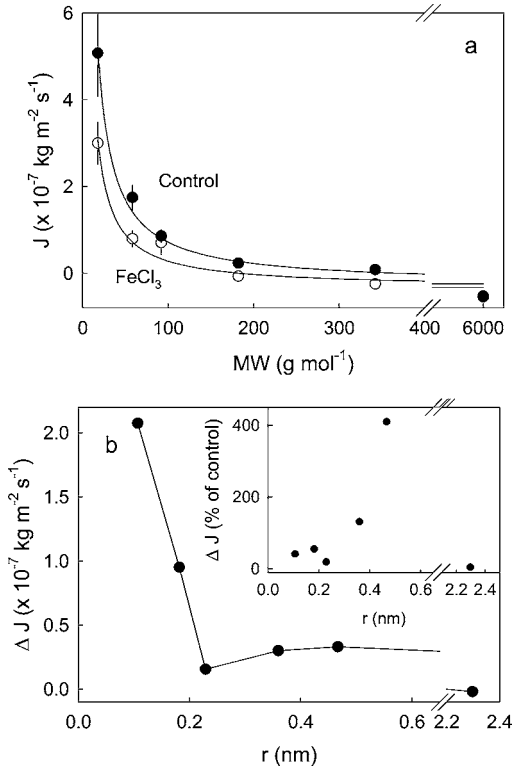

Figure 5. (**a**) Effect of osmotica of differing molecular weights (MW) on the water flux (*J*) through the exocarp of intact Sam sweet cherry fruit. Fruit was pretreated with water or 10 mM $FeCl_3$ and subsequently incubated in isotonic solutions of osmotica of different MW. Osmotica and their respective molecular weights and hydrodynamic radii (*r*) were as follows: NaCl, MW 58, r_{Na^+} 0.18×10^{-9} m; glycerol, MW 92, *r* 0.23×10^{-9} m; mannitol, MW 182, *r* 0.36×10^{-9} m; sucrose, MW 342, *r* 0.47×10^{-9} m; PEG 6000, MW 6000, *r* 2.3×10^{-9} m. Water (H_2O, MW 18, *r* 0.11×10^{-9} m) was included as control. (**b**) $FeCl_3$ induced decrease in water flux (ΔJ) as affected by hydrodynamic radii of osmotica. The *J* was calculated by subtracting the *J* after preincubation in $FeCl_3$ (○) from that in the water control (●). The main graph gives ΔJ on an absolute scale, and the inset gives it on a relative scale as percent of *J* in the absence of $FeCl_3$.

are strongly acidic (pH 2.4 at 10 mM $FeCl_3$). During penetration of the exocarp, the ferric ion is exposed to a microenvironment of increasing pH until the typical pH of the plant's apoplast is encountered (pH 5.5; *14*). As pH increases, condensation occurs and colloidal gels and, finally, precipitates are formed that comprise complex hydrated amorphous ferric-oxides and -hydroxides (most likely FeO(OH); *15*). The data reported in this paper are consistent with this hypothesis. First, the decrease in water transport always required (*1*) the presence of a pH gradient between the $FeCl_3$ donor and the aqueous receiver solution (**Table 2)** and (*2*) the absence of substances that form complexes or chelates with the ferric ion thereby preventing precipitation. For example, citric acid is known to form such complexes, and in the presence of citrate buffer in the receiver, $FeCl_3$ had no effect on the P_d for water transport regardless of pH (**Figure 3b**). Formation of a ferric citrate complex would also account for (i) the partial reversal of the Fe-dependent decrease in P_d when replacing the water donor and receiver by citrate buffer (phase IV in **Figure 3c**) and (ii) the absence of an effect of ferric citrate on water uptake (Weichert, unpublished data). The absence of an effect of $FeCl_3$ on water diffusion when it is added to the donor and receiver solutions simultaneously is also consistent with the above hypothesis (**Table 2**). Under these

Table 5. Calculated Resistance (*R*) of Fe-Precipitates to Penetration of NAA, 2,4-D, and H_2O through the Exocarp of Sweet Cherry Fruit[a]

		R ($\times 10^8$ s m^{-1})			
substance/source	pH	$R^{exocarp}$	R^{tot}	R^{precip}	R^{precip} (% of R^{tot})
NAA (**Table 4**)	2.2	0.13	0.16	0.03	18
	6.2	1.12	2.63	1.51	57
2,4-D (**Table 4**)	2.2	0.18	0.23	0.05	23
	6.2	0.99	2.13	1.14	53
H_2O (**Figure 1**)	5.5	0.99	2.80	1.81	65
H_2O (**Figure 2**)	5.5	0.64	1.44	0.81	56

[a] Resistance of Fe-precipitates (R^{precip}) was calculated using the resistors in series analogy where the total resistance of exocarp plus Fe-precipitates (R^{tot}) equaled the sum of the resistances of exocarp ($R^{exocarp}$) and precipitates (R^{precip}). The resistances (*R* in s m^{-1}), in turn, represented the inverses of the respective permeabilities (P_d in m s^{-1}).

conditions, the donor and receiver pH values are both strongly acidic. Since there is no pH-gradient, precipitation does not occur and there is no effect on water transport.

The ferric oxides and hydroxides formed upon precipitation represented a barrier particularly for penetration of polar substances, including water (**Figure 1a** and **b)**. First, $FeCl_3$ increased the E_a for water diffusion through stomatous ES (+11.6 kJ mol^{-1}; **Table 3**). Second, $FeCl_3$ decreased the permeability of the organic acids NAA and 2,4-D in a pH-dependent manner. Interestingly, the decrease in P_d was larger for the dissociated, more polar anion than the nondissociated, more lipophilic acid (**Table 4**). Assuming that the precipitates formed a transport barrier arranged in series to the CM, the resistance of the precipitates may be calculated using the resistor in series analogy (*16*), where total resistance (R^{tot}) represents the sum of the individual serial resistances, i.e., exocarp ($R^{exocarp}$) and Fe-precipitates (R^{precip}). The resistances (*R*), in turn, equal the inverse of the respective permeabilities (P_d; eq 2).

$$R^{tot} = R^{exocarp} + R^{precip} = \frac{1}{P_d^{exocarp}} + \frac{1}{P_d^{precip}} \qquad (2)$$

Performing the calculations revealed that R^{tot}, $R^{exocarp}$, R^{precip}, and the relative contributions of R^{precip} to R^{tot} were similar for (i) NAA and 2,4-D at either pH and for (ii) water and the anions of both acids, but they were markedly larger for the anion as compared to the nondissociated acid (**Table 5**). These data suggest that (i) the precipitates represented a major resistance to transport of the polar anions and water and that (ii) the NAA and 2,4-D-anion and water must have penetrated the exocarp along the same pathway.

Site of Action. The data presented in this paper confirm earlier observations that the effect of $FeCl_3$ on water transport is closely related to stomata. First, positive linear and significant relationships between the $FeCl_3$ induced decreases in permeability in self-diffusion of water (P_d; **Figure 2c**) and in osmotic water uptake (P_f; *3*) and the stomatal density of ES were obtained. Intercepts of regression lines representing the P_d of a hypothetical astomatous ES were not significantly different from zero, predicting the absence of an effect of $FeCl_3$ in the absence of stomata (**Table 1**). Second, the energy of activation for water transport was linearly and negatively related to stomatal density in the absence and presence of $FeCl_3$ (**Figure 4c**). The slopes of linear regression lines fitted through plots of E_a vs d_{sto} were −17.3 and −12.5 kJ mol^{-1} per stoma per mm^2 in the absence and presence of $FeCl_3$, respectively, indicating a 28% decrease in the dependence of E_a on stomatal density by $FeCl_3$. In

contrast, intercepts of regression lines were again similar in the presence (E_a = 61.5 kJ mol^{-1}) and absence of $FeCl_3$ (E_a = 63.5 kJ mol^{-1}, **Figure 4c**). Third, there was no effect of $FeCl_3$ on P_d in astomatous ES, CM, CM-ECW, or DCM of sweet cherry, tomato, or pepper fruit.

Theoretically, the $FeCl_3$-dependent decrease in P_d of the stomatal apparatus could be accomplished by one or several of the following events: (i) a direct plugging of the stomatal pore, (ii) differential wettability of the CM surface above guard cells, (iii) a localized gradient in apoplastic pH that results in preferential precipitation of $FeCl_3$ at the stomatal apparatus, and/or (iv) decreased permeability of polar penetration pathways that are preferentially located at or in the vicinity of the stomatal apparatus.

Plugging of the stomatal pore (i) is unlikely to occur. Scanning electron microscopy and EDX analysis did not reveal any coating or plugging of the stomatal pore that was associated with Fe (Schroeder, Bukovac, and Knoche, unpublished data). Also, it is generally assumed that penetration of the stomatal pore by water is prevented by the wetting characteristics of the leaf or fruit surface, the high surface tension of water, and the morphology of cuticular ledges of guard cells (*17*). In sweet cherries, the critical surface tension of the fruit surface averaged 24.9 mN m^{-1}, which is markedly lower than that of water (72 mN m^{-1}), making mass flow through open stomata unlikely (*11*). Some publications, however, report on penetration of stomata by aqueous solutions which could be accounted for by condensation of water droplets on the surface of the stomatal pore and subsequent formation of a continuous water film (*18*, *19*). An alternative explanation that would explain a localized stomatal effect relates to differences in wettability between the stomatal apparatus and the CM surface in between stomata (ii). The fine structure of the surface above guard cells often differs from that of the remaining surface, and this could cause differential wetting. In sweet cherry fruit, however, water droplets form a contact angle of 92.4° with a dry surface, indicating a fairly easy-to-wet surface (*11*). In our diffusion cell system, wetting was probably further facilitated due to extended exposure to donor solutions, making differential wetting less likely. The third hypothesis (iii) would require spatial pH-gradients between the cell wall space underlying the CM above guard and accessory cells and that of epidermal cells in the area between stomata. Such gradients would be difficult to generate and maintain in the absence of diffusion barriers in the cell wall space and, therefore, are difficult to visualize. Thus, there is no direct evidence in sweet cherries to support any of the first three hypotheses.

The fourth hypothesis (iv) offers the most likely explanation for the decreased P_d and its association with stomata, i.e., effects of $FeCl_3$ on polar pathways. Such pathways have long been postulated (*20*−*22*) and recently received renewed interest ("aqueous pores"; *23*−*26*). Polar pathways are preferentially located above guard cells, at cuticular ledges of the stomatal apparatus, and above anticlinal cell walls of epidermal cells (*20*, *22*). The close association of polar pathways with the stomatal apparatus would be consistent with the significant relationship between the Fe-dependent decrease in water permeability and stomata. Two further arguments support this hypothesis.

First, $FeCl_3$ had a markedly larger effect on diffusion of the dissociated vs the nondissociated species of NAA and 2,4-D (**Table 4**). For example, the nondissociated species of NAA is lipophilic (partition coefficient $K_{CM/buffer}$ = 189 for NAA at pH 2.2; *27*) and, therefore, would be expected to penetrate the CM along the lipophilic pathway by a partitioning/diffusion mechanism. In contrast, the NAA anion is excluded from this pathway based on polarity ($K_{CM/buffer}$ = 7 for NAA at pH 6.2; *27*) but would be accommodated by the polar pathways, since the molecular dimensions are smaller than the exclusion limit of the polar pathway in the sweet cherry exocarp (MW_{NAA} 186 g mol^{-1}; *6*). Since water molecules penetrate along the same path as the NAA anion (see discussion above), the precipitates formed must have decreased water uptake by decreasing the permeability of the polar pathway.

Second, we observed significant water uptake by sweet cherry fruit incubated in isotonic solutions with water fluxes depending on molecular weight and, hence, the dimensions of the osmotica in this (**Figure 5a**) and our earlier study (*6*). Since the osmotica were polar and solutions were isotonic, the driving force for this water uptake originated from size-dependent penetration of the osmotica along the polar pathway (*6*). Treating fruit with $FeCl_3$ decreased, but did not eliminate, this size-dependent penetration completely (**Figure 5a**). The decrease in water flux was inversely related to the molecular dimensions of the osmotica (**Figure 5b**). Furthermore, for osmotica having molecular dimensions below the exclusion limit of the polar pathway (*6*), the percentage decrease in water flux increased markedly as molecular weight increased (**Figure 5b**, inset). Hence, the resistance formed by the precipitates must have increased as the size of the osmotica increased. This effect would be consistent with a decrease in the size exclusion limits of the polar pathway by the precipitates.

In summary, our data provide evidence for decreased permeability of polar pathways across the sweet cherry fruit exocarp by pH-dependent formation of ferric precipitates, most likely viscous oxides and hydroxides. Unfortunately, the usefulness of the Fe-effect in horticultural practice is limited (*4*). Nevertheless, decreasing the permeability of polar pathways by a pH-dependent precipitation reaction is an attractive strategy for reducing water uptake and, hence, fruit cracking. Since gas exchange during respiration is expected to be largely independent of polar pathways, undesirable side effects on fruit physiology are less likely to occur. Such effects would inevitably be associated with "coating strategies", that result in a nonspecific increase in the diffusive resistance of the sweet cherry exocarp. Furthermore, a precipitate formed within the polar pathways is largely protected from resolubilization by rain, which would eliminate the need for repeated spray applications. It should be kept in mind that the Fe-dependent decrease in water uptake represents a contact mode of action that is limited to the exocarp, while the parallel pathway for water uptake along the pedicel/fruit juncture remains unaffected (*4*). Thus, the strategy offered could prove useful, but it is not the "golden bullet" that will resolve the problem of sweet cherry fruit cracking.

ABBREVIATIONS USED

A, surface area; CM, cuticular membrane(s); DCM, dewaxed CM; d_{sto}, number of stomata per unit surface area; E_a, energy of activation; ECW, epicuticular wax; ES, exocarp segment(s); *F*, flow per unit time; *J*, flux per unit area and time; MW, molecular weight; P_d, permeability coefficient for self-diffusion.

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LITERATURE CITED

(1) Christensen, J. V. Rain-induced cracking of sweet cherries: Its causes and prevention. In *Cherries Crop Physiology, Production and Uses*; Webster, A. D., Looney, N. E., Eds.; CAB International: Wallingford, U.K., 1996; pp 297−327.

(2) Pennell, D.; Webster, A. D. Sweet cherries: Protection of fruit from bird and rain damage. In *Cherries Crop Physiology, Production and Uses*; Webster, A. D., Looney, N. E., Eds.; CAB International: Wallingford, U.K., 1996; pp 393−407.

(3) Beyer, M.; Peschel, S.; Weichert, H.; Knoche, M. Studies on water transport through the sweet cherry fruit surface: VII. Fe^{3+} and Al^{3+} reduce conductance for water uptake. *J. Agric. Food Chem*. **2002**, *50*, 7600−7608.

(4) Weichert, H.; v. Jagemann, C.; Peschel, S.; Knoche, M.; Neumann, D.; Erfurth, W. Studies on water transport through the sweet cherry fruit surface: VIII. Effect of selected cations on water uptake and fruit cracking. *J. Am. Soc. Hortic. Sci.* **2004**, *129*, 781−788.

(5) Beyer, M.; Lau, S.; Knoche, M. Studies on water transport through the sweet cherry fruit surface: IX. Comparing permeability in water uptake and transpiration. *Planta* **2005**, *220*, 474−485.

(6) Weichert, H.; Knoche, M. Studies on water transport through the sweet cherry fruit surface: X. Evidence for polar pathways across the exocarp. *J. Agric. Food Chem*. **2006**, *54*, 3951−3958.

(7) Orgell, W. H. The isolation of plant cuticle with pectic enzymes. *Plant Physiol*. **1955**, *30*, 78−80.

(8) Yamada, T.; Wittwer, S. H.; Bukovac, M. J. Penetration of ions through isolated cuticles. *Plant Physiol*. **1964**, *39*, 28−32.

(9) Silcox, D.; Holloway, P. J. A simple method for the removal and assessment of foliar deposits of agrochemicals using cellulose acetate film stripping. In *Aspects of applied biology 11. Biochemical and physiological techniques in herbicide research*; Association of Applied Biologists, Warwick; Nottingham Trent Polytechnic, Nottingham, U.K., 1986; pp 13−17.

(10) Bukovac, M. J.; Petracek, P. D. Characterizing pesticide and surfactant penetration with isolated plant cuticles. *Pestic. Sci.* **1993**, *37*, 179−194.

(11) Peschel, S.; Beyer, M.; Knoche, M. Surface characteristics of sweet cherry fruit: stomata number, distribution, functionality and surface wetting. *Sci. Hortic.* **2003**, *97*, 265−278.

(12) Bukovac, M. J.; Petracek, P. D.; Fader, R. G.; Morse, R. D. Sorption of organic compounds by plant cuticles. *Weed Sci*. **1990**, *38*, 289−298.

(13) Beyer, M.; Peschel, S.; Knoche, M.; Knörgen, M. Studies on water transport through the sweet cherry fruit surface: IV. Regions of preferential uptake. *HortScience* **2002**, *37*, 637−641.

(14) Marschner, H. *Mineral Nutrition of Higher Plants*; Academic Press: London, 1995.

(15) Greenwood, N. N.; Earnshaw, A. *Chemie der Elemente*; VCH-Verlagsgesellschaft: Weinheim, Germany, 1990; pp 1383 and 1394.

(16) Schönherr, J. Water permeability of isolated cuticular membranes: the effect of cuticular waxes on diffusion of water. *Planta* **1976**, *131*, 159−164.

(17) Schönherr, J.; Bukovac, M. J. Penetration of stomata by liquids: Dependence on surface tension, wettability, and stomatal morphology. *Plant Physiol*. **1972**, *49*, 813−819.

(18) Eichert, T.; Goldbach, H. E.; Burkhardt, J. Evidence for the uptake of large anions through the stomatal pores. *Bot. Acta* **1998**, *111*, 461−466.

(19) Eichert, T.; Burkhardt, J. Quantification of stomatal uptake of ionic solutes using a new model system. *J. Exp. Bot.* **2001**, *52*, 771−781.

(20) Franke, W. Role of guard cells in foliar absorption. *Nature* **1964**, *202*, 1236−1237.

(21) Jyung, W. H.; Wittwer, S. H.; Bukovac, M. J. The role of stomata in the foliar absorption of Rb by leaves of tobacco and tomato. *Proc. Am. Soc. Hortic. Sci.* **1965**, *86*, 361−367.

(22) Schönherr, J.; Bukovac, M. J. Preferential polar pathways in the cuticle and their relationship to ectodesmata. *Planta* **1970**, *92*, 189−201.

(23) Schönherr, J. Calcium chloride penetrates plant cuticles via aqueous pores. *Planta* **2000**, *212*, 112−118.

(24) Schönherr, J.; Schreiber, L. Size selectivity of aqueous pores in astomatous cuticular membranes isolated from *Populus canescens* (Aiton) Sm. leaves. *Planta* **2004**, *219*, 405−411.

(25) Schlegel, T. K.; Schönherr, J.; Schreiber, L. Size selectivity of aqueous pores in stomatous cuticles of *Vicia faba* leaves. *Planta* **2005**, *221*, 648−655.

(26) Schreiber, L. Polar paths of diffusion across plant cuticles: new evidence for an old hypothesis. *Ann. Bot*. **2005**, *95*, 1069−1073.

(27) Shafer, W. E.; Morse, P.; Bukovac, M. J. Effect of pH and temperature on sorption of auxin by isolated tomato fruit cuticles. *HortScience* **1988**, *23*, 204−206.

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