Studies on Water Transport through the Sweet Cherry Fruit Surface: VIII. Effect of Selected Cations on Water Uptake and Fruit Cracking

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ABSTRACT. Water uptake through the exocarp of intact sweet cherry [Prunus avium (L.)] fruit was determined gravimetrically in an immersion assay (25 °C). Fruit with sealed pedicel/fruit juncture were incubated in water during the first interval (0 to 0.75 hour) and in 10 mM salt solutions of selected cations during the second (0.75 to 1.5 hours) and third interval (1.5 to 2.25 hours) of an experiment. Rates of water uptake (F) were calculated for first, second and third intervals (F1, F2 and F3, respectively) and salt effects indexed by the ratios F2/F1 and F3/F1. AgNO3 (F2/F1 = 0.65), NaCl (0.70), BaCl2 (0.67), CdCl2 (0.69), CuCl2 (0.42), HgCl2 (0.58), and the salts of trivalent cations AlCl3 (0.50), EuCl3 (0.58), and FeCl3 (0.49), significantly decreased water uptake into mature ‘Sam’ fruit as compared to the water control (0.87). KCl (0.82), NH4Cl (0.85), CaCl2 (0.75), MgCl2 (0.88), MnCl2 (0.81), and ZnCl2 (0.72) had no effect, LiCl (1.00) increased uptake. Similar data were obtained for F3/F1. The effect of FeCl3 on water uptake was independent of the presence of CaCl2, AlCl3, or CuCl2, as sequential or simultaneous treatment with these salts reduced water uptake to the same extent as with FeCl3 alone. Increasing FeCl3 concentration up to 1 mM decreased uptake, higher concentrations had no further effect. FeCl3, and CaCl2, to a smaller extent decreased water uptake in developing ‘Regina’ sweet cherry fruit (55 to 91 days after full bloom). FeCl3 had no significant effect on water uptake along the pedicel/fruit juncture, but markedly reduced uptake through the exocarp of all cultivars investigated (‘Burlat’, ‘Early Rivers’, ‘Hedelfinger’, ‘Knauffs’, ‘Regina’, ‘Sam’, ‘Summit’, and ‘Van’). Effects of CaCl2 on water uptake were limited to ‘Burlat’, ‘Early Rivers’, and ‘Hedelfinger’. CaCl2, and FeCl3, both decreased fruit cracking, but FeCl3, was more effective. The mode of action of mineral salts in decreasing water uptake and fruit cracking and their potential for field use are discussed.

Rain-induced fruit cracking is a severe limitation to sweet cherry production world-wide. Fruit cracking is thought to be related to increased turgor resulting from water uptake into the fruit (Considine and Kriedemann, 1972; Sekse, 1998). Spray application of salt solutions and incubating fruit in salt solutions were reported to reduce fruit cracking under field and laboratory conditions. Most of these studies focused on Ca-salts (e.g., Bangerth, 1968; Callan, 1986; Christensen, 1996; Glenn and Poovaiah, 1989; Lang and Flore, 1999; Verner, 1937). Effects of Ca-salts on fruit cracking were inconsistent. While cracking was decreased in some experiments (Callan, 1986; Lang and Flore, 1999), there was little or no effect in others (Koffmann et al., 1996; Looney, 1985). Less information is available on other salts. Bullock (1952) found cations in general, and the Ca, Cu, Fe, Al, Th, and U cations in particular, to be more effective in reducing cracking than anions. Similar findings were reported for Ca, Fe, and Al (Bangerth, 1968; Christensen, 1996). The authors hypothesized that reduced cracking resulted from cations cross-linking cell wall constituents, thereby improving mechanical properties of the exocarp. Recently, Beyer et al. (2002a) demonstrated that FeCl3, Fe(NO3)3, Fe2(SO4)3, and AlCl3, but not CaCl2, markedly reduced water uptake by decreasing conductance of the fruit exocarp, and this effect may have contributed to reduced cracking. There is little published data on effects of other cations or interactions of cations on water uptake. If the mechanistic basis for salt effects on water uptake differed, a combined application of effective salts may yield superior performance than of either salt alone. Furthermore, from a practical point of view, it would be important to know whether salt effects are restricted to certain cultivars or specific stages of fruit development. Such information would be helpful in assessing the potential usefulness of field applications of mineral salts for reducing cracking.

The objectives of the present study therefore were to: 1) establish effects of cations and their interaction on water uptake and 2) characterize the effect of selected cations on water uptake and fruit cracking in selected sweet cherry cultivars.

Materials and Methods

Plant material. Sweet cherry fruit [‘Burlat’, ‘Early Rivers’, ‘Hedelfinger’, ‘Knauffs’, ‘Regina’, ‘Sam’, ‘Summit’, ‘Van’ all...
Water uptake assays

**Effect of cations.** Effects of univalent (Ag, K, Li, Na, NH₄), divalent (Ba, Ca, Cd, Cu, Hg, Mg, Mn, Sr, Zn), and trivalent cations (Al, Eu, Fe) on water uptake through the exocarp of mature ‘Sam’ sweet cherry fruit were studied using an immersion assay (25 °C) and sequential treatments performed on an individual fruit basis. During the initial incubation period (0 to 0.75 h), deionized water was used as the donor. At 0.75 h, the water donor was replaced by a 10 mm salt solution (equivalent to an osmotic potential Φ = –0.10 MPa, calculated for a salt of a trivalent cation) that served as the donor for the two subsequent intervals (0.75 to 1.5 h, 1.5 to 2.25 h). Except for Ag, which was used as the nitrate, all other cations were used as chlorides because these are readily water soluble and the chloride anion does not interact with water conductance of the exocarp (Beyer et al., 2002a). Solutions were prepared shortly before use. Water uptake was restricted to the fruit exocarp by removing the pedicel by gently pulling and sealing the hole above the stony endocarp with silicone rubber (Dow Corning 3140 RTV coating; Dow Corning Corp., Midland, Mich.). This procedure eliminated uptake along the parallel pathway of the pedicel/fruit juncture (Beyer et al., 2002b). Water uptake was determined gravimetrically (BP 211 D; Sartorius AG, Göttingen, Germany). Briefly, fruit were removed from incubation solutions, blotted with soft tissue paper, then weighed and returned to the incubation vessel. Rates of uptake during first (F₁, 0 to 0.75 h), second (F₂, 0.75 to 1.5 h), and third intervals (F₃, 1.5 to 2.25 h) were calculated and salt effects indexed by the ratio of flow rates (F₂/F₁ or F₃/F₁) after replacing the water donor (F₁) by a salt solution (F₂, F₃). Using this procedure control and treatment effects are established sequentially on an individual fruit basis, thereby reducing fruit to fruit variation in response. Since fruit surface area and driving forces for water uptake remain essentially constant, effects of cations on the F₂/F₁ or F₃/F₁ ratio reflect effects on water permeability of the exocarp.

**Interactions among salts.** Interactions among CaCl₂/FeCl₃, CuCl₂/FeCl₃, H₂O/NaCl, and AlCl₃/FeCl₃ on water uptake through the exocarp of mature ‘Sam’ fruit were established using the following sequences of donor solutions: H₂O/salt1/salt2, H₂O/NaCl/salt2, H₂O/NaCl/salt1/salt2, H₂O/NaCl/salt1, H₂O/NaCl/salt1/salt2, H₂O/NaCl/salt1/salt2, H₂O/NaCl/salt1. Concentrations of individual salts were 10 mm, except where both salts were used simultaneously (5 mm each). F₂/F₁, F₃/F₁ and F₂/F₁/F₃ were calculated and analyzed for interactions among salts on water uptake.

**Time course.** The time course of water uptake into mature ‘Regina’ sweet cherry fruit was monitored during a 5-h incubation period in donor solutions containing CaCl₂ or FeCl₃. Water uptake was restricted to the exocarp by removing the pedicel by gently pulling and sealing the hole above the endocarp with silicone rubber. Osmolality of donor solutions, as determined by vapor pressure osmometry (model 5520; Wescor Inc., Logan, Utah), was maintained approximately constant. Molar concentrations and osmotic potentials were 30 and 25 mm and –0.18 and –0.19 MPa for CaCl₂ and FeCl₃, respectively. Water uptake was determined at 0.075, 1.5, 3, and 5 h (n ≥ 16).

**Concentration response.** The concentration response of CaCl₂ and FeCl₃ on water uptake into mature ‘Regina’ fruit was established at 0, 0.025, 0.25, 1, 2.5, and 25 mm using the procedure described above. The pedicel/fruit juncture remained open (n = 17 and 7 for CaCl₂ and FeCl₃, respectively).

**Duration of incubation.** The effect of duration of incubating mature ‘Summit’ sweet cherry fruit in 10 mm FeCl₃ on water uptake through the exocarp was studied using the following experimental design. Initially (0 to 0.75 h), fruit were incubated in water. The second interval (0.75 to 1.5 h) comprised immersing fruit for 0 and 45 min, 1 and 44 min, 5 and 40 min, 15 and 30 min, 30 and 15 min, and 45 and 0 min in 10 mm FeCl₃ and H₂O, respectively. In the third interval (1.5 to 2.25 h), H₂O only served as the donor again. To avoid carryover from the FeCl₃ solution into H₂O when changing solutions during the second interval, fruit were removed from the FeCl₃ solution and blotted before incubation in H₂O resumed.

**Water uptake along pedicel/fruit juncture.** Penetration along the pedicel/fruit juncture in absence and presence of FeCl₃ (10 mm) was studied using mature ‘Sam’ fruit that were sealed entirely with silicone rubber except for the pedicel/fruit juncture. Preliminary experiments established that the sum of rates of water uptake into fruit with pedicel/fruit juncture sealed (7.8 ± 1.1 mg·h⁻¹, n = 10) plus the rate of uptake into fruit that was sealed, except for the pedicel/fruit juncture (8.5 ± 0.8 mg·h⁻¹, n = 20), approximately corresponded to uptake into non-sealed control fruit (18.2 ± 3.3 mg·h⁻¹, n = 12 (H. Weichert, unpublished data)). These data indicate that there was little effect of silicone rubber on fruit turgor and, hence, driving force for water uptake must have remained essentially constant. The effect of FeCl₃ was established using sequential treatments as described above and the salt effect indexed by the F₂/F₁ and F₃/F₁ ratios.

**Effects of cultivars.** To address a potential salt × cultivar interaction, effects of CaCl₂ (30 mm) and FeCl₃ (25 mm) on water uptake were established in ‘Burlat’, ‘Early Rivers’, ‘Heidelberg’, ‘Knauffs’, ‘Regina’, ‘Sam’, ‘Summit’, and ‘Van’. Water uptake was restricted to the exocarp by sealing the pedicel/fruit juncture. Effects of salts were established by performing sequential treatments on an individual fruit basis and salt effects indexed by the ratio F₃/F₁ as described above. To allow uptake through the exocarp to be related to whole fruit uptake, the contribution of penetration along the pedicel/fruit juncture was quantified in a separate experiment. Pedicels (5 mm in length) and receptacle were coated with silicone rubber (“control”). In a second group, the pedicel was removed and the hole above the endocarp sealed. Uptake along the pedicel/fruit juncture was calculated by difference.

**Effect of fruit development.** Response of developing ‘Regina’ fruit to CaCl₂ (30 mm) and FeCl₃ (25 mm) was established between stage II (55 DAFB) and the mature stage III (91 DAFB). The pedicel (5 mm in length) was sealed as described above; the pedicel/fruit juncture remained open (n = 10).

**Fruit cracking.** Two samples of 25 fruit each (‘Summit',...
‘Hedelfinger’, ‘Van’) were incubated in solutions of CaCl2 or FeCl3 at 25 °C. The pedicel at full length remained attached to the fruit. No silicone rubber was applied. At 2, 4, 6, and 10 h, fruit were inspected by naked eye for macroscopically visible cracks. Fruit with one or several cracks were removed from solution and the experiments continued. Cracking indices (CI) were calculated according to Christensen (1996) for each of the two samples using Eq. 1:

\[
CI = (5a + 3b + c) \cdot 100
\]

where a, b, and c represent the number of fruit cracked at 2, 4, and 6 h. To establish relationships between fruit cracking and water uptake, effects of salts on water uptake were quantified in parallel experiments on fruit from the same harvest. Pedicels were coated with silicone rubber to facilitate blotting; the pedicel/fruit juncture remained not coated. The initial number of fruit incubated was 10, of which a minimum of four (‘Van’), eight (‘Summit’), or nine (‘Hedelfinger’) remained macroscopically crack-free in the incubation assay.

**Scanning Electron Microscopy (SEM).** Epidermal segments were excised from mature ‘Van’ and ‘Hedelfinger’ fruit used for determining cracking indices. Segments were fixed by incubating for 1 h in 3% (w/v) glutaraldehyde in phosphate buffer at pH 7.4, followed by 1 h in 1% (w/v) osmium tetroxide in 40 mM acetate-veronal buffer containing 20% (v/v) 0.1 M HCl (pH 7.4; Palade, 1952). Subsequently, samples were dehydrated in an acetone series. Specimens were critical-point-dried in CO2 (CPD Palade, 1952). Subsequently, samples were dehydrated in an acetone series. Specimens were critical-point-dried in CO2 (CPD Palade, 1952). Specimens were critical-point-dried in CO2 (CPD Palade, 1952) and viewed using a JSM6340F (Jeol, Tokyo, Japan) SEM at accelerating voltages of 5 or 8 kV.

**Data Analysis.** Following water uptake experiments, fruit were inspected for macroscopically visible cracks. Data from fruit that were cracked were excluded from the analysis. The specified number of single fruit observations refers to the minimum number of fruit that remained crack free (by macroscopic inspection) during experiments. Where meaningful, data were subjected to analysis of variance (ANOVA). ANOVA, multiple comparisons of means, and regression analysis were carried out using the Statistical Analysis System software package (version 8.02; SAS Institute Inc., Cary, N.C.). Data in figures are presented as means ± se of means.

**Results.**

Effects on rates of water uptake differed among cations (Table 1). Salts of univalent cations had little effect except for LiCl, AgNO3, and NaCl, which increased (LiCl) or decreased (AgNO3 and NaCl) uptake, respectively. Chlorides of the divalent cations Cd, Cu, Hg, and Sr consistently decreased uptake. BaCl2 and ZnCl2 reduced uptake in only one of the two intervals. There was no effect of CaCl2, MgCl2, or MnCl2. All chlorides of trivalent cations significantly reduced uptake. CuCl2, HgCl2, AlCl3, and FeCl3 were most effective in decreasing water uptake among the salts tested.

The effect of FeCl3 on water uptake was independent of the presence of CaCl2, CuCl2, or AlCl3 (data not shown). Sequential or simultaneous treatments with CuCl2, or AlCl3 and FeCl3 had no significant effect on FII/FI, FIII/FI, or FIII/FII as compared to treatment with either of the salts alone (data not shown). However, sequential or simultaneous application of HgCl2, and FeCl3, decreased FII/FI, FIII/FI, and FIII/FII to a somewhat larger extent than either of the salts alone (Table 2). This effect, however, was small and only occasionally significant. Based on these data, toxicological considerations, and potential for field use, subsequent experiments focused on FeCl3 and CaCl2.

Water uptake into sweet cherry fruit increased linearly with time up to 5 h (Fig. 1A). FeCl3 decreased the rate of water uptake, but CaCl2 had no effect (Fig. 1B).

Increasing the concentration of FeCl3 up to 1 mm decreased rates of water uptake (Fig. 2). Concentrations of FeCl3 > 1 mm produced little additional effect. Again, CaCl2 had no effect on water uptake.

A minimum incubation period in FeCl3 of 0.08 h (5 min) was necessary to induce a detectable decrease in water uptake (Fig. 3). When fruit were exposed to FeCl3 for 0.25 h or longer, rates of uptake remained at the decreased level during subsequent incubation in water.

Uptake along the parallel pathway of the pedicel/fruit juncture was only little affected by FeCl3. The 17.5% and 13.4% decrease in FII/FI and FIII/FI as compared to the water control was nonsignificant (P = 0.055 and 0.113 for FII/FI and FIII/FI, respectively).

Rates of water uptake differed between cultivars (Table 3). Highest rates were obtained in ‘Van’, ‘Hedelfinger’, and ‘Burlat’, the lowest in ‘Early Rivers’, ‘Sam’, and ‘Summit’. Restricting penetration to the exocarp by sealing the pedicel/fruit juncture significantly decreased uptake on average by 46%.

CaCl2 decreased water uptake through the exocarp only in ‘Burlat’, ‘Early Rivers’, and ‘Hedelfinger’ (Table 4). In contrast, FeCl3 was effective in essentially all cultivars except for ‘Knauffs’ (P = 0.06). Across all cultivars, the FII/FI ratio averaged 0.62 and 1.06 for FeCl3, and H2O, respectively, equivalent to a 42% reduction in uptake through the exocarp.

### Table 1. Effect of selected salts (10 mM) on rates of water uptake (F) through the exocarp of mature ‘Sam’ sweet cherry fruit. Effect of salts were indexed by the ratio of flow rates (FII/FI or FIII/FI) after replacing the water donor (F, 0 to 0.75 h) by a salt solution (F, 0.75 to 1.5 h; FII, 1.5 to 2.25 h).

| Sequence of donor | in interval I, II, and III | n | FII/FI (ratio) | FIII/FI (ratio) |
|-------------------|---------------------------|---|---------------|----------------|
| H2O/H2O/H2O       | 50                        | 0.87 ± 0.01a | 0.91 ± 0.01a  |
| H2O/AgNO3/AgNO3   | 9                         | 0.65 ± 0.01b | 0.75 ± 0.01a  |
| H2O/KCl/KCl       | 7                         | 0.82 ± 0.01a | 0.91 ± 0.01a  |
| H2O/LiCl/LiCl     | 21                        | 1.00 ± 0.01b | 1.03 ± 0.01b  |
| H2O/NaCl/NaCl     | 8                         | 0.70 ± 0.01b | 0.82 ± 0.01a  |
| H2O/NaHCl/NaHCl   | 10                        | 0.85 ± 0.01a | 0.90 ± 0.01a  |
| H2O/BA/BA/Cl2     | 7                         | 0.67 ± 0.01b | 0.77 ± 0.01a  |
| H2O/CaCl2/CaCl2   | 20                        | 0.75 ± 0.01a | 0.84 ± 0.01a  |
| H2O/CdCl2/CdCl2   | 8                         | 0.69 ± 0.01b | 0.63 ± 0.01b  |
| H2O/CuCl2/CuCl2   | 7                         | 0.42 ± 0.01b | 0.36 ± 0.01b  |
| H2O/HgCl2/HgCl2   | 7                         | 0.58 ± 0.01b | 0.38 ± 0.01b  |
| H2O/MgCl2/MgCl2   | 18                        | 0.88 ± 0.01a | 1.03 ± 0.01a  |
| H2O/MnCl/MnCl2    | 16                        | 0.81 ± 0.01a | 0.79 ± 0.01a  |
| H2O/SrCl2/SrCl2   | 14                        | 0.69 ± 0.01b | 0.68 ± 0.01b  |
| H2O/ZnCl2/ZnCl2   | 8                         | 0.72 ± 0.01a | 0.75 ± 0.01a  |
| H2O/AlCl3/AlCl3   | 17                        | 0.50 ± 0.01b | 0.50 ± 0.01b  |
| H2O/EuCl3/EuCl3   | 12                        | 0.58 ± 0.01b | 0.62 ± 0.01b  |
| H2O/FeCl3/FeCl3   | 54                        | 0.49 ± 0.01b | 0.40 ± 0.01b  |

*Mean within columns followed by the letter b differ significantly from the water control by Dunnett’s t test, P = 0.05. FII, FIII, and FIII for the water control averaged 2.6 ± 0.1, 2.3 ± 0.1, and 2.4 ± 0.2 mg·h⁻¹, respectively.*
Table 2. Interaction of HgCl₂ with FeCl₃ on rates of water uptake (F) through the exocarp of mature ‘Sam’ sweet cherry fruit. Effect of salts were indexed by the ratio of flow rates (FII/FI, FIII/FI, FIII/FII) calculated from flow rates during interval I (FI, 0 to 0.75 h), II (FII, 0.75 to 1.5 h) and III (FIII, 1.5 to 2.25 h). Concentrations of salts were 10 mM except when used simultaneously (5 mM each).

| Sequence of donor in interval I/II/III | n  | FII / FI | FIII / FI | FIII / FII |
|----------------------------------------|----|----------|-----------|-----------|
| H₂O/H₂O/H₂O                            | 10 | 0.93 a   | 0.98 a    | 1.08 a    |
| H₂O/FeCl₃/FeCl₃                        | 9  | 0.49 bc  | 0.44 bc   | 0.93 ab   |
| H₂O/FeCl₃/HgCl₂                        | 10 | 0.47 bc  | 0.36 bc   | 0.83 ab   |
| H₂O/HgCl₂/HgCl₂                        | 10 | 0.67 b   | 0.47 b    | 0.71 bc   |
| H₂O/FeCl₃/FeCl₃                        | 10 | 0.61 bc  | 0.27 cd   | 0.45 c    |
| H₂O/FeCl₃ + HgCl₂/FeCl₃ + HgCl₂        | 8  | 0.42 c   | 0.26 d    | 0.68 bc   |

Means separation within columns by Tukey’s Studentized range test, P = 0.05. FI, FII, and FIII for pooled water controls averaged 2.6 ± 0.2, 2.4 ± 0.2, and 2.5 ± 0.2 mg·h⁻¹, respectively.

Fig. 1. Effect of CaCl₂ and FeCl₃ on the time course of water uptake into mature ‘Regina’ sweet cherry fruit. (A) Cumulative uptake. (B) Rate of uptake (F).

Fig. 2. Effect of concentration of (A) CaCl₂ and (B) FeCl₃ on water uptake into mature ‘Regina’ sweet cherry fruit. Main graphs: Ratio of flow rates. Insets: Absolute rates of uptake (F). Effect of salts were indexed by the ratio of flow rates (FII/FI or FIII/FI) after replacing the water donor (FI, 0 to 0.75 h) by a salt solution (FII, 0.75 to 1.5 h; FIII, 1.5 to 2.25 h). Horizontal solid and dashed lines in insets represent FII/FI and FIII/FI ratios for the water control, respectively.

Fig. 3. Effect of duration of incubation of mature ‘Summit’ fruit in FeCl₃ on water uptake through the exocarp. Rate of water uptake from a water donor during the first (0 to 0.75 h) and third interval (1.5 to 2.25 h) is referred to as F₁ and F₃. During the second interval (0.75 to 1.5 h), fruit were incubated for 0 and 45 min, 1 and 44 min, 5 and 40 min, 15 and 30 min, 30 and 15 min, and 45 and 0 min in 10 mM FeCl₃ and H₂O, respectively, and the rate of uptake is referred to as F₂. The effect of FeCl₃ was indexed by the ratios F₂/F₁ and F₃/F₁. Main graph: Change in rates of uptake during phase II (F₂/F₁) and III (F₃/F₁) vs. duration of incubation in FeCl₃. Insets: Absolute rates of water uptake during the second and third intervals.
There was no consistent change in water uptake between 55 and 91 DAFB, when mass of ‘Regina’ fruit increased from 1.8 ± 0.0 (stage II) to 9.2 ± 0.1 g (stage III; Fig. 4A and B). Normalizing data for uptake in the water control revealed that CaCl$_2$, and FeCl$_3$ to a larger extent, decreased water uptake throughout fruit development. The effect of CaCl$_2$ remained about constant, but the effect of FeCl$_3$ increased up to 70 DAFB and then decreased towards maturity (Fig. 4C).

When incubating ‘Summit’ (Fig. 5A and C), ‘Van’ (Fig. 5E), or ‘Hedelfinger’ (data not shown) fruit in water, percentage of cracked fruit increased rapidly and approached 100% within 10 h. CaCl$_2$ decreased cracking at 2.5 and 25 mM, but FeCl$_3$ was more effective at both concentrations (Fig. 5A). The lowest effective FeCl$_3$ concentrations were 1 mM (‘Summit’) and 2.5 mM (‘Van’ and ‘Hedelfinger’), and higher concentrations resulted in greater response (Fig. 5 C and E; Table 5). Effects of salt concentration on water uptake into ‘Summit’ (CaCl$_2$, FeCl$_3$), ‘Van’ (FeCl$_3$), or ‘Hedelfinger’ (FeCl$_3$) were qualitatively similar to those obtained with ‘Regina’ (data not shown, for ‘Regina’ see Fig. 2). Since data on water absorption were generated on fruit from the same batch as those used for determining cracking, cracking was analyzed as a function of the amount of water taken up. Fruit incubated in CaCl$_2$ sustained higher water uptake without cracking than control fruit. This effect was even more pronounced with FeCl$_3$. The amount of water taken up at a given percentage of fruit cracking increased with increasing FeCl$_3$ concentrations (Fig. 5 B, D, and F).

It is important to note that appearance of fruit incubated in FeCl$_3$, but not in CaCl$_2$, AlCl$_3$, or water, changed during the course of a cracking experiment. In the first 2-h incubation interval, small

Table 3. Rates of water uptake (F, 0 to 3 h) through exocarp and pedicel/fruit juncture in selected sweet cherry cultivars. Uptake through exocarp plus pedicel/fruit juncture was determined in fruit having the pedicel except for the pedicel/fruit juncture sealed with silicone rubber, uptake through the exocarp only in fruit with pedicel removed and hole above stony endocarp sealed. Penetration along the pedicel/fruit juncture was calculated by difference (n ≥ 4).

| Cultivar | Exocarp plus pedicel/fruit juncture | Exocarp only | Pedicel/fruit juncture |
|----------|-------------------------------------|--------------|------------------------|
| Burlat   | 23.6                                | 6.3          | 17.9 ab                |
| Early Rivers | 10.4                                | 6.6          | 8.5 cd                |
| Hedelfinger | 24.7                                | 15.7         | 10.4 a              |
| Knauffs  | 13.8                                | 5.8          | 10.7 bd               |
| Regina   | 14.9                                | 9.2          | 11.3 bc              |
| Sam      | 11.8                                | 5.3          | 8.6 cd              |
| Summit   | 11.8                                | 5.4          | 8.8 cd                   |
| Van      | 25.1                                | 18.3         | 8.2 bc              |
| Mean$_{cultivar}$ | 16.6 a                      | 8.9 b          |                         |

*Main effect cultivar and sealing pedicel/fruit juncture significant at P = 0.0001. Interaction cultivar × sealing nonsignificant (P = 0.52). Mean separation by Tukey’s Studentized range test, P = 0.05.

Table 4. Effect of CaCl$_2$ (30 mM) and FeCl$_3$ (25 mM) on rates of water uptake (F) through the exocarp in selected sweet cherry cultivars. Uptake was confined to the exocarp by removing the pedicel and sealing the hole above the stony endocarp with silicone rubber. Effects of salts were indexed by the ratio of flow rates (F$_{II}$/F$_{I}$) after replacing the water donor (F$_{I}$, 0 to 0.75 h) by a salt solution (F$_{II}$, 0.75 to 1.5 h; n ≥ 6).

| Cultivar | H$_2$O | CaCl$_2$ | FeCl$_3$ |
|----------|--------|---------|----------|
| Burlat   | 1.28 a | 0.92 b  | 0.66 c   |
| Early Rivers | 1.08 a | 0.87 b | 0.72 c   |
| Hedelfinger | 1.06 a | 0.89 b | 0.47 c   |
| Knauffs  | 0.98 a | 0.73 a  | 0.61 a   |
| Regina   | 1.13 a | 1.15 a  | 0.50 b   |
| Sam      | 1.00 a | 0.84 a  | 0.51 b   |
| Summit   | 0.87 a | 0.87 a  | 0.57 b   |
| Van      | 0.95 a | 0.88 a  | 0.48 b   |
| Grand mean | 1.06  | 0.97 a  | 0.62     |

*Main effects of cultivar and salts, and the interaction between cultivar and salts, are all significant. Mean separation within cultivars by Tukey’s Studentized range test, at P = 0.05.

Fig. 4. (A) Time course of change in mass of developing ‘Regina’ sweet cherry fruit between 55 and 91 d after full bloom (DAFB). Arrows indicate duration of stages II and III of fruit development. (B, C) Effect of CaCl$_2$ and FeCl$_3$, on absolute (B) and relative rates of water uptake (C).
black spots formed on the surface of some fruit. Generally, there were no spots in proximate portions of cheek and suture regions, but spot density increased towards the stylar end. Also, black spots developed in the pedicel cavity adjacent to the pedicel/fruit junction. The area of these spots increased with incubation time and FeCl₃ concentration until significant portions of the fruit surface, particularly in stylar end and pedicel cavity regions, were discolored black at 10 h. Macroscopically, the fruit surface in these areas appeared intact. However, inspection by SEM revealed a fine network of closely spaced cracks that were limited to the cuticle and that did not extend into the exo- and mesocarp (Fig. 6B). In contrast, the cuticle in non-discolored adjacent to discolored regions of the same fruit was crack-free (Fig. 6A).

Larger, macroscopically visible cracks, that occasionally developed after longer periods of incubation in FeCl₃, also differed from those of control fruit (Fig. 6 C vs. D). Cracks in control fruit extended deeply into the mesocarp, sometimes as deep as the stony endocarp, but those from fruit in FeCl₃ solutions were shallow and confined to cuticle and outermost cell layer(s) of the exocarp (Fig. 6 C vs. D).

**Discussion**

Our data demonstrate that some mineral salts markedly decrease water uptake and reduce cracking. Among the most effective salts were CuCl₂, HgCl₂, AlCl₃ and FeCl₃. Effects of CaCl₂ on water uptake, however, were inconsistent. In some cultivars rates of uptake were reduced, but not in others (Tables 1 and 4).

The rate of water uptake (F) across the sweet cherry fruit exocarp is a composite quantity depending on several factors (Eq. 2; Nobel, 1999, modified):

\[
F = A \cdot J \cdot \rho = A \cdot L_w \cdot \rho \cdot \Delta \Psi
\]  

In this equation, F (kg·s⁻¹) equals the product of the surface area (A in m²), the volume flux density of water (J in m³·m⁻²·s⁻¹) and the density (ρ in kg·m⁻³) of water. The J, in turn, corresponds to the product of the water conductivity coefficient (Lw in m·s⁻¹·MPa⁻¹) of the exocarp and the gradient in water potential (ΔΨ in MPa) across the fruit surface. Lw is related to the conductance for water uptake (guptake in m·s⁻¹) used in our earlier study (Beyer and Knoche, 2002; alternate expression: filtration or osmotic permeability, Pf in m·s⁻¹; Nobel, 1999; Schönherr, 1982) by Eq. 3:

\[
L_w = g_{uptake} \cdot \frac{V_w}{RT} = P_f \cdot \frac{V_w}{RT}
\]

where \( V_w \) (in m³·mol⁻¹) represents the partial molar volume of water, R (in m³·MPa⁻¹·mol⁻¹·K⁻¹) the universal gas constant, and T (in K) the absolute temperature. Based on Eq. 2, effects on water uptake must be accounted for by effects on A, Lw, and/or ΔΨ. Since A remained essentially constant, ΔΨ and/or Lw of the exocarp must have changed. Furthermore, in non-sealed fruit, the pedicel/fruit juncture represents a significant parallel pathway for water uptake (Beyer et al., 2002b) that could also be affected by salts.
EFFECTS ON PERMEABILITY OF THE CHERRY FRUIT EXOCARP. Earlier studies established that AlCl₃, EuCl₃, and FeCl₃, but not CaCl₂, decreased conductance of the sweet cherry exocarp (Beyer et al., 2002a). These effects were related to the cation and the chloride anion had no effect. Beyer et al. (2002a) hypothesized that formation of Al and Fe oxides and hydroxides caused by a pH dependent precipitation reaction may be responsible for the marked decrease in conductance. In an aqueous solution of CuCl₂, Cu(OH)₂ would form upon hydrolysis. Since the solubility product of Cu(OH)₂ is low and likely to be exceeded at the pH encountered in the apoplast of the exocarp, precipitation would occur resulting in decreased conductance. Similarly, effects of AgNO₃ and HgCl₂ may also be accounted for by a precipitation reaction. First, deposits of reduced Ag were demonstrated in the stomatal pore of pear leaves following treatment with AgNO₃ (Greene and Bukovac, 1974). Second, HgCl₂ is readily reduced to HgCl (Franke, 1961) and solubility of HgCl₂ is low. Further support for this hypothesis comes from early work on foliar uptake, where mercurial deposits were observed after treatment of leaves with HgCl₂ (Franke, 1961, 1964). The sites, where these deposits occurred, represent regions in the cuticle that are preferentially permeable to polar substances such as the above electrolytes and possibly, water (Schönherr and Bukovac, 1970). Wade (1988) hypothesized that HgCl₂ reduced uptake by inhibiting metabolism. However, in our earlier studies, carbonylcyanide and NaN₃ did not decrease water transport through excised exocarp segments (Beyer and Knoche, 2002; Knoche et al., 2000). Furthermore, since water transport is a physical process, effects on metabolism are unlikely to affect water uptake. It is interesting to note that mercurials are effective inhibitors of water transport through aquaporins (Tyerman et al., 1999). However, to our knowledge aquaporins have not been identified in plant cuticles. Since water permeability of the sweet cherry fruit exocarp is several orders of magnitude lower than that of the plasma membrane (10⁻⁸ vs. 10⁻¹⁴ m⁻¹·s⁻¹ for exocarp vs. plasma membranes, respectively; Beyer and Knoche, 2002; Nobel, 1999), effects of HgCl₂ on aquaporins in plasma membranes would not explain the marked reduction in water flow rates through the exocarp.

The effects of NaCl, BaCl₂, CdCl₂, SrCl₂, and ZnCl₂ that occasionally were significant are unlikely to be accounted for by precipitation, since solubility products of the respective hydroxides are sufficiently high. The mechanism of decreasing water penetration is currently unknown for these salts and may possibly be related to altered mechanical properties of the fruit (see below).

EFFECT ON THE DRIVING FORCE FOR WATER UPTAKE (ΔΨ). According to van’t Hoff’s law (Nobel, 1999), the osmotic pressure of a dilute solute solution is proportional to its concentration and, for electrolytes, proportional to the concentration of ions. Thus, salts decrease the osmotic potential of the donor solution thereby decreasing ΔΨ. In our experiment (Table 1), salts of trivalent cations should have the largest effect on the osmotic potential and these were indeed among the most effective (Table 1). Assuming complete dissociation and ideal behavior, the maximum decrease in ΔΨ due to osmotic effects would amount to –0.10 MPa for a 10 mm solution of a salt of a trivalent cation. At a water potential of –1.42 MPa (‘Napoleon’, Andersen and Richardson, 1982), the maximum decrease in ΔΨ due to osmotic effects would be about 7% of the water control (for water FII/FI and FIII/FI were 0.87 and 0.91, respectively). Hence, ratios of FII/FI and FIII/FI lower than 0.80 and 0.84, respectively, cannot be accounted for by osmotic effects and must have a different basis (Table 1). Also, osmotic effects would be non-specific and hence, would occur with any salt present in the donor solution.

Alternatively, salts may have altered the ΔΨ by altering the fruit’s water potential. The sweet cherry fruit exocarp is markedly stained during fruit development (Knoche et al., 2001, 2004; Tukey and Young, 1939) and the turgor pressure generated by the strained exocarp lowers the driving force for water uptake. Increased rigidity of cell walls as a result of a cross-linking of cell wall components by divalent or trivalent cations could therefore decrease water uptake. Several arguments support this hypothesis. First, effects of CaCl₂ on fruit firmness are well established (Johnston et al., 2002) and also documented for sweet cherry (Lidster et al., 1978, 1979). Second, analysis of cracking as a function of water uptake demonstrated that for a given amount of water uptake percentage of cracked fruit from CaCl₂ and even more so from FeCl₃, treatments was always lower than in the control (vertical comparison: Fig. 5 B, D, and F). Accordingly, fruit from CaCl₂ and FeCl₃ treatments survived higher uptake at a given percentage of cracked fruit than the water control (horizontal comparison; Fig. 5 B, D, and F). If salt effects were limited to water uptake, a single relationship for salt and control treatments would be obtained. This, however, was not the case. Third, cracks that occasionally also formed in fruit incubated in FeCl₃ were always shallow and rarely extended into the mesocarp, while those in control fruit often extended deeply into the mesocarp (Fig. 6 C and D).

EFFECTS ON PENETRATION ALONG THE PEDICEL/FRUIT JUNCTURE. FeCl₃ had little effect on water uptake along this pathway since penetration into fruit that was sealed except for the juncture was...
reduced by only 17.5% (FII/F) and 13.4% (FIII/F). The absence of a larger effect on penetration along the juncture may also account for the decrease in effectiveness of FeCl₃ in developing ‘Regina’ fruit, since juncture penetration markedly increases towards maturity (Beyer et al., 2002).

**Practical Implications.** From a practical point of view, the effect of FeCl₃, but unlikely that of HgCl₂, AlCl₃, or other heavy metals, may be useful provided a Fe salt suitable for spray application can be identified. Our data demonstrate that FeCl₃ and possibly other Fe³⁺ salts are effective in decreasing water uptake and reducing fruit cracking in all cultivars investigated. However, some caution is needed. First, our data were obtained in laboratory assays where entire surfaces of whole fruit (this study) or excised exocarp segments (Beyer et al., 2002a) were exposed to Fe containing, donor solutions. Following spray application in the field only a limited portion of the fruit surface would be in contact with spray solution. Based on the proposed mode of action, Fe effects on water uptake would be limited to those portions of the fruit surface in direct contact with solution making maximum coverage an essential requirement for a detectable response. Second, the discoloration observed on fruit incubated in FeCl₃, is critical from a consumer’s point of view. At present it is not known whether other Fe salts have the same limitation. This would be expected, if the precipitation products formed in the exocarp were the primary cause for discolored fruit. Last, phytotoxicity of the acidic FeCl₃ solution and formation of spray residues on fruit are potential problems that would have to be resolved before application of Fe salts in the field can be evaluated as a potential cultural treatment to reduce water uptake and cracking in sweet cherry fruit.

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