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In Strawberries, Calcium Uptake and Water Soaking Are Negatively Related

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Abstract. Water soaking is a common disorder of field-grown strawberries, and its incidence can be severe. Calcium (Ca) sprays reduce susceptibility to water soaking. Vascular import of Ca to developing strawberry fruit decreases during development as a result of progressive xylem dysfunction. Calcium sprays can raise Ca levels in late-stage strawberry fruit. The objective of this study was to quantify Ca uptake through the fruit skin. Submerging fruit in calcium chloride ($CaCl_2$) solutions increased both the mass of Ca per fruit and the mass of Ca per fruit dry mass. There was no effect of $CaCl_2$ solution concentration on fruit water uptake. Fruit mass increases exponentially during development. When in a $CaCl_2$ solution, water soaking was increased, but less severely, rather than when in pure water. Fruit Ca content and fruit size were related positively, but the Ca/dry mass ratio was independent of fruit size. Both were consistently higher for fruit incubated in $CaCl_2$ than for control fruit incubated in water. There was no preferential Ca uptake through the calyx or through the abscission scars from petals or stamens. Both fruit Ca content and the fruit Ca/dry mass ratio were related positively to fruit $CaCl_2$ concentration and were increased by incubation in solutions of Ca salts. There was no effect of temperature on fruit Ca content or the Ca/dry mass ratio. At all temperatures, fruit incubated in $CaCl_2$ had greater Ca contents and Ca/dry mass ratios than fruit incubated in water. Inducing microcracking in the cuticle had no significant effect on the water uptake rate, on the Ca content, or on the Ca/dry mass ratio. Across all experiments, Ca content and the Ca/dry mass ratio correlated negatively with water soaking. In summary, Ca is taken up from a Ca salt solution in contact with the fruit skin, and fruit Ca content and susceptibility to water soaking are related negatively.

Strawberry is a highly perishable fruit as a result of its especially thin skin and the soft texture of its flesh (Darrow 1966). In very many fruit-crop species, fruit calcium (Ca) content is a critical determinant of fruit quality, with both pre- and postharvest performance being increased by elevated levels of fruit Ca. Although, in botanical terms, a strawberry is not strictly a fruit, this same behavior applies also in strawberry (Cieniawska et al. 2023; Makus and Morris 1998). Recent evidence demonstrates that Ca can also reduce the susceptibility of strawberries to water soaking (WS) (Hurtado and Knoche 2022). WS is a common disorder of field-grown strawberries that impairs fruit quality by reducing the shelf life, compromising fruit appearance, and increasing fruit rot (Herrington et al. 2009, 2011; Hurtado and Knoche 2021).

Calcium is taken up from the soil by the roots and is transported to the aboveground organs in the xylem stream. The xylem flows are mostly driven by transpiration (Kalcsits et al. 2017; Marschner 1995). Strawberry fruit Ca content (mass per fruit) increases during development, but the Ca/dry mass ratio decreases. The Ca/dry mass ratio decrease occurs consistently in the skin, the pith, the flesh, and the achenes (Hurtado et al. 2025). The only exception is in the calyx, where both the Ca content and the Ca/dry mass ratio increase during development. The decrease in Ca/dry mass ratio in developing strawberry fruit results from 1) a progressive loss of xylem functionality that begins at the distal end of the fruit and progresses to the proximal end (Winkler et al. 2021), and 2) a marked increase in fruit dry mass that "dilutes" the fruit Ca already taken up (Telias et al. 2006). Hence, similar to apples (Drazeta et al. 2004; Griffith and Einhorn 2023; Lang 1990; Schlegel and Schönherr 2002), kiwifruit (Dichio et al. 2003), and sweet cherry (Grimm et al. 2017; Winkler et al. 2020), the Ca content and Ca/dry mass ratios of strawberries cannot be increased by broadcast fertilization of the soil. Instead, to increase fruit Ca, the Ca must be applied directly to the fruit as a spray. Unfortunately, little is known about Ca uptake through the strawberry fruit surface from a spray. Thus, the objective of our study was to quantify Ca uptake through the strawberry fruit skin and to identify the factors affecting this uptake.

Materials and Methods

Plant material. Strawberry fruit (*Fragaria ×ananassa* Duch.) cvs. Clery, Flair, and Florentina were harvested from commercial high tunnels at Laatzen, Germany (lat. 52°16′N, long. 9°50′E), and from growth chambers on the Herrenhausen Campus of the Leibniz University, Hannover, Germany, in the 2022 and 2023 growing seasons. 'Flair' and 'Clery' were grown in the tunnels; 'Clery' and 'Florentina', in a growth chamber. The temperature and relative humidity (RH) of the growth chamber were set at 20/16 °C and 60%/80%, respectively, during a 16/8-h day/night photoperiod.

Individual fruit were harvested at commercial ripeness [> 80% of the fruit surface red (Mitcham 2025)], unless specified otherwise, and were selected carefully for uniformity of size,

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Table 1. Calcium (Ca) content and Ca/dry mass ratio of halves of cv. Clery strawberry with and without water-soaking damage after 24 h in deionized water. The effect of water soaking was established by incubating fruits in deionized water for 24 h, with their longitudinal axis horizontal such that they were part submerged. Half the fruit was below the surface and half above the surface (this served as the control). Water soaking was quantified using a five score rating scheme where score 0 = no water soaking; score 1 = <10% of the surface area water-soaked; score 2 = 10% to 35%; score 3 = 35% to 60%; and score 4 = >60% of the surface area water-soaked.

Treatment	Water soaking (rating)	Ca content (mg per half fruit)	Ca/dry mass ratio ($mg \cdot g^{-1}$ DM)
Above water (control)	0.0 ± 0.0 a[i]	0.86 ± 0.01 a	2.41 ± 0.06 a
Below water (treatment)	3.2 ± 0.2 b	0.75 ± 0.03 a	2.32 ± 0.02 a

[i] Means within columns followed by the same letter are not significantly different, Tukey's studentized range test, $P = 0.05$.

shape, and color, and the absence of visual defects. Mean fruit mass was 18.1 ± 0.2 g/fruit.

General procedure. The calyx region of a strawberry fruit is a zone of preferential water uptake resulting from the presence of multiple cuticular microcracks and the presence of scars following the abscission of petals and stamens (Hurtado and Knoche 2023a). Unless specified otherwise, the calyx was removed carefully and the resulting wound was sealed using a fast-curing nonphytotoxic silicone rubber (Silicone rubber, SE 9186 Clear; Dow Corning, Tokyo, Japan) (Hurtado et al. 2021). Fruit were incubated individually in beakers containing 100 mL of 10 mM calcium chloride ($CaCl_2$) or deionized water for up to 16 h. The pH of the 10 mM $CaCl_2$ solution was pH 6.7. Fruit were held submerged to a depth of ~1.5 cm using a soft polypropylene foam plug. Unless specified otherwise, Ca uptake, water uptake, and WS were quantified.

After incubation, the fruit were rinsed with deionized water and, thereafter, with 10 mM citric acid to eliminate any residual Ca present on the fruit surface (Winkler and Knoche 2021a). Fruit were then frozen at –20 °C. Subsequently, fruit were freeze-dried for 3 to 4 d. Samples were then dried at 103 °C for 15 d and ground using a ball mill (MM 400 mill; Retsch, Haan, Germany). The resulting powder was redried for a minimum of 3 d at 103 °C. An aliquot of 100 mg was ashed in a muffle furnace (L24/11/B180; Nabertherm, Lilienthal, Germany) at 500 °C. When the ashing was incomplete, as indicated by the presence of dark-black ash, the ash was taken up using a few drops of 1 N hydrogen chloride (HCl) and then re-ashed. The ash was taken up in 2 mL of 1 N HCl and 8 mL deionized water and filtered (MN 640 M; Macherey-Nagel, Dueren, Germany). To prevent potential interference with phosphorus during Ca measurement by atomic absorption spectroscopy (AAS), 1% lanthanum chloride was added to the extract (Fishman and Downs 1996). Analysis of the solutions was carried out by AAS (1100B; Perkin Elmer, Shelton, CT, USA) equipped with a hollow cathode lamp for Ca–magnesium and an air–acetylene flame. Solutions were diluted as needed to achieve a concentration within the measuring range (0–4 $mg \cdot L^{-1}$) of the AAS. There were three replications, with one replicate consisting of 8 to 10 fruit. Calcium uptake was calculated as the Ca content, on a whole-fruit basis (mg Ca/fruit) or as the Ca/dry mass ratio ($mg \cdot g^{-1}$ dry mass).

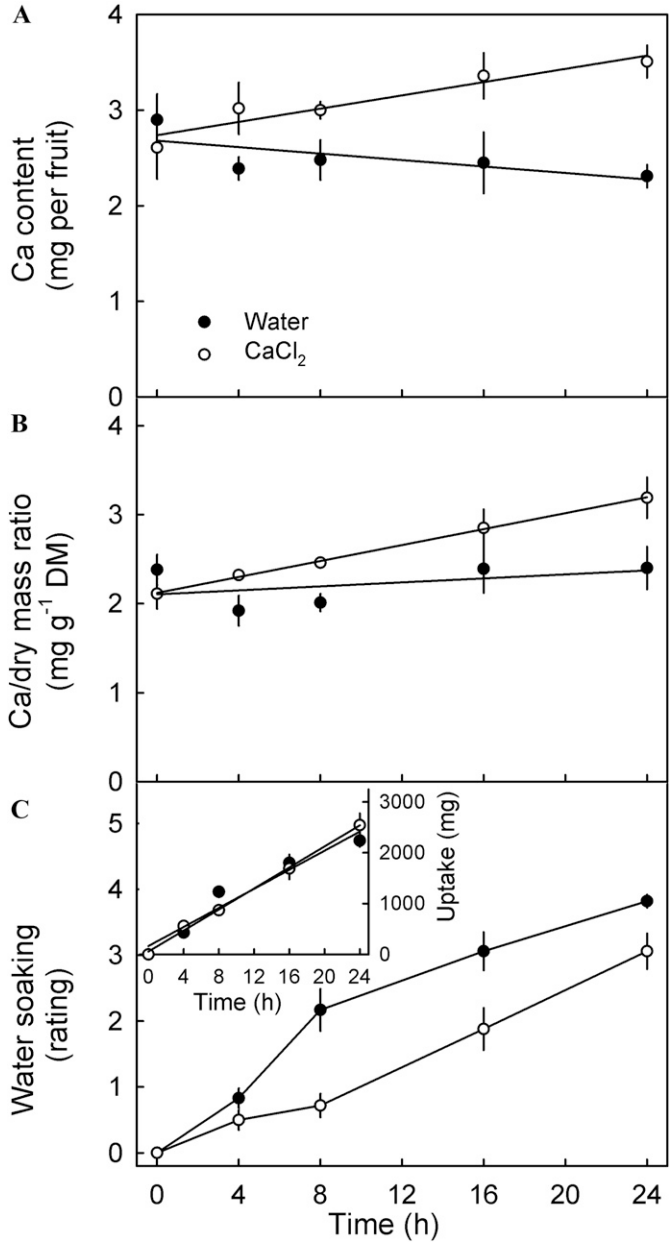

Fig. 1. Time course of change in (**A**) Ca content, (**B**) Ca/dry mass ratio, and (**C**) area affected by water soaking of 'Florentina' strawberry fruit immersed in deionized water or in 10 mM of $CaCl_2$. Inset in **C**: time course of water uptake. Water soaking was quantified using a five score rating scheme where score 0 = no water soaking; score 1 = <10% of the surface area water-soaked; score 2 = 10% to 35%; score 3 = 35% to 60%; and score 4 = >60% of the surface area water-soaked.

Water uptake was determined gravimetrically before and after incubation. WS was quantified using a 5-point rating scale (Hurtado and Knoche 2021). The rating scale was: score 0 = no water soaking; score 1 = <10% of the surface area water-soaked; score 2 = 10% to <35% water-soaked; score 3 = 35% to 60% water-soaked; score 4 = >60% of water-soaked (Hurtado and Knoche 2021) (Supplemental Fig. 1). There were 20 individual fruit replicates for WS and water uptake assessment. Preliminary experiments carried out using ripe 'Clery' raised in the growth chamber established that WS had no effect on the fruit Ca content or on the Ca/dry mass ratio (Table 1).

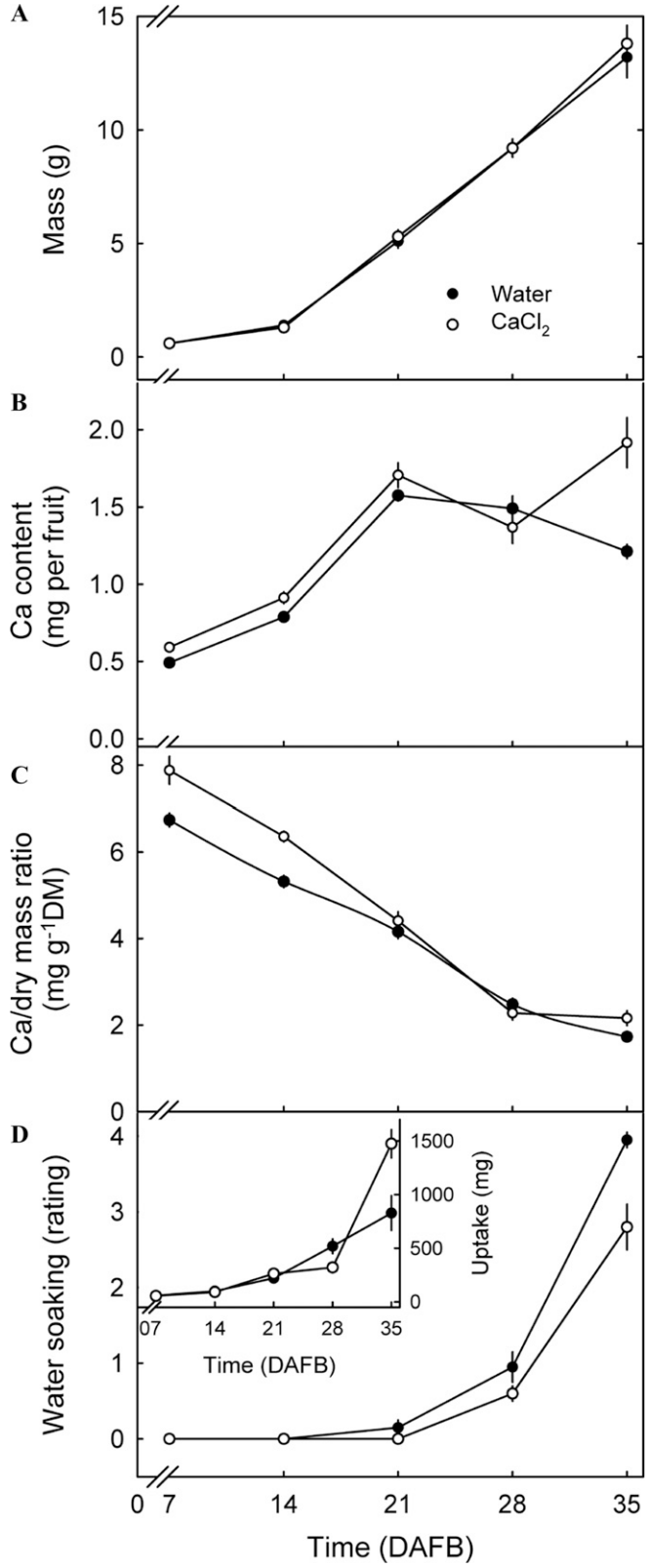

Fig. 2. Developmental time course of (**A**) change in mass, (**B**) Ca content, (**C**) Ca/dry mass ratio, and (**D**) area affected by water soaking of 'Clery' strawberry fruit immersed in deionized water or in 10 mM of $CaCl_2$ for 16 h. Inset in **D**: developmental time course of water uptake. Water soaking was quantified using a five score rating scheme where score 0 = no water soaking; score 1 = <10% of the surface area water-soaked; score 2 = 10% to 35%; score 3 = 35% to 60%; and score 4 = >60% of the surface area water-soaked. DAFB = days after full bloom.

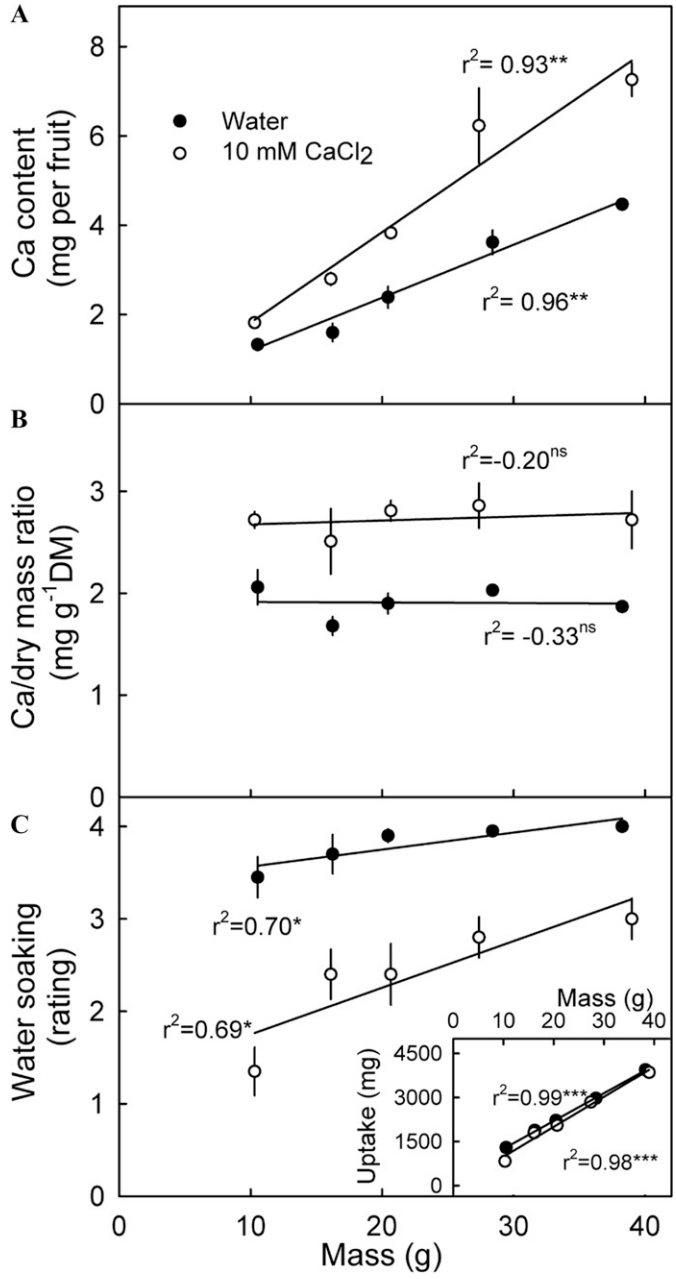

Fig. 3. Fruit size effect on change in (**A**) Ca content, (**B**) Ca/dry mass ratio, and (**C**) area affected by water soaking of 'Flair' strawberry. Inset in **C**: effect of fruit size on water uptake. Significance levels (P values < 0.05): *** = 0.001, ** = 0.01, * = 0.05; ns = nonsignificant. Water soaking was quantified using a five score rating scheme where score 0 = no water soaking; score 1 = <10% of the surface area water-soaked; score 2 = 10% to 35%; score 3 = 35% to 60%; and score 4 = >60% of the surface area water-soaked.

Experiments. The time course of Ca uptake after submerging ripe 'Florentina' fruit from the growth chamber in 10 mM $CaCl_2$ was established after 0, 4, 8, 16, and 24 h of incubation. Fruit submerged in deionized water served as the control. Calcium uptake and WS were quantified as described earlier.

The developmental time course of Ca uptake was established in 'Clery' from the growth chamber at 7, 14, 21, 28, and 35 d after full bloom (DAFB). Fruit were incubated in 10 mM $CaCl_2$ or deionized water for 16 h. Fruit color was quantified using a colorimeter (CM-2600 d; Konica Minolta, Tokyo, Japan).

Table 2. Effect of the presence or absence of a calyx on Ca content and Ca/dry mass ratio, water soaking, and water uptake of 'Clery' strawberry fruit incubated for 16 h in deionized water and in 10 mM $CaCl_2$. 'Treated' refers to fruit with the calyx removed and the wound sealed with silicone rubber; 'Control' refers to fruit with calyx present. 'Calyces' refers to the organs removed from the controls after incubation. These were measured separately. Water soaking was quantified using a five score rating scheme where score 0 = no water soaking; score 1 = <10% of the surface area water-soaked; score 2 = 10% to 35%; score 3 = 35% to 60%; and score 4 = >60% of the surface area water-soaked.

Treatment	Solution	Ca content (mg per fruit/calyx)	Ca/dry mass ratio ($mg \cdot g^{-1}$ DM)	Water soaking (rating)	Water uptake (mg)
Control	Water	2.09 ± 0.17	2.04 ± 0.18	2.5 ± 0.2	1249.3 ± 106.6
Treated	Water	1.91 ± 0.11	1.96 ± 0.09	2.5 ± 0.2	1170.1 ± 99.7
Mean	—	2.00 ± 0.10 a[i]	2.00 ± 0.07 a	2.5 ± 0.2 a	1209.7 ± 68.2 a
Control	$CaCl_2$	2.50 ± 0.14	2.42 ± 0.07	1.7 ± 0.2	791.8 ± 77.2
Treated	$CaCl_2$	2.45 ± 0.14	2.38 ± 0.03	1.9 ± 0.2	814.1 ± 99.7
Mean	—	2.47 ± 0.10 b	2.40 ± 0.07 b	1.8 ± 0.2 b	803.0 ± 68.2 b
Calyces	Water	0.41 ± 0.03 ns	17.63 ± 0.34 ns	—	—
Calyces	$CaCl_2$	0.51 ± 0.05	20.43 ± 1.77	—	—

[i] Means within "solution" followed by the same letter are not significanlty different, Tukey's studentized range test, $P = 0.05$.
ns = Nonsignificant effect of calyx Ca content after incubation.

Color, Ca uptake, and WS were measured at each stage of development.

The effect of fruit size of ripe 'Flair' grown in the tunnel on Ca uptake was investigated by selecting fruit of different classes of fruit mass and subsequent incubation in 10 mM $CaCl_2$ or deionized water for 16 h. The fruit-mass classes were <13, 13–20, 20–25, 25–35, and >35 g.

Earlier studies established that water is taken up preferentially in the calyx region as a result of cuticular microcracks and abscission zones of petals and stamens (Hurtado and Knoche 2023a). To quantify the role of these discontinuities in the cuticle in Ca uptake, ripe 'Clery' fruit from the growth chamber with calyx (only the cut end of the peduncle sealed, but with the petal and stamen scars exposed) and fruit with the calyx removed [the petal and stamen scars sealed with silicone rubber (SE 9186 Clear; Dow Corning)] were incubated for 16 h in 10 mM $CaCl_2$ or deionized water. Calcium uptake, water uptake, and WS were quantified.

The effects of the $CaCl_2$ concentration on Ca uptake, water uptake, and WS of tunnel-grown ripe 'Clery' were determined by incubating fruit for 16 h in 0, 1, 3, 10, 30, or 100 mM $CaCl_2$.

The effects of different Ca-salt anions on Ca uptake, water uptake, and WS were determined by incubating ripe 'Clery' fruit for 16 h in 10 mM Ca-acetate, Ca-formate, Ca-propionate, Ca-lactate, Ca-heptagluconate, calcium sulfate ($CaSO_4$), calcium nitrate [$Ca(NO_3)_2$], or $CaCl_2$. Deionized water was used as the control.

The effects of the pH of the incubation solution on Ca uptake, water uptake, and WS were studied in ripe 'Clery' grown in a tunnel. The pH values of the 10 mM $CaCl_2$ solutions were adjusted to pH 2, 4, 7, or 10 using 1 N HCl for acidic pHs or 1 N potassium hydroxide for alkaline pHs. Deionized water (pH 6.0) served as the control.

The effects of temperature on Ca content, water uptake, and WS were studied by incubating ripe 'Clery' fruit from a growth chamber for 16 h in 10 mM $CaCl_2$ or deionized water at 2, 12, 22, or 35 °C. To detect potential confounding effects of temperature on membrane leakage, the anthocyanin absorbances of the representative incubation media were quantified at 520 nm using a spectrophotometer (Specord 210; Analytik Jena, Jena, Germany). After the experiment and before absorbance measurement, the pH values of the incubation media were adjusted to pH 2.5 by adding citric acid to a final concentration of 39 mM. This concentration matches the citric acid concentration of the juice from ripe strawberries (Herrmann 2001). The standardization was needed because the absorbance of an anthocyanin solution depends on pH (Brouillard et al. 1997).

The effects of microcracking on Ca uptake were investigated by inducing microcracking in ripe 'Clery' fruit from a growth chamber. Microcracks were induced by exposing fruit for 24 h to high RH (~100%) above water. Exposure to low RH (~15%) above dry silica gel served as the nonmicrocracked control (Hurtado and Knoche 2023b). Direct contact of the fruit surface with water or silica gel was prevented by placing fruit on a soft foam plug positioned on a stainless steel grid above the water or by placing the silica gel in a closed box. After the induction period, fruit were incubated in 10 mM $CaCl_2$ or deionized water for 16 h. Calcium uptake, water uptake, and WS were quantified as described earlier. The effect of the induction period on cuticular microcracking at high or low RH was established by fluorescence microscopy using the procedure described by Knoche and Peschel (2006). Briefly, a stainless steel washer (inner ⌀ = 6.4 mm) was mounted on the fruit surface in the equatorial region of a ripe strawberry using a fast-curing epoxy glue (UHU Plus Schnellfest; Bolton Adhesives, Bühl, Germany). The washer effectively prevented any strain relaxation of the fruit skin. Acridine orange at 0.1% (w/w) (Carl Roth, Karlsruhe, Germany) was applied to the center of the washer for 5 min. Thereafter, the dye solution was removed, and the epidermal skin segments were rinsed thoroughly in deionized water and viewed using fluorescence microscopy (MZ10F; Leica Microsystems, Wetzlar, Germany). Calibrated images were taken (Camera DP73, Olympus, Tokio, Japan; GFP-plus filter, 480/440-nm excitation, ≥510-nm emission wavelength, Leica) and microcracking of the cuticle was quantified as the percentage of the fruit surface area infiltrated with the fluorescent tracer acridine orange. Acridine orange does not penetrate an intact cuticle; hence, dye penetration is limited to the cuticular microcracks. Tissue infiltrated with acridine orange exhibits orange, yellow, and green fluorescence (Peschel and Knoche 2005). The infiltrated area was quantified using image analysis (cellSens Dimension 1.16 Desktop; Olympus Soft Imaging Solutions, Münster, Germany). Infiltration was assessed on an individual

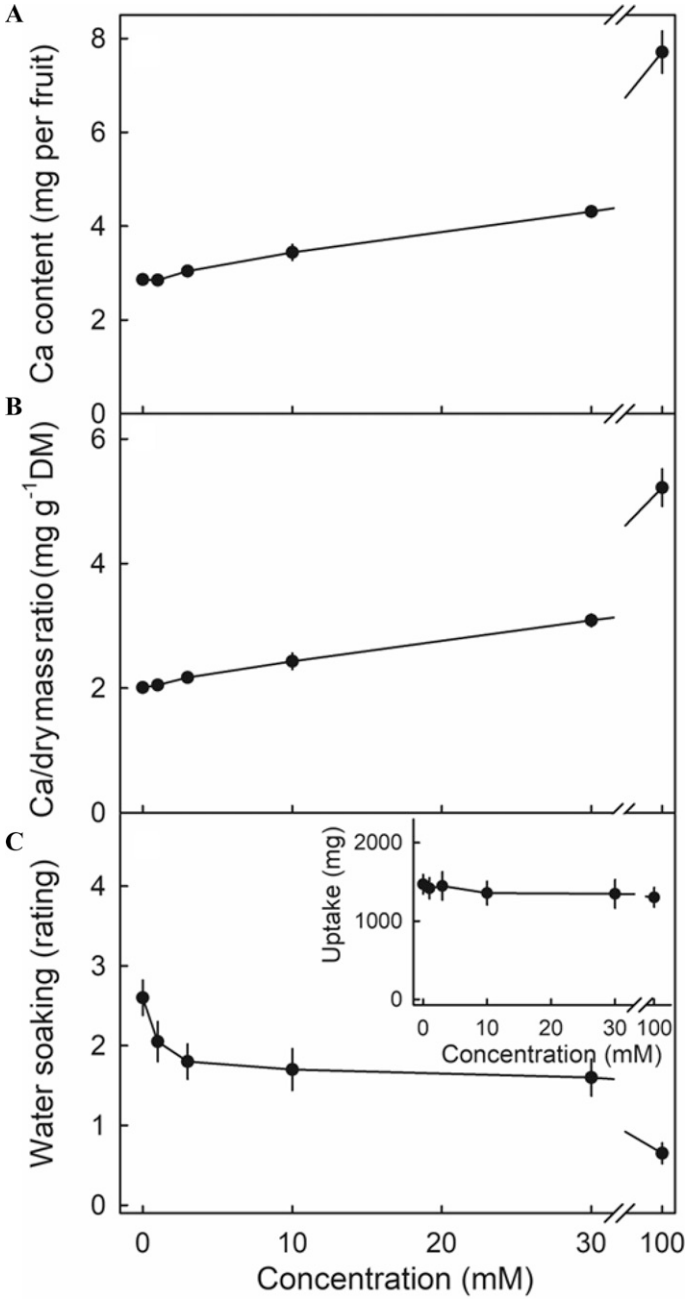

Fig. 4. Effect of the concentration of $CaCl_2$ on change in (**A**) Ca content and (**B**) Ca/dry mass ratio, and (**C**) area affected by water soaking of 'Clery' strawberry. Inset in **C**: effect of $CaCl_2$ concentration on water uptake. Water soaking was quantified using a five score rating scheme where score 0 = no water soaking; score 1 = <10% of the surface area water-soaked; score 2 = 10% to 35%; score 3 = 35% to 60%; and score 4 = >60% of the surface area water-soaked.

fruit basis before and after microcrack induction. There were 16 individual fruit replicates.

Data analysis. All experiments were conducted and analyzed using completely randomized designs. Data were analyzed by analysis of variance (ANOVA). When ANOVA assumptions were not met, the nonparametric Kruskal-Wallis test was used. Means were compared using Tukey's studentized range tests, the pairwise Dunnett's test, and the pairwise Mann-Whitney U test ($P < 0.05$). All analyses were done using RStudio (R ver. 4.1.0; R Foundation for Statistical Computing, Vienna, Austria). Data are presented as means ± standard error.

Results

Calcium content and the Ca/dry mass ratio of fruit incubated in a 10 mM $CaCl_2$ solution increased linearly with time, but remained constant for fruit incubated in water (Fig. 1A and B). WS and water uptake also increased with time (Fig. 1C). Fruit incubated in $CaCl_2$ developed less WS than fruit incubated in water (Fig. 1C, main graph). There was no significant difference in water uptake between fruit incubated in $CaCl_2$ and fruit incubated in water (Fig. 1C, inset).

Fruit mass increased with time (Fig. 2A). The effect of 10 mM $CaCl_2$ on Ca content and Ca/dry mass ratio was inconsistent. Incubation in $CaCl_2$ increased the fruit Ca content only at full ripeness (Fig. 2B), whereas the Ca/dry mass ratio of fruit increased at early stages (7 and 14 DAFB) and at full ripeness, but not at 21 DAFB (Fig. 2C). Susceptibility to WS increased during development and was always less for fruit incubated in $CaCl_2$ compared with fruit incubated in water (Fig. 2D). Water uptake increased exponentially during development for both treatments (Fig. 2D, inset).

Calcium content increased consistently with fruit mass, whereas the Ca/dry mass ratio remained constant. Fruit incubated in 10 mM $CaCl_2$ contained more Ca and had a higher Ca/dry mass ratio than that of the water control (Fig. 3A and B). WS increased with fruit mass and was consistently less severe for fruit incubated in $CaCl_2$ compared with that incubated in deionized water. There was no difference in water uptake between fruit incubated in $CaCl_2$ and fruit incubated in deionized water (Fig. 3C). In both cases, water uptake related positively to fruit mass (Fig. 3C, inset).

The fruit calyx had no significant effect on Ca content, Ca/dry mass ratio, WS, or water uptake. Calcium chloride at 10 mM increased the Ca content and Ca/dry mass ratio consistently, and decreased WS and water uptake regardless of the presence of the calyx (Table 2).

The fruit Ca content and the Ca/dry mass ratio related positively to the $CaCl_2$ concentration of the incubation solution (Fig. 4A and B). WS decreased as the concentration of Ca increased up to 100 mM (Fig. 4C, main graph). There was no effect of $CaCl_2$ concentration on water uptake (Fig. 4C, inset).

In general, incubating strawberries in solutions of selected organic and inorganic Ca salts at 10 mM increased the Ca content and the Ca/dry mass ratio and decreased WS compared with the water control (Table 3). The only exceptions were $Ca(NO_3)_2$ and $CaSO_4$, in which the effect on the fruit Ca content was not significant compared with the untreated control. There was no significant effect of any of the Ca salts on water uptake.

The pH of a 10 mM $CaCl_2$ solution had a marked effect on Ca content, Ca/dry mass ratio, WS, and water uptake. Strawberries incubated in $CaCl_2$ at pH 2 were entirely water-soaked and cracked; the fruit lost its integrity at this pH. The incubation solution was colored, indicating extensive leakage of anthocyanins released from bursting cells. Meaningful measurements of Ca content, Ca/dry mass ratio, and water uptake were not possible on fruit incubated at pH 2. At pH 4 and pH 7, $CaCl_2$ incubation increased Ca content, but the increase of the Ca/dry mass ratio was not significant (Table 4). With the exception of a pH of 2, $CaCl_2$ decreased WS at all other pH values compared with the

Table 3. Effect of selected organic and inorganic Ca salts on Ca content and Ca/dry mass ratio, water soaking, and water uptake of ripe 'Clery' strawberry. Fruit was incubated for 16 h at a salt concentration of 10 mM. Water soaking was quantified using a five score rating scheme where score 0 = no water soaking; score 1 = <10% of the surface area water-soaked; score 2 = 10% to 35%; score 3 = 35% to 60%; and score 4 = >60% of the surface area water-soaked.

Salt solution	Ca content (mg per fruit)	Ca/dry mass ratio (mg·g^{-1} DM)	Water soaking (rating)	Water uptake (mg)
Calcium formate	3.42 ± 0.19 b[i]	2.91 ± 0.15 b	1.2 ± 0.2 b	777.3 ± 68.2 ns
Calcium acetate	3.44 ± 0.09 b	2.69 ± 0.09 b	1.5 ± 0.2 b	912.6 ± 109.9
Calcium heptagluconate	3.07 ± 0.14 b	2.67 ± 0.04 b	1.7 ± 0.2 b	972.3 ± 102.8
Calcium lactate	3.12 ± 0.12 b	2.79 ± 0.11 b	1.4 ± 0.2 b	683.7 ± 52.5
Calcium propionate	3.23 ± 0.15 b	2.72 ± 0.09 b	1.5 ± 0.2 b	822.6 ± 101.1
Calcium nitrate	2.73 ± 0.25 a	2.68 ± 0.11 b	1.2 ± 0.3 b	902.5 ± 114.5
Calcium chloride	3.14 ± 0.06 b	2.77 ± 0.04 b	1.3 ± 0.2 b	1198.5 ± 132.6
Calcium sulfate	2.91 ± 0.25 a	2.71 ± 0.08 b	1.1 ± 0.2 b	1121.7 ± 185.9
Water	2.34 ± 0.11 a	2.21 ± 0.06 a	2.8 ± 0.3 a	1058.8 ± 88.2

[i] Means within columns followed by the same letter as the "water" control are not significantly different from the control, Dunnett's test at $P = 0.05$. ns = Nonsignificant. Means do not differ significantly from the control.

water control. The pH values of the $CaCl_2$ solution between pH 4 and pH 10 had no effect on water uptake compared with the water control.

There was no significant effect of temperature on Ca content and Ca/dry mass ratio. Regardless of temperature, the Ca content and Ca/dry mass ratio were consistently greater for fruit incubated in 10 mM $CaCl_2$ than for fruit incubated in deionized water (Fig. 5A and B). There was no consistent effect of temperature on WS (Fig. 5C). Fruit incubated in $CaCl_2$ was less water-soaked than fruit incubated in water (Fig. 5C). This effect was related in part to a marked decrease in cell bursting, as indexed by the marked decrease in anthocyanin leakage of fruit in $CaCl_2$ compared with that in the water control (Fig. 5A, inset). There was no consistent effect of temperature on water uptake (Fig. 5C, inset).

Microcracking of the cuticle increased in fruit held at high RH compared with low RH. The increase had no significant effect on water uptake, Ca content, or Ca/dry mass ratio. Interestingly, $CaCl_2$ at 10 mM reduced WS only in fruit that were previously induced to have a microcracked cuticle (Table 5).

Across all experiments, fruit Ca content and Ca/dry mass ratio both correlated negatively to fruit susceptibility to WS (Fig. 6A and B).

Discussion

From our findings we infer that 1) Ca penetration through the strawberry skin is a physical process that is unaffected by temperature; 2) with incubation in different Ca salts, there is little difference in penetration or in the effects on WS among them; and 3) higher Ca/dry mass ratios result in reduced WS.

Ca uptake is a physical process. The primary pathway of Ca uptake into strawberry fruit from a dilute solution was through the fruit skin. The calyx and the abscission scars of stamens and petals do not play a role in Ca uptake. The latter are inevitably also sealed when sealing the cut surface of the calyx using silicone rubber. The decreased water uptake in fruit incubated in $CaCl_2$ most likely resulted from a reduction in microcracking, as indexed by the lower scores for WS. WS is always associated with cuticular microcracking (Hurtado and Knoche 2021). The Ca concentration change (and, hence, the osmotic potential change) is too small relative to the water potential of the fruit to affect the water potential gradient significantly, which is the driving force for water uptake. The osmotic potential of a 10 mM $CaCl_2$ solution (equivalent to an osmolarity of 30 mmol·kg^{-1}) is approximately –0.07 MPa. This is only about 7% of the water potential of a ripe strawberry, approximately –1.0 MPa (Hurtado et al. 2021). A reduction in water uptake rate of 7% is unlikely to be detected by our methods given the relatively high fruit-to-fruit variability of the water uptake rate.

That Ca penetration through the strawberry fruit skin is a physical process we infer from the following observations. First, Ca uptake increased linearly with time, indicating that the rate of penetration remained constant and occurred under essentially steady-state conditions. The area of the absorbing surface (fruit surface area) remained essentially constant during the experiments, and likewise the water potential difference driving penetration. The driving force for Ca penetration corresponds to the difference in Ca concentration in the incubation solution less

Table 4. Effect of the pH of the $CaCl_2$ incubation solution (all at 10 mM, for 16 h) on Ca content, Ca/dry mass ratio, water soaking, and water uptake of ripe 'Clery' strawberry. Water soaking was quantified using a five score rating scheme where score 0 = no water soaking; score 1 = <10% of the surface area water-soaked; score 2 = 10% to 35%; score 3 = 35% to 60%; and score 4 = >60% of the surface area water-soaked.

Solution	pH	Ca content (mg per fruit)	Ca/dry mass ratio (mg·g^{-1} DM)	Water soaking (rating)	Water uptake (mg)
$CaCl_2$	2	1.96 ± 0.11 a[i]	3.43 ± 0.10 b	3.8 ± 0.2 c[ii]	119.2 ± 94.2 b
$CaCl_2$	4	2.68 ± 0.09 b	3.18 ± 0.15 a	1.5 ± 0.3 b	700.0 ± 96.9 a
$CaCl_2$	7	2.74 ± 0.07 b	3.11 ± 0.09 a	1.5 ± 0.2 b	568.6 ± 89.5 a
$CaCl_2$	10	2.40 ± 0.03 a	3.01 ± 0.04 a	1.4 ± 0.2 b	543.7 ± 71.0 a
Water	6	2.27 ± 0.08 a	2.84 ± 0.11 a	2.7 ± 0.3 a	728.1 ± 89.9 a

[i] Means within columns followed by the same letter as the "water" control are not significantly different from the control, Dunnett's test at $P = 0.05$.
[ii] Means within columns followed by the same letter are not significantly different, Tukey's studentized range test, $P = 0.05$.

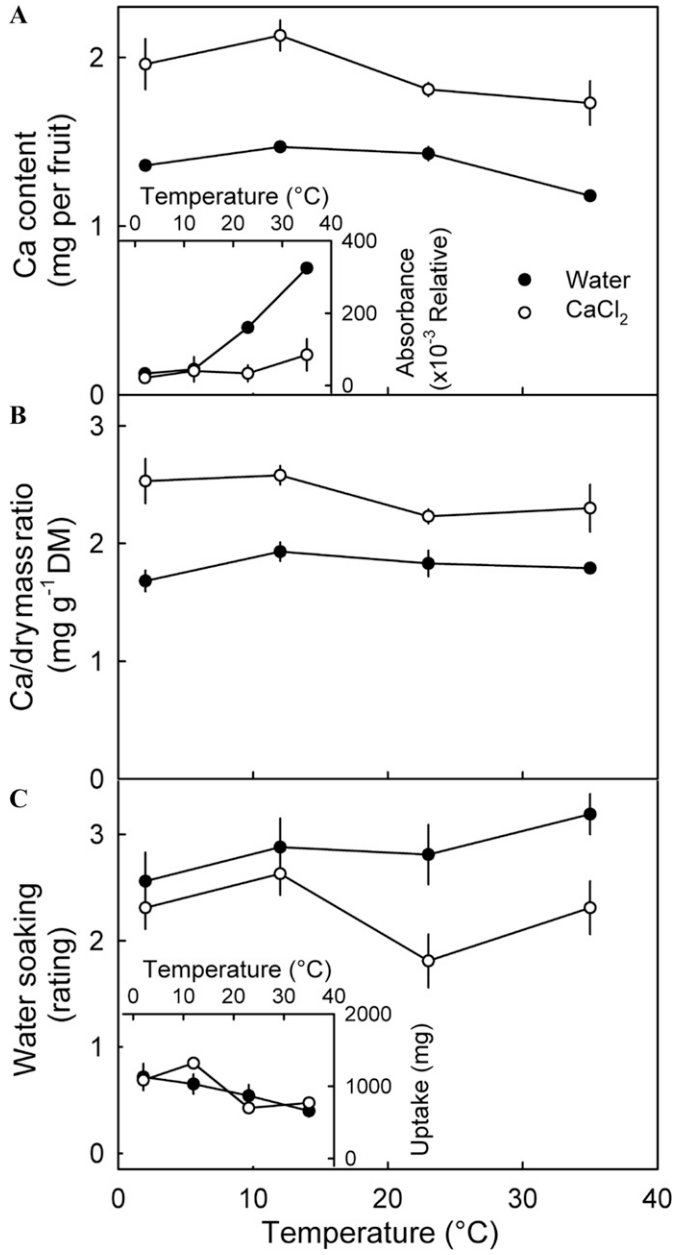

Fig. 5. Effect of incubation temperature on (**A**) change in area affected by water soaking, (**B**) Ca content, and (**C**) Ca/dry mass ratio of 'Clery' strawberry fruit immersed in deionized water or in 10 mM of $CaCl_2$. Insets: (**A**) Effect of temperature on water uptake and (**B**) leakage of anthocyanin in the incubation solution. Water soaking was quantified using a five score rating scheme where score 0 = no water soaking; score 1 = <10% of the surface area water-soaked; score 2 = 10% to 35%; score 3 = 35% to 60%; and score 4 = >60% of the surface area water-soaked.

that in the fruit skin. A constant concentration difference is not surprising given the relatively large volume of the incubation solution and the low amount of Ca penetration into the fruit. Second, Ca uptake increased linearly as the concentration of Ca in the incubation solution increased. This is typical for physical processes during which active transport mechanisms via carrier molecules are not involved and, hence, cannot be saturated. Third, Ca uptake related positively to fruit mass. As fruit mass increases, the fruit surface area and, hence, the cross-sectional area for penetration increases. Fourth, Ca uptake was largely independent of temperature in the range of 5 to 35 °C. This is typically the case when uptake occurs by viscous flow through openings such as polar pathways and/or microcracks in the cuticle. Earlier studies established that Ca uptake occurs via polar pathways (Schönherr 2000; Schönherr and Bukovac 1970; Schreiber 2005). Polar pathways are polar regions in a hydrated cuticle that do not represent physical holes. In these regions, polar functional groups from cuticle constituents orient upon hydration and form an aqueous continuum across the cuticle. This aqueous continuum allows penetration of polar substances up to a certain maximum size limit (Schönherr 1976; Weichert and Knoche 2006). The Ca cation is highly polar. Also, the sizes of the Ca cation and the accompanying anion are smaller than the size exclusion limit of polar pathways in the strawberry fruit cuticle (Hurtado et al. 2021). The size exclusion limit was estimated at 1500 g·mol^{-1}. These arguments suggest that Ca uptake from the dilute solutions is a physical process.

The previous arguments also explain why there was little difference in penetration of the different Ca salts. To maintain electrical neutrality, for every Ca cation penetrating, the corresponding anions must also penetrate in their stochiometric ratios. The anions of the Ca salts investigated differed in size but were all smaller than the size exclusion limit of the polar pathways. Thus, penetration from the dilute solutions was essentially the same. It is important to note that this may differ under field conditions, where penetration occurs from spray droplets subjected to a drying process. Here, only the initial penetration during the first minutes after application occurs from the liquid phase. But, after droplet drying, the dry droplet residue serves as the donor for penetration. Under these conditions, the amount of moisture maintained in the droplet residue determines the mobility of the Ca ion in the deposit (Winkler and Knoche 2021b).

More Ca uptake results in less WS. In this study with strawberries, and also in our earlier one (Hurtado and Knoche 2022), an increase in fruit Ca/dry mass ratio is associated with a decrease in susceptibility to WS. This effect is likely the result of an improvement in the mechanical properties of the cell wall in response to an increase in cell wall Ca. Indeed, most fruit Ca is associated with the cell wall and is thus apoplastic. Calcium is known to crosslink the cell wall constituents (Demarty et al. 1984; Jarvis 1982). As a consequence, adequate cell wall Ca both prevents cell wall swelling and increases cell-to-cell adhesion (Schumann et al. 2022). Furthermore, adequate Ca reduces membrane leakage, as indexed by reduced anthocyanin leakage from flesh disks (Hurtado and Knoche 2022). In WS, cell bursting occurs, and this releases citric and malic acids from the cells (symplast) into the cell wall free space (apoplast), where their presence 1) extracts complex Ca from adjacent cell walls and 2) increases the permeability of the plasma membranes. Thus, there is a chain reaction—a domino effect—in which cell bursting causes further cell bursting. Moreover, the structure of the cell walls begins to loosen, as indexed by cell wall swelling, and cell-to-cell adhesion reduces. In this way, WS spreads cell-by-cell across the fruit surface (Hurtado and Knoche 2021). These same arguments also account for rain cracking in sweet cherries (Schumann et al. 2019). However, in the presence of free Ca ions, this chain of events is disrupted, leading to a decrease in susceptibility to cracking in sweet cherry fruit and, similarly, to a decrease in WS in strawberry (Hurtado and Knoche 2021; Winkler et al. 2024).

Table 5. Effect of cuticular microcracks in 'Clery' strawberry on Ca content and Ca/dry mass ratio, water soaking, and water uptake after immersion in deionized water or in 10 mM $CaCl_2$. Microcracking was previously induced by incubation of fruit at high relative humidity (RH) for 24 h. Fruit held for the same period at low RH was used as the low-microcracking control. Water soaking was quantified using a five score rating scheme where score 0 = no water soaking; score 1 = <10% of the surface area water-soaked; score 2 = 10% to 35%; score 3 = 35% to 60%; and score 4 = >60% of the surface area water-soaked.

RH (%)	Microcrack increase in infiltration area (%)	Solution	Ca content (mg per fruit)	Ca/dry mass ratio ($mg \cdot g^{-1}$ DM)	Water soaking (rating)	Water uptake (mg)
100	2.6 ± 0.4 a[i]	Water	2.43 ± 0.05 ns	2.68 ± 0.11 ns	3.2 ± 0.2 a[ii]	894.9 ± 101.6 ns
100	2.6 ± 0.4 a	$CaCl_2$	2.60 ± 0.20	2.95 ± 0.13	1.7 ± 0.3 b	724.6 ± 123.2
15	0.6 ± 0.4 b	Water	2.62 ± 0.07	3.00 ± 0.17	1.8 ± 0.3 a	644.6 ± 52.1
15	0.6 ± 0.4 b	$CaCl_2$	2.63 ± 0.07	3.10 ± 0.05	2.2 ± 0.3 a	572.0 ± 58.4

[i] Means within columns followed by the same letter as the "water" control are not significantly different from the control, pairwise Mann-Whitney U test at $P = 0.05$.
[ii] Means within RH followed by the same letter are not significanlty different, Tukey's studentized range test, $P = 0.05$.
ns = Nonsignificant. Means do not differ significantly from the control.

Conclusion

Our results demonstrate that Ca is taken up through the strawberry fruit skin from dilute solutions of a range of Ca salts. Furthermore, Ca reduces the susceptibility of strawberry fruit to WS. The experimental system used in our study investigated penetration under well-defined, steady-state conditions in which the entire fruit surface was in contact with a dilute solution of a Ca salt at a constant concentration. This system allowed us to establish basic penetration characteristics of Ca salts. However, our experimental system differs from spray application in the field, where only a fraction of the fruit surface is in contact with spray droplets, which are subjected to a drying process. Under these conditions, the concentration of the droplet solution and its contact area with the fruit surface change rapidly. Our findings now have to be tested under field conditions to identify useful application schemes and formulations that maximize Ca/dry mass ratio and reduce WS under field conditions.

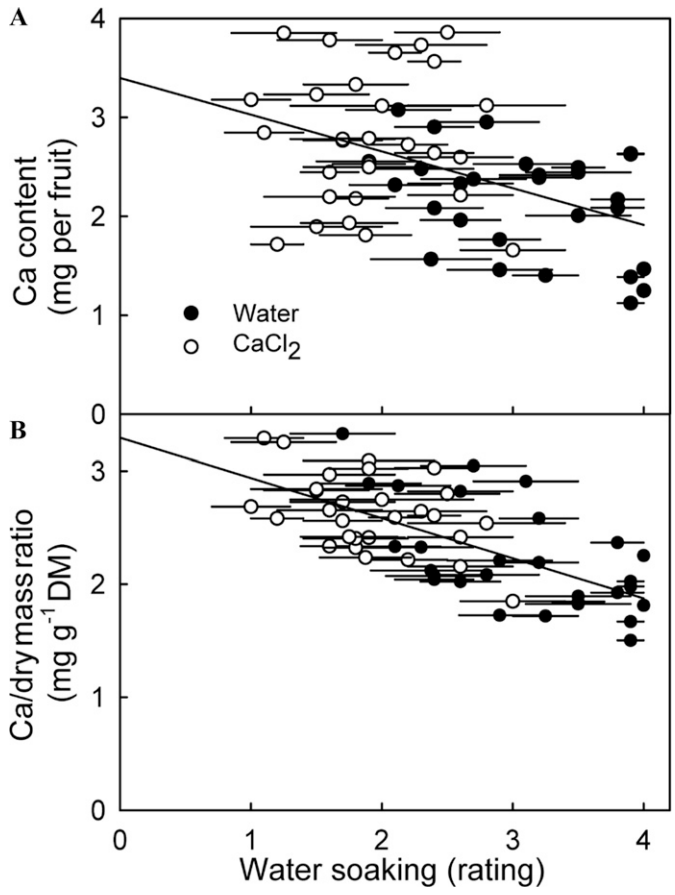

Fig. 6. Relationship between (**A**) Ca content or (**B**) Ca/dry mass ratio, and the area affected by water soaking (WS). Each data point represents the mean water soaking of 10 fruits from where Ca was measured. The regression equations were: Ca content (mg per fruit) = 3.40 (± 0.26) − 0.37 (± 0.10) × WS (score), $r^2 = 0.21$*** and Ca/dry mass ratio ($mg \cdot g^{-1}$ DM) = 3.29 (± 0.14) − 0.35 (± 0.14) × WS (score), $r^2 = 0.46$***.

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