Rain cracking mechanisms in sweet cherry fruit: Drivers and pathways of water uptake

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Zusammenfassung

Das Platzen von Süßkirschen (*Prunus avium* L.) während oder kurz nach Regenfällen führt nahezu weltweit zu Problemen bei der Produktion. Der Mechanismus des Platzens ist trotz intensiver Forschung noch weitgehend unbekannt. Auch Mechanismen und Wege der Wasseraufnahme sind nicht vollständig geklärt. Ziele dieser Arbeit waren: (1) den Einfluss von Äpfelsäure auf die Wasseraufnahme und das Platzen von Süßkirschen zu identifizieren, (2) den Xylemstrom in sich entwickelnden Süßkirschen zu quantifizieren, (3) die Beziehung zwischen der Wasseraufnahme entlang unterschiedlicher Wege und dem Platzen zu untersuchen (4) und die Hypothese zu überprüfen, ob die Süßkirsche sich wie ein ideales Osmometer bezüglich des Wassertransportes verhält.

Die Wasseraufnahme über die Fruchthaut wurde gravimetrisch und über den Stiel volumetrisch mittels Potometrie bestimmt. Das Platzen wurde durch Inkubation von Süßkirschen in Wasser und Auszählen geplatzter Früchte zu unterschiedlichen Zeitpunkten bestimmt und als T_{50} (Zeit bis zum Platzen von 50% der Früchte) sowie WU_{50} (aufgenommene Wassermenge bis zum Platzen von 50% der Früchte) angegeben. Im Platztest wurden Früchte in Äpfelsäurelösungen verschiedener Konzentrationen und pH-Werte inkubiert. Die Wasseraufnahmewege wurden u.a. durch Abkleben unterschiedlicher Regionen der Frucht oder durch direkte Injektion von Wasser mittels Injektionsnadel und Perfusor modifiziert. Das osmotische Potential der Inkubationslösung wurde durch Einsatz von Kirschsaft, künstlichem Kirschsaft oder Lösungen von Glucose, Saccharose und Polyethylen Glykol 6000 (PEG 6000) variiert.

Die Inkubation von Früchten in eigenem Saft führte im Platztest im Vergleich zu einer Inkubation in Wasser zu einer reduzierten T_{50} und WU_{50}. Der Effekt war auf die Äpfelsäure als Bestandteil des Kirschsaftes zurückzuführen und konnte auch mit anderen organischen Säuren nachgestellt werden. Dabei war der pH-Wert der Inkubationslösung für das Platzen der Kirschen ausschlaggebend. Mit sinkendem pH-Wert nahmen T_{50} und WU_{50} ab.

Der Xylemstrom durch den Stiel der Süßkirsche nahm von Beginn der Phase III an bis zur Reife hin von anfangs 24.9 ± 1.1 µl h^{-1} auf 5.2 ± 0.2 µl h^{-1} bei der Reife kontinuierlich ab. Überraschenderweise konnte der Einstrom nicht durch den Einsatz

hypertonischer Saccharoselösungen (bis zu -10 MPa) gestoppt werden, es verblieb ein Reststrom von 1.2 – 3.8 µl h^{-1}. Der Xylemstrom hatte keinen Einfluss auf das Platzen.

Durch Manipulation der Wege der Wasseraufnahme (z.B. Versiegeln der Stielgrube, Abschleifen der Kutikula oder Injektion von Wasser mittels einer Kanüle) und nachfolgenden Wasseraufnahme- und Platztests konnte gezeigt werden, dass das „critical turgor pressure model“ – welches postuliert, dass Früchte bei Erreichen eines kritisches Turgordruckes durch Wasseraufnahme platzen – nicht zur Erklärung des Platzens von Süßkirschen genutzt werden kann.

Die Süßkirsche ist kein ideales Osmometer. So hat die Fruchthaut einen Reflektionskoeffizienten (σ) < 1 für kleinere Osmolyte wie Glukose, Fruktose oder Sorbitol. Gelöste kleine Osmolyte diffundieren je nach chemischem oder osmotischem Gradienten aus der Inkubationslösung durch die Fruchthaut in die Frucht (Influx) oder aus der Frucht in die Inkubationslösung (Efflux). Der Ausfluss von Osmolyten (81% des gesamten Effluxes waren Glukose, Fruktose und Sorbitol) war bei Inkubation in Wasser mit der Netto-Wasseraufnahme korreliert.

Auf der Grundlage dieser Arbeit und der Ergebnisse in der Literatur wurde daher ein neues Modell zur Erklärung des Platzens von Süßkirschen formuliert. Dieses Modell wird nachfolgend als Reißverschlussmodell („zipper model“) bezeichnet. Nach diesem Modell ist der Ablauf des Platzens wie folgt:

1) Die lokale Wasseraufnahme durch Mikrorisse führt zum Platzen von Zellen im Bereich der Mikrorisse. 2) Als Folge des Platzens tritt Zellinhalt in den Apoplasten aus. Der Zellsaft von Kirschen enthält hohe Konzentrationen von Äpfelsäure. Äpfelsäure zerstört Membranen und schwächt Zellwände, so dass der lokale Defekt sich ausweitet. Zudem extrahiert Äpfelsäure Calcium aus den Zellwänden und fördert damit die Quellung. 3) Gequollene Zellwände sind mechanisch instabil. Die Spannung innerhalb der Fruchthaut führt dazu, dass diese gequollenen, instabilen Zellwände reißen und diese Zellen wiederum ihren Zellinhalt in den Apoplasten abgeben. Dieser Prozess setzt sich fort und es kommt zu einem Auftreten von Makrorissen und dem Platzen der Frucht.

Schlüsselwörter: Platzen, Wasseraufnahme, Zipper Modell

Summary

The cracking of fruit of sweet cherry (*Prunus avium* L.) during or after rainfall is a serious production problem in almost all countries where this high-value crop is grown. The mechanism of cracking is still largely unknown or misunderstood, despite extensive research. Also, the mechanisms and pathways of fruit water uptake have not been completely clarified. Uncertainties include: the contribution of water uptake via the xylem to rain cracking or the reason for water uptake when a fruit is incubated in its own juice. The objectives of this work were: (1) to identify the effect of malic acid on water uptake and rain cracking of sweet cherry fruit, (2) to quantify the xylem flows in developing sweet cherry fruit, (3) to investigate the relations between water uptake via various different pathways and rain cracking, and (4) to evaluate the implicit hypothesis that a sweet cherry fruit acts as an ideal osmometer.

Water uptake via the fruit skin was determined gravimetrically and via the pedicel using a potometer. Cracking was determined by incubating fruit in water for different times, counting the numbers of cracked fruit, and quantifying the results as a T_{50} (the time to 50% cracking) and a WU_{50} (the amount of water taken up at 50% cracking). In the cracking assay, fruits were also incubated in malic acid solutions of different concentration and pH values. The water uptake path was controlled by sealing different regions of the fruit or by direct injection of water using a hypodermic needle and a perfusor. The osmotic potential of the incubation solution was varied using expressed sweet cherry juice, artificial sweet cherry juice, and various solutions of glucose, sucrose, and polyethylene glycol 6000 (PEG 6000).

Incubation of fruit in their own juice reduced the T_{50} and WU_{50} values compared with fruit incubated in pure water. The effect could be attributed to malic acid, which is a major component of the juice. The behavior can be reproduced using alternative organic acids. The pH of the incubation solution was also crucial. Decreases in pH led to decreases in the values of T_{50} and WU_{50}.

The xylem flow via the pedicel of sweet cherry fruit decreased continuously from the beginning of stage III development towards fruit maturity from 24.9 ± 1.1 µl h^{-1} to 5.2 ± 0.2 µl h^{-1}. Surprisingly, the xylem flow could not be stopped by using hypertonic sucrose solutions (down to -10 MPa), a flow between 1.2 – 3.8 µl h^{-1} still remained. The xylem flow had no effect on cracking.

By manipulating the pathways of water uptake (e.g. by sealing the pedicel cavity or by abrading the cuticle, or by injection of water using a hypodermic needle) in water uptake and cracking experiments, it was shown that the “critical turgor pressure model” – which suggest that fruit crack after reaching a critical turgor pressure by water uptake – fails to explain the cracking of sweet cherry fruit.

The sweet cherry fruit is not an ideal osmometer. The fruit skin has a reflection coefficient (σ) < 1 for smaller osmolytes like glucose, fructose, or sorbitol. A solute diffuses along the gradient of its chemical potential or osmotic gradient of the incubation solution into the fruit (inflow) or out of the fruit into the incubation solution (outflow). The outflow of osmolytes (81% of the total outflow were glucose, fructose, and sorbitol) was correlated with the water uptake when incubating fruit in water.

Based on this work and on a review of the literature, a new model to explain sweet cherry fruit cracking is proposed. This model is called the “zipper model”. According to this model, the process of cracking is:
1) Local water uptake through microcracks in the cuticle leads to bursting of cells in the locality. 2) As a consequence, cell contents enter the apoplast. The cell contents include high concentrations of malic acid. Malic acid destroys membranes and weakens cell walls. Hence, the local defect spreads from cell to cell. Moreover, malic acid extracts calcium from the cell walls and promotes cell-wall swelling. 3) Swollen cell walls are mechanically weak. The natural growth-induced tensions in the fruit skin causes rupture of these swollen (weakened) cell walls, releasing further amounts of malic acid into the apoplast. A chain reaction propagates the cell failure forming a macrocrack.

Keywords: cracking, water uptake, zipper model

Abbreviations

A	Fruit surface area
C_i	Water-vapor concentration inside of the fruit
C_0	Water-vapor concentration in the surrounding atmosphere
ΔC	Difference in water-vapor concentration between C_i and C_0
Ca	Calcium
Ca^{2+}	Calcium ions
$CaCl_2$	Calcium chloride
CI	Cracking index
CM	Cuticular membrane
D_2O / DHO	Deuterium-labelled water
DAFB	Days after full bloom
F	Rate of water uptake or transpiration
$FeCl_3$	Ferric chloride
J	Flux density
H_2SO_4	Sulfuric acid
K_2HPO_4	Dipotassium phosphate
LD_{50}	Dose of a toxic compound resulting in 50% mortality
LVDT	Linear variable displacement transducers
NaCl	Sodium chloride
P	Pressure on whole fruit basis
p	Permeance of the fruit skin
PE	Polythene
PEG 6000	Polyethylene glycol 6000
P_f	Permeability of the fruit surface
r	Radius of the fruit
R	Gas constant
RH	Relative humidity
t	Thickness of the skin
T	Absolute temperature
T_{50}	Time to 50% cracking
THO / ^3H_2O	Tritium-labelled water
V_w	Molar volume of water

WU_{50}	Water uptake at 50% cracking
s	Mechanical stress
σ	Reflection coefficient
Ψ^{flesh}	Water potential of the flesh
Ψ_{Π}^{flesh}	Osmotic potential of the flesh
Ψ^{fruit}	Fruit water potential
Ψ_{Π}^{fruit}	Osmotic potential of the fruit juice
Ψ_{Π}^{skin}	Osmotic potential of the skin
$\Psi_{\Pi}^{solution}$	Osmotic potential of the incubation solution
Ψ_{P}	Turgor
$\Delta\Psi$	Difference between apparent Ψ^{fruit} and $\Psi_{\Pi}^{solution}$

Contents

1. General introduction to the phenomenon of cracking

Rain cracking is a major problem in sweet cherry production in most of the countries of the world where this high-value crop is grown (Christensen, 1996). Rain cracking refers to the appearance of highly-visible 'macrocracks' in the fruit skin during or soon after rainfall (or extended periods of surface wetness from dew, etc). Sweet cherry fruit are most susceptible to rain cracking during the three-week period leading up to harvest (Christensen, 1973). Percentages of cracking of up to 90% of the fruit in an orchard have been reported (Christensen, 1996). Beginning with about 25% of cracked fruit, the cost of harvest of sweet cherry orchards becomes uneconomical (Looney, 1985). The reason being the high labor cost associated with selection of non-cracked fruit during picking and later during grading in the packhouse. Badly cracked fruit are not saleable as the crack opens up the vulnerable flesh to rapid deterioration due to pathogen entry and drying. In addition, the quality of the non-cracked fruit from the same orchard is usually severely compromised due to the presence of 'microcracks' (microscopic cracks) in the cuticle. Both microcracks and macrocracks impair the barrier functions of the fruit skin to water movement and present entry ports for fruit rot pathogens including: *Monilia*, *Botrytis*, and *Alternaria* (Børve et al., 2000; Børve and Stensvand, 2003; Thomidis and Exadaktylou, 2013). Infections then spread rapidly from a few cracked fruit to neighboring, non-cracked fruit. In addition, microcracks limit storability of fruit due to increased rates of water loss. They increase transpiration (Knoche et al., 2002; Beyer et al., 2005) and compromise fruit appearance due to a loss of gloss and a decrease in firmness. Also, after a few days of storage/transit, the postharvest disorder "orange peel" (syn. alligator skin, lizard skin) develops, which limits access to lucrative high-quality export markets.

Rain cracking is not limited to sweet cherry production. It also occurs in other soft and fleshy fruit including grapes (Considine and Kriedemann, 1972; Clarke et al., 2010), tomatoes (Peet, 1992; Lichter et al., 2002), litchi (Li et al., 2001; Mitra et al., 2014), bell peppers (Aloni et al., 1998), pomegranates (Galindo et al., 2014; Saei et al., 2014), currants (Khanal et al., 2011), and plums (Mrozek and Burkhard, 1973; Milad and Shackel, 1992).

This introduction provides a short overview of the problem of rain cracking. It is not intended as a comprehensive review of the literature that can be found elsewhere

(Sekse, 1995a, 1998, 2008; Christensen, 1996; Sekse et al., 2005; Simon, 2006; Balbontín et al., 2013; Khadivi-Khub 2015; Knoche and Winkler, 2017). This overview merely serves the purpose of providing some background information that is helpful to better understand the phenomenon of rain cracking of sweet cherry fruit.

1.1 Morphology of the sweet cherry drupe

The sweet cherry fruit develops from a single carpel. The margins of the carpel fuse and form the suture. The proximal portion of the fruit extends from the pedicel cavity to the equatorial region, the distal portion from the equatorial region to the stylar scar at fruit apex (Fig. 1A). The side opposite to the suture is referred to as the cheek, the 'lateral' sides as the two shoulders (Fig. 1B).

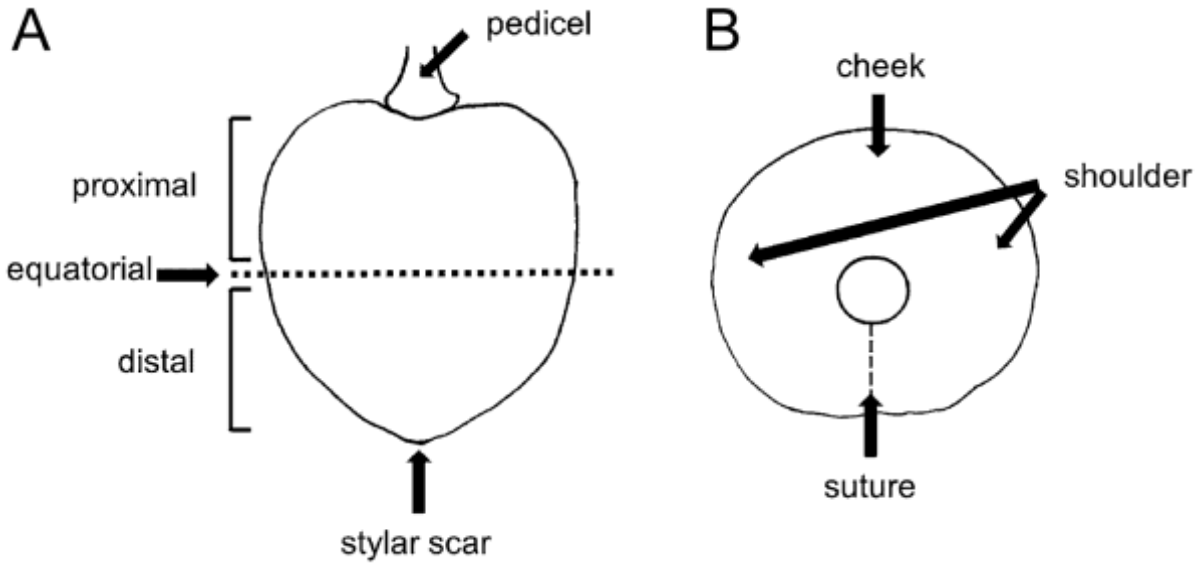

Fig. 1: Schematic drawing illustrating the terminology used to describe different regions on a sweet cherry fruit. A) View from the front; B) view from the top. Modified after Grimm et al. (2017).

The fruit skin (exocarp) of the sweet cherry fruit comprises the cuticular membrane (CM) and two underlying cellular layers – the epidermis and the hypodermis (Fig. 2). The CM consists primarily of cutin and waxes and is very thin – about 1 µm thick in a mature sweet cherry fruit (Peschel and Knoche, 2012). The epidermis comprises a single layer of collenchyma cells of diameter about 25 µm, with thick cell walls (3.2 ± 0.1 µm). There are no trichomes or hairs on the sweet cheery skin and stomata occur

at very low density (0.0 to 1.7 mm^{-2}) and are non-functional in mature fruit (Peschel et al., 2003). Immediately under the epidermis is the hypodermis of thickness about 50 to 100 µm. The hypodermis comprises from 2 to 7 cell layers of collenchyma (Glenn and Poovaiah, 1989). Under the skin (i.e. immediately adjacent to the hypodermis) is the flesh (mesocarp). It comprises very large (diameter 227 ± 2.9 µm), thin-walled, isodiametric, parenchyma cells (Yamaguchi et al., 2004). The vascular system (xylem and phloem) is embedded in the flesh and does not extend into the skin. At maturity, macerated tissue surrounding the pit can be observed in many cultivars, indicating a loss of cell integrity.

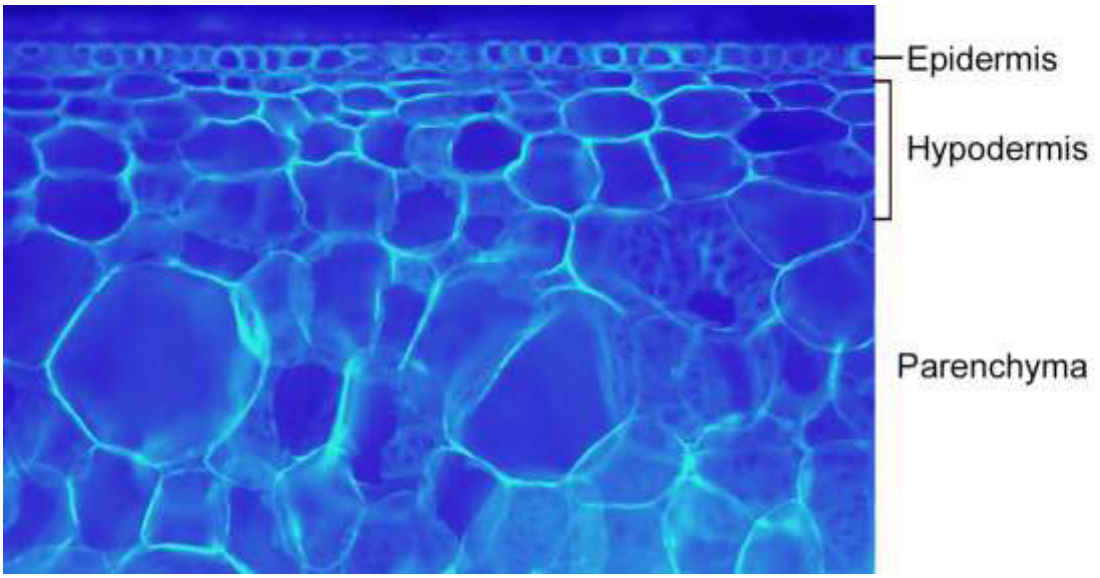

Fig. 2: Cross-section of the fruit skin of a mature sweet cherry stained with calcofluor white (modified after Knoche and Winkler (2017)).

1.2 Fruit growth and development

A sweet cherry fruit growth follows the classic, double-sigmoid growth pattern of stone fruit. Development is usually described in terms of being into three stages (Lilleland and Newsome, 1934; Tukey, 1934). Stages I and III are characterized by rapid increases in fruit fresh mass and surface area, whereas the intermediate stage II is indicated by a lag phase, when growth slows down. In stage I, growth of the pericarp is mostly by cell division. Mass increases during stage I from very low to about 1.5 to 2.5 g per fruit. Stage II is characterized by development of the endocarp (the pit). At the transition from stage II to III the pit hardens and the skin color changes from green to yellow. Stage III is dominated by cell extension of the

mesocarp and a color change to red or black in the non-yellow cultivars (Serrano et al., 2005). In addition, the flesh osmotic potential decreases rapidly (i.e. the osmotic concentration increases rapidly) from about -0.7 MPa (Knoche et al., 2004) to below -4 MPa at maturity (Knoche et al., 2014). Knoche et al. (2001) reported maximum fresh mass growth rates of about 0.54 g d^{-1} and surface area growth rates of about 0.96 mm^{-2} d^{-1} during stage III.

1.3 Cracking

Before discussing details of the quantification and the mechanistic basis of cracking it is useful to briefly consider the different types of cracking in the literature.

1.3.1 Microcracks

Microcracks are small cracks, which are limited to the cuticle and do not extend into the cellulosic walls of the underlying epidermis or hypodermis. They cannot usually be detected by the naked eye. However, they are detectable under a light microscope after infiltration of the cracks with the fluorescent tracer acridine orange (Fig. 3). A detailed description of the method is given by Peschel and Knoche (2005). Microcracks occur at high frequency in the pedicel cavity and stylar scar regions of virtually all fruit grown in non-protected environments. Their frequency increases during stage III (Peschel and Knoche, 2005). Formation of microcracks is increased by high atmospheric humidity and by the presence of free water on the fruit surface (Knoche and Peschel, 2006). It is likely, microcracks can propagate to form macrocracks (Glenn and Poovaiah, 1989), but definitive evidence for this is lacking. Interestingly, microcracks do not impair the mechanical properties of the fruit skin indicating that it is the epidermal and hypodermal cell layers that form the structural ‘backbone’ of the fruit (Brüggenwirth et al., 2014).

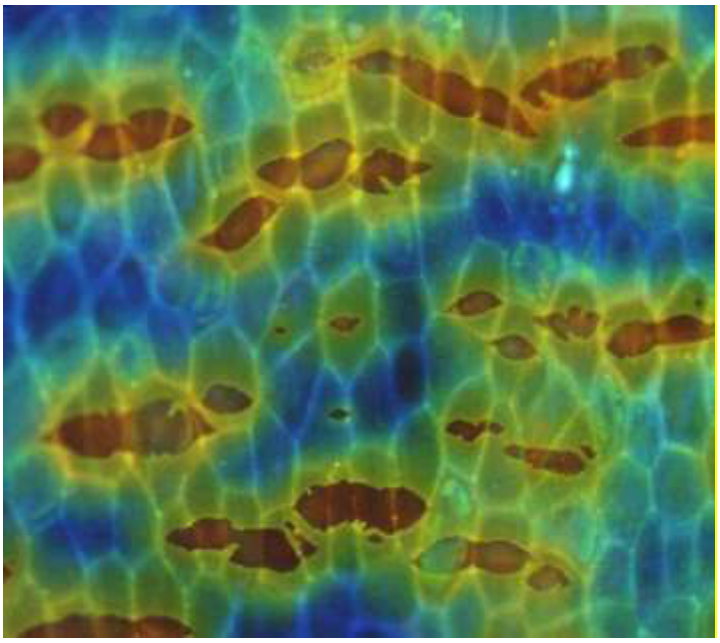

Fig. 3: Microcracks in the cuticle of a mature sweet cherry fruit after staining with acridine orange (modified after Knoche and Winkler (2017)).

1.3.2 Macrocracks

Macrocracks are not limited to the cuticle, but extend into the fruit skin, possibly into the flesh and in severe cases right down to the pit. They are clearly visible to the naked eye. Macrocracks often gape due to the presence of elastic strain in the skin (Grimm et al., 2012).

1.3.3 Position of cracks

There are three different kinds of macrocracks reported in the literature: a) apical cracks (sometimes also described as nose cracks (Simon, 2006)) (Fig. 4A), b) pedicel or stem end cracks at or next to the pedicel cavity (Fig. 4B), and c) side cracks which are irregular and usually long and deep (Fig. 4C). Fruit with small apical and pedicel end cracks can be sold on the domestic market at a lower price, but fruit with side cracks are usually without commercial value.

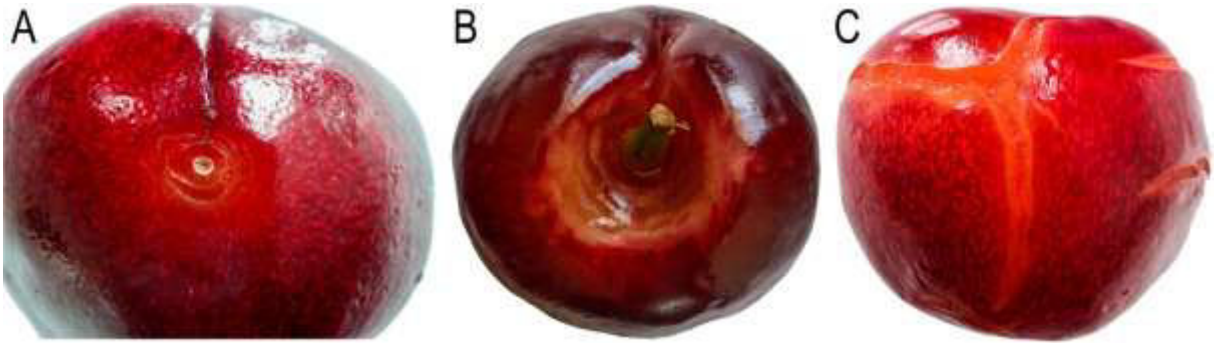

Fig. 4: (A) Apical cracks in stylar scar region, (B) pedicel or stem end crack in the pedicel cavity region, and (C) side cracks in cheek and shoulder regions (modified after Knoche and Winkler, 2017).

It has been observed – but never described in detail – that cracking usually starts at the apical end or in the cavity region where the density of microcracks is highest (Peschel and Knoche, 2005). Consequently, side cracks are thought to represent elongation of cracks that originated from the stylar scar or pedicel end regions (Verner and Blodgett, 1931; Glenn and Poovaiah, 1989). In the field, this may be related to higher water uptake in these regions compared to the remaining fruit surface. In addition, both regions are characterized by extended periods of surface wetness due to the presence of a hanging water droplet at the stylar end and a puddle in the pedicel cavity. Extended periods of wetness allow more water uptake in these regions. The pedicel/fruit junction (Beyer at al., 2002) and the apical end (Glenn and Poovaiah, 1989) are preferential water uptake sites which may explain why these sites are most susceptible to cracking. Furthermore, due to the architecture of the fruit, these regions are also exposed to the highest stresses (Considine and Brown, 1981).

In contrast to the above arguments, Measham et al. (2010) state that vascular water uptake flow is the relevant factor in side cracking. This claim is based on the observation that irrigating the soil while keeping the canopy dry resulted in large side cracks. However, when applying the same amount of water to the canopy using overhead sprinklers, only small cracks developed in the cavity region or the stylar scar region of the fruit.

1.4 Measurements of cracking

Studies on the mechanism of cracking require procedures to reproducibly assess cracking susceptibility of the fruit. In principle, cracking may be assessed in the orchard or in the laboratory.

1.4.1 Measuring of cracking in the orchard

1.4.1.1 Measuring of cracking in the orchard after rainfall

The easiest and most practical method of quantifying cracking in the orchard is to simply count the frequency of cracked fruit after rainfall. The percentages of cracked fruits are then calculated and compared. However, there are several problems with this method: Timing, duration, and intensity of rain cannot be controlled. The stage of maturity has a marked effect on cracking susceptibility but this is impossible to standardize. Also, several short rainfall events may produce different results from a single, long rainfall event. Wind and light modify wetness duration and the humidity in and around the tree. Temperature also affects cracking susceptibility. These factors are impossible to standardize and to control, so it is also impossible to make direct comparisons between cultivars that differ in time of maturation (Christensen, 1972b).

1.4.1.2 Measurement of cracking under artificial rain

A more precise method is to quantify cracking under artificial rain using potted trees and overhead sprinklers installed in a rain shelter or tunnel (Quero-Garcia et al., 2014). Rainfall can be simulated and times and durations and intensities can be controlled. Performing this experiment using potted trees in a growth chamber, is logistically challenging but allows all environmental factors to be controlled. It is important to use rain water or deionized water in such experiments because even low concentrations of calcium may affect cracking (Lang et al., 1998).

1.4.2 Measurement of cracking in the laboratory – the cracking index

Determining the cracking index (CI) is the classical procedure for measuring cracking sensitivity in the lab. The test was established first by Verner and Blodgett (1931) and later modified by Christensen (1972b). Briefly, 50 fruit without defects are immersed in deionized water at constant temperature. After 2, 4, and 6 hours fruit are removed

from the incubation solution and checked for cracks. Non-cracked fruit are returned to the solution while cracked fruit are eliminated. The number of cracked fruit is counted. The CI is calculated from:

$$CI = \frac{(5a+3b+c)*100}{250} \quad \text{(Eq. 1.1)}$$

In this equation, a, b, and c represent the numbers of fruit cracked after 2, 4, and 6 hours, respectively. Rapid cracking of fruit has a larger effect on the CI as compared to the same percentage of cracking after more extended exposure. Hence, the same numbers of cracked fruit may result in different CI values. Detailed instructions for performing cracking assays are given by Christensen (1972b, 1996). In cracking assays performed in this dissertation, cracking is characterized by the time to reach half maximum cracking (T_{50}; Knoche, 2015; chapter 4 (Winkler et al., 2015)).

Weichert et al. (2004) determined the intrinsic cracking susceptibility in a modified cracking test. In their test, water uptake is quantified on the same batch of fruit and the rate of water uptake is calculated. This rate is usually constant during the typical duration of a cracking experiment. From the rate of uptake and the time course of cracking, the amount of water taken up at any one time is simply calculated by multiplying the duration of incubation by the uptake rate. Cracking is now described as a function of the amount of water taken up. In analogy to the T_{50}, the amount of water uptake at 50% cracking (the WU_{50}) may be calculated. The WU_{50} is suitable in characterizing the mechanical architecture of the fruit used in these assays, since it is standardized for water uptake. The WU_{50} is used among others tests for comparison of different organic acids, acids with different concentrations and pHs, different cultivars and mechanical modifications of the fruit skin in the present dissertation in chapters 4 (Winkler et al., 2015) and 5 (Winkler et al., 2016b).

1.4.3 Disadvantages of cracking tests

Despite the many advantages of laboratory-based cracking tests, some disadvantages and problems should be noted. 1) CI of the same cultivars determined at different sites are often poorly correlated or are not correlated at all. The same cultivars may have a high CI at one site, but a low CI at another site. For example, the CI for ‘Van’ in Oregon, USA, is 12 (Zielinski, 1964), but it is 92 in Norway (Sekse, 1987; see compilation in Christensen (1996)). 2) CI varies between seasons. It is

recommended to quantify the CI over several (2 to 3) years and then calculate the average (Christensen, 1996). This variation may be caused by environmental effects that affect water uptake characteristics and/or the mechanical constitution of the fruit (i.e. temperature, cumulative rainfall, and number of periods of surface wetness). 3) Up to now it is not known whether the cracking-behavior of laboratory-based immersion tests mimics the cracking susceptibility of the fruit on the tree. The lack of a hydraulic connection between fruit and tree in a laboratory-based immersion assay is a likely source of artefact if water uptake through the xylem and the phloem vasculature of fruit and pedicel contribute to cracking. This has not yet been investigated critically as far as I am aware. Functionality of the xylem in mature fruit is investigated in this dissertation in chapter 3 (Winkler et al., 2016a). To the best of my knowledge there is only a single study comparing cracking assays in the field to laboratory-based immersion assays (Quero-Garcia et al., 2014). The authors reported significant correlations for cracking of the apical end and pedicel cavity region between their field and their laboratory tests, but their correlations were not significant for the side cracks.

Based on the above arguments, laboratory-based tests are particularly useful for head to head comparisons of treatments within the same batch of fruit, such as carried out in this dissertation (chapters 2 (Winkler et al., 2015) and 4 (Winkler et al., 2016b)). Further, the intrinsic cracking susceptibility offers a useful and straightforward approach to assessing the mechanical properties of a fruit relevant to cracking on a whole-fruit basis. Whether these determinations reflect the cracking susceptibility of fruit in the field depends on the role of the xylem and phloem transport in cracking. The xylem portion of this question is addressed in chapter 3 of this dissertation (Winkler et al., 2016a). The phloem contribution merits further investigation.

1.5 Factors affecting cracking

Many studies have been published investigating a wide range of factors affecting cracking. The results are summarized in Table 1. For more detailed information, the reader is referred to the original references. When these data are needed for discussion in the subsequent chapters, they will be discussed in greater detail there.

What can be concluded here is that these empirical studies often report inconsistent and in some cases even contradictory data. Typical examples are the effects of water uptake or firmness on cracking. Because these studies only describe single aspects of cracking, they are of limited usefulness in identifying the underlying mechanisms.

Table 1: Factors affecting the cracking susceptibility of sweet cherry fruit. Data are compiled from the literature (Knoche and Winkler, 2017).

Factor	Levels	Susceptibility to cracking	Reference
Fruit size	2.8 – 10 g	Increased susceptibility in large fruited cultivars, high variability between cultivars	Tucker, 1934; Christensen, 1975; Yamaguchi et al., 2002
Water uptake		No, or only weak correlation with susceptibility	Kertesz and Nebel, 1935; Christensen, 1972a
		Positive correlation between rate of uptake and cracking	Belmans and Keulemans, 1996; Yamaguchi et al., 2002
Firmness	Fruit firmness (kg) 1 – 3.6, grade 3 – 10	No correlation	Tucker, 1934; Christensen, 1975
	Flesh firmness (g) 29.1 – 148.1	Positive correlation between firmness and rate of cracking	Yamaguchi et al., 2002
Temperature	1 – 48°C	Higher temperature, faster cracking	Bullock, 1952; Richardson, 1998
Osmolarity	10.1 - 20% sugar	Positive correlation with susceptibility	Verner and Blodgett, 1931
	12.8 – 26.4% soluble solids	Lack of, or a weak correlation	Tucker, 1934; Christensen, 1972c; Moing et al., 2004
Skin	Skin per gram soluble solids	Higher mass of cell walls in less susceptible cultivars	Tucker, 1934
	Thickness of inner epidermal cell wall	Positive correlation between thickness of inner epidermal cell wall and susceptibility	Kertesz and Nebel, 1935
	Thickness of cuticle and epidermal cell wall (µm) 7.5 – 12.5	Thicker cuticle and thicker epidermal cell wall in less susceptible cultivars	Belmans et al., 1990
	Cuticle thickness (µm)	Thicker cuticle in less-susceptible cultivars	Belmans and Keulemans,

	0.9 – 4.02		1996; Demirsoy and Demirsoy, 2004
Cell size	Size of subepidermal cells	Smaller cell size in more susceptible cultivars	Kertesz and Nebel, 1935
		Higher variability of cell size in flesh of susceptible cultivars	
		Larger cells (longitudinally and latitudinally) in susceptible cultivars	Yamaguchi et al., 2002
Stomata	Density of stomata	No correlation	Christensen, 1972c; Glenn and Poovaiah, 1989
Rootstock	Colt, MaxMa 14, Maxma 97	Differences in cracking rates between cherries of different rootstocks after 4 and 24 h immersion in water	Simon et al., 2004
	Colt, F12/1	More cracking on Colt than on F12/1	Cline et al., 1995a,b
Calcium salts	Immersion test, overhead sprinkler	Decreased susceptibility	Verner, 1937; Christensen, 1972d; Glenn and Poovaiah, 1989; Meheriuk et al., 1991; Brown et al., 1995; Fernandez and Flore, 1998; Lang et al., 1998; Wójcik et al., 2013; Erogul, 2014
		No, or minor effect	Looney, 1985; Koffmann et al., 1996
Aluminium, iron and copper salts	$Al_2(SO_4)_3$, $Al_3(PO_4)_2$, $CuSO_4$, $AlCl_3$, $FeCl_3$, $Fe(NO_3)_3$, $Fe_2(SO_4)_3$	Decreased water uptake and less cracking	Bullock, 1952, Christensen, 1972d, Beyer et al., 2002a
Growth regulators	4 × 10 ppm GA_3, 1 × 10 ppm GA_3	Increased cracking	Cline and Throught, 2007
	GA_3 at 20 ppm	Decreased cracking	Demirsoy and Bilgener,

	NAA at 0.5, 1, and 2 ppm	Decreased cracking	1998 Yamamoto et al., 1992; Demirsoy and Bilgener, 1998
Fungicides	Borax, Captan, Maneb	No effect	Christensen, 1972d
Coatings	Vaporgard at 2% 14 d or 7 d before harvest	No, or negative effect of tested coatings	Richardson, 1998
	Mobileaf, SureSeal, RainGard, Mixture T1+T2[1]	Decreased susceptibility	Davenport et al., 1972; Meland et al., 2014; Kaiser et al., 2014; Torres et al., 2014; Dumitru et al., 2015

[1]T1) 1% calcium chloride, 1% zinc sulphate, 0,1% polyphenols extracted from *Vitis vinifera* seeds and 0.1% humic acid extracted from lignite

T2) solution obtained of galactomannan extracted from seeds of *Gleditsia triacanthos*, 1%; calcium chloride, 1%; zinc sulphate, 1%; polyphenols extracted from *Vitis vinifera* seeds 0.1% and humic acid extracted from lignite 0.1%. For details see Dumitru et al., 2015.

1.6 Mechanism of water uptake

The rate of osmotic water uptake (F) depends on the liquid water permeability of the fruit surface (P_f), the fruit surface area (A), and the gradient in water potential ($\Delta\Psi$, MPa) between the fruit's water potential (Ψ^{fruit}) and that of the incubation solution ($\Psi_{\Pi}^{solution}$). In the case of rainwater, the osmotic potential of the incubation solution would be very close to 0 MPa.

The fruit's water potential equals the sum of the hydrostatic pressure, i.e. the turgor of the flesh (Ψ_P) and of the osmotic potential (Ψ_{Π}^{fruit} or π, MPa) of the juice. The gravitational and matric potentials are insignificant. The osmotic potential of the juice extracted from the fruit using a spaghetti or a garlic press, reflects the osmotic properties of the flesh's symplast, because the volume of the apoplast of the fruit and fruit skin is very small relative to that of the flesh (predominantly, the cells are thin-walled and large). Recently published data from our group indicates that the turgor of a mature sweet cherry fruit is very low (ranging 8 to 60 kPa; Knoche et al., 2014; Schumann et al., 2014) and is negligible (three orders of magnitude lower) compared to the osmotic potential of a mature fruit (-1.5 to -4.1 MPa; chapter 2; Knoche et al., 2014). Hence, the water potential of the fruit essentially equals the osmotic potential of its flesh.

The permeability of the fruit skin is a composite quantity that depends on the cross-sectional areas for transport and the water permeabilities of the individual pathways along which water uptake occurs. For a detailed review the reader is referred to Knoche (2015). Water uptake occurs through several parallel pathways: the pedicel/fruit junction (Beyer et al., 2002b; Weichert et al., 2004), microcracks in the cuticle (Peschel and Knoche, 2005), polar pores in the cuticle (Weichert and Knoche, 2006), mechanical defects such as insect holes, and through the cuticle. There is no evidence for mass flow of water through open stomata in the absence of a hydrostatic pressure or powerful surfactants (Peschel et al., 2003; Peschel and Knoche, 2012).

In addition, water inflow into the fruit may occur through the pedicel via the vascular system of xylem and phloem (Hovland and Sekse 2004a,b; Measham et al., 2010, 2014; Brüggenwirth et al., 2016).

Although all empirical evidence indicates that water uptake is involved in cracking, attempts to establish relationships between the amount of water uptake (or the determinants of uptake discussed above) and cracking have not proved successful. The relationships obtained are weak and not always significant (Christensen, 1972a; Peschel and Knoche, 2012). These relationships are now re-investigated in greater detail in chapter 5 (Winkler et al., 2016b) of this dissertation.

1.7 Mechanical properties of the fruit skin

The mechanical properties of the fruit skin are a critical factor in cracking. Recently, a number of papers have been published that address these properties in greater detail (Brüggenwirth et al., 2014; Brüggenwirth and Knoche, 2016a-d). In these studies a biaxial tensile test was employed where an excised skin segment of the fruit was pressurized from its inner surface. The pressure and the extent of bulging were monitored and fracture strains, fracture pressures, and the moduli of elasticity were quantified. Using this test, the following findings were established:

Epidermis and hypodermis represent the mechanical ‘backbone’ of the fruit skin. The cuticle has only a negligible effect on the skin’s mechanical properties (Brüggenwirth et al., 2014). Fracture pressures of the fruit skin are of the same order of magnitude as the turgor reported for cells of the outer mesocarp and also for the whole fruit (Knoche et al., 2014; Brüggenwirth and Knoche, 2016b). However, fracture strains usually exceed those estimated from water uptake rates by several orders of magnitude (Christensen, 1972a; Brüggenwirth et al. 2014; Brüggenwirth and Knoche, 2016d). Only when strain rates were lowered to values comparable to those during uptake, did fracture strains of excised skins decrease markedly so values approximated to those calculated for intact fruit (Brüggenwirth and Knoche, 2016d).

The fruit skin becomes significantly strained during stage III growth due to a rapid increase in surface area and a lack of cuticle deposition (Knoche et al., 2004; Grimm et al., 2012; Lai et al., 2016). Excising a skin segment releases the tension in the skin as indicated by a decrease in the surface area of the segment. Also cutting into the fruit causes the cut to gape (Grimm et al., 2012). The release of strain is time dependent, indicating elastic and also viscoelastic behavior (Grimm et al., 2012; Brüggenwirth et al., 2014).

1.8 The current concept of fruit cracking

The conventional concept of fruit cracking is based on the idea that the fruit cracks when water uptake causes the turgor to increase beyond the critical turgor pressure that strains the skin beyond its limit of extensibility (Considine and Kriedemann, 1972). This model is also referred to as the "critical turgor pressure model" (Considine and Kriedemann, 1972; Measham et al., 2009). This model was introduced for grape berries and assumes a soft, fleshy fruit. Because, barring the pit, sweet cherries and grape berries have similar mechanical architectures, this model has also been used to explain rain cracking of sweet cherry (Sekse, 1995a; Sekse et al., 2005; Measham et al., 2009).

The flesh of the cherry is thought to generate the pressure that subjects the skin to tensional forces causing stress in and strain of the skin. The amount of stress (s) is proportional to the radius of the fruit (r), the pressure on a whole fruit basis (p), and inversely related to the thickness of the skin (t; Considine and Brown, 1981).

$$s = \frac{P \cdot r}{2t} \qquad \text{(Eq. 1.2)}$$

Water uptake during rain would then increase fruit (flesh) volume and pressure that stresses and strains the skin. When the fracture strain is exceeded at critical turgor pressure (syn. fracture pressure) the skin cracks.

This concept offers a logical explanation for cracking. It is consistent with the presence of a strained fruit skin as indexed by strain relaxation of excised skin (Grimm et al., 2012), the gaping of fruit after incision (Grimm et al., 2012), and at least some portion of this strain being elastic (Brüggenwirth et al., 2014). The elastic portion of the strain generates a proportional internal pressure. Sekse (1995b) reported that cracking of sweet cherry fruit is also affected by irrigation. This observation suggests that water uptake via the vascular system (xylem and phloem) as well as via the fruit surface (wet skin) are involved in cracking and that the fruit's water balance is the critical determinant.

However, several arguments indicate that cracking may be more complex than this simple concept implies. 1) There was no detectable change in turgor when fruit was allowed to transpire or to take up water even up to the limit of cracking (Knoche et al., 2014). 2) The fracture strains observed in biaxial tensile tests of excised skins largely exceeded those calculated from water uptake rates and half-times to cracking indicated the excised skin is more extensible in the tensile tests as compared to the skin when still on the fruit (Brüggenwirth et al., 2014; Winkler, unpublished data). 3)

Cracking of sweet cherries has been observed despite negative net water uptake rates (Knoche and Peschel, 2006).

1.9 Gap of knowledge

Despite of the significant economic importance of rain cracking of cherry fruit and despite many years of intense research effort, cracking is still poorly understood and the cracking mechanism largely unknown.

Results have been published that appear contradictory and inconsistent with the "critical turgor pressure model". Should the critical turgor concept be wrong, an alternative explanation must be thought to explain rain cracking of sweet cherry fruit.

Key questions and phenomena that are poorly understood and that require detailed research include the following:

(1) The pathways and mechanisms of water uptake through the sweet cherry fruit surface have been studied extensively (Christensen, 1972a; Beyer and Knoche, 2002; Beyer et al., 2002a,b, 2005; Weichert et al., 2004; Weichert and Knoche, 2006a,b). Yet, some puzzling findings remain that question the view that a sweet cherry fruit behaves like a simple osmometer with respect to water uptake (Weichert and Knoche, 2006a). For example, relationships between the osmotic potential of a fruit and the corresponding water uptake rate are surprisingly weak. But these should be highly significant, based on theoretical considerations (Christensen, 1972a). Also, turgors calculated from rates of water uptake using PEG 6000 as osmolyte are unrealistic high, i.e., several fold higher than the air pressure in a car tire. Further, why do sweet cherries incubated in their own juice gain weight and apparently take up water? The water potential of the fruit skin is less negative than that of the flesh and one would expect either no transport of water into the fruit or even a loss of water from the fruit's skin to the incubation solution (Grimm and Knoche, 2015). These observations were reproducible and bring into serious question the simple view that the fruit behaves like an ideal osmometer.

(2) Measham et al. (2010) hypothesized that the "side cracking" of fruit was due to water inflow via the vascular system. This hypothesis requires (i) a re-direction of xylem flows from non-transpiring leaves during rain towards the fruit and (ii) the negative osmotic potential of mature fruit to "drive" water inflow into the fruit when transpiration is absent. Besides, the xylem vascular system must be

conductive. To our knowledge, there is only limited information on water inflows via the xylem under transpiring and non-transpiring conditions. We therefore studied water inflow to the fruit via the xylem vascular system under transpiring and non-transpiring conditions (chapter 3 (Winkler et al., 2016a)).

(3) The most commonly cited explanation for sweet cherry cracking is the critical turgor pressure model. This model, however, is at odds with a number of recent findings including a negligibly low turgor, the absence of detectable effects of transpiration, or water uptake (up to the point of cracking) on turgor (Knoche et al., 2014). Further, the limit of extensibility of the excised skin is about 20% in the elastometer (Brüggenwirth et al. 2014), whereas in submersion tests fracture strains are in the range of about 0.1 to 2.7% (Winkler, unpublished data). Finally, Knoche and Peschel (2006) observed cracking of fruit having negative rates of net water uptake. We therefore investigated relationships between water uptake and cracking in greater detail (chapter 5 (Winkler et al., 2016b)).

(4) Mature sweet cherry fruit have a negligible low turgor (Knoche et al., 2014). In post veraison grape berries turgor is also low (Bernstein and Lustig, 1981, 1985; Lang and Düring, 1990; Thomas et al., 2006, 2008) and this has been explained by the presence of solutes in the apoplast (Wada et al., 2008, 2009). Apoplastic solutes reduce the gradient in osmotic potential between apoplast and symplast thereby decreasing turgor (Lang and Düring, 1990; Wada et al., 2008, 2009). In fact, when osmotic potentials of apoplast and symplast are equal, a turgor would be absent. Apoplastic solutes may arise from a loss of compartmentation during maturation (Lang and Düring, 1991), significant cell death in the maturing fruit (Tilbrook and Tyerman, 2008), enzymatic cell-wall degradation while ripening (Kondo and Danjo, 2001), or the bursting of individual cells due to heterogeneity in water potential components between individual cells (Grimm and Knoche, 2015). As a consequence, the cell contents come into contact with the cell wall. Sweet cherry symplast contains malic acid at concentrations of 70 mM (Herrmann, 2001) and the juice of the fruit is highly acidic (pH 3.6 – 3.9; Serradilla et al., 2011; Hayaloglu and Demir, 2015). Data for tomato indicate that incubating fruit in acidic buffers markedly promoted cracking, while incubating in neutral or basic buffers inhibited cracking (Lichter et al., 2002). What effect exposure

of the tissue to low pH and/or to high concentrations of malic acid has, is unknown. We therefore investigated the effects of malic acid on water uptake and cracking (chapter 4 (Winkler et al., 2015)).

1.10 Objectives

The objectives of this work were to

(1) Test the hypothesis that a sweet cherry fruit is an ideal osmometer with respect to water transport (chapter 2);
(2) Quantify xylem inflow into developing sweet cherry fruit under transpiring and non-transpiring conditions (chapter 3 (Winkler et al., 2016a));
(3) Establish the effects of malic acid on water uptake and cracking of sweet cherry fruit (chapter 4 (Winkler et al., 2015));
(4) Investigate the relationships between water uptake and cracking by manipulating water uptake along the various pathways and to confirm or reject the “critical turgor pressure model” (chapter 5 (Winkler et al., 2016b)).

2. A sweet cherry fruit: An ideal osmometer?

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Contribution of authors

M. Knoche attracted the funding. A. Winkler and M. Knoche planned the experiments. A. Winkler and E. Grimm performed the experiments. A. Winkler and M. Knoche analyzed the data and wrote the manuscript.

A sweet cherry fruit: An ideal osmometer?

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ABSTRACT

Osmotic water uptake through the fruit skin is an important factor in rain cracking of sweet cherries. The objective was to establish whether a sweet cherry behaves like an ideal osmometer, where: (1) water uptake rates are negatively related to the fruit's osmotic potential (Ψ_{Π}^{fruit}), (2) a change in osmotic potential of the incubation solution ($\Psi_{\Pi}^{solution}$) results in a proportional change in the water uptake rate, (3) the value of $\Psi_{\Pi}^{solution}$ yielding a zero uptake rate is numerically equal to the value of Ψ^{fruit}. Also, in the absence of significant fruit turgor (Ψ_P), this $\Psi_{\Pi}^{solution}$ value also equals that of Ψ_{Π}^{fruit}, and (4) the fruit's cuticular membrane (CM) is permeable to water only.

The value of Ψ_{Π}^{fruit} and the rate of water uptake were only weakly related. Surprisingly, incubating a fruit in juice from the same batch of fruit, in isotonic artificial juice comprising five major osmolytes of the juice, or in isotonic glucose solution all resulted in significant water uptake. Decreasing the $\Psi_{\Pi}^{solution}$ decreased rates of uptake and for even lower values caused water loss from the fruit to the solution. The apparent Ψ^{fruit} (at zero water uptake) was always more negative than the Ψ_{Π}^{fruit} throughout development. When incubating excised flesh discs in a series of glucose solutions, the apparent water potential of the flesh discs (Ψ^{flesh}) matched the Ψ_{Π}^{fruit}. Plasmolysis of epidermal cells indicated that the skin's osmotic potential (Ψ_{Π}^{skin}) was less negative than the Ψ_{Π}^{fruit}. Holding fruit for up to 48 d at ~100% RH slightly reduced, but did not eliminate the difference between Ψ_{Π}^{skin} and Ψ_{Π}^{fruit}. Similarly, transpiration (4 d at ~0% RH and 22°C) did not eliminate the difference between Ψ_{Π}^{skin} and Ψ_{Π}^{fruit}. Water uptake from deionized water was paralleled by the outflow of glucose, fructose, and sorbitol and of anthocyanins into the incubation solution. Our data indicate that a sweet cherry is not an ideal osmometer. This is in part due to the CM having a reflection coefficient (σ) for the above moieties somewhat less than σ < 1. These findings have consequences for gravimetric determinations of water uptake.

Rain cracking severely limits sweet cherry production in nearly all regions where this high-value crop is grown and especially when rainfall occurs during the harvest period (Christensen, 1996). Osmotic water uptake through the fruit surface into the flesh is considered to be the primary cause of rain cracking (Christensen, 1996; Winkler et al., 2016). There is no published evidence that the pit plays any significant role in either water uptake or in rain cracking.

In the now familiar 'osmometer' model, the fruit is regarded as a small, thin-walled vessel containing a sugary solution of negative osmotic potential (i.e., the semi-fluid flesh) surrounded by a semipermeable membrane (i.e., the CM). As with an osmometer, the rate of osmotic water uptake (*F*; $g \cdot s^{-1}$) by a cherry fruit equals the product of the flux density (*J*; $g \cdot m^{-2} \cdot s^{-1}$) times the surface area (*A*; m^2). Meanwhile, *J* may be expressed as the product of the osmotic water permeability (P_f; $m \cdot s^{-1}$) and the water potential difference ($\Delta\Psi$) between the inside of the fruit (Ψ^{fruit}) and the outside solution ($\Psi_{\Pi}^{solution}$). Based on the gas laws, this is multiplied by the molar volume of water (V_w) divided by the product of the gas constant (R) and the absolute temperature (T) ($\frac{V_w}{R \cdot T}$) (House, 1974; Kramer and Boyer, 1995; modified), hence we can write:

$$F = J \cdot A = P_f \cdot \Delta\Psi \cdot \mathrm{A} \cdot \frac{V_w}{R \cdot T}$$

Since for rain water the value of $\Psi_{\Pi}^{solution}$ is very close to zero, the value of $\Delta\Psi$ during rain is determined solely by Ψ^{fruit}. Meanwhile, the value of Ψ^{fruit} equals the sum of a number of water potential components: the principal ones being the internal pressure (i.e., the fruit Ψ_P (relative to atmospheric pressure)) and the average tissue Ψ_{Π}^{fruit}. Recent studies have established that values of fruit and cell Ψ_P of mature cherry fruit are negligibly low relative to values of the Ψ_{Π}^{fruit} (Knoche et al., 2014; Schumann et al., 2014). Thus, for a mature sweet cherry fruit either outside in the rain or inside immersed in pure water, we can safely introduce the simplification that $\Delta\Psi$ is numerically equal to Ψ_{Π}^{fruit}.

From the above, one would expect close and positive relationships between *F* and *A*, P_f, and $\Delta\Psi$, when a fruit is incubated in deionized water in the laboratory. Furthermore, expressing *F*/*A* as the *J* eliminates *A* as a source of variability and this

should yield close relationships of *J* with Ψ_{Π}^{fruit} and/or P_f. Also, by decreasing $\Delta\Psi$ by increasing the osmolyte concentration in the incubation solution one would anticipate a simple proportional decrease in *F* and/or *J*. Furthermore, because osmotic activity is a colligative property, the relationship obtained should be independent of the osmolyte used.

To the best of our knowledge, relationships between *F* or *J* and $\Delta\Psi$ or P_f have not been investigated in sweet cherry. The only results we are aware of reveal highly variable relationships between *F* (expressed as a % weight increase) and the value of Ψ_{Π}^{fruit} (Christensen, 1972).

The objective of our study was to establish whether sweet cherry behaves like an ideal osmometer. To classify as an ideal osmometer, the following criteria should be fulfilled: (1) *F* and *J* should be negatively related to Ψ_{Π}^{fruit}, (2) a change in $\Psi_{\Pi}^{solution}$ should result in a corresponding change in *F*, (3) the value of $\Psi_{\Pi}^{solution}$ yielding zero net uptake should exactly match that of $\Psi_{\Pi}^{fruit}$and should be independent of the osmolyte used, (4) the CM should be permeable only to water but not to any osmolytes present in the fruit or in the incubation solution, and (5) the value of Ψ_{Π}^{fruit} should be uniform within the fruit.

MATERIAL AND METHODS

PLANT MATERIAL. Sweet cherry fruit of the cultivars Adriana, Burlat, Hedelfinger, Merchant, Sam, Samba, Schneiders Späte Knorpel, and Regina and the sour cherry cultivars Achat, Morellenfeuer, and Ungarische Traubige were picked from greenhouse-grown or field-grown trees grafted on 'Gisela 5' rootstocks (*P. cerasus* L. x *P. canescens* Bois) at the Horticultural Research Station of the Leibniz University in Ruthe, Germany (lat. 52°14'N, long. 9°49'E). The only exception was 'Regina' used in the outflow-experiment, which was harvested from a commercial orchard in Ohndorf, Germany (52°36'N, 9°35'E) and held for up to two weeks at 2°C. Fruit were selected for uniformity of size, color, and freedom from defects. The pedicels were cut flush with the receptacle. Pedicel end and receptacle as well as the pedicel/fruit junction were sealed using a fast curing silicone rubber (Dow Corning SE 9186; Dow Corning Corp., Midland, MI). The silicone was allowed to cure for about one hour.

Sealing the pedicel/fruit junction restricted water uptake to the fruit surface (Beyer et al., 2002).

DETERMINING WATER UPTAKE, THE APPARENT Ψ^{fruit}, AND THE CALCULATED Ψ_P .

Water uptake and water loss were determined gravimetrically as changes in fruit mass. Fruit were weighed, incubated individually in various solutions, removed usually after 45 and 90 min, carefully dried using tissue paper, re-weighed, and re-incubated. The value of *F* was calculated as the slope of a linear regression fitted through a plot of cumulative water uptake vs. time. Fruits that cracked in the course of an experiment were excluded from the analyses.

The apparent Ψ^{fruit} was obtained by determining *F* from osmolyte solutions at concentrations ranging from hypertonic to hypotonic. We refer to the Ψ^{fruit} as the apparent Ψ^{fruit} because it is quantified indirectly by determining water uptake and loss. Briefly, a linear regression was fitted through a plot of *F* vs. the $\Psi_{\Pi}^{solution}$. Because these relationships were not linear, the regression was limited to those data lying close to the x-axis intercept (i.e., close to the point of zero net change in mass). The intercept was calculated from the regression equation and corresponds to the $\Psi_{\Pi}^{solution}$ that resulted in constant fruit mass. Under these conditions, there is no net movement of water indicating that the apparent Ψ^{fruit} equals the $\Psi_{\Pi}^{solution}$ (Weichert and Knoche, 2006a).

A fruit Ψ_P is classically calculated as the difference between Ψ^{fruit} and Ψ_{Π}^{fruit}. We refer to this Ψ_P as the calculated Ψ_P .

DETERMINING Ψ_{Π}^{skin}.

The value of Ψ_{Π}^{skin} was determined using plasmolysis (Grimm and Knoche, 2015). Briefly, skin sections were prepared using a razor blade, the sections were momentarily rinsed in isotonic sucrose solution to remove any juice from the cut surface and blotted with tissue paper. The sections were immediately transferred to a glass microscope slide, covered with a cover slip, and incubated in a sucrose solution ($\Psi_{\Pi}^{solution}$) for 30 min. The sucrose solutions used, ranged in concentration from –5.0 to 0 MPa. Afterwards, slides were viewed at ×40 (BX-60; Olympus Europa GmbH, Hamburg, Germany) in transmitted light. Calibrated images were taken (DP73;

Olympus). The numbers of plasmolyzed cells and the total numbers of cells were counted and the percentage of plasmolyzed cells was calculated. The values of Ψ_{Π}^{fruit} and $\Psi_{\Pi}^{solution}$ were determined by water vapor pressure osmometry (VAPRO® 5520 and 5600; Wescor, Logan, UT).

EXPERIMENTS.

Relationships between Ψ_{Π}^{fruit} and *F* from deionized water were investigated using 'Adriana', 'Burlat', 'Hedelfinger', 'Merchant', 'Sam', 'Samba', 'Schneiders Späte Knorpel', and 'Regina' fruit. Fruit juice was extracted using a garlic press and the value of Ψ_{Π}^{fruit} was determined (VAPRO® 5520 and 5600; Wescor). The number of individual fruit was 172. There were no consistent differences among cultivars, which allowed the data for all cultivars to be pooled.

The effect of varying $\Psi_{\Pi}^{solution}$ of artificial juice or of polyethylene glycol 6000 (PEG 6000; Merck Eurolab, Darmstadt, Germany) on *F* and on the apparent Ψ^{fruit} was quantified in 'Adriana'. The artificial juice and the PEG 6000 solutions were prepared at values of $\Psi_{\Pi}^{solution}$ ranging from -5.1 to 0 MPa. The lowest (most negative) $\Psi_{\Pi}^{solution}$ were hypertonic relative to the Ψ_{Π}^{fruit} and so resulted in negative rates of water uptake (i.e., water losses). Sweet cherry juice extracted from the same batch of fruit served as a control. The number of replicates was ten.

The effect of different cultivars on the calculated fruit Ψ_P was studied in 'Adriana', 'Regina', 'Sam', and 'Samba'. Fruit were incubated in PEG 6000 solutions ranging from -5.4 to 0 MPa. Values of Ψ_{Π}^{fruit} were determined by vapor pressure osmometry (VAPRO® 5520 and 5600; Wescor). The number of replicates was ten.

The effect of incubating fruit in expressed fruit juice on water uptake was studied in 'Achat', 'Adriana', 'Hedelfinger', 'Morellenfeuer', 'Regina', 'Samba', 'Schneiders Späte Knorpel', and 'Ungarische Traubige'. Juice was extracted from the same batch of fruit. Values of Ψ_{Π}^{fruit} were determined by vapor pressure osmometry (VAPRO® 5520 and 5600; Wescor). Fruit incubated in deionized water served as controls. The number of replicates was ten.

The effect of heat treatment to inactivate any enzymes present in the juice was studied in 'Adriana'. Rates of water uptake were determined by incubating fruit in juice heated for 5 min to 60°C, then cooled to laboratory temperature before use. Juice that had not been heat-treated served as control. Juice was prepared from the

same batch of fruit as described above. As an additional control, an artificial juice was prepared, comprising the five major osmolytes present in real juice. This was isotonic to the real fruit juice used in this experiment (Herrmann, 2001). The osmolytes in the artificial juice and their relative contributions to total osmolarity were: glucose (41.2%), fructose (37.5%), sorbitol (7.3%), malic acid (6.7%), and potassium as potassium malate (5.4%). These five osmolytes accounted for 98% of the osmotically active components of sweet cherry juice (Herrmann, 2001; Winkler et al., 2015). Values of $\Psi_{\Pi}^{solution}$ were determined (VAPRO® 5520 and 5600; Wescor). The number of replicates was 15.

The effect of the pH of the sweet cherry juice on the rate of water uptake was investigated in 'Regina'. Fruit were incubated in juice extracted from the same batch of fruit or in isotonic artificial juice. Both juices were used at their 'native' pH (4.2) or titrated to pH 7 using KOH. The number of replicates was 15.

The effect of the developmental stage on the apparent Ψ^{fruit} was investigated in 'Regina' at 32, 52, 71, and 91 days after full bloom (DAFB). The *F* from glucose solutions ranging in $\Psi_{\Pi}^{solution}$ from -5.8 to 0 MPa were determined. The number of replicates was ten.

To identify whether the skin has an effect on the apparent Ψ^{fruit}, the latter was determined by incubating flesh discs excised from 'Regina' sweet cherry in glucose solutions differing in osmolarity. Flesh cylinders having diameters of 8 mm were excised from the equatorial region of the fruit's cheek using a biopsy punch and cut to discs of 2 mm thickness using parallel-mounted razorblades. The discs were incubated in multiwell plates (well diameter 24 mm) containing glucose at $\Psi_{\Pi}^{solution}$ ranging from -5.9 to 0 MPa. Calibrated photographs were taken after 0, 15, and 30 min of incubation at 1× using a binocular microscope equipped with a camera (MZ6, Leica, Wetzlar, Germany; camera DP73; Olympus). The area of the discs was quantified using image analysis software (cellSens 1.7.1; Olympus). The *F* values were calculated from the change in disc area during the first incubation interval. It was assumed that the disc tissue was isotropic and hence, would have undergone a change in thickness (not measured), proportional to the change in area (measured). Whole fruit from the same batch and incubated in the same solutions served as controls. The minimum number of replicates was six for discs and seven for whole fruit.

To establish whether the history of transpiration of the fruit alters the apparent Ψ^{fruit}, the effect of the water vapor concentration deficit during a holding period before an experiment was studied. Mature 'Schneiders Späte Knorpel' fruit were preconditioned by incubation above dry silica gel (~0% RH) or above water (~100% RH) at 22°C for 4 d. Subsequently, the apparent Ψ^{fruit} was determined in glucose solutions ($\Psi_{\Pi}^{solution}$) ranging from -4.9 to 0 MPa. Fresh fruit without a holding period and fruit held at ~100% RH for 4 d were used as controls. The number of replicates was ten.

To identify whether the water vapor concentration deficit during a holding period induced a gradient in Ψ_{Π}^{fruit} within the fruit, 'Adriana' fruit was allowed to transpire for up to 154 h above dry silica gel (~0% RH) and the mass loss quantified. Fruit incubated above water (~100% RH) served as controls. The values of Ψ_{Π}^{fruit} from the flesh were determined. Tissue cylinders ranging in depth from the skin to the pit were excised from the equatorial region using a biopsy punch of 8 mm diameter. The cylinders were then cut into four discs, each of about 1.5 mm thickness. The discs represented the entire transect of the flesh from skin to pit. The discs were squeezed to liberate their juice and this was measured immediately (VAPRO® 5520 and 5600; Wescor). In a second experiment, plasmolysis of epidermal cells was quantified by incubating skin sections in sucrose solutions ($\Psi_{\Pi}^{solution}$) ranging from -5.0 to 0 MPa (Grimm and Knoche, 2015). This procedure yields a high-resolution estimate of the Ψ_{Π}^{skin} only. We are not aware of any other method to assess this value. The history of transpiration of the fruit was varied. In one experiment fruit was preconditioned by excluding any transpiration by incubation for up to 48 days above water (~100% RH at 22°C, 'Regina'). In another experiment fruit was incubated for 4 d above dry silica gel (~0% RH, 22°C, 'Schneiders Späte Knorpel') to maximize transpiration. Freshly sampled fruit were used as control. The number of replicates was ten.

Leakage of osmolytes from the fruit into the incubation solution was investigated by incubating 'Regina' fruit for up to 74 h in beakers containing 60 ml of deionized water. Water uptake was quantified gravimetrically. All fruit were carefully inspected macroscopically and fruits that cracked in the course of the experiment were discarded. Following removal of the fruit from solution, the incubation solution was lyophilized, its dry mass determined gravimetrically, and the residue taken up in

2.5 ml of deionized water. Subsequently, soluble solids were determined by refractometry. There was a linear relationship between the amount of solute outflow and the content of soluble solids (r^2=0.99***, data not shown). Any leakage of anthocyanin that may have occurred was quantified by determining the absorption at 520 nm using a spectrophotometer (Specord 210; Analytik Jena, Jena, Germany) after adjusting the pH to pH 3.5 using malic acid. Finally, sucrose, glucose, fructose, and sorbitol in the incubation solution were quantified by HPLC. Briefly, a 20 µl aliquot was injected onto a HC-75 (Ca^{2+}, 9 µm 7.8 x 305 mm; Hamilton Company, Reno, NV) column of an HPLC system (LC-CaDI 22-14, HPLC compact pump 2250; Bischoff, Leonberg, Germany) equipped with a refractive index detector (RI detector 8020; Bischoff) and a column heater (Variotherm 880; Bischoff). The flow rate was 0.4 ml min^{-1}, column temperature 80°C, and deionized water was used as a mobile phase.

DATA ANALYSIS. Data are presented as means ± SE. Where error bars are not visible in a graph, they are smaller than the data symbols. Exception is Fig. 1, where data points for individual fruit are shown. Data were examined using analysis of variance or regression analysis. Pairwise comparisons of treatment means were carried out using Tukey's Studentized range test ($P \leq 0.05$, package multcomp 1.3-1, procedure glht, R version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria). Regression analysis was performed using R (version 3.3.2) and SigmaPlot (version 12.5; Systat Software, San Jose, CA). Significance of coefficients of determination (r^2) at $P \leq 0.05$, 0.01, and 0.001 is indicated by *, **, and ***, respectively.

RESULTS

The relationships between *F* or *J* and *A* or Ψ_{Π}^{fruit} were significant, but highly variable as indicated by coefficients of determination ranging from r^2 = 0.12*** to r^2 = 0.23***. Variability was particularly high for larger *A* and larger driving forces as indexed by more negative Ψ_{Π}^{fruit} (Fig. 1A and B). There was little difference in variability between the relationships of *A* or Ψ_{Π}^{fruit} with *F* and *J*.

Water uptake from artificial sweet cherry juice and from PEG 6000 solutions increased with time during a 1.5 h incubation at an approximately constant rate (Fig. 2A and B). Making the $\Psi_{\Pi}^{solution}$ more negative decreased rates of water uptake. At equal Ψ_{Π}^{fruit}, the *F* from artificial juice exceeded that from iso-osmolar PEG 6000 (Fig. 2C). The only exceptions were hypertonic solutions, where *F* from artificial juice were equal to those from PEG 6000 or higher. Interestingly, the apparent Ψ^{fruit} averaged -2.7 MPa for fruit incubated in artificial juice, but -1.4 MPa for that incubated in PEG 6000 (Fig. 2C). At equal tonicity there was no difference in *F* from artificial juice and juice extracted from the same batch of fruit. When calculating the fruit Ψ_P, an excessively high calculated fruit Ψ_P was obtained from Ψ^{fruit} determined in PEG 6000 (Ψ^{fruit} =-1.4 MPa, Ψ_{Π}^{fruit} = -2.6 MPa, calculated fruit Ψ_P = 1.2 MPa). However, a negative fruit Ψ_P was calculated for Ψ^{fruit} determined in artificial juice (Ψ^{fruit} =-2.7 MPa, Ψ_{Π}^{fruit} = -2.2 MPa, Ψ_P = -0.5 MPa). Most surprisingly, fruit incubated in its own juice or in artificial juice of the same tonicity as the fruit's juice had a positive *F* (Fig. 2C).

The extremely high fruit Ψ_P calculated from Ψ^{fruit} determined in PEG 6000 was not unique to a specific cultivar, but was also obtained in other sweet cherry cultivars (Table 1). Similarly, water uptake from its own juice was also observed in other sweet cherry cultivars and also in two sour cherry cultivars. However, one sour cherry cultivar decreased slightly in mass (Table 2).

Artefacts during incubation in juice that might result from enzymatic activity in the juice or from the acidity of the solution that may have changed the $\Psi_{\Pi}^{solution}$ can be excluded as factors. First, heating juice to 60°C produced the same *F* (1.3 ± 0.2 $mg \cdot h^{-1}$) as that from non-heated juice (1.0 ± 0.1 $mg \cdot h^{-1}$) or from artificial juice of the same tonicity (1.4 ± 0.1 $mg \cdot h^{-1}$). Also, the pH of natural juice or that of artificial juice had no effect on *F*. For natural juice of pH 4.2 and pH 7.0, the *F* were 1.1 ± 0.2 $mg \cdot h^{-1}$ and 1.2 ± 0.2 $mg \cdot h^{-1}$, respectively, and for artificial juice 1.1 ± 0.2 $mg \cdot h^{-1}$ at pH 4.2 and 1.3 ± 0.2 $mg \cdot h^{-1}$ at pH 7.0.

Water uptake (mass gain) decreased, becoming a water loss (mass loss) at all developmental stages as the value of $\Psi_{\Pi}^{solution}$ became more negative (Fig. 3). The apparent Ψ^{fruit} and the Ψ_{Π}^{fruit} became more negative as development progressed

(Fig. 3A-E). Interestingly, the fruit's apparent Ψ^{fruit} was always more negative than the Ψ_{Π}^{fruit} implying a negative calculated fruit Ψ_P . The difference between the apparent Ψ^{fruit} and the Ψ_{Π}^{fruit} increased up to 71 DAFB and remained constant at about -1.3 MPa, thereafter (Fig. 3F). At equal tonicity rates of uptake did not differ between fruit incubated in juice and in glucose solutions (Fig. 3D).

Water uptake into flesh discs levelled off after 0.25 h indicating a decrease in *F* (Fig. 4A), whereas cumulative uptake into whole fruit increased linearly (i.e., a constant rate of uptake) up to 2 h (Fig. 4B). The apparent water potentials were -2.8 MPa (Ψ^{flesh}) and -4.1 MPa (Ψ^{fruit}) for fruit from the same batch (Fig. 4C). Thus, the apparent Ψ^{flesh} approximately matched the Ψ_{Π}^{fruit} (-3.1 MPa), whereas the apparent Ψ^{fruit} was far more negative than the Ψ_{Π}^{fruit} (Fig. 4C).

The difference between the Ψ_{Π}^{fruit} determined in the osmometer and the apparent Ψ^{fruit} determined in the incubation assays did not depend on the transpiration history of the fruit. Holding fruit for 48 h at ~100% RH slightly increased the Ψ_{Π}^{fruit} from -2.7 to -2.6 MPa and the Ψ_{fruit} from -3.8 to -3.7 MPa. Fruit held at ~0% RH lost 1.4 g water and decreased in Ψ_{Π}^{fruit} from -2.7 to -3.3 MPa and in the apparent Ψ^{fruit} from -3.8 to -4.4 MPa. The difference between the Ψ_{Π}^{fruit} and the apparent Ψ^{fruit} in both treatments and the control remained constant at 1.1 MPa (Table 3). Holding fruit under transpiring conditions (~0% RH) for up to 154 h produced a mass loss of up to 2.2 g and a proportional decrease of the Ψ_{Π}^{fruit} as compared to fruit held at 100% RH (data not shown). However, despite of the high mass loss at ~0% RH, there was no detectable gradient in Ψ_{Π}^{fruit} between the fruit's outer and inner flesh. When holding fruit under non-transpiring conditions (~100% RH), mass and Ψ_{Π}^{flesh} remained constant (data not shown).

The percentage of plasmolyzed epidermal cells increased in a sigmoidal fashion as $\Psi_{\Pi}^{solution}$ decreased (Fig. 5). The osmotic potential at 50% plasmolysis represented the Ψ_{Π}^{skin}, which was always less negative than the Ψ_{Π}^{flesh}, as indexed by the Ψ_{Π}^{fruit}. Holding fruit for up to 48 d at ~100% RH slightly reduced, but did not eliminate the difference between Ψ_{Π}^{flesh} and Ψ_{Π}^{skin}, the former being approximately equal to the Ψ_{Π}^{fruit} (Table 4). Fruit held at ~0% RH for 4 d lost 1.4 g and had slightly

more negative Ψ_{Π}^{flesh} and Ψ_{Π}^{skin} (Fig. 5B). The difference between the Ψ_{Π}^{flesh} and Ψ_{Π}^{skin}, however, remained constant.

Water uptake increased with time when incubating fruit in deionized water (Fig. 6A, r^2 = 0.90***). The increase in water uptake was closely paralleled by the outflow of solutes from the fruit into the incubation solution as indexed by the increase in soluble solids (Fig. 6B, r^2 = 0.99***). Coefficients of determination for the relationship between osmolyte outflow and water uptake were higher (r^2 = 0.99***) than those for osmolyte outflow and time (r^2 = 0.80***). Also, anthocyanin leakage increased exponentially at high rates of water uptake, indicating the bursting of cells and vacuoles (Fig. 6B inset, r^2 = 0.89***). The HPLC analyses revealed an outflow of glucose, fructose, and sorbitol that was linearly related to water uptake (Fig. 6C, all r^2 = 0.99***). The sum of these three sugars accounted for ~80% of the total outflow of osmolytes (Fig. 6C inset). There was no outflow of sucrose (data not shown).

DISCUSSION

A sweet cherry is more complex than a simple osmometer

Our results demonstrate that a sweet cherry fruit functions like an ideal osmometer only in some aspects - but clearly not in all. Consistent with being an ideal osmometer is the response of sweet cherry fruit to varying $\Psi_{\Pi}^{solution}$. For a given osmolyte or a mixture of osmolytes, decreasing the $\Psi_{\Pi}^{solution}$ (making it more negative) decreased water uptake and *vice versa* for increases. This response was obtained for all osmolytes investigated, including for PEG 6000, artificial juice, and glucose. It is also consistent with observations reported for grapes (Becker and Knoche, 2011) and Ribes berries (Khanal et al., 2011).

However, inconsistent with an ideal osmometer were the following responses: First, the apparent Ψ^{fruit} determined from the point of zero net change in mass depended on the osmolyte used in this and an earlier study (Weichert and Knoche, 2006a). PEG 6000 yielded a less negative apparent Ψ^{fruit} than artificial juice. This response was not related to the low pH of the artificial juice, but occurred also when the pH was adjusted to pH 7.0. Second, the difference between the apparent Ψ^{fruit} and the $\Psi_{\Pi}^{solution}$, i.e., the $\Delta\Psi$, is considered an estimate of the fruit Ψ_P . However, when

subtracting the apparent Ψ^{fruit} determined in PEG 6000 solutions from the $\Psi_{\Pi}^{solution}$, excessively high values for the fruit Ψ_P are obtained (therefore referred to here as the calculated fruit Ψ_P). Literature data for other fruit crops also yielded values of the same order of magnitude (Table 5). However, experimentally determined values of fruit and cell Ψ_P using pressure probes with mature fruit are negligibly low relative to the $\Psi_{\Pi}^{solution}$ (<0.1 MPa for grapes, see Thomas et al., 2006, 2008; 0.03 to 0.05 MPa for sweet cherry, see Knoche et al., 2014; Schumann et al., 2014). For grape berries, the lack of a significant measured cell Ψ_P despite the very negative Ψ_{Π}^{fruit} was accounted for by the presence of apoplastic solutes (Wada et al., 2008, 2009), possibly as a result of a compartmental breakdown (Lang and Düring, 1991). Whether the same explanation applies to sweet cherry is not known. Thirdly, and most surprisingly, we detected water uptake from juice extracted from the same batch of fruit. This effect was reproducible across all sweet cherry and two of three sour cherry cultivars. Also, it was not caused by any enzymatic reactions in the juice that could have altered its composition or osmolarity. Furthermore, at equal tonicity there was no difference in water uptake between natural and artificial juice or glucose-only solutions. These observations are particularly difficult to explain. This is because, at first sight, one would expect a fruit incubated in its own juice to represent an isotonic system. Such a system should – at best – yield only a very slight decrease in fruit mass with time due to a very low value of fruit Ψ_P . In the complete absence of fruit Ψ_P one would expect no change at all in fruit mass. Finally, there is clear evidence for leakage of osmolytes from the fruit into the incubation solution.

Thus, the hypothesis that a sweet cherry is an ideal osmometer must be rejected. Some other factors are clearly involved and a more complex model must be postulated.

The CM has a reflection coefficient <1 for small osmolytes.

An ideal osmometer allows penetration of the solvent, but totally excludes penetration by solutes (osmolytes). The results for cherry, however, demonstrate that the CM allows some passage of osmolytes from the fruit into the incubation solution. In membrane studies, the permeability to solutes relative to the permeability to solvents

is characterized by the dimensionless σ. Thus, for a cherry, the value $\sigma = 1$ implies a membrane permeable only to water. While a $\sigma < 1$ would imply diffusional inflow and outflow of osmolytes depending on the magnitude and direction of any chemical potential gradients in addition to diffusional inflow and outflow of water depending on the magnitude and direction of water potential gradient. Because for a given membrane the σ depends on the size of the osmolyte, the values of σ differ for each solute species. For osmolytes too large to penetrate, the sweet cherry CM has a σ = 1 and for those below some size exclusion limit a $\sigma < 1$.

The leakage of glucose (180 $g \cdot mol^{-1}$), fructose (180 $g \cdot mol^{-1}$), and sorbitol (182 $g \cdot mol^{-1}$) from the fruit into the incubation solution indicates that the size of these solutes is below the exclusion limit of the CM and hence, the value of σ for these osmolytes is $\sigma < 1$. These findings are consistent with the size exclusion limits of the sweet cherry CM estimated earlier (Weichert and Knoche, 2006a). The largest penetrating osmolyte was sucrose that had a σ = 0.74 (MW 342 $g \cdot mol^{-1}$), the smallest non-penetrating osmolyte was PEG 1500 (MW 1500 $g \cdot mol^{-1}$) (Weichert and Knoche, 2006a). Consequently, the PEG 6000 (Mean MW 6000 $g \cdot mol^{-1}$) also used in the present study is size-excluded ($\sigma = 1$).

It is interesting to note the outflow of glucose, fructose, and sorbitol was closely correlated to the water movement, suggesting identical pathways for water uptake and osmolyte outflow. In principle, two pathways may be thought of. First, polar pathways that are present in sweet cherry (Weichert and Knoche, 2006a). Polar pathways result from the orientation of polar functional groups in the hydrated CM (Schönherr, 2006). They bypass the lipophilic CM and allow viscous flow and diffusion of polar substances and water in an aqueous continuum across the CM (Weichert and Knoche, 2006a; Schönherr, 2000, 2006). Second, microcracks in the CM that form upon exposure of the strained CM to water (Knoche and Peschel, 2006). The relative contribution of microcracks to the movement of water and osmolytes across the skin is unknown, but expected to be highly variable.

These findings have consequences for experimental determinations of the Ψ^{fruit} where fruit is incubated in solutions of osmolytes at different tonicity and the change in fruit mass is recorded. Two situations must be distinguished, i.e., fruit incubated in solutions of osmolytes that (1) exceed the size exclusion limit of the CM ($\sigma = 1$) or (2) are below the size exclusion limit ($\sigma < 1$).

The first situation would be typical for fruit incubated in PEG 6000 solutions, where $\sigma = 1$ (Fig. 2 and Table 1). The osmolytes present in the sweet cherry including glucose, fructose, and sorbitol, were size permitted ($\sigma < 1$; Weichert and Knoche, 2006a). For these osmolytes a chemical potential gradient exists from the fruit (high concentration) to the solution (low concentration). Thus, an outflow of osmolytes will occur when incubating fruit in PEG 6000 solutions (Fig. 7A). Meanwhile, water movement (uptake, loss) will depend on magnitude and direction of the water potential gradient. As a consequence of the outflow of osmolytes, the apparent Ψ^{fruit} determined by monitoring the change in fruit mass gravimetrically is less negative than the "true" Ψ^{fruit} that reflects water transport only. Also, when subtracting the Ψ_{Π}^{fruit} from the apparent Ψ^{fruit}, an unrealistically high calculated Ψ_P is obtained (Fig. 2 and Table 1). It is worth noting that the artefactual high calculated Ψ_P that may be estimated for fruit of other species when incubated in PEG 6000 (grape and Ribes berries (Becker and Knoche, 2011; Khanal et al., 2011), would probably also be accounted for by $\sigma < 1$.

The second situation – fruit incubated in solutions of osmolytes below the size exclusion limit – is mimicked for example by fruit incubated in its own juice, in artificial juice, or in glucose (Fig. 7B). Solutions of these osmolytes were selected, because they are identical to most or at least one of the major osmolytes of the sweet cherry juice. Also, these osmolytes were recovered in the outflow (Table 2 and Fig. 2-4; Herrmann, 2001) indicating that the $\sigma < 1$. Thus, the osmolytes would penetrate along their gradients in chemical concentration and in Ψ^{fruit}. In hypotonic solutions, outflow of osmolytes would occur out of the fruit into the incubation solution. As the $\Psi_{\Pi}^{solution}$ is decreased and becomes more negative and hypertonic, the outflow from the fruit would decrease, cease, and then become an inflow of osmolytes into the fruit. As a consequence, the concurrent diffusion of the osmolytes would decrease the slope of the relationship between the gravimetrically determined flow and the $\Psi_{\Pi}^{solution}$ compared to that between the flow of water only and the $\Psi_{\Pi}^{solution}$. Because the relationship is not displaced, the x-axis intercept and hence, the Ψ^{fruit} remain constant.

These arguments demonstrate that the sweet cherry CM has a $\sigma < 1$ for low molecular weight osmolytes and that this accounts for both osmolyte outflow and

inflow. The movement of osmolytes occurred in gravimetrically detectable amounts. Furthermore, an outflow of osmolytes from the fruit accounted for the excessively high Ψ_P calculated from apparent Ψ^{fruit} determined in PEG 6000.

Active uptake of solutes from apoplast into symplast

Experiments in this and earlier studies (Grimm and Knoche, 2015) established that the Ψ_{Π}^{skin} is less negative than Ψ_{Π}^{fruit}. The data herein demonstrate that this gradient remained essentially constant despite of very significant transpiration or despite of extended holding periods under non-transpiring conditions. Thus, the fruit represented a fairly stable two compartment system of skin and flesh. If a less negative Ψ_{Π}^{skin} (irrespective of its mechanistic basis) was driving water uptake, the juice of the flesh would resemble a hypertonic solution relative to the skin. Under these conditions, we would expect a fruit incubated in its own juice to lose water from the skin to the incubation solution. Instead, water uptake occurred consistently and regardless of the type of osmolyte used (e.g., natural juice with and without heating or pH adjustment, isotonic artificial juice, or isotonic glucose solutions). A conceivable explanation for this reproducible observation would be an active mechanism of uptake of osmolytes from the apoplast into the symplast following penetration of the CM. An active accumulation of osmolytes in the skin would result in a more negative Ψ_{Π}^{skin} and hence, a steepening of the water potential gradient that drives osmotic water uptake into the fruit. To the best of our knowledge, however, there is no conclusive or direct evidence supporting a role of active water uptake. Wade (1988) investigated the effects of metabolic inhibitors on water uptake and cracking. Of the five inhibitors investigated, NaF (-43% after 16 h at 50 mM), NaN_3 (-10% after 16 h at 2 mM), and $AgNO_3$ (-43% after 16 h at 10 mM) significantly reduced water uptake consistent with a role of active processes in water uptake. However, decreased water uptake in the presence of $AgNO_3$ was also reported by Weichert et al. (2004), but attributed to a precipitation reaction that plugged polar pathways across the CM (Weichert and Knoche, 2006b). Further, recent experiments investigating the effects of NaN_3 and CCCP on water uptake did not yield consistent or conclusive data (A. Winkler, unpublished data). Thus, direct evidence for active water uptake remains to be established.

The role of the cell wall

It may be argued that the phenomenon of water uptake from its own juice was an artefact resulting from the juice not being representative due to the extraction by pressing. Following pressing, the residue in the press largely comprises cell walls. The water potential associated with the cell wall residue is missed when sampling the juice for osmometry. Cell walls swell and swelling is a typical characteristic of ripening fruit including sweet cherry (Redgwell et al., 1992, 1997; Grimm and Knoche, 2015). Swelling results from water partitioning into the cell wall (Redgwell et al., 1997). A preferential movement of water into the cell wall implies a more negative water potential of the cell wall relative to the symplast. However, there was no difference in osmolarity and – in the absence of cell Ψ_P – no difference in water potential between the cell wall pellet and the supernatant juice when separating a homogenate of sweet cherry fruit (minus pit) by centrifugation into juice and cell wall fractions (C. Schumann, unpublished data). Also, the water potential of apoplast and symplast are expected to be in equilibrium (Knoche et al., 2014; Schumann et al., 2014). Thus, at present the cell wall is considered to be an unlikely candidate for the unknown driving force.

However, the cell wall could still play a role in water uptake from fruit incubated in its own juice provided that swollen cell walls of some cells generated a significant boundary layer resistance for water movement. The plasmolysis experiments in this and our earlier study revealed a marked difference in osmotic potentials between the onset of plasmolysis as indexed by the first cell plasmolyzing and the completion of plasmolysis as indexed by the last cells plasmolyzing (Grimm and Knoche, 2015). This indicates considerable heterogeneity within the population of cells in a fruit (Grimm and Knoche, 2015). Assuming cells to be in water potential equilibrium, this response range reflects the range in cell Ψ_P . Also, our earlier study indicates that cell walls swell essentially instantaneously when the cell Ψ_P decreases. Hence, when incubating fruit in more negative $\Psi_\Pi^{solution}$, the Ψ_P of cells having a less negative osmotic potential resulting in the swelling of cell walls. In contrast, cells having a more negative osmotic potential remain turgescent and with non-swollen cell walls. If swollen cell walls represented a significant boundary layer resistance in water movement compared to non-swollen cell walls, diffusive resistance would increase for uptake into cells having a less negative osmotic potential, but not or less for those

that are more negative. Since the Ψ_{Π}^{fruit} represents the driving force for water uptake, cell wall swelling would introduce a bias in permeability towards cells having a more negative osmotic potential. It is the latter fraction of the population of cells that remains to have a lower diffusive resistance (high permeability) and a driving force exceeding (more negative) the average osmolarity of the juice. This phenomenon would account for water uptake from its own juice. Clearly, these arguments are highly speculative and direct supporting evidence is needed in further studies.

Practical implications

Our data have important implications for research on water relations in sweet cherry. The gravimetrically measured change in fruit mass during incubation represents a net change comprising the flow of water plus that of osmolytes. The osmolytes present in the fruit are low molecular weight solutes. For these, $\sigma < 1$, possibly resulting in outflow of osmolytes from the fruit into the incubation solution. In addition, an inflow of osmolytes from the incubation solution to the fruit may also occur when the osmolytes in the incubation solution are sufficiently small to allow passage through the CM ($\sigma < 1$). Quantifying water uptake by incubating a fruit in an aqueous solution and determining the change in mass is a convenient and precise method to study water movement. This technique is used in practically all studies in sweet cherry and other fruit. The question arising now is how large the errors in typical incubation assays are, if the change in mass is not solely due to water movement, but also to the movement of osmolytes into and out of the fruit. A first estimate of the magnitude of the error may be obtained from the outflow experiments in our study. The gross uptake rate determined from the change in fruit mass amounted to 8.6 ± 0.6 $mg \cdot h^{-1}$ of which 1.3 ± 0.1 $mg \cdot h^{-1}$ was attributed to osmolyte outflow. This yields a net rate of water uptake of 9.8 ± 0.6 $mg \cdot h^{-1}$. In this experiment, the osmolyte outflow accounted for 14% of the total weight change recorded.

A robust procedure to quantify the outflow of osmolytes during a standard incubation in deionized water is to directly determine the dry mass of the lyophilized incubation solution. In our experiment, the dry mass of the incubation solution was very closely related to a more sophisticated HPLC analysis of carbohydrates (glucose and fructose $r^2 = 0.99^{***}$, sorbitol $r^2 = 0.97^{***}$).

Alternative procedures to quantify water flow directly would require labelled water, e.g., THO, DHO, or D_2O. Due to safety considerations (THO) and the requirement for complicated analyses by NMR or mass spectrometry (DHO; D_2O) the use of labeled water represents a considerable obstacle in experimentation. Furthermore, a non-destructive monitoring of water movement on an individual fruit basis in repeated measures designs is technically impossible. Thus, gravimetry is likely to remain the standard procedure for quantifying water uptake into sweet cherry and other soft and fleshy fruit.

Conclusions

A sweet cherry is more complex than a simple osmometer. First, the CM has a $\sigma < 1$ for a number of common solutes including glucose, fructose, and sorbitol. Second, the fruit resembles a two-compartment system, where an active component may modify the Ψ . In addition, the swelling of cell walls may represent a significant boundary layer resistance that results in confounding between the cell's water permeability and the osmotic potential of this cell as the driving force for water uptake. Cells having a high osmotic potential (less negative) would have a low water permeability. These subjects merit further study.

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Table 1. Osmotic potential (Ψ_{Π}^{fruit}), apparent fruit water potential (Ψ^{fruit}), and the calculated fruit turgor (Ψ_P) of selected cultivars of sweet cherry. Fruit were incubated in PEG 6000 solutions ranging in osmotic potential from -5.4 to 0 MPa. The rate of water uptake was determined gravimetrically. The apparent Ψ^{fruit} was calculated as the point of zero mass change. The calculated Ψ_P was determined as the difference between Ψ_{Π}^{fruit} and the apparent Ψ^{fruit}.

Cultivar	Ψ_{Π}^{fruit} [mean ± SE (MPa)]	apparent Ψ^{fruit} [mean ± SE (MPa)]	calculated Ψ_P [mean ± SE (MPa)]
Adriana	-2.1 ± 0.1	-1.4 ± 0.0	0.7 ± 0.1
Regina	-3.4 ± 0.1	-3.0 ± 0.2	0.4 ± 0.3
Sam	-3.0 ± 0.1	-2.6 ± 0.3	0.4 ± 0.3
Samba	-2.8 ± 0.1	-2.4 ± 0.0	0.4 ± 0.3
Grand mean	-2.8 ± 0.2	-2.4 ± 0.3	0.5 ± 0.1

Table 2. Osmotic potentials (Ψ_{Π}^{fruit}) and rates of water uptake (*F*) from deionized water and from juice extracted from selected cultivars of sweet (*Prunus avium*) and sour cherry (*Prunus cerasus*). Cherry juice was extracted from the same batch of fruit.

			F [mean ± SE ($mg \cdot h^{-1}$)]	
Species	Cultivar	Ψ_{Π}^{fruit} (MPa)	Water	Juice
Prunus avium	Adriana	-1.5	4.4 ± 0.4	1.0 ± 0.1
	Hedelfinger	-2.9	12.0 ± 1.1	3.2 ± 0.4
	Regina	-2.6	8.6 ± 1.1	1.3 ± 0.2
	Samba	-2.4	9.9 ± 1.1	1.5 ± 0.1
	Schneiders Späte Knorpel	-2.6	15.1 ± 2.5	1.4 ± 0.2
Prunus cerasus	Achat	-3.0	7.4 ± 0.8	-0.1 ± 0.0
	Morellenfeuer	-2.8	21.6 ± 1.8	2.8 ± 0.2
	Ungarische Traubige	-2.9	11.5 ± 1.1	0.3 ± 0.1
Grand mean		-2.5	11.3 ± 0.6	1.4 ± 0.1

Table 3. Osmotic potential (Ψ_{Π}^{fruit}), apparent fruit water potential (Ψ^{fruit}), and the calculated turgor (Ψ_P) of sweet cherry fruit held for 4 d at ~100% RH or ~0% RH at 22°C. Fruit were incubated in glucose solutions ranging in osmotic potential from -5.8 to 0 MPa. The rate of water uptake was determined gravimetrically. The apparent Ψ^{fruit} was calculated as the point of zero mass change. The Ψ_P was calculated as the difference between Ψ_{Π}^{fruit} and the apparent Ψ^{fruit}.

Treatment	RH (%)	Ψ_{Π}^{fruit} [mean ± SE (MPa)]	apparent Ψ^{fruit} [mean ± SE (MPa)]	calculated Ψ_P [mean ± SE (MPa)]
0 d	-	-2.7 ± 0.1	-3.8 ± 0.1	-1.1 ± 0.1
4 d	100	-2.6 ± 0.1	-3.7 ± 0.3	-1.1 ± 0.3
4 d	0*	-3.3 ± 0.1	-4.4 ± 0.3	-1.1 ± 0.4

*Mass loss averaged 1.4 g

Table 4. Difference between the osmotic potential of the flesh (Ψ_{Π}^{flesh}) and the skin (Ψ_{Π}^{skin}) of mature sweet cherry fruit after holding up to 48 d at ~100% RH or for 4 d at ~0% RH at 22°C. The Ψ_{Π}^{skin} is estimated from the osmotic potential of the incubation solution yielding 50% plasmolysis of the epidermal cells (see Fig. 5).

Treatment	RH (%)	Ψ_{Π}^{flesh} [mean ± SE (MPa)]	Ψ_{Π}^{skin} [mean ± SE (MPa)]	Ψ_{Π}^{flesh} - Ψ_{Π}^{skin} [mean ± SE (MPa)]
0 d	-	-2.6 ± 0.1	-1.9 ± 0.1	-0.7 ± 0.1
19 d	100	-2.4 ± 0.1	-1.7 ± 0.0	-0.7 ± 0.1
48 d	100	-2.2 ± 0.1	-1.8 ± 0.0	-0.4 ± 0.1
0 d	-	-2.7 ± 0.1	-1.8 ± 0.1	-0.9 ± 0.1
4 d	0*	-3.0 ± 0.0	-2.3 ± 0.1	-0.8 ± 0.1

*Mass loss averaged 1.4 g.

Table 5: Osmotic potential (Ψ_{Π}^{fruit}), apparent fruit water potential (Ψ^{fruit}), and the calculated fruit turgor (Ψ_P) of different Ribes species and grape cultivars from the literature. The rate of water uptake was determined gravimetrically by incubating fruit in PEG 6000 solutions with different osmotic potentials. The Ψ^{fruit} was calculated as the point of zero mass change. The Ψ_P was calculated as the difference between Ψ_{Π}^{fruit} and Ψ^{fruit}.

Species	Cultivar	Ψ_{Π}^{fruit} [mean ± SE (MPa)]	Ψ^{fruit} [mean ± SE (MPa)]	Ψ_P [mean ± SE (MPa)]	Reference
Ribes nigrum	-*	-2.7 ± 0.0	-2.1 ± 0.2	0.5 ± 0.2	Khanal et al., 2011
Rives uva-crispa	-	-1.9 ± 0.0	-1.2 ± 0.0	0.7 ± 0.1	
Ribes × nigridolaria	-	-2.5 ± 0.0	-1.9 ± 0.2	0.6 ± 0.2	
Vitis vinifera	Chardonnay	-3.9 ± 0.0	-1.7 ± 0.1	2.3 ± 0.1	Becker and Knoche, 2011
Vitis vinifera	Müller-Thurgau	-3.5 ± 0.0	-2.3 ± 0.1	1.2 ± 0.1	
Vitis vinifera	Riesling	-3.7 ± 0.0	-1.6 ± 0.0	2.1 ± 0.0	

* cultivar unknown

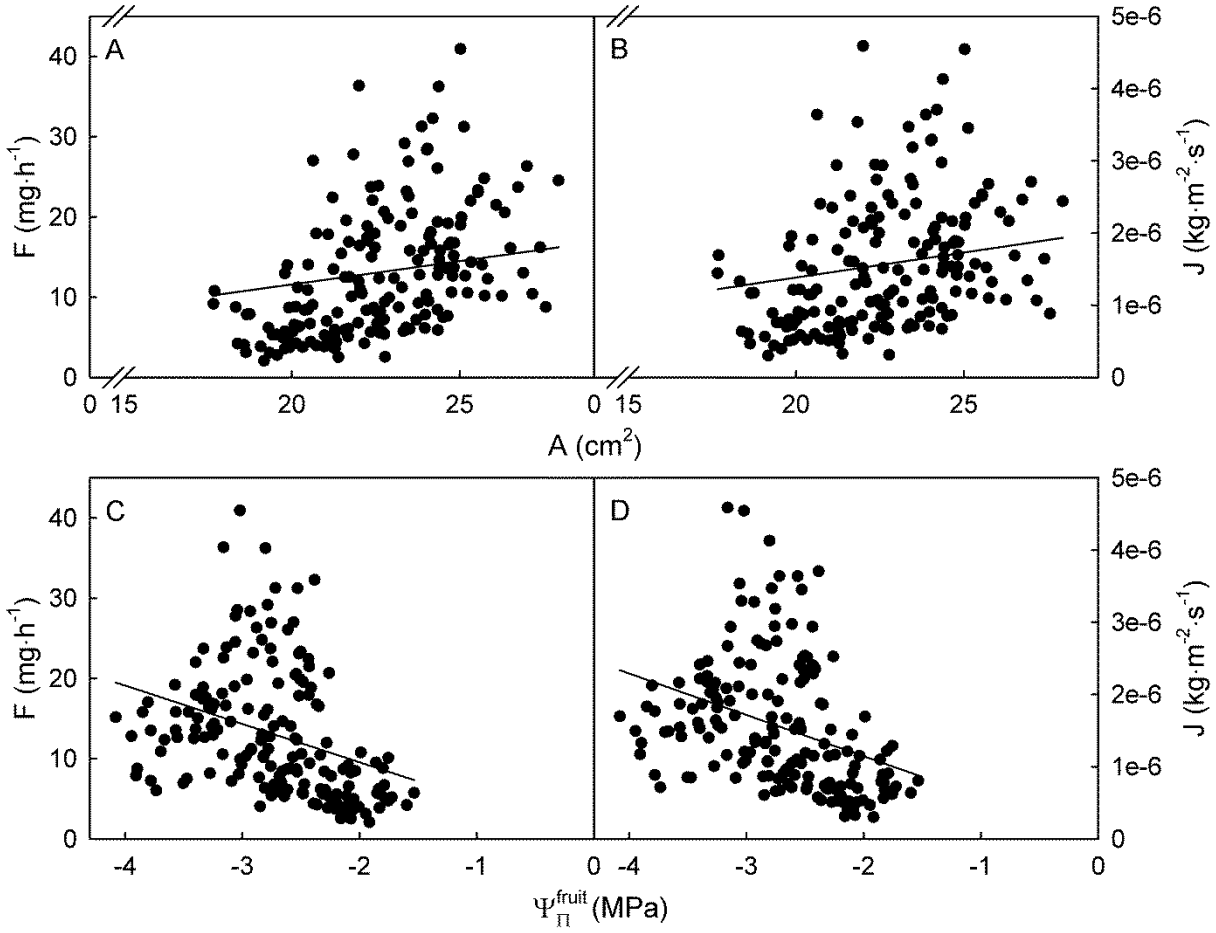

Fig. 1. Relationship between the rate of water uptake (*F*; g·h^{-1}; A and C) or the flux in water uptake (*J*; kg m^{-2} s^{-1}; B and D) and the surface area (*A*; cm²) or the osmotic potential (Ψ_{Π}^{fruit}; MPa) of mature sweet cherry fruit. The equations of the linear regression lines were: *F* (g·h^{-1}) = 1.71 (± 0.24) × *A* (cm²) – 25.86; r² = 0.22 (A); *J* (×10^{-6} kg m^{-2} s^{-1}) = 0.15 (± 0.03) × *A* (cm²) – 1.75; r² = 0.13 (B); *F* (g·h^{-1}) = -4.78 (± 0.20) × Ψ_{Π}^{fruit} (MPa); r^2 = 0.76 (C); *J* (×10^{-6} kg m^{-2} s^{-1}) = -0.57 (± 0.02) × Ψ_{Π}^{fruit} (MPa); r²=0.78 (D). The intercept terms for C and D were not significant. Data symbols represent individual fruit.

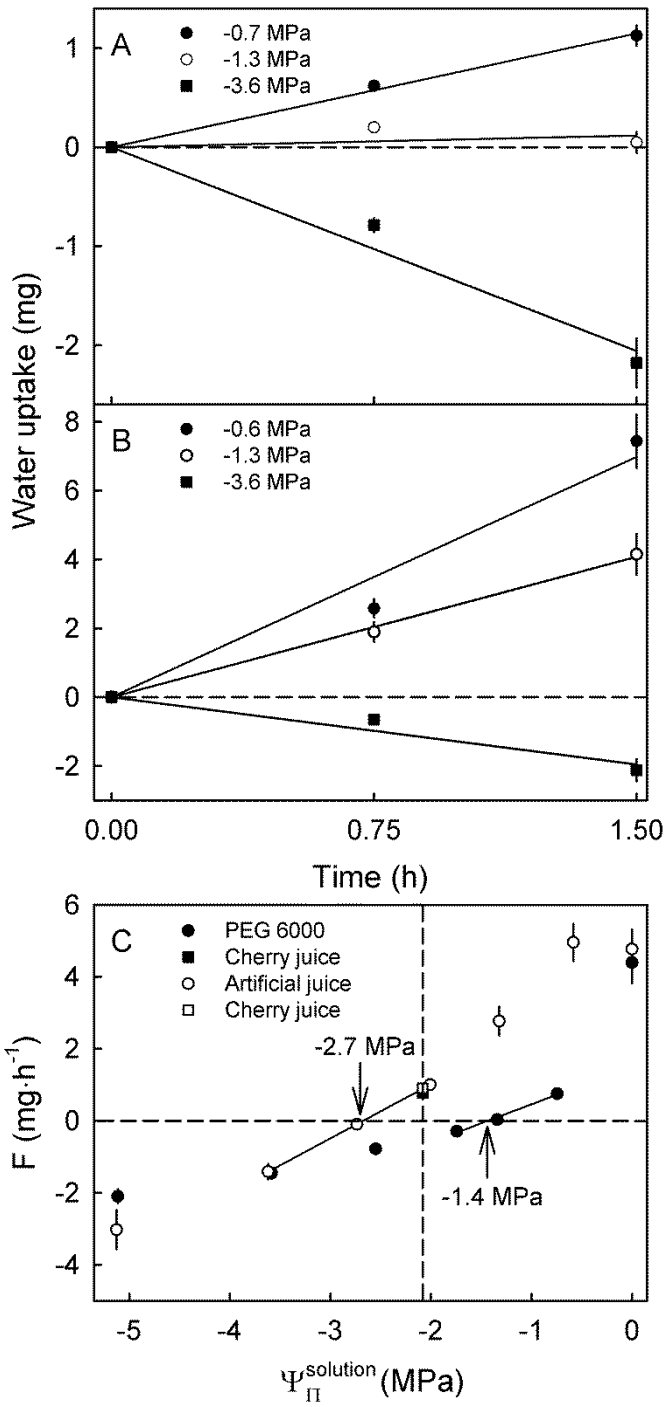

Fig. 2. Effect of the osmotic potential ($\Psi_{\Pi}^{solution}$) of polyethylene glycol 6000 (PEG 6000) (A) and artificial or natural sweet cherry juice (B) on the time course of water uptake and rates of water uptake (*F*) (C). Artificial cherry juice was prepared using the five most abundant osmolytes of sweet cherry fruit, i.e., glucose, fructose, sorbitol, malic acid, and potassium malate. The apparent fruit water potential (Ψ^{fruit}) equals the $\Psi_{\Pi}^{solution}$ causing no change in fruit mass. The apparent Ψ^{fruit} is indicated by arrows. The dashed vertical line represents the Ψ_{Π}^{fruit}.

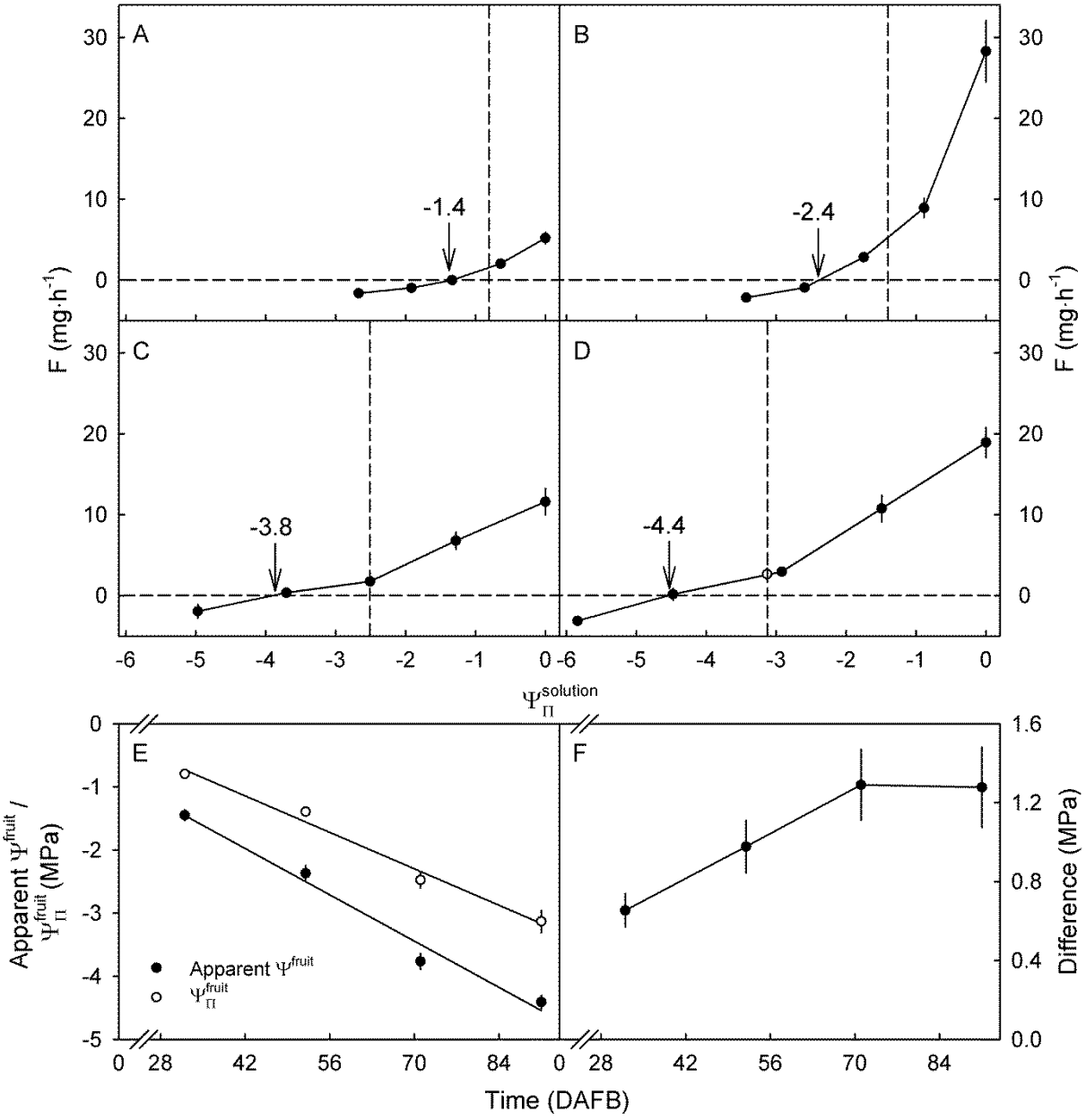

Fig. 3. Effect of the osmotic potential of glucose incubation solutions ($\Psi_{\Pi}^{solution}$) on the rates of water uptake (*F*) and the apparent fruit water potential (Ψ^{fruit}) of developing sweet cherry fruit [(A) 32, (B) 52, (C) 71, and (D) 91 days after full bloom (DAFB)]. The open circle in (D) represents water uptake from juice extracted from the same batch of fruit. (E) Apparent Ψ^{fruit} and osmotic potentials (Ψ_{Π}^{fruit}) in the course of development. (F) Difference between apparent Ψ^{fruit} and Ψ_{Π}^{fruit} in the course of development. The apparent Ψ^{fruit} equals the $\Psi_{\Pi}^{solution}$ causing no change in fruit mass and is indicated by vertical arrows (A-D). Vertical dashed lines in A-D indicate the Ψ_{Π}^{fruit}.

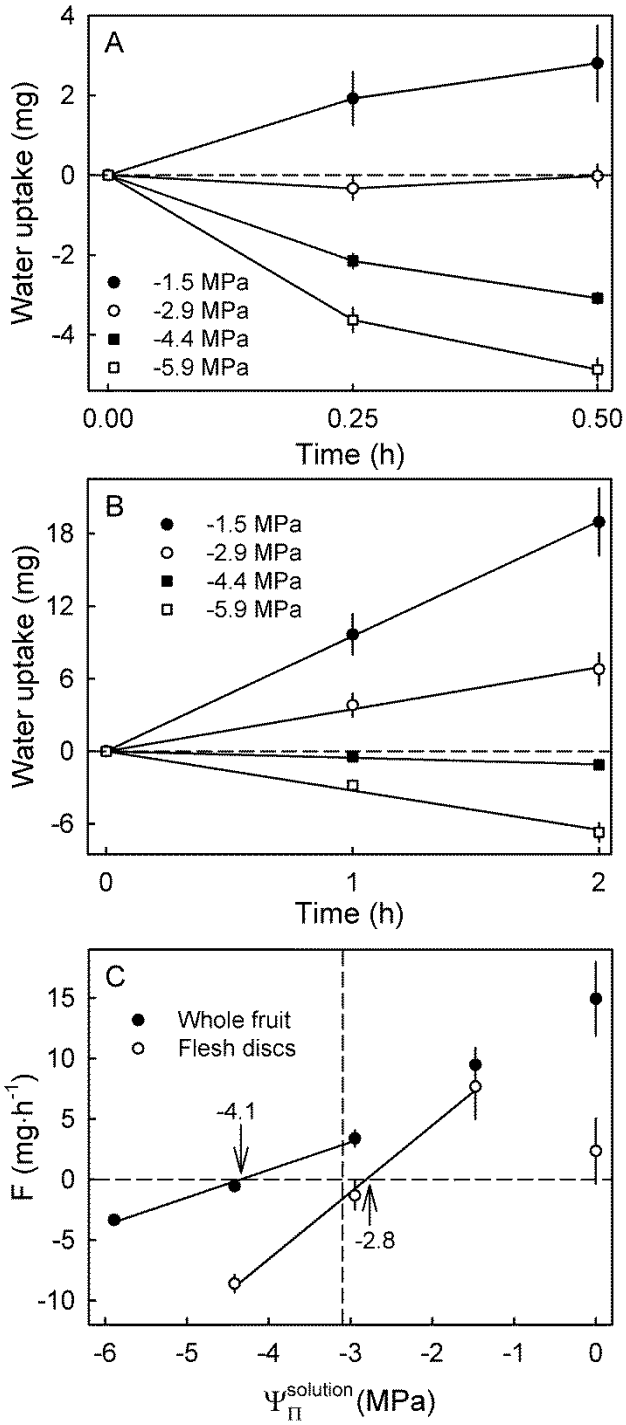

Fig. 4. Time course of water uptake into discs excised from the flesh of mature sweet cherry fruit (A) and whole fruit (B). (C) Rates of water uptake (*F*) as affected by the osmotic potentials of the glucose incubation solutions ($\Psi_{\Pi}^{solution}$). The apparent fruit water potential equals the water potential of a solution causing no change in fruit mass and is indicated by arrows. The dashed vertical line represents the osmotic potential of the fruit.

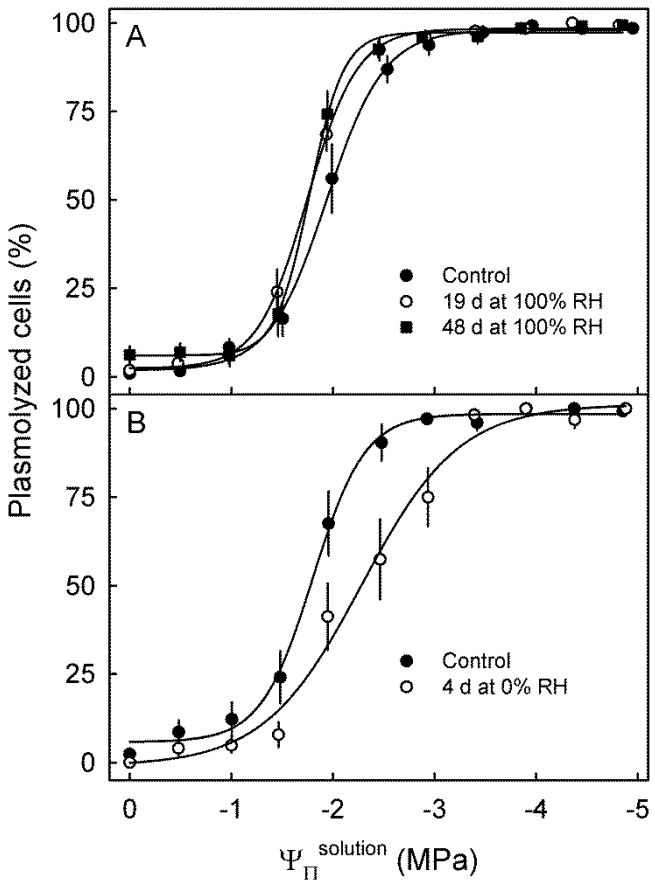

Fig. 5. Effect of the osmotic potential of sucrose incubation solutions ($\Psi_{\Pi}^{solution}$) on the percentage of plasmolyzed epidermal cells of sweet cherry fruit held under non-transpiring conditions (A) or transpiring conditions (B). Non-transpiring and transpiring conditions were imposed on the fruit to equilibrate (non-transpiring) or to induce a gradient (transpiring) in the osmotic potential between fruit and skin. Fruit was held under non-transpiring conditions (~100% RH) for up to 48 d or under transpiring conditions (~0% RH) for 4 d.

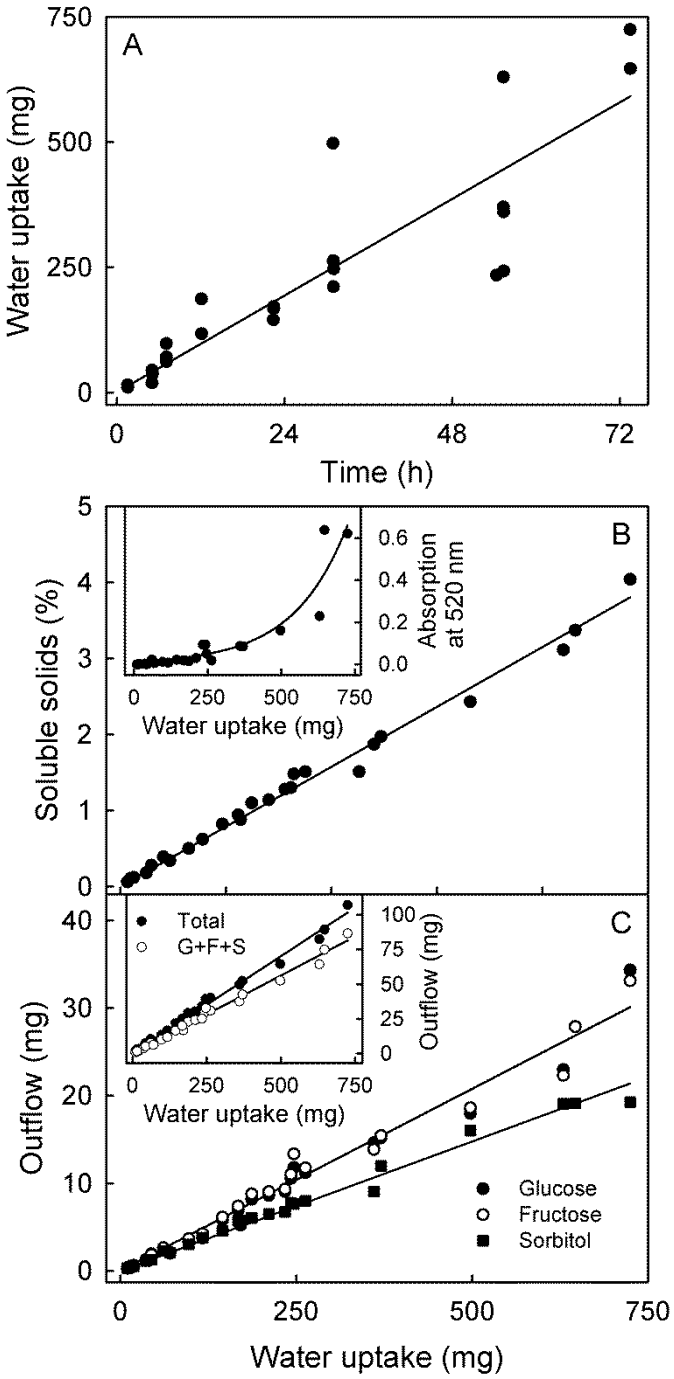

Fig. 6. Time course of water uptake (A). Outflow of osmolytes from the sweet cherry fruit into the incubation solution as affected by water uptake. The incubation solution was concentrated by lyophilizing and then subjected to osmometry (B). Anthocyanin-leakage as a function of water uptake (B inset). Outflow of the three main osmolytes of the sweet fruit as a function of water uptake (C). Total outflow of osmolytes and the sum of the three main osmolytes glucose (G), fructose (F), and sorbitol (S) as affected by water uptake (C inset).

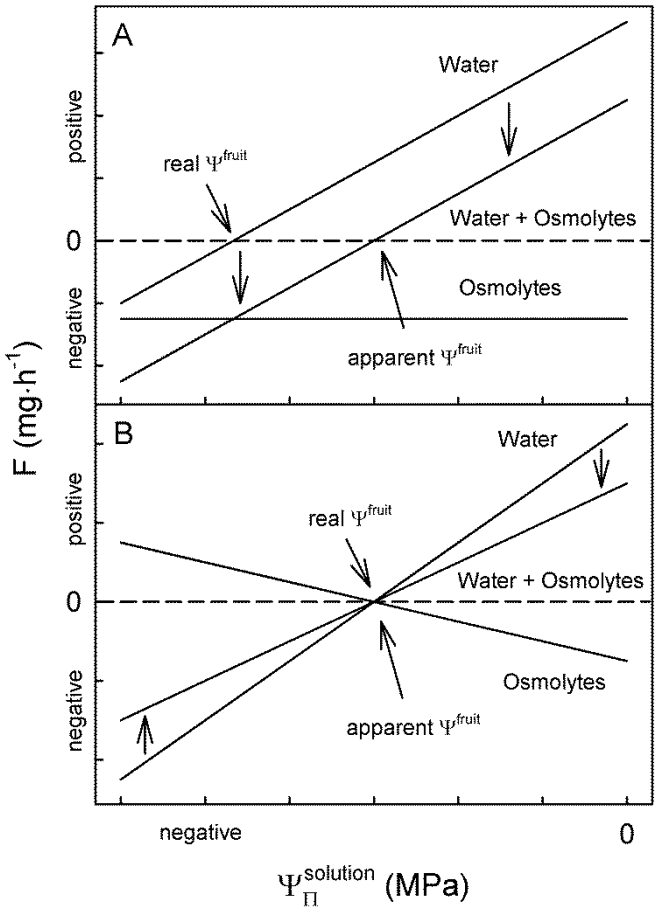

Fig. 7. Simulated change in fruit mass due to inflow and outflow of water and osmolytes into and out of the fruit when incubated in solutions ranging from hypertonic to hypotonic osmolarity ($\Psi_{\Pi}^{solution}$). (A) Parallel displacement of the relationship caused by the hypothetical outflow of low molecular weight osmolytes ($\sigma < 1$) from the fruit into the incubation solution. There is no inflow of osmolytes from the incubation solution into the fruit when the osmolyte is size-excluded ($\sigma = 1$) due to its large molecular weight as would be the case for fruit incubated in PEG 6000. The water potential (Ψ^{fruit}) equals the $\Psi_{\Pi}^{solution}$ causing no change in fruit mass and is indicated by arrows. The measured apparent Ψ^{fruit} is higher (less negative) than the real Ψ^{fruit}. (B) Change in slope of the relationship due to the outflow of osmolytes from fruit into a hypotonic solution and to inflow of osmolytes from a hypertonic solution into the fruit. The osmolytes in fruit and in solution are identical. This situation mimics sweet cherries incubated in artificial juice comprising the most abundant osmolytes of the fruit (all $\sigma < 1$). The measured apparent Ψ^{fruit} is identical to the real Ψ^{fruit}.

3. Fruit apoplast tension draws xylem water into mature sweet cherries

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Contribution of authors

M. Knoche attracted the funding. A. Winkler, M. Brüggenwirth, and M. Knoche planned the experiments. A. Winkler, M. Brüggenwirth, and N.S. Ngo performed the experiments. A. Winkler, M. Brüggenwirth, and M. Knoche analyzed the data and wrote the manuscript. A. Winkler, M. Brüggenwirth, N.S. Ngo, and M. Knoche revised and edited the publication.

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Fruit apoplast tension draws xylem water into mature sweet cherries

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ABSTRACT

Rain cracking of sweet cherry fruit (*Prunus avium* L.) is likely related to fruit water balance. Most research on fruit water balance has focused on water transfers through the fruit skin (e.g. osmotic inflows, transpiration outflows) with little work on the water flows through the xylem and phloem vascular systems of the pedicel. The objectives here were to use a potometer and a pressure probe to study xylem sap flows in the pedicels of fruit isolated from the tree by a cut at the proximal end of the fruit + pedicel.

Xylem water inflow rates were constant for up to 8 h but then slowed. The pressure probe established the presence of a tension (a negative pressure) in the pedicel xylem. Xylem sap flow rates and xylem tensions were instantly, totally and permanently eliminated if the fruit was detached from the pedicel. Progressive removal of slices of the fruit flesh beginning at the distal end (stylar scar) and work towards the proximal end (pedicel) gradually decreased sap flow rates and tensions. The length of the pedicel had only a marginal effect on flows. Fruit mass and osmotic pressure of the flesh increased sigmoidally during stage II (pit development) and stage III (final swell) of fruit development. Flow rates and tensions both increased to maxima at early stage III, and both decreased thereafter. Flow rates and tensions were higher at ~0% relative humidity (RH) than at ~100% RH. The flow rate difference at ~0% and at ~100% RH decreased during development. There was no difference in flow rate of fruit at ~100% RH and fruit submerged in water. During early development, xylem flow in the pedicel was inversely related to RH. As development proceeded, xylem flow became progressively less dependent on RH. Abrading the cuticle or slashing the skin had no effect on xylem sap inflow. There was no relationship between the tension in the xylem and the osmotic pressure of the expressed juice of the flesh (r^2 = 0.13). Feeding the cut end of the pedicel xylem with sucrose solutions of increasing osmotic pressure decreased xylem inflow and tension up to osmotic pressures of about 2.5 MPa. Beyond 2.5 MPa, some inflow and tension remained detectable. The results establish that xylem flow is likely drawn into the fruit from the tree by apoplastic tension in the fruit, resulting from osmotic water uptake from apoplast to symplast and from transpiration. Minor contributions may result from cell wall swelling within the flesh. Indirect evidence suggests that decreased conductance of the xylem accounts for the decrease in flow during stage III. These findings render the possibility of a significant role for xylem transport in fruit cracking unlikely.

1. Introduction

Cracking of sweet cherry fruit severely limits production in many areas of the world where this high-value crop is produced, and can result in catastrophic economic loss (Christensen, 1996). Due to the coincidence of cracking and rainfall, this phenomenon is commonly referred to as 'rain cracking' and excessive water uptake is presumed to be causal. Water uptake may occur through the fruit skin (osmotic uptake through a wet skin) and/or through the vascular systems of the pedicel (in the xylem and/or phloem). The mechanisms and pathways of water transfer through the skin have been examined (Beyer and Knoche, 2002; Beyer et al., 2002a,b, 2005; Weichert et al., 2004; Weichert and Knoche, 2006a,b), but there are only a few studies of the vascular transport systems through the pedicel (Hovland and Sekse, 2004a,b; Measham et al., 2014; Brüggenwirth and Knoche, 2015; Knoche et al., 2015). Hence, the role of vascular flow in fruit cracking is unknown, although some indirect evidence suggests it may contribute: (A) Cracking is occasionally observed under rain shelters, when contact of fruit skins with liquid water is presumably excluded (Børve et al., 2003; Thomidis and Exadaktylou, 2013) and uptake of water from the vapour phase (under high RH conditions) is very slow (Beyer et al., 2005). (B) Sweet cherries generate a negative apoplastic pressure (a tension) which draws water through the pedicel even when the

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fruit is held at high RH so little or no transpiration can occur (Knoche et al., 2015). Little is known about the mechanisms, driving forces, and pathways of pedicel xylem sap flow. Such information, however, is needed if we are to properly assess the role of pedicel xylem water uptake in cracking.

The objectives of this study were (1) to identify driving forces and pathways for water uptake through the pedicel and (2) to establish the effects of fruit development and other selected factors on xylem sap flow through the pedicel.

2. Material and methods

2.1. Plant material

Fruit from the sweet cherry cultivars 'Adriana', 'Burlat', 'Dönissens Gelbe', 'Earlise', 'Early Korvic', 'Fabiola', 'Flamengo Srim', 'Frühe Rote Mecklenburger', 'Gill Peck', 'Hedelfinger', 'Jacinta', 'Kordia', 'Merchant', 'Querfurter Königskirsche', 'Regina', 'Sam', 'Samba', 'Schneiders Späte Knorpel', 'Staccato', and 'Sweetheart' were sampled from greenhouse-grown or field-grown trees grafted on 'Gisela 5' rootstocks (*P. cerasus* L. × *P. canescens* Bois) at the Horticultural Research Station of the Leibniz University in Ruthe, Germany (lat. 52°14′N, long. 9°49′E). Fruit was also sampled of the cultivar 'Bing', from trees grafted on *P. mahaleb* rootstocks in a commercial orchard near Rancagua, Chile (lat. 34°13′S, long. 70°73′W). Fruit was detached by cutting under water, the proximal 1 cm of the pedicel end submerged in water and transferred to the laboratory located within a short (<300 m) walking distance. Only fruit free of macroscopically visible defects and of uniform development (based on colour and size) were used. Commercial maturity was judged based on colour, size and taste.

2.2. General experimental procedures

2.2.1. Potometry

Water flow through the pedicel was quantified using a potometer (Hovland and Sekse, 2004a,b; Knoche et al., 2015). Unless otherwise mentioned, pedicels were recut under water using a razor blade, to leave a 20 mm length of pedicel still attached to the fruit. A water-filled low density polythene (PE) tubing (inner diameter 1.6 mm, length 40 mm) was connected to the pedicel and the tubing/pedicel junction sealed using a fast-curing epoxy glue (UHU plus schnellfest; UHU, Bühl/Baden, Germany). Unless specified otherwise, fruit were incubated in 0.75 l PE-boxes either above dry silica gel (~0% RH) to maximise transpiration, or above water (~100% RH) to minimise transpiration. Boxes remained closed with a tight fitting lid for the duration of the experiment. The tubing was guided through a port in the PE-box and connected to the graduated capillary of a potometer. The capillary comprised 600 mm of Teflon-tubing (inner diameter 0.5 mm) mounted on a mm ruler. Unless stated otherwise, to increase the visibility of the water:air meniscus in the tubing, a 0.01% (w/v) aqueous methylene blue solution was used. Preliminary experiments established that for Adriana sweet cherry fruit, potometer flow rates were not significantly ($p = 0.19$) affected by using dyed water. Thus, for fruit in atmospheres at ~0% and ~100% RH the flows with 0.01% methylene blue were, respectively, 4.4 ± 0.2 and $3.5 \pm 0.2\ \mu l\ h^{-1}$, with 0.1% acid fuchsin they were 4.5 ± 0.2 and $4.0 \pm 0.3\ \mu l\ h^{-1}$ and with pure water they were 4.4 ± 0.4 and $4.0 \pm 0.3\ \mu l\ h^{-1}$. To optimise meniscus visibility, 0.01% aqueous methylene blue was used in all experiments. In the ~0% RH treatment, transpiration was restricted to that from the fruit surface by sealing the pedicel cavity using epoxy-glue (UHU plus schnellfest; UHU) and by wrapping the pedicel in aluminium foil. For fruit held at ~100% RH, these were wrapped in damp tissue paper and this surrounded by aluminium foil. The position of the meniscus against the scale was read every 30 min, over a 2 h period. The volume of water (mm^3 or μl) moving through the pedicel during the time interval between readings was calculated by multiplying the distance (mm) travelled by the meniscus by the cross-sectional area of the tube (mm^2). Preliminary experiments with mature fruit of 'Bing' showed no effect of time of day of sampling on flow rates. Thus, for fruit held at ~0% and ~100% RH, at 7:15am (shortly after sunrise) the flow rates were 10.8 ± 0.8 and $7.1 \pm 0.7\ \mu l\ h^{-1}$, and in the warmest part of the day (around 2pm) they were 10.5 ± 1.0 and $6.5 \pm 0.6\ \mu l\ h^{-1}$, respectively.

In some cases, fruit transpiration was also quantified after the potometric measurement. For this the pedicel was cut flush with the receptacle and the receptacle and stem/fruit juncture were sealed using fast-curing epoxy glue (UHU plus schnellfest; UHU). Fruit were incubated in a PE-box as described above and transpiration quantified by weighing fruit at 0, 2, and 4 h. The rate of transpiration (F, $kg\ s^{-1}$) corresponded to minus the slope of the linear regression of fruit mass vs. time. The permeance (p, $m\ s^{-1}$) was calculated from Eq. (1).

$$p = \frac{F}{A \times \Delta C} \quad (1)$$

In this equation A (m^2) is the fruit surface area and ΔC ($g\ m^{-3}$) the difference in water-vapour concentration between the inside (C_i) of the fruit and the surrounding atmosphere (C_o). Because the relative humidity above dry silica gel is close to zero (Geyer and Schönherr, 1988), ΔC is numerically equal to the water-vapour concentration inside the fruit, which is usually taken to be equal to the saturated water-vapour concentration at the temperature of the fruit (Nobel, 1999).

2.2.2. Pressure probe

A modified pressure probe was used to quantify the tension in the xylem of detached cherry fruit (Knoche et al., 2015; Steudle, 1993). The instrument comprised a glass tube (inner diameter 0.3 mm) where the proximal end was filled with silicone oil (Wacker AK 10, Wacker Chemie, Munich, Germany) and connected to a pressure sensor (26PCGFA6D; Honeywell Sensing and Control, Golden Valley, MN, USA). The distal end was filled with degassed, deionised water and connected to the pedicel using a cyanoacrylate adhesive (Loctite 406; Henkel Loctite, Munich, Germany). The pedicel was re-cut under water to a length of 20 mm. Initial experiments established that cavitation and embolism occurred as indexed by the appearance of air bubbles when fruit was held at ~0% RH above dry silica gel during measurement of pressure (Brüggenwirth, unpublished data). Therefore, all tensions were quantified at ~100% RH by wrapping fruit and pedicel in damp tissue paper. The time course of change in tension was recorded until a constant value was attained.

2.3. Experiments

The time course of potometric flow through the pedicel, also the development of tension in the pedicel of early stage III and of mature 'Regina' sweet cherry fruit, were established at ~100% RH. For the long-term potometer experiment, a tube of length 100 mm and inside diameter 1.6 mm was used. This was refilled as necessary. The minimum number of replications was nine. Fruit used for measurement of tension were sampled, placed on a test tube such that the proximal 1 cm of the pedicel was submerged in water contained within the test tube, while the fruit surface was exposed to a water-vapour-saturated atmosphere. Fruit were removed from this setup at 0, 4, 8, and 24 h and their tensions determined using a pressure probe. The number of replications was 10.

Whether flows and tensions were generated by the fruit or by the pedicel was established for flows in fruit of 'Bing', and for

tensions in fruit of 'Regina' held at ~100% RH. For flows, after establishment of steady state flow (>2 h), fruit were detached but the pedicel left connected to the potometer. For tensions, after establishment of a steady value (>12 min), fruit were detached but the pedicel left connected to the pressure probe. Flows and tensions were monitored for further periods of 2 h and 12 min, respectively. The minimum number of replications was 10.

To identify the region of the fruit that generates the tension, distal sections of 'Sam' fruit at early stage III and at maturity were cut away progressively beginning at the distal (stylar scar) end. Cuts were made (i) half-way between stylar scar and pit, (ii) across the equatorial plane of the fruit, (iii) half-way between pit and stem cavity, and (iv) at the pedicel such that the pedicel was detached from the fruit (see Fig. 4 inset). The minimum number of replications was eight.

The effect of pedicel length on flow and tension was studied in 'Frühe Rote Mecklenburger' at early stage III and at maturity. Pedicel length was 5 and 50 mm. The minimum number of replications was 10.

The developmental time course of change in flow and tension was determined in 'Sam' between 20 DAFB and 75 DAFB (maturity) at both, ~0% and ~100% RH. In addition, the rate of transpiration of the fruit was quantified and the permeance (p) calculated for fruit held at ~0% RH. The osmotic pressure of juice extracted from de-pitted fruit using a garlic press was measured by water vapour pressure osmometry (VAPRO 5520 and 5560; Wescor, Logan, UT). The minimum number of replications was 10.

The effect of submerging fruit on flow was studied in fruit of 'Bing' at early stage III and at maturity. Fruit held at ~0% or ~100% RH served as control. The minimum number of replications was 10.

The effect of RH on pedicel xylem flow was studied by incubating fruit of developing 'Sam' above dry silica gel (~0% RH), or above saturated slurries of $CaCl_2$ (28% RH; Wexler, 1995), NaCl (75% RH; Wexler, 1995), or above water (~100% RH). Following potometry, rates of transpiration of fruit (without pedicels) were quantified at the different RHs. The experiment was repeated at four developmental stages: 29, 43, 57 and 71 DAFB. The minimum number of replications was eight.

The effects of abrading the fruit cuticle, and of slashing the fruit skin on pedicel water flow at maturity was studied in 'Merchant'. The effects of abrading the cuticle on tension were studied in 'Samba' and the effects of slashing the fruit skin on tension were studied in 'Frühe Rote Mecklenburger'. The cuticle was abraded using sandpaper (grain 800, K800; EMIL LUX GmbH & Co. KG, Wermelskirchen, Germany). The skin was slashed by cutting the fruit surface in longitudinal and latitudinal directions using parallel mounted razorblades (distance between blades 5 mm, depth of cut 2 mm). Upon cutting, the fruit 'gaped' indicating release of significant elastic strain in the skin (Brüggenwirth, unpublished). The minimum number of replications was 10.

Potential relationships between pedicel water flow rates at ~0% RH and those at ~100% RH were investigated by comparing flow rates across cultivars. Flows were quantified in mature fruit of: 'Adriana', 'Burlat', 'Dönissens Gelbe', 'Earlise', 'Early Korvic', 'Fabiola', 'Frühe Rote Mecklenburger', 'Gill Peck', 'Hedelfinger', 'Jacinta', 'Merchant', 'Querfurther Königskirsche', 'Regina', 'Sam', 'Samba', 'Staccato', and 'Sweetheart'.

The effects of the osmotic pressure of the feeding solution on flow through the pedicel and tension in the pedicel were quantified in 'Sam' at 28, 42, 56 and 70 DAFB. Osmotic pressure was varied using feeding sucrose solutions having osmotic pressures of 0, 1.2, 2.5, 4.9 and 9.8 MPa. The minimum number of replications was eight.

The relationship between flesh osmotic pressure and pedicel tension was investigated in 'Dönissens Gelbe', 'Flamengo Srim', 'Gill Peck', 'Hedelfinger', 'Kordia', 'Regina', 'Sam', 'Schneiders Späte Knorpel', 'Staccato' and 'Sweetheart'. The minimum number of replications was nine.

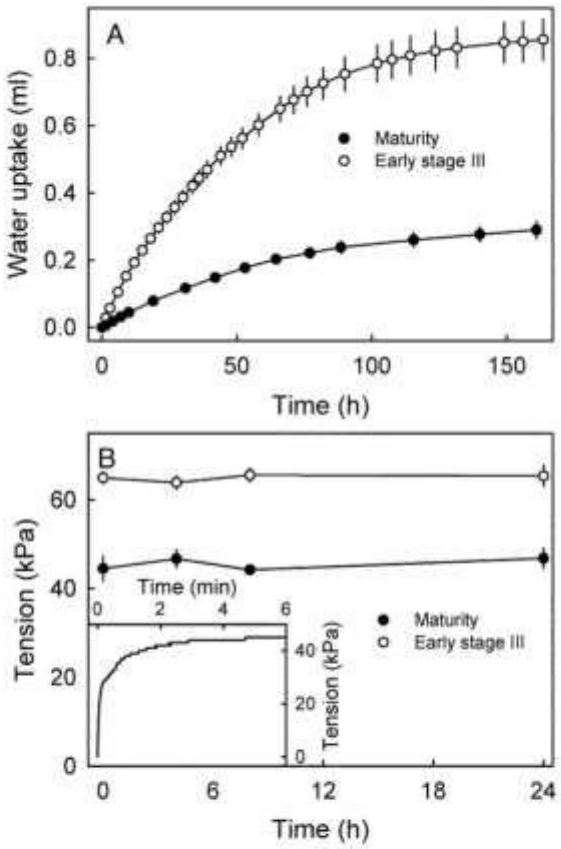

Fig. 1. (A) Time course of potometric flow through the pedicel and (B) development of tension in the pedicel of early stage III and mature sweet cherry fruit. For pressure measurements, fruit was held with pedicel tip submerged in water for up to 24 h. Inset in (B): Representative short-term time course of change in tension after connecting the pedicel (with fruit attached) to the pressure probe. Flows and tensions were determined on fruit held at ~100% RH.

2.4. Data analysis

Data are presented as means ± SE. Where error bars are not visible in a graph, they are smaller than the data symbols. Data were examined by analysis of variance and regression. Pairwise comparisons of treatment means with the non-treated controls were performed using Dunnett's test or Tukey's Studentised range test ($P \leq 0.05$, package multcomp 1.3-1, procedure glht, R 3.0.2; R Foundation for Statistical Computing, Vienna, Austria). Regression analysis was performed using R (version 3.0.2). The significance of coefficients of determination (r^2) at $P \leq 0.05$, 0.01 and 0.001 is indicated by *, ** and ***, respectively.

3. Results

The time course of water uptake through the pedicel was linear during an initial 8 h period, but levelled off thereafter, approaching an asymptote after about 160 h (Fig. 1A). By 160 h, at ~100% RH, early stage III fruit and mature fruit had taken up 0.9 ± 0.1 ml and 0.3 ± 0.0 ml of water, respectively. When a fruit was connected to the pressure probe, the probe equilibrated with a negative pres-

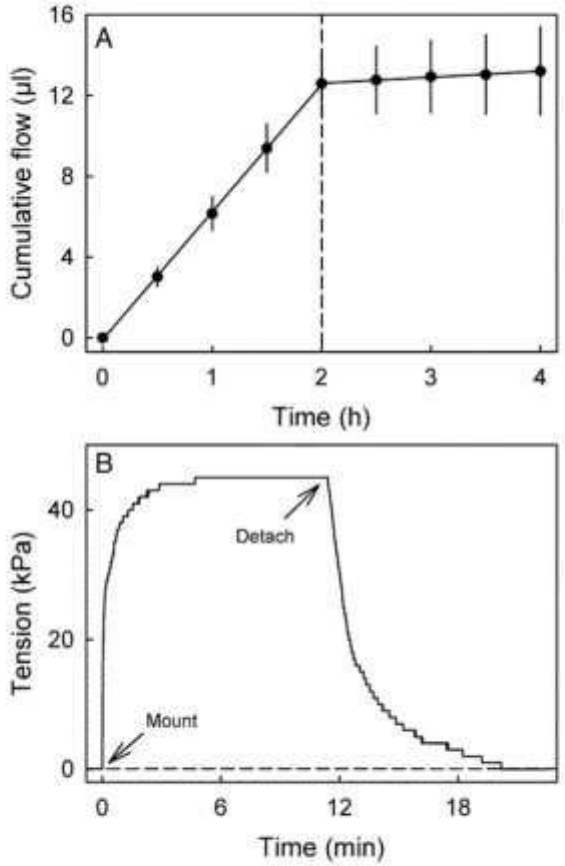

Fig. 2. (A) Effect of detaching fruit from pedicel on cumulative vascular flow through the pedicel and (B) the tension in the pedicel of mature sweet cherry fruit. For flow determinations, fruit were detached from their pedicels after 2 h. For tension determinations, fruit were detached from their pedicels after 12 min. Tension is expressed relative to atmospheric pressure. Flow was determined using potometry, tension using a pressure probe. Fruit was held at ~100% RH.

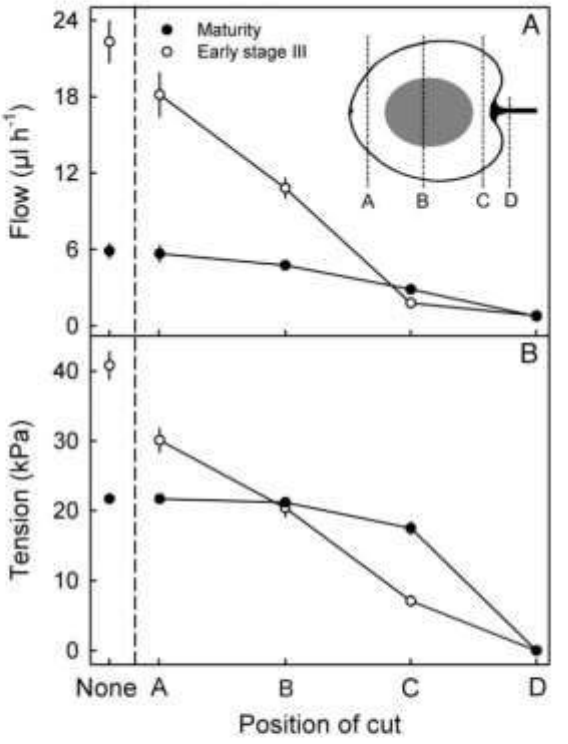

Fig. 3. Effect of progressive removal of tissue sections from the stylar end of the fruit on vascular flow and tension in the pedicel of early stage III and mature sweet cherry fruit. The 'None' on the x-axis refers to the un-cut control, and A to D to the various positions when progressively excising slices from the fruit beginning at the stylar end (see sketch in A). Flow was determined using potometry, tension using a pressure probe. Fruit was held at ~100% RH.

sure within about 5 min, indicating the presence of tension in the xylem of the pedicel (Fig. 1B, inset). Holding fruit for up to 24 h with the pedicel end submerged in water (thereby allowing continuing water uptake through the pedicel) had no effect on the tension recorded. This applied for fruit both in early stage III and at maturity. Tension was always higher (more negative) during early stage III, than at maturity (Fig. 1B).

Detaching the fruit from its pedicel immediately stopped the flow through the pedicel (Fig. 2A) and decreased the tension to the atmospheric pressure recorded before mounting the fruit on the pressure probe (Fig. 2B).

Progressively excising distal portions of the fruit, beginning at the stylar end and working towards the pedicel end, steadily decreased water flow rates and tension in early stage III fruit (Fig. 3). However, in mature fruit, flow was reduced only if the cut was between the pit and the stem cavity. Removing the stylar end of the fruit or cutting across the fruit's equatorial plane had no additional effects (Fig. 3). Again, there was no detectable flow, nor any tension, when the entire fruit was removed from the pedicel (Fig. 3).

Slightly lower flow rates and tensions were measured in long, as compared to short, pedicels but these differences were usually non-significant. The only exception was the flow at ~0% RH, and only during early stage III, when longer pedicels exhibited significantly reduced flow rates (Table 1).

Fruit mass and the osmotic pressure of the expressed juice increased sigmoidally from early stage II (20 DAFB) until maturity (Fig. 4A). Flow rates decreased somewhat from 20 to 27 DAFB, then increased to a maximum at 48 DAFB and thereafter decreased until maturity. Flow rates at ~0% RH consistently exceeded those at ~100% RH. The difference in flow rates at the two relative humidities decreased during fruit development (Fig. 4B). Rates of transpiration through the fruit surface increased steadily between 20 DAFB and maturity. From 55 DAFB onwards, rates of fruit transpiration exceeded the water flows through the pedicel (~0% RH; Fig. 4B and C). The permeance of the fruit surface (calculated from transpiration rate) peaked at around 40 DAFB but fell during stage III. Throughout development changes in tension paralleled those in flow rate (Fig. 4D), with flow and tension being linearly related (Fig. 4D, inset).

Regardless of developmental stage, there was no difference in water flow rate in the pedicels of fruit submerged in water, vs of fruit wrapped in damp tissue paper and aluminium foil (~100% RH). Both flow rates were lower than those of fruit in dry air (~0% RH) (Table 2).

Flow through the pedicel and the rate of transpiration through the fruit surface depended on the RH, but for vascular flow there was a clear interaction between the effect of RH and the stage of development. In stage II fruit, vascular flow decreased as RH increased. However, as development proceeded, vascular flow became less and less dependent on RH as indexed by a decrease

Table 1
Effect of the pedicel length on xylem flow and xylem tension in pedicels of immature and mature sweet cherry fruit. Flow was quantified using potometry and fruit incubated at ~0% or ~100% RH. Tension was determined using a pressure probe and fruit held at ~100% RH. Data are means ± SE.

Stage	Pedicel length (mm)	Flow (μl h^{-1})		Tension (kPa)
		0% RH	100% RH	
Early stage III	5	15.2 ± 0.7[z]	10.6 ± 0.6	49.6 ± 2.5
	50	13.1 ± 1.0*	8.8 ± 0.7	45.5 ± 2.7
Maturity	5	8.1 ± 0.5	7.1 ± 0.3	26.8 ± 0.9
	50	8.0 ± 0.4	5.8 ± 0.3	26.2 ± 1.7

[z]Significance of difference of flow or pressure to 5 mm control indicated by *, Dunnett test at $P \leq 0.05$.

Table 2
Effect of submerging fruit in water on vascular flow through the pedicel of mature sweet cherry. Flow was determined using potometry and fruit incubated at ~0% or ~100% RH or submerged in water. Data are means ± SE.

Treatment	Flow (μl h^{-1})	
	Early stage III	Maturity
0% RH	21.1 ± 2.1 a[z]	7.3 ± 0.5 a
100% RH	12.1 ± 0.8 b	4.6 ± 0.4 b
Submerged	13.4 ± 1.8 b	3.4 ± 0.3 b

[z]Mean separation within columns by Tukey's Studentised range test at $P \leq 0.05$.

Table 3
Effect of abrading the fruit cuticle, or slashing the fruit skin, on xylem flow and xylem tension in the pedicels of mature sweet cherry fruit. Flow was quantified using potometry and fruit incubated at ~0% or ~100% RH. Tension was determined using a pressure probe and fruit held at ~100% RH. Data are means ± SE.

Treatment	Flow (μl h^{-1})		Tension (kPa)
	0% RH	100% RH	
Control	7.1 ± 0.5[z]	7.7 ± 0.6	22.7 ± 1.1
Cuticle abraded	8.4 ± 0.6	8.0 ± 0.6	22.6 ± 1.2
Control	7.8 ± 0.7	7.5 ± 0.5	25.3 ± 2.2
Skin slashed	7.7 ± 0.6	6.9 ± 0.6	25.7 ± 2.2

[z]None of the comparisons significantly different from the control, Dunnett test at $P \leq 0.05$.

in the slope of the flow vs. RH relationships (Fig. 5A–D). In contrast, transpiration rate decreased with increasing RH, throughout development to maturity (Fig. 5A–D).

Neither abrading the cuticle nor slashing the skin had any effect on pedicel flow or tension regardless of RH (Table 3).

The effect of RH on rate of vascular flow did not differ among cultivars. Across a total of 17 cultivars, flows at ~0% RH always exceeded those at ~100% RH. The relationship among cultivars between the flows at the two RHs was linear and significant (Slope: 0.77 ± 0.02, $r^2 = 0.70^{***}$; Fig. 6).

Feeding the pedicel with sucrose solutions of increasing osmotic pressure decreased flow and tension up to osmotic pressures of about 2.5 MPa at all developmental stages (Fig. 7). For osmotic pressures above about 2.5 MPa there was little additional effect. There was no relationship between the osmotic pressure of the juice expressed from the flesh, and the effect of sucrose on flows and tensions.

There was no significant relationship between the tension measured using the pressure probe and the osmotic pressure of the juice expressed from the fruit ($r^2 = 0.13$). Cultivars differed in osmotic pressure of the juice and in tension in their pedicel (Table 4). The highest (most negative) tension was detected in 'Regina', the lowest (least negative) in 'Kordia'.

Table 4
Osmotic pressure of the flesh of the fruit and tension in the xylem of the pedicel of selected sweet cherry cultivars at maturity. Tension was quantified using a pressure probe and fruit held at ~100% RH. Data are means ± SE.

Cultivar	Osmotic Pressure (Mpa)	Tension (kPa)
Dönissens Gelbe	2.4 ± 0.2	15.7 ± 3.5
Flamengo Srim	2.8 ± 0.1	20.1 ± 2.9
Gill Peck	3.5 ± 0.1	29.5 ± 4.2
Hedelfinger	2.7 ± 0.1	30.4 ± 5.4
Kordia	2.4 ± 0.1	13.7 ± 2.4
Regina	3.5 ± 0.1	33.9 ± 6.1
Sam	2.9 ± 0.1	23.1 ± 1.9
Schneiders Späte Knorpel	2.8 ± 0.1	33.1 ± 3.7
Staccato	2.9 ± 0.0	20.0 ± 2.6
Sweetheart	2.8 ± 0.1	24.2 ± 2.9
Grand mean	3.0 ± 0.1	24.4 ± 1.3

4. Discussion

New and important findings of our study are:

(1) Vascular flow in the xylem of the pedicel is due to a difference in pressure between the tree end (atmospheric) and the fruit end (negative relative to atmospheric – i.e. a tension).
(2) Tension in the distal xylem is the primary factor causing this flow.
(3) Tension is caused primarily by osmotic uptake of apoplast water by the flesh cells and will also likely be affected by loss of water by transpiration.
(4) Xylem flow rates decrease markedly during stage III development.

4.1. Pedicel vascular flow is generated by the fruit and occurs in the xylem

The tension as determined by a pressure probe (Steudle, 1993) and the associated vascular flow as measured by a potometer, are properties of the fruit – not that of the pedicel (Fig. 2A and B). Furthermore, the flows and tensions recorded were those in the xylem – not those of the phloem. First, detaching the fruit from the pedicel caused an instantaneous cessation of water flow in the pedicel and also eliminated the tension in the pedicel (Fig. 2). Second, pedicel length had no effect on flow and tension (Table 1) indicating that the resistance to flow in the pedicel xylem must have been small relative to that within the fruit. Third, the sieve elements of the phloem are blocked within minutes after wounding by the deposition of callose (Engleman and Esau, 1964; Nobel, 1999). Fourth, the fruit was detached from the spurs and hence, a functional source for the uploading of carbohydrates into the phloem as required by the Münch mechanism of phloem translocation was absent (Taiz et al., 2015). Thus, the primary conducting system in potometry is the xylem.

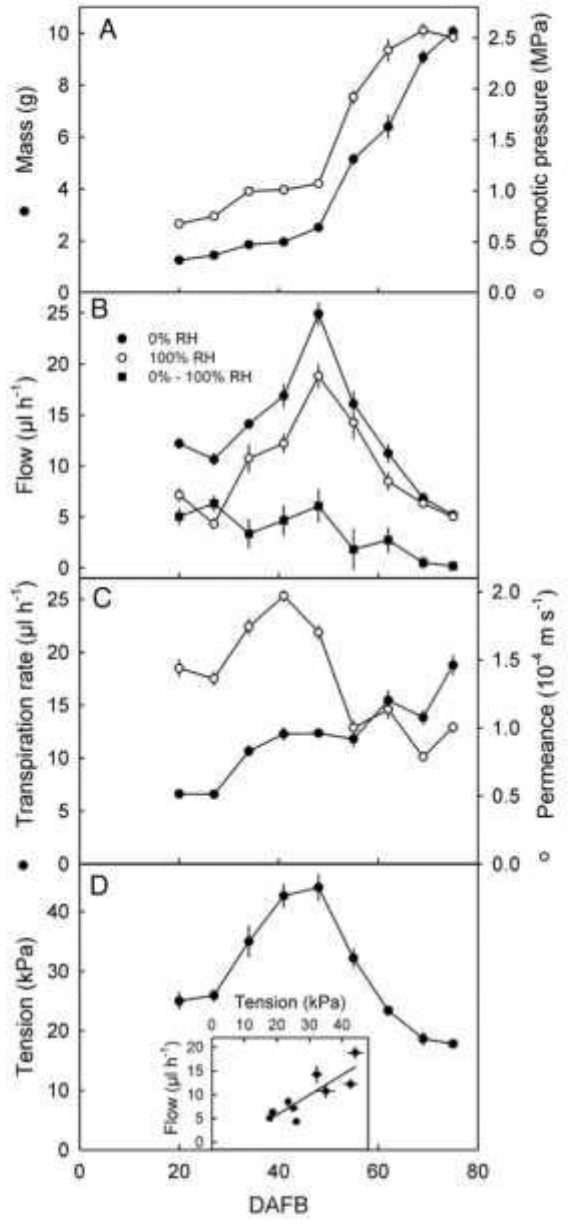

Fig. 4. (A) Developmental time course of change in mass and osmotic pressure of the juice, (B) vascular flow through the pedicel of sweet cherry fruit held at ~0% and ~100% RH, (C) rate of transpiration and permeance of the fruit surface, and (D) tension in the pedicel. Transpiration and tension were determined at ~0 and ~100% RH. Inset: Relationship between flow at 100% RH and tension. The regression equation was: Flow at 100% RH ($\mu l\ h^{-1}$) = 0.34 (±0.03) * tension (kPa), $r^2 = 0.69^{***}$. The flow through the pedicel was determined using potometry and fruit held at 0% or 100% RH, tension using a pressure probe. Fruit was held at ~100% RH. The x-axis scale is in days after full bloom (DAFB).

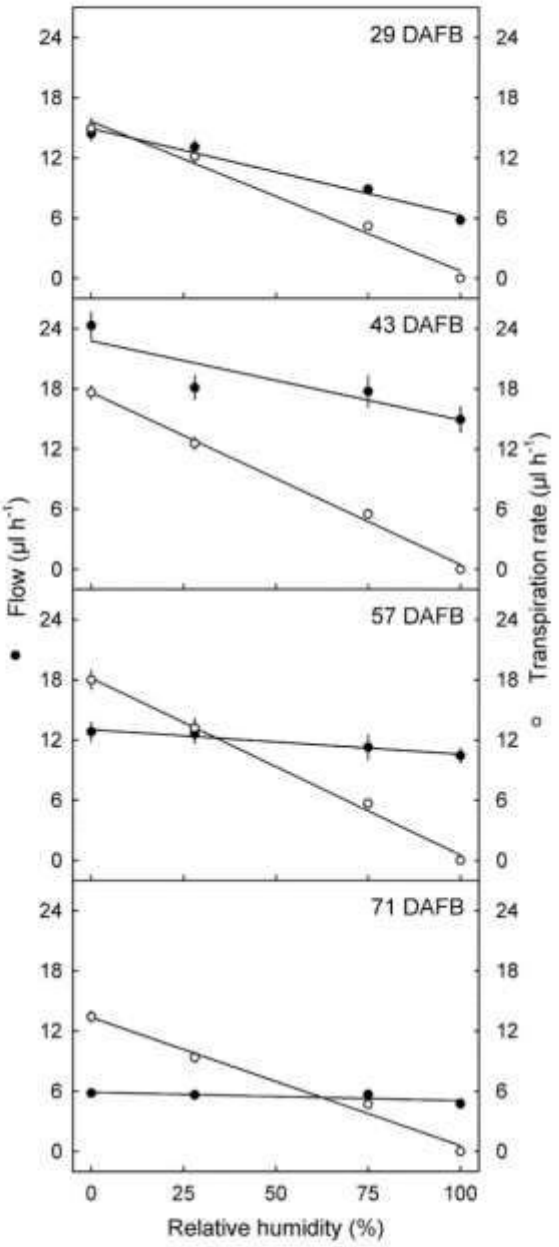

Fig. 5. Effect of relative humidity (RH) on vascular flow through the pedicel and the rate of transpiration through the fruit surface of sweet cherry at different stages of developments as indexed by the days after full bloom (DAFB). Flow was determined using potometry. Transpiration was quantified gravimetrically. Fruit were held above dry silica gel (~0% RH), or above saturated slurries of $CaCl_2$ (28% RH) or NaCl (75% RH) or above water (~100% RH).

4.2. Tension in the xylem is the primary factor causing vascular flow

In physical terms, the xylem represents a system of in-parallel pipes where the flow through any one pipe follows the Hagen Poiseuille law and the total flow through a bundle of pipes equals the sum of the flows through the individuals (Nobel, 1999). For a given geometry and number of pipes the Hagen Poiseuille law predicts flow to be proportional to the difference in hydrostatic pressure along the pipe. This was indeed the case in our study where the tension in the xylem and the flow were linearly related (Fig. 4D, Inset).

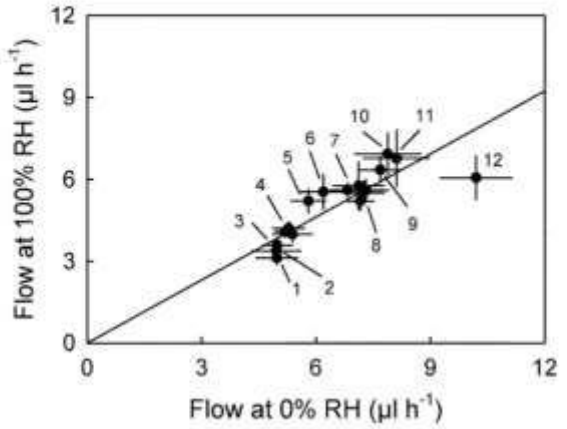

Fig. 6. Relationship between vascular flow at ~100% RH and ~0% RH across 17 cultivars of sweet cherry fruit at maturity. Flow was determined using potometry. The regression equation was: Flow at 100% RH ($\mu l\,h^{-1}$) = 0.77 (±0.02) * flow at 0% RH ($\mu l\,h^{-1}$), $r^2 = 0.70^{***}$. Cultivars were 'Fabiola' (1), 'Hedelfinger' (2), 'Dönnisens Gelbe' (3), 'Staccato', 'Adriana', 'Sam' (4), 'Querfurter Königskirsche' (5), 'Sweetheart' (6), 'Early Korvic' (7), 'Gill Peck', 'Earlise', 'Regina', 'Frühe Rote Mecklenburger' (8), 'Samba' (9), 'Merchant' (10), 'Burlat' (11), and 'Jacinta' (12).

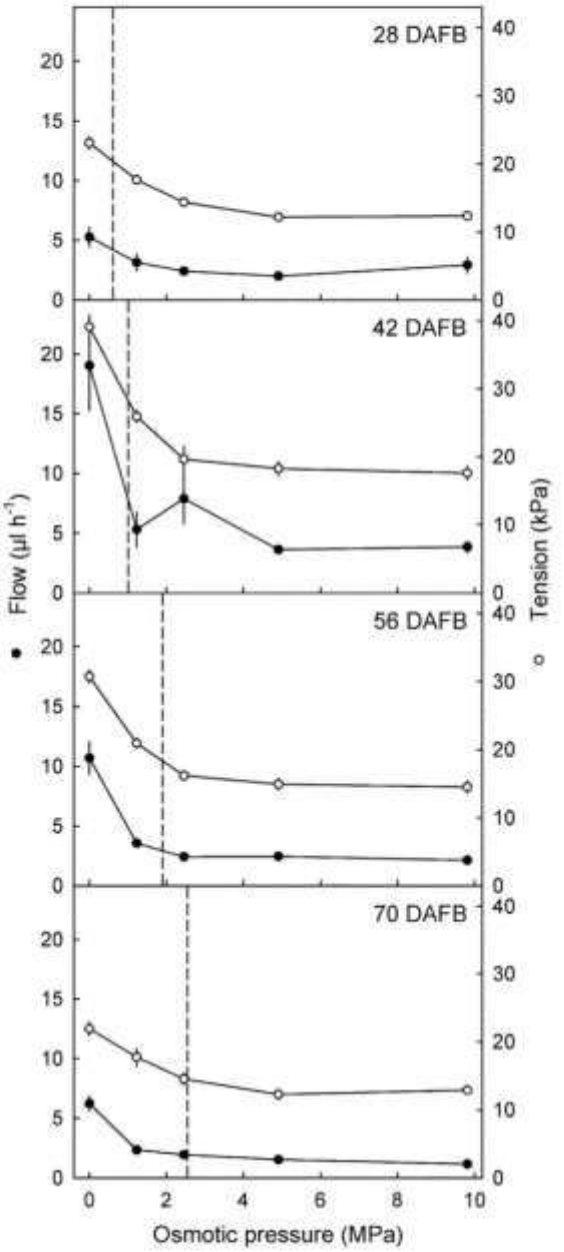

Fig. 7. Effect of the osmotic pressure of sucrose solutions fed to the cut surfaces of the pedicels of developing sweet cherry fruit on vascular flow and the tension. Measurements were made at different stages of development as indexed by the days after full bloom (DAFB). Flows and tensions were quantified using a potometer and a pressure probe, respectively. Fruit were held at ~100% RH. Vertical dashed lines indicate the osmolarity of juice expressed from fruit of the same developmental stage.

Mechanistically, the difference in tension between the cut end of the pedicel (atmospheric pressure) and that at the fruit end of the pedicel (the pressure of the fruit apoplast) represents the 'driving force' for flow in potometry. This difference probably resulted from tension in the fruit apoplast caused by water uptake into the fruit symplast and by the loss of water during transpiration. During stage III osmolytes accumulate in the symplast causing its osmotic potential to becoming more negative (from −1.1 MPa at the end of stage II, to −2.6 MPa at maturity). Because the turgor of the parenchyma cells remains essentially constant and very low at about 50 kPa throughout stage III (Knoche et al., 2014; Schumann et al., 2014) and because symplast and apoplast are likely to be in water potential equilibrium, the fruit apoplast should have an osmotic potential close to that of the symplast. Thus, a gradient in tension is likely to be present between the xylem at the pedicel's cut end and its distal ends in the apoplast. This gradient would pull water into the fruit's apoplast and subsequently from the apoplast into the symplast. Of course in the intact system (tree + pedicel + fruit), the pressure in the tree end of the pedicel xylem will vary diurnally from values close to atmospheric just pre-dawn, to values close to −1.5 MPa or even lower in the mid-afternoon (depending on the weather). Hence the flows recorded in the isolated part-system (pedicel + fruit) being studied here, while usefully indicative of the fruit's and pedicel's xylem conductance properties and also of the properties of the flesh apoplast:symplast, should not be taken as indicating the flows likely to take place *in vivo*. Here, we would be more likely to see a lower inflow (tree → fruit) or even an outflow of xylem sap (fruit → tree) during the day and a higher inflow of sap (tree → fruit) at night (Lang and Volz, 1993, 1998).

Fruit transpiration is an additional factor causing tension and flow in the xylem when the fruit is held in a non-saturated atmosphere. Throughout our experiments, flows were higher under high-transpiring conditions (~0% RH) than they were under low-transpiring conditions (~100% RH). Nevertheless, although a significant contributor to apoplast tension, transpiration was not the only or even the dominant contributor. First, for mature fruit the measured transpiration flow accounted (on average across the 17 cultivars) for only about 35% of the measured flow in the pedicel xylem (Fig. 6). Second, there was no effect of abrading or interrupting the cuticle on xylem tension or flows (Table 3). Both treatments can reasonably be assumed to have greatly increased transpiration.

While osmotic water uptake and transpiration account for most of the tension, a low, clearly detectable flow remained even when feeding hypertonic sucrose solutions to the pedicel and the fruit was held at ~100% RH. Based on vessel diameters (Brüggenwirth

and Knoche, 2015) and pedicel length these flow rates were sufficiently high for the sucrose solution to arrive within a few minutes in the fruit's apoplast in the vicinity of the flesh cell membranes where an osmotic effect would be felt. Because solutions were hypertonic, the continuing inflow at 100% RH is surprising and its mechanistic basis is unclear. It may be speculated that the inflow of hypertonic sucrose concentrations induced plasmolysis in the flesh cells surrounding the distal ends of the xylem vessels. Plasmolysis, in turn, is accompanied by significant swelling of cell walls (Grimm and Knoche, 2015). Thus, the cell walls represent a compartment that becomes available for water inflow only after the turgor was eliminated. Swelling of cell walls is a phenomenon frequently observed in maturing soft and fleshy fruit including sweet cherry (Redgwell et al., 1997; Grimm and Knoche, 2015). Further studies are needed to evaluate this hypothesis.

4.3. Flow in the xylem decreases towards maturity

Observed changes in flow in the course of development must be accounted for by changes in the 'driving force' for flow and/or in the conductance of the xylem. Until 48 DAFB (early stage III) increases in flow rate may be attributed to increases in the tension gradient as the osmotic potential of the fruit symplast decreases, whereas the osmotic potential of the apoplast lags behind. The hypothetical lagging behind of the osmotic potential of the apoplast is inferred from the transient peak in turgor at the stage II/stage III transition (Schumann et al., 2014). However, after 48 DAFB, turgor must be negligibly low (Schumann et al., 2014) so the decreased flow must be attributed to decreased xylem conductance. Because the xylem conductance of the pedicel remains essentially constant throughout stage III (Brüggenwirth and Knoche, 2015), it is the conductance of the xylem within the fruit that must have decreased. Direct supporting evidence comes from the observed decreases in flow rate associated with the progressive, sequential sectioning of the fruit, starting from the stylar end (Fig. 3). In early stage III fruit, sectioning resulted in a proportional decrease in flow rate and tension indicating a functional xylem throughout the fruit. However, at maturity the gradient in tension and the water flow were limited to the proximal pedicel end of the fruit. Thus, the more distal equatorial portion and the stylar end of the fruit had no additional effect. Decreased conductance of the fruit xylem offers a plausible explanation for the effective functional isolation of the xylem in the distal portion of the fruit (Fig. 3). A decrease in conductance of the fruit xylem is not unique for sweet cherry, but has also been reported in apple (Lang, 1990; Drazeta et al., 2004), grape (Düring et al., 1987; Greenspan et al., 1994), and kiwifruit (Dichio et al., 2003). At present the mechanistic basis for the decreased xylem conductance in sweet cherry is unknown. Potential explanations include a physical disruption within the fruit caused by growth as demonstrated for grape berries (Lang and Düring, 1991; Coombe and McCarthy, 2000) and apples (Drazeta et al., 2004).

4.4. Practical implications

The xylem flow rates into the mature fruit were low and only slightly affected by changes in fruit transpiration or submersion of the fruit in water. From the flow rates measured in this and our earlier study (Brüggenwirth et al., 2016), it seems unlikely that xylem flows contribute significantly to cracking in mature sweet cherry. Clearly, use of a potometer to measure xylem sap flows in these fruit requires they be detached from the tree. This creates the potential for experimental artefacts. However, flows obtained using this technique were closely related ($r = 0.82^{***}$) and of the same order of magnitude as the xylem flows calculated from diameter changes of still-attached fruit during high humidity nights that were measured by linear variable displacement transducers using the method described by Lang and Thorpe (1989), Lang (1990), Morandi et al. (2010) and Brüggenwirth et al. (2016). That potometric xylem flows were slightly larger than those determined by LVDT (on average 1.5-fold; for comparison see Brüggenwirth et al., 2016) may be accounted for by the following argument. In potometry, the water is fed to the pedicel end at atmospheric pressure, whereas in attached fruit, the xylem at the pedicel end is hydraulically connected to the xylem of the tree and, hence, exposed to a strongly negative water potential in daytime. Thus, the "driving force" for vascular flow is expected to be somewhat larger for detached fruit (by potometry) than for attached fruit (by LVDT). We expect differences between flows measured in detached fruit and those in attached fruit will be smaller for well-irrigated trees at night and larger for droughted trees in the middle of a warm day.

5. Conclusion

Our study demonstrates that pedicel xylem flow is driven by a gradient in tension caused by osmotic water uptake from the fruit apoplast into the fruit symplast and by transpiration. Furthermore, there is some evidence that the conductance of the xylem decreases during stage III. The consequence of decreased xylem conductance is that the fruit apoplast becomes hydraulically isolated from the tree (Tyerman et al., 2004). From a physiological point of view, this isolation prevents (1) uncontrolled osmotic uptake of water via the xylem into the fruit that would potentially cause fruit cracking and (2) a backflow of apoplastic solution out of the fruit into the tree. This flow would carry along with it dissolved carbohydrates and other osmolytes from the cell wall free space of the fruit into the vascular system of the pedicel, the spur, the branch and, ultimately, the tree.

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Contribution of authors

M. Knoche attracted the funding. A. Winkler and M. Knoche planned the experiments. A. Winkler and M. Ossenbrink performed the experiments. A. Winkler and M. Knoche analyzed the data and wrote the manuscript. A. Winkler, M. Ossenbrink, and M. Knoche revised and edited the publication.

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Malic Acid Promotes Cracking of Sweet Cherry Fruit

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Abstract. **When mature sweet cherries (*Prunus avium* L.) came into contact with sweet cherry juice, cracking dramatically increased. The objectives of our study were: 1) to quantify the cracking of fruit in cherry juice, 2) to determine which constituent(s) of the juice especially promote cracking and, 3) to establish its/their mode of action in promoting cracking. Artificial juice was made up as an aqueous solution of the same five pure chemicals and at the same relative concentrations as the five major osmolytes of real sweet cherry juice. Artificial and real juice was used at half-isotonic concentrations as the real juice from that batch of fruit. Cracking of sweet cherries placed in either artificial or real juice was more rapid and occurred for lower net water uptakes than of fruit placed in half-isotonic polyethylene glycol 6000. The crack-promoting component in sweet cherry juice was malic acid. Further tests with malic acid, and other organic acids, and with different concentrations of malic acid, with and without pH control, and with the enantiomers of malic acid, showed the effects were primarily related to the pH of the incubation solution. Leakage of anthocyanin from discs of flesh was increased in the presence of malic acid and greater in hypotonic than hypertonic solutions, suggesting that malic acid increases the permeability of the plasma membrane and tonoplast and weakens the cell walls. Malic acid may be an important link (amplifier) in a reaction chain that begins with the bursting of individual epidermal cells and ends with the formation of macroscopic skin cracks.**

Rain cracking is a problem for sweet cherry production in all countries where this very high-value crop is grown (Christensen, 1996). Despite considerable research effort the mechanistic basis of the phenomenon is still poorly understood. The fruit's water relations are thought to play a critical role in cracking (Considine and Kriedemann, 1972; Measham et al., 2009; Sekse, 1995, 2008; Sekse et al., 2005). Recent investigations have established that stage III sweet cherry fruit have a surprisingly low turgor (Knoche et al., 2014; Schumann et al., 2014). The mechanism by which low turgor is maintained despite a massive accumulation of carbohydrates and, hence, a very negative osmotic potential is at present unknown. Barring the pit, a sweet cherry has a similar mechanical constitution as a grape (*Vitis vinifera* L., *Vitis labrusca* L.), which is also very vulnerable to rain cracking (Considine and Brown, 1981). As in sweet cherries, grape turgor is very low in postveraison fruit (Lang and Düring, 1990; Wada et al., 2008, 2009). The low turgor in grapes is accounted for by the accumulation in the apoplast of solutes at osmotic concentrations closely matching those in the symplast. The rough balance in the osmotic potentials in symplast and apoplast results in similar pressures in these two compartments and thus flaccidity (Wada et al., 2008). This conclusion is based on the following experimental evidence. First, at harvest maturity there is no significant difference in osmolarity between apoplastic fluid extracted from grapes via the stalk using a pressure bomb and that of the expressed juice of crushed fruit (almost totally symplastic in origin—very large, thin-walled flesh cells), whereas in immature grapes they are substantially different (Lang and Düring, 1991). This finding is interpreted as being the result of a loss of compartmentation during maturation (Lang and Düring, 1991). Second, Tilbrook and Tyerman (2008) observed significant cell death within an intact, mature grape berry using microscopy and vitality staining. The membrane's loss of osmotic competence allows diffusion of symplastic solutes into the apoplast. Third, using tissue centrifugation (Wada et al., 2008) and a pressure plate apparatus (Wada et al., 2009) the grape apoplast was selectively sampled. The subsequent compositional analyses also revealed that symplastic solutes accumulate in the apoplast of mature grapes (Wada et al., 2008, 2009).

For sweet cherries, comparable, direct evidence for the presence of apoplastic solutes that would account for the lack of turgor is not yet available. However, grape berry and sweet cherry are morphologically and physiologically similar with respect to cracking and therefore the above explanation may also apply to sweet cherry. First, the bursting of cells as a consequence of excessive water uptake possibly through microcracks (Glenn and Poovaiah, 1989; Peschel and Knoche, 2005) and of significant cell-wall degradation during maturation (Kondo and Danjo, 2001) would result in solute leakage into the apoplast. Second, we observed macerated tissue surrounding the pit of many sweet cherry cultivars at maturity (M. Knoche, unpublished data). We interpret this observation as the loss of compartmentation in this region of the fruit.

The question arises what would be the consequences of such leakage? In the work we report here, we observe a surprising and dramatic increase in cracking when sweet cherry fruit are brought into direct contact with the expressed juice of sweet cherries.

The objectives of this study were to investigate what consequences the leakage of cell contents into the fruit's apoplast might have. We were particularly interested in 1) quantifying cracking of sweet cherry fruit when incubated in different osmotica, including in artificial sweet cherry juice containing the same five dominant chemical moieties found in real cherry juice; 2) identifying any "active," crack-promoting constituent in real cherry juice; and 3) establishing its mode of action in increasing cracking.

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Material and Methods

Plant material. 'Adriana' and 'Sam' sweet cherry fruit were harvested at commercial maturity based on color, size, and taste from greenhouse-grown or field-grown trees grafted on 'Gisela 5' rootstocks (*Prunus cerasus* L. × *Prunus canescens* Bois) at the Horticultural Research Station of the Leibniz University in Ruthe (lat. 52°14′N, long. 9°49′E). Fruit were cultivated according to current European regulations for integrated fruit production. In a limited number of experiments, off-season 'Bing' fruit was obtained from a market. Here rootstock and harvest date are unknown. Fruit of uniform size and color were randomly selected. All fruit were free from visible defects. To restrict water uptake to the fruit surface, the pedicels were cut about 5 mm above the pedicel/fruit juncture and the juncture and the pedicel end sealed using silicone rubber (3140 RTV Coating; Dow Corning, Midland, MI). Thereafter fruit was placed in cold storage overnight at 2 °C. Next morning the silicone had cured and fruit were equilibrated to room temperature for one hour or so before initiating experiments.

General experimental procedure. Cracking susceptibility was determined using a modified protocol for determining the cracking index first described by Verner and Blodgett (1931) and adapted by Christensen (1996). Briefly, two groups of 25 fruit each were incubated in 500 mL of treatment solutions at 22 °C. Fruit were removed from treatment solutions at regular time intervals, inspected for macroscopically visible cracks (by the naked eye) and the noncracked fruit returned to the incubation solution. Visual inspection detects cracks that extend into the flesh, but not microcracks limited to the cuticle. The experiment was ended after the last fruit cracked or fruit began to rot. There was no detectable change in osmolarity of the incubation solution caused by fruit cracking in the course of an experiment. Also, pH remained essentially constant (within 0.5 pH units) in presence of organic acids without pH adjustment. However, pH of nonbuffered solutions [i.e., those containing water only, carbohydrates, polyethylene glycol (PEG) 6000, or those of organic acids when titrated to pH values > pH 6] decreased by up to 3 pH units during incubation due to cracking.

Water uptake was quantified gravimetrically. Fruit was incubated in treatment solutions at 22 °C, removed from solution usually at 30 and 60 min after incubation, carefully blotted with tissue paper, weighed, and then reincubated. The rate of water uptake was calculated on an individual-fruit basis as the slope of a linear regression through a paired dataset of cumulative water uptake vs. time. As individual fruits cracked during the course of the experiment, these were excluded from further incubation and measurement. The only exception was for fruit incubated in 300 mM malic acid without pH control, where all fruit had cracked within the first 30 min of incubation. The minimum number of individual fruit replicates was 11.

Experiments. Water uptake and fruit cracking were compared in 'Sam' sweet cherries following incubation in PEG 6000, real sweet cherry juice extracted from the same batch of fruit using a spaghetti press, or in artificial juice comprising the five most abundant osmolytes of sweet cherries (Herrmann, 2001). Together, these five osmolytes account for 98% of the osmolarity of sweet cherry juice. Relative contributions to total osmolarity and absolute final molar concentrations were 41.2% and 432 mM for glucose, 37.5% and 394 mM for fructose, 7.3% and 77 mM for sorbitol, 6.7% and 70 mM for malic acid, and 5.4% and 56 mM potassium as potassium malate (Herrmann, 2001). The PEG 6000 was used for comparison. All solutions were prepared and diluted as necessary such that the final incubation solutions were half-isotonic to the real juice as determined by water vapor pressure osmometry (VAPRO® 5520 and 5600; Wescor, Logan, UT). Fruit incubated in deionized water served as controls. Cracking was quantified at selected time intervals as described above. In a subsequent experiment, the relative contributions of the individual osmolytes of cherry juice to cracking was identified by incubating 'Sam' sweet cherry in solutions containing only one of the osmolytes at half of its respective concentration in the juice. Concentrations and osmolarities of the various constituents were: 15.9 mM and 0.1 MPa malic acid, 98.1 mM and 0.5 MPa glucose, 89.4 mM and 0.4 MPa fructose, 17.5 mM and 0.1 MPa sorbitol, and 18.3 mM and 0.1 MPa potassium malate. Fruit incubated in half-isotonic artificial juice (1.2 MPa) and in

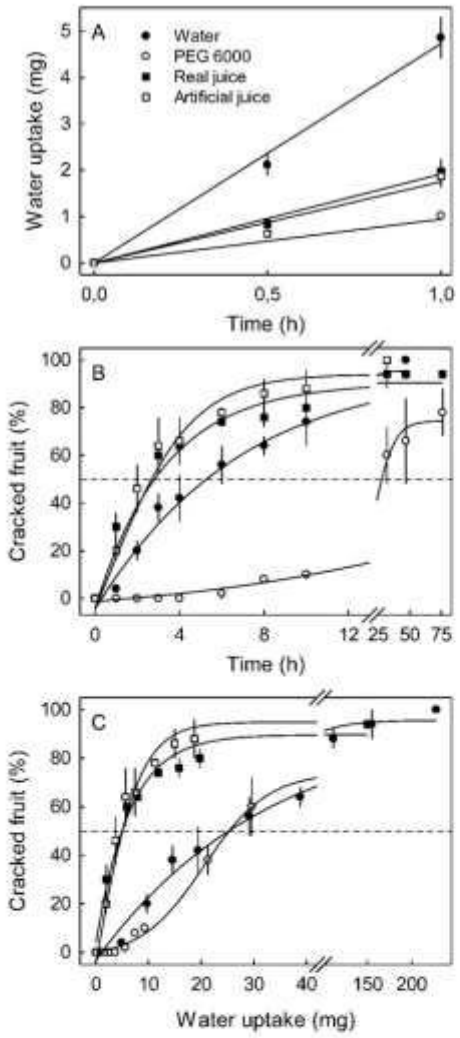

Fig. 1. Time course of water uptake (**A**) and cracking of 'Sam' sweet cherry fruit (**B**). Percentage of cracked fruit as a function of water uptake (**C**). Dashed horizontal line indicates 50% cracking (**B** and **C**).

Table 1. Effect of sweet cherry juice, artificial juice, polyethylene glycol 6000 (PEG 6000), and deionized water on water uptake and cracking of 'Sam' sweet cherry fruit. Artificial juice comprised the five most abundant osmolytes reported in real sweet cherry juice [glucose, fructose, sorbitol, potassium malate, and malic acid (Herrmann, 2001)]. All solutions were prepared half-isotonic (1.2 MPa) relative to the osmolarity of the expressed juice of this batch of sweet cherries (2.4 MPa). Cracking was indexed by the amount of water taken up for 50% of fruit to crack (WU_{50}) or by the time to 50% fruit cracking (T_{50}).

Treatment	Rate of water uptake [mean ± SE (mg·h^{-1})]	Cracking	
		WU_{50} [mean ± SE (mg)]	T_{50} [mean ± SE (h)]
Water	4.9 ± 0.4[z]	26.1 ± 5.6	5.4 ± 1.1
PEG 6000	0.9 ± 0.1*	28.9 ± 6.3	31.2 ± 6.8
Sweet cherry juice	2.0 ± 0.3*	4.9 ± 0.4	2.5 ± 0.2
Artificial juice	1.9 ± 0.2*	4.7 ± 1.2	2.5 ± 0.6

[z]Significance of difference of rate of water uptake to water control indicated by *, Dunnett test at $P \leq 0.05$.

Table 2. Effect of the most abundant osmolytes in sweet cherry fruit on water uptake and cracking. The concentrations and osmolarities correspond to those in a half-isotonic 'Sam' sweet cherry juice. Cracking was indexed by the amount of water taken up for 50% of fruit to crack (WU_{50}) or by the time to 50% fruit cracking (T_{50}).

Treatment	Osmolarity (MPa)	Rate of water uptake [mean ± SE (mg·h^{-1})]	Cracking	
			WU_{50} [mean ± SE (mg)]	T_{50} [mean ± SE (h)]
Water	0.0	3.5 ± 0.4[z]	23.9 ± 1.1	6.8 ± 0.3
Artificial juice	1.2	2.1 ± 0.2*	7.5 ± 1.5	3.6 ± 0.7
Malic acid	0.1	4.8 ± 0.5	9.2 ± 2.6	1.9 ± 0.5
Glucose	0.5	3.6 ± 0.3	41.0 ± 4.7	11.4 ± 1.3
Fructose	0.4	3.3 ± 0.2	24.2 ± 7.4	7.3 ± 2.2
Sorbitol	0.1	3.8 ± 0.4	25.7 ± 1.6	6.8 ± 0.4
Potassium malate	0.1	3.9 ± 0.5	26.2 ± 9.1	6.7 ± 2.3

[z]Significance of difference of rate of water uptake to water control indicated by *, Dunnett test at $P \leq 0.05$.

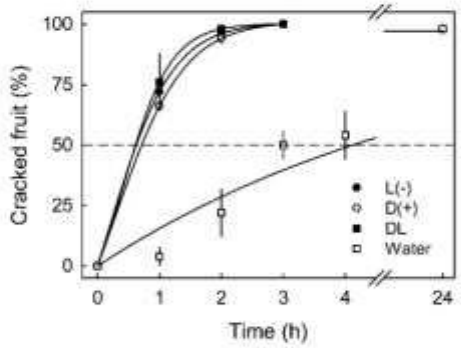

Fig. 2. Effect of L(−) and D(+) enantiomers or a racemic mixture (DL) of both enantiomers of malic acid on cracking of 'Bing' sweet cherry fruit. Dashed horizontal line indicates 50% cracking.

deionized water served as controls. Cracking was assessed regularly as described previously.

Possible differences among the effects of L(−)- and D(+)-enantiomers or a racemic mixture (DL) of malic acid on cracking were identified by incubating 'Bing' sweet cherries in 70 mM L(−), D(+) and DL malic acid and monitoring fruit cracking. This concentration corresponds to the malic acid concentration present in real and in our artificial juice (Herrmann, 2001). Fruit were inspected at regular time intervals. Because there were no significant differences in cracking between the enantiomers (see Results section), the racemic mixture of DL malic acid was used in all subsequent experiments.

To control whether the effects on water uptake and cracking were specific for malic acid, a range of mono, di- and tricarboxylic acids (all at 70 mM, except for fumaric acid at 40 mM due to its lower solubility limit) were screened for their effects on water uptake and cracking using 'Bing' sweet cherry fruit. The acids investigated comprised malic, tartaric, succinic, fumaric, maleic, formic, acetic, propionic, and citric acids. Deionized water served as control. Cracking was monitored by regular inspection.

The effect of malic acid concentration (1, 3, 10, 30, 100, and 300 mM) with and without pH control on water uptake and cracking were established in 'Sam' sweet cherries. In the first experiment, the concentration effect was investigated without pH control (range pH 1.9–3.3). In the second (subsequent) experiment, pH was maintained constant between concentrations by adjusting all solutions to pH 3.3 using KOH. Fruit incubated in pure water served as control. Water uptake and fruit cracking were monitored as described above.

The effect of pH on water uptake and cracking was studied in 'Adriana' sweet cherries incubated in malic or citric acid or in phosphate buffer. For malic and citric acid (70 mM), pHs were adjusted to pH 3, 4, 5, 6, and 7 using KOH. The pH values < pH 3 were achieved by using malic and citric acid at their respective pH (pH 2.3 and pH 2.1 at 70 mM for malic and citric acid, respectively). The phosphate buffer (70 mM K_2HPO_4) was prepared by titration using H_2SO_4. Fruit incubated in deionized water served as control. Fruits were inspected for cracks up to 97 h.

To identify the mechanistic basis of the effect of malic acid on fruit cracking, its effect on membrane leakage in the presence and absence of osmotic stress was studied in 'Bing' sweet cherry. The leakage of anthocyanins from discs of sweet cherry flesh served as an indicator of membrane damage. In the first experiment, the time course of anthocyanin efflux was established. Discs (8 mm diameter, 2 mm thick, three discs per replicate, $n = 10$) were excised from the cheek of a fruit using a biopsy punch and parallel razorblades. Discs were blotted,

Table 3. Effect of selected mono, di- and tricarboxylic acids (concentrations all 70 mM, except fumaric acid 40 mM) on water uptake and cracking of 'Bing' sweet cherry fruit. Cracking was indexed by the amount of water taken up for 50% of fruit to crack (WU_{50}) or by the time to 50% fruit cracking (T_{50}).

Treatment	Water uptake rate [mean ± SE ($mg \cdot h^{-1}$)]	Cracking	
		WU_{50} [mean ± SE (mg)]	T_{50} [mean ± SE (h)]
Water	47.2 ± 3.3[z]	233.7 ± 14.3	4.9 ± 0.3
Formic acid	57.1 ± 11.3	141.4 ± 7.2	2.5 ± 0.1
Acetic acid	52.1 ± 10.9	129.6 ± 17.6	2.5 ± 0.3
Propionic acid	41.4 ± 3.9	124.9 ± 3.4	3.0 ± 0.1
Succinic acid	40.8 ± 7.7	99.6 ± 4.2	2.4 ± 0.1
Malic acid	59.1 ± 7.2	126.6 ± 4.5	2.1 ± 0.1
Tartaric acid	50.0 ± 6.8	97.5 ± 8.6	2.0 ± 0.2
Maleic acid	52.5 ± 8.3	94.5 ± 0.9	1.8 ± 0.0
Fumaric acid	61.2 ± 5.9	63.9 ± 8.5	1.0 ± 0.1
Citric acid	53.8 ± 7.2	99.7 ± 7.4	1.9 ± 0.1

[z]All rates of water uptake not significantly different from water control, Dunnett test at $P \leq 0.05$.

rinsed and incubated in sucrose solution (osmolarity 3.1 MPa) with and without 70 mM malic acid (osmolarity 3.0 MPa). These solutions were about isotonic to juice extracted from the same batch of 'Bing' fruit (3.0 ± 0.2 MPa). After 1, 2, 4, 8, and 24 h, discs were removed from the incubation solution and the incubation medium sampled for anthocyanin content. To correct for differences in pH between the different incubation solutions, malic acid at a final concentration of 70 mM was also added to the controls (after removal of the discs and before photometry) such that control and treatment solutions contained the same amount of malic acid and hence, had the same pH of pH 2.3. This was necessary, because the absorption spectrum of anthocyanin depends on pH. The absorption at 520 nm was determined using a photometer (Specord 210; Analytik Jena, Jena, Germany). In the second experiment, osmotic stress was imposed by incubating discs in sucrose solutions having osmolarities of 0.9, 1.7, 2.6, 3.4, 4.3, and 5.2 MPa with and without 70 mM malic acid. At low sucrose concentrations, we expect cell walls to be strained due to increased turgor following water uptake from the hypotonic donor solutions. If malic acid weakened cell walls so they were more likely to rupture, anthocyanin efflux would be higher into hypotonic than into hypertonic solutions. Discs were removed from the incubation solutions after 8 h and the anthocyanin leakage quantified as described above. The number of replicates was 10.

Data analysis. Data are presented as means ± SE. When error bars are not visible in a graph, they are smaller than the plotting symbols. Data on rates of water uptake were log-transformed, subjected to analysis of variance and mean comparisons performed using the Dunnett test (packet multcomp 1.3–1, procedure glht, R 3.0.2; R Foundation for Statistical Computing, Wien, Austria). Linear and nonlinear regression analyses were performed using SigmaPlot (version 12.5; Systat Software, San Jose, CA). Fruit cracking typically increased with time in a sigmoidal manner. This relationship is best described by a four-parameter sigmoidal regression model:

$$Cracking\ (\%) = y_0 + \frac{a}{1 + e^{\frac{-(time(h) - x_0)}{b}}}$$

Equating the percentage of cracking to 50% and solving for time allowed the half time (T_{50} in hours) of fruit cracking to be estimated from:

$$T_{50}(h) = -ln\left(\frac{a}{50 - y_0} - 1\right) * b + x_0$$

A high T_{50} represents fruit that cracks slowly. The percentage of fruit cracking was also expressed as a function of water uptake by simply replacing time (in hours) by the amount of water taken up (in milligrams) in the preceding equations (Weichert et al., 2004). The amount of water uptake at any one time is calculated as the product of the rate of uptake multiplied by the duration of incubation in water assuming that cumulative water uptake increases linearly with time. This is a fair assumption for the time periods of incubation in our experiments (Beyer et al., 2005). In analogy with a semilethal dose of a toxic compound resulting in 50% mortality (LD_{50}), the water uptake required for 50% of the fruit to crack (WU_{50} in milligrams) is obtained from the T_{50}. This WU_{50} characterizes the mechanical stability of a fruit in a given solution. Because the WU_{50} is corrected for the amount of water taken up, it is a useful parameter in comparing cracking susceptibility of genotypes, treatments, and conditions. A high WU_{50} is interpreted as fruit having a high cracking resistance or a low cracking susceptibility irrespective of its water uptake rates. Thus, the WU_{50} is a measure of the "intrinsic" cracking resistance of a fruit in a given system.

Results

Cumulative water uptake increased linearly with time (Fig. 1A). Incubating fruit in real or artificial juice or in a PEG 6000 solution decreased rates of uptake as compared with the deionized water control. There were no differences in rates of water uptake between real and artificial juice, but both rates were higher than that with PEG 6000 (Fig. 1A; Table 1). The percentage of cracked fruit increased rapidly with time and was always higher for fruit incubated in solutions containing real or artificial juice followed by that of fruit incubated in water or PEG 6000 (Fig. 1B; Table 1). Calculating water uptake at 50% cracking revealed there was little difference in WU_{50} between fruit incubated in PEG 6000 or in deionized water (Fig. 1C; Table 1). However, WU_{50} was dramatically lower for fruit incubated in real or artificial juice. Again, there was no difference in WU_{50} between real and artificial juice (Fig. 1C; Table 1).

Compared with deionized water, water uptake was essentially independent of the presence of any of the major osmolytes

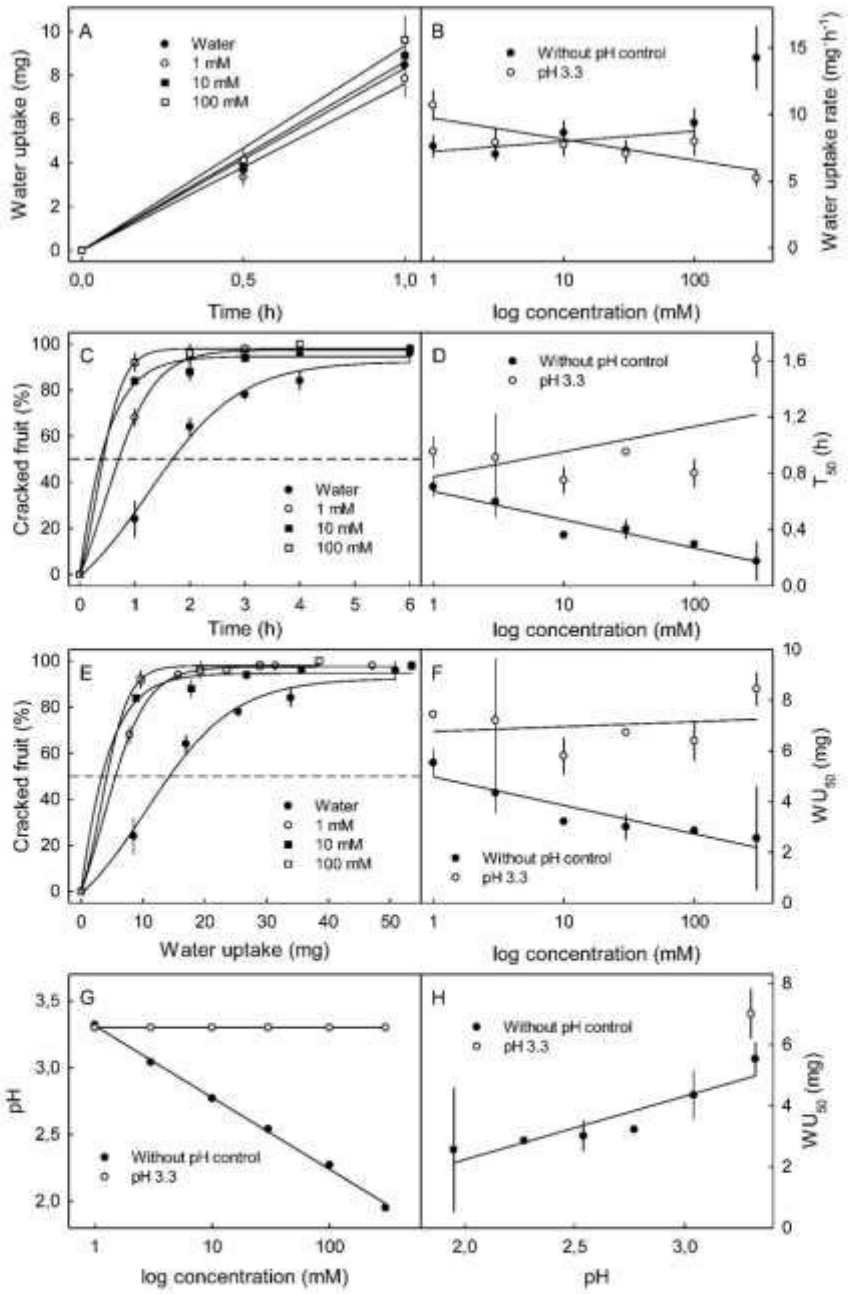

Fig. 3. Effect of concentration of malic acid on water uptake and cracking of 'Sam' sweet cherry fruit. (**A**) Time course of water uptake. (**B**) Rate of water uptake as a function of concentration. Solutions were used with (pH 3.3) or without pH control. The 300 mM malic acid treatment without pH control was excluded from regression analysis, because all fruit had cracked and the high rate of uptake may have resulted from cracking. (**C**) Time course of cracking. (**D**) Time to 50% cracking (T_{50}) as a function of concentration. (**E**) Fruit cracking as a function of the amount of water taken up. (**F**) Water uptake to 50% cracking (WU_{50}) as a function of concentration. (**G**) pH values of malic acid at different concentrations with (pH 3.3) or without pH control. (**H**) WU_{50} as a function of pH of the malic acid solutions. Dashed horizontal lines in C and E indicate 50% cracking.

in sweet cherry juice (Table 2). Also, most osmolytes examined (glucose, fructose, sorbitol, and potassium malate) had little effect on the time to 50% cracking or on the intrinsic cracking susceptibility (Table 2). However, fruit was markedly more susceptible to cracking as indexed by decreases in WU_{50} and T_{50}, when incubated in malic acid. Indeed, there was little difference in WU_{50} and T_{50} between malic acid and artificial juice containing malic acid at the same molar concentration (Table 2). Interestingly, compared with the water control, incubation in potassium malate had no effect on WU_{50} or T_{50}.

The effect of malic acid on cracking was independent of the enantiomer (Fig. 2). Compared with the water control, the L(−)- and D(+)-enantiomers and the racemic mixture (DL) of malic acid increased cracking to the same extents.

There was little effect of the organic acids on the rate of water uptake but, compared with the water controls, all mono, di- and tricarboxylic acids increased cracking as indexed by decreases in WU_{50} and T_{50} (Table 3). Fumaric acid gave the lowest WU_{50} and T_{50}, formic acid the highest and malic acid was intermediate (Table 3).

Varying the concentration of malic acid had little effect on the time course of water uptake (Fig. 3A). Only the highest malic acid concentration markedly increased the rate of uptake without pH control (pH 1.9). There was little difference in T_{50} between the lower malic concentrations (Fig. 3A and B). When pH was held constant, rates of water uptake decreased only slightly as the malic acid concentration increased. Fruit cracking, as indexed by decreasing T_{50} was positively related to malic acid concentration without pH control, but not when pH was maintained constant. Only the highest malic acid concentration increased T_{50} (Fig. 3C and D). The WU_{50} decreased with increasing malic acid concentration without pH control, whereas there was no effect of concentration when pH was held constant (Fig. 3E and F). Plotting the effects of malic acid concentration on the WU_{50} as a function of pH revealed that the effects were essentially a linear function of pH for all concentrations with and without pH adjustment (Fig. 3G and H).

The pHs of malic acid and citric acid solutions had little effect on rates of uptake, but not when incubated in phosphate buffer. Here, uptake decreased as pH of the phosphate buffer increased (Fig. 4A and B). Fruit cracking

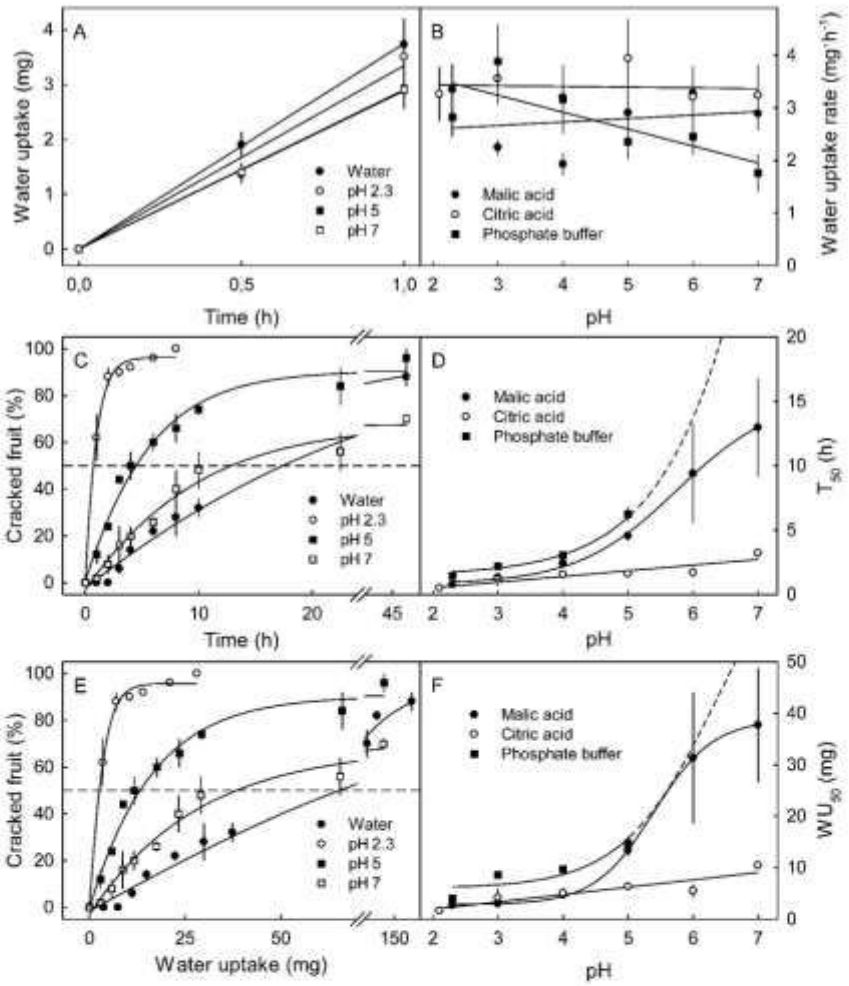

Fig. 4. Effect of pH of malic acid, citric acid, and phosphate buffer on cracking of 'Adriana' sweet cherry fruit. (**A**) Time course of water uptake from malic acid. (**B**) Rates of water uptake as affected by pH. (**C**) Time course of fruit cracking. (**D**) Time to 50% cracking (T_{50}) as affected by pH of the incubation solutions. (**E**) Fruit cracking as affected by the amount of water taken up. (**F**) Effect of solution pH on the amount of water uptake needed for cracking of 50% of fruit (WU_{50}). Dashed horizontal lines in **C** and **E** indicate 50% cracking. Dashed lines in **D** and **F** indicate extrapolated regression lines above pH 5.

as indexed by T_{50} was most severe at low pH, and decreased as pH increased (Fig. 4C and D). Only in citric acid did the T_{50} remain low with rising pH. Qualitatively similar relationships were obtained between WU_{50} and pH. The effects of malic acid and phosphate buffer were essentially identical (Fig. 4E and F). The lower T_{50} for citric acid at higher pHs, indicates that fruit are more susceptible to cracking, compared with malic acid or phosphate buffer of corresponding pHs (Fig. 4F).

When discs of cherry flesh were incubated in isotonic sucrose solutions, anthocyanin efflux into the solution increased with time in the presence of malic acid, but markedly less in its absence (Fig. 5A). The increase in anthocyanin leakage in the presence of malic acid was greater in hypotonic solutions (lower osmolarities) and tended to decrease as osmolarity increased (Fig. 5B). It is worth noting that in hypertonic sucrose solutions (where the bathing solution had higher osmolarity than juice from the flesh), anthocyanin leakage was still increased by malic acid, whereas only little anthocyanin efflux was observed in the absence of malic acid (Fig. 5B).

Discussion

Our results demonstrate that sweet cherry juice causes rapid fruit cracking for low amounts of water uptake. The effect of the juice on cracking can be reproduced using an artificial juice composed of the five most abundant osmolytes in real juice which include malic acid. It can also be reproduced using malic acid on its own (Fig. 1; Table 1). Thus, malic acid is clearly identified as the major crack-promoting component in sweet cherry juice.

A comparison of the enantiomers of malic acid (Fig. 2), of various related mono, di-, and tricarboxylic acids (Table 3), the effects of concentration of malic acid with and without pH control (Fig. 3) and that of pH (Fig. 4) demonstrates that cracking is largely independent of the type of acid and is instead primarily related to solution pH. The only deviations occurred with malic acid and citric acid at pH > 4. These pHs are outside of the typical buffer range of these acids and hence, pH control at the site of action in the fruit's apoplast (pH 3.6) may have been insufficient.

Increased cracking at low pH has also been reported for cherry tomatoes (Lichter et al., 2002). As in sweet cherry, cracking in tomato decreases as pH rises. The mechanistic basis for increased cracking at low pH is not known. Several factors may be involved including effects on the cell walls and/or plasma membranes. Malic acid also increased anthocyanin leakage into a hypotonic sucrose solution, indicating increased membrane permeability compared with the control. Furthermore, the increase in leakage was greater as osmolarity decreased (water potential became less negative and turgor increased), suggesting a weakening of the cell walls and the bursting of cells. Potential mechanisms of cell-wall weakening include 1) the cleavage of neutral sugar side-chains of cell-wall pectin that would weaken the cross-linking between the cellulosic cell wall and the pectins (Brummel, 2006), 2) a pH-dependent activation of polygalacturonases (Chun and Huber, 1998), and/or 3) a desorption, and complexation of cell-wall bound Ca^{2+} (Glenn and Poovaiah, 1989). These reactions weaken the cell-to-cell adhesion and decrease cell wall cross-linking (Demarty et al., 1984; Hepler, 2005).

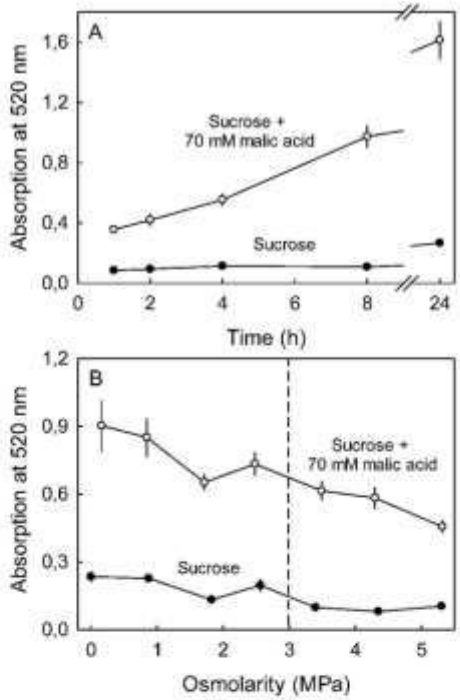

Fig. 5. (**A**) Time course of anthocyanin leakage from discs excised from the flesh of mature 'Bing' sweet cherry fruit in the absence and presence of malic acid. (**B**) Effect of osmolarity of the incubation solution on anthocyanin leakage in the presence and absence of malic acid. The vertical dashed line indicates the osmolarity of the fruit.

We do not have an explanation for the effect of glucose on cracking (Table 2). Osmotic effects on water uptake can be excluded because the WU_{50}, which is corrected for water uptake, increased. Also, the osmolarity of the glucose solution was too low to alter the driving force for water uptake and consequently, there was no significant effect on rates of water uptake. Compared with the control treatment, fruit incubated in glucose solution required more water to crack which would be interpreted as mechanically more robust, less susceptible fruit. Direct effects of glucose on the mechanical properties of the fruit skin that forms the structural backbone of the sweet cherry (Brüggenwirth et al., 2014), are unlikely, particularly within the short time period of the experiment. Any indirect effects for example on strain of the fruit skin would be essentially osmotic and, therefore, equally expected for fructose. Fructose was used in the same experiment at similar osmolarity, but had no effect on the WU_{50} or T_{50} (Table 2). Clearly, further experiments are necessary to reproduce and clarify this effect in greater detail.

Practical consequences. The scenario described here may occur also in sweet cherry fruit under orchard conditions. First, there is evidence that bursting of individual cells precedes macroscopic cracking (Glenn and Poovaiah, 1989) and also that loss of membrane integrity occurs, at least in the flesh surrounding the pit. Second, microcracks in the cuticle impair its barrier function, resulting in increased water uptake (Knoche and Peschel, 2006) and exposure of cells to water results in cell bursting and release of anthocyanins (Simon, 1977). The bursting of individual cells would also release malic acid into the apoplast. The appearance of malic acid in the apoplast, even at low concentration, will increase membrane permeability and weaken cell walls. This response may initiate a chain reaction that could contribute to macroscopic fruit cracking.

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5. Rain cracking in sweet cherries is not due to excess water uptake but to localized skin phenomena

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Contribution of authors

M. Knoche attracted the funding. A. Winkler and M. Knoche planned the experiments. A. Winkler, S. Peschel, and K. Kohrs performed the experiments. A. Winkler and M. Knoche analyzed the data and wrote the manuscript. A. Winkler, S. Peschel, K. Kohrs, and M. Knoche revised and edited the publication.

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Rain Cracking in Sweet Cherries is not Due to Excess Water Uptake but to Localized Skin Phenomena

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Additional index words. *Prunus avium*, perfusor, splitting, strain, turgor

Abstract. **Rain cracking of sweet cherry (*Prunus avium* L.) fruit is commonly thought to result from excessive net water uptake. This excess increases flesh turgor, which then strains and eventually ruptures the skin at the weakest point. This idea—the critical turgor hypothesis—assumes the fruit comprises a semifluid flesh, held under pressure by a taut skin. The objectives of this study were to test the validity of this popular hypothesis. We investigated the effects of 1) the different pathways of water uptake and 2) the fruit's water balance on cracking. Incubating fruit of 19 cultivars in water resulted in rapid fruit cracking. The time to 50% cracking (T_{50}) averaged 7.5 ± 1.3 hours with considerable variability between cultivars (T_{50} range from 1.5 to 18.6 hours). The amount of water taken up at 50% cracking (WU_{50}) averaged 96.5 ± 17.6 mg (WU_{50} range from 17.7 to 331.5 mg). There was no correlation between either the T_{50} or the WU_{50}, and the rate of water uptake. Also, there was no correlation between the values of T_{50} (r = 0.58) and only a weak correlation between the values of WU_{50} (r = 0.80*) determined in different years. Comparing the value of WU_{50} under incubation vs. under perfusion revealed a 3.9- to 38-fold higher WU_{50} under perfusion (397.6 to 1840 mg) than under incubation (48.8 to 102.6 mg). This marked dissimilarity remained, regardless of pretreatments with isotonic polyethylene glycol (PEG) 6000 to induce microcracking or by manipulation of skin wetness during perfusion. Sealing the pedicel/fruit junction markedly decreased the rate of water uptake under incubation. It had no effect on the T_{50}, and it markedly decreased the WU_{50}. Similarly, manually induced skin defects greatly increased the rate of water uptake but, with few exceptions, had no effect on the T_{50}, whereas, the WU_{50} had increased. The location on the fruit surface of the resulting cracks was not related to the region of the skin in which the manual defect was induced. Allowing the fruit to transpire increased both, the T_{50} and the WU_{50}. Interestingly, the amount of water lost by transpiration exceeded the amount that was subsequently required to cause cracking up to 5-fold. Incubating fruit with their stylar ends immersed in water, whereas their remaining surfaces were in air of 0%, 28%, 75%, or 100% relative humidity (RH) resulted in net losses of water of up to 5.9 ± 0.7 mg·h^{-1}, nevertheless their stylar ends still cracked. All our results indicate rain cracking in sweet cherries is a localized phenomenon that is not related to the net fruit water balance (the critical turgor hypothesis) but is the result of more local exposure of the fruit skin to liquid-phase water (the zipper hypothesis).**

Rain cracking severely limits sweet cherry production in all regions of the world where rainfall occurs immediately before and during the harvest period (Christensen, 1996). Rain cracking is thought to be related to an excessively positive water balance resulting from 1) uptake of surface water through the fruit skin (Christensen, 1996), 2) via the vasculature of the pedicel (Measham et al., 2010; Sekse et al., 2005), and coupled with 3) the much-reduced transpiration of a wet fruit under humid conditions (Knoche and Measham, 2016).

A commonly cited model of rain cracking of soft and fleshy fruit is the critical turgor model originally proposed by Considine and Kriedemann (1972) for grape (*Vitis vinifera* L.) berries. This concept assumes the flesh of the berry to be held under compression by an elastically strained skin. Upon water uptake, the pressure in the fruit rises. When the fruit pressure (synonymous with fruit turgor and with flesh turgor) exceeds some critical threshold; i.e., the critical turgor pressure, the fruit skin is strained beyond its limit of extensibility and it cracks (Considine and Kriedemann, 1972). Although developed for grapes, the critical turgor model has also been used to account for rain cracking in sweet cherries (Measham et al., 2009; Sekse, 1995a, 1998a; Sekse et al., 2005). This is not unreasonable, given the similar sizes, shapes, textures, and external architectures of grapes and sweet cherries (notwithstanding the multiple small seeds of the one and the single large pit of the other).

The critical turgor model offers a logical conceptual framework for discussion of rain cracking in sweet cherry, which is consistent with the following empirical and experimental observations: 1) Sweet cherry fruit comprise two distinct tissues—the flesh and the skin. There is no published evidence (of which we are aware) that suggests the pit plays any role in cracking. The cellular components of the skin form the load-bearing structure implied in the critical turgor model (Brüggenwirth et al., 2014). 2) The sweet cherry skin is markedly strained (Grimm et al., 2012; Knoche et al., 2004). A portion of this strain is elastic (Brüggenwirth et al., 2014). According to Hook's law, elastic strain is caused by a proportional stress and this (in a near spherical fruit) can be simply related to a biaxial strain and a proportional internal pressure. 3) Rain cracking is reported to be affected by irrigation (Sekse, 1995b) and also occurs occasionally in fruit grown under rain shelters. These observations suggest water uptake can occur along different parallel pathways—e.g., both via vascular sap flow (xylem and phloem) in the pedicel and also via surface water uptake through a wet skin (osmotic uptake)—both of these pathways potentially contributing to rain cracking

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(Cline et al., 1995). 4) Conversely, skin cracking is markedly decreased if surface water uptake is decreased, e.g., by incubating fruit in aqueous $FeCl_3$ (Beyer et al., 2002; Weichert et al., 2004). Whereas the above observations are consistent with the critical turgor model (Considine and Kriedemann, 1972), the low turgor pressure recently reported in mature sweet cherry and—in particular—the lack of response of turgor to water uptake and transpiration (Knoche et al., 2014; Schumann et al., 2014) are not consistent with the critical turgor model.

The objective of the present study was to test the validity of the critical turgor model for sweet cherry rain cracking. We specifically investigated the effects of the route of water uptake and of the fruit's water balance on cracking. The pathway of water uptake was varied by 1) perfusing sweet cherry fruit; 2) by inducing microcracking and surface defects as parallel pathways for water uptake; and 3) by sealing the pedicel/fruit junction. The fruit's water balance was varied by allowing whole fruit or selected regions of the fruit surface to transpire before conducting cracking assays.

Material and methods

Plant material. Mature sweet cherry fruit of the cultivars Burlat and Summit for the perfusion experiments were harvested from a commercial orchard near Höhnstedt, Germany (lat. 51°30′N, long. 11°44′E) and from an experimental orchard in Marquardt, Germany (lat. 52°31′N, long. 12°51′E; Bundessortenamt, Prüfstelle Marquardt). Fruit used for the cracking assays (i.e., of the cultivars Adriana, Burlat, Dönissens Gelbe, Earlise, Early Korvic, Fabiola, Flamengo Srim, Gill Peck, Hedelfinger, Kordia, Merchant, Querfurther Königskirsche, Rainier, Regina, Sam, Samba, Schneiders Späte Knorpel, Staccato, and Sweetheart) were from greenhouse grown or field-grown trees at the Horticultural Research Station of the Leibniz University in Ruthe, Germany (lat. 52°14′N, long. 9°49′E). Most cultivars were grafted on 'Gisela 5' rootstocks (*Prunus cerasus* L. × *Prunus canescens* Bois). The only exceptions were 'Burlat' and 'Summit' used in the perfusion experiments. These two cultivars were grafted on 'Mazzard' (*P. avium*). Fruit of uniform size and color without visible defects were selected at commercial maturity from a minimum of three trees per cultivar. Unless specified otherwise, the pedicels were cut flush with the receptacle and the pedicel end, the receptacle and the pedicel/fruit junction were sealed with silicone rubber (3140 RTV coating; Dow Corning, Midland, MI). The silicone rubber was allowed to cure overnight. This procedure limited fruit water uptake to the skin surface only. Preliminary experiments established that holding fruit overnight at 2 °C had no effect on water uptake and cracking (A. Winkler, unpublished data).

Intrinsic cracking test. Unless otherwise specified, the intrinsic cracking susceptibility was determined as described by Weichert et al. (2004) and Winkler et al. (2015). Briefly, two groups of 25 fruit each were incubated in deionized water. Fruit were removed from the water at regular intervals and checked for macroscopically visible skin cracks. Cracked fruits were removed and noncracked fruits were reincubated in water. Water uptake was determined gravimetrically in a separate experiment on fruit from the same batch. Fruits were weighed, incubated in deionized water, removed after 45 and 90 min, blotted with tissue paper, reweighed, and reincubated. The rate of water uptake was calculated on an individual fruit basis as the slope of a linear regression fitted through a plot of cumulative fruit mass vs. time. The number of individual fruit replicates was 15. From these data, the time to 50% cracking and the amount of water uptake at 50% cracking were calculated (Winkler et al., 2015).

Perfusion test. In the perfusion assays, a fruit was perfused through a hypodermic needle inserted through a silicone rubber septum that covered the receptacle and sealed the pedicel/fruit junction (for a sketch see Lang and Düring, 1990). The needle was positioned such that the tip was close to the pit on the cheek of the fruit. The custom built perfusor comprised a total of 30 disposable syringes connected via rigid polytetrafluoroethylene tubing to the hypodermic needles inserted in the fruit. The rate of perfusion was 44.9 mg·min^{-1}. Fruit was perfused at ambient temperature up to the point of cracking. At this time, the volume injected into the fruit was read from the syringe. Cracking was assessed by visual inspection. Preliminary experiments established that it was technically impossible to perfuse sweet cherry fruit through the vascular system of the pedicel, probably because of the high hydraulic resistance of the vasculature within the fruit (Brüggenwirth and Knoche, 2015). For data analysis, the cumulative frequency of the number of fruit cracked was plotted against cumulative water uptake. This relationship follows a sigmoidal pattern. A logistic regression model was fitted and the WU_{50} calculated as the x coordinate at the point of inflection.

Experiments. Potential relationships between the rate of water uptake and the T_{50} and WU_{50} were established by quantifying cracking and water uptake in 'Adriana', 'Burlat', 'Dönissens Gelbe', 'Earlise', 'Early Korvic', 'Fabiola', 'Flamengo Srim', 'Gill Peck', 'Hedelfinger', 'Kordia', 'Merchant', 'Querfurther Königskirsche', 'Rainier', 'Regina', 'Sam', 'Samba', 'Schneiders Späte Knorpel', 'Staccato', and 'Sweetheart' sweet cherry.

Cracking following immersion in deionized water and following perfusion through the receptacle was studied in 'Burlat'. In the immersion assay, the rate of water uptake and the time course of cracking were determined and the T_{50} and WU_{50} calculated as described earlier. The minimum number of replications for the perfusion test was 36.

The role of microcracks in the cuticle on water uptake and cracking was investigated in 'Summit' sweet cherry fruit. Microcracks were induced by incubating fruit for 24 h in isotonic PEG 6000 solution. Tonicity was established by water vapor pressure osmometry (VAPRO® 5520 and 5600; Wescor, Logan, UT) of juice extracted from fruit of the same batch. Incubating fruit in isotonic PEG 6000 increases the frequency of microcracks in the strained sweet cherry cuticle (Knoche and Peschel, 2006). Thereafter, the time course of cracking following incubation in water, the rate of water uptake, and cracking following perfusion was determined as described earlier. Fruit not incubated in PEG 6000 served as control. The minimum number of replicates for the perfusion test was 39.

The effect of fruit surface wetness during perfusion was determined in 'Summit'. Fruit was perfused in air and also in a PEG 6000 solution that was isotonic to the fruit's own juice. The minimum number of replicates was 36. For comparison, the WU_{50} was determined on fruit from the same batch following incubation.

The effect of water uptake along the pedicel/fruit junction was studied in 'Samba', 'Sam', 'Burlat', and 'Regina'. The pedicel was cut to a length of 5 mm and left open or sealed using a fast-curing silicone (SE 9186, Dow Corning, Midland, MI).

The silicone was allowed to cure for ≈1 h. Time courses of cracking and water uptake were established and the T_{50} and WU_{50} calculated.

The effect of simulated skin defects on water uptake and cracking was studied in 'Early Korvic'. Skin defects were simulated by manually puncturing the fruit skin in the cheek region using a double-edged blade. Depth and width of the cuts were 0.8 × 0.8, 2.1 × 1.7, and 3.6 × 2.6 mm. Fruit having an intact skin served as control. Subsequently, water uptake and cracking were determined and the T_{50} and WU_{50} calculated as described earlier.

The effect of a nonintact cuticle on water uptake and cracking was investigated in 'Kordia'. A circular area, ≈5 mm in diameter, on the cuticle of the cheek or around the stylar scar region was abraded using abrasive paper (K240; Emil Lux, Wermelskirchen, Germany). Unabraded fruit served as control. Time courses of cracking and water uptake were established and the T_{50} and WU_{50} calculated.

The effect of manipulating the fruit water balance by transpiration was investigated in 'Early Korvic'. The pedicel/fruit junction was sealed using a fast-curing silicone (SE 9186). The silicone was allowed to cure for ≈1 h. After curing, fruit were held for 48 h above water in polyethylene boxes [100% relative humidity (RH)] or above saturated slurries of NaCl [75% RH (Wexler, 1995)] or $CaCl_2$ [28% RH (Wexler, 1995)]. The amounts of water transpired were determined gravimetrically. Thereafter, the time course of cracking and water uptake were quantified and the T_{50} and WU_{50} calculated.

The effect of partial wetting of the fruit surface was quantified in 'Sam'. Fruit was incubated with their stylar ends immersed in deionized water in multiwell plates (well diameter 24 mm), whereas the skin in the equatorial plane and the stem end were exposed to air of different RHs by placing the multiwell plates in polyethylene boxes above dry silica gel (0% RH), saturated slurries of $CaCl_2$ [28% RH (Wexler, 1995)] or NaCl [75% RH (Wexler, 1995)], or above pure water (100% RH). The net change in mass was quantified gravimetrically. Cracking was determined by regular visual inspection and the T_{50} and WU_{50} calculated. The number of replicates was 10.

Data analysis. Data are presented as means ± SE. Where error bars are not visible in a graph, they are smaller than the plotting symbols or data for individual fruit are shown (Fig. 2B). Data on rates of water uptake were log transformed before analysis of variance (ANOVA). Data were examined using Student's *t* test, ANOVA, or regression analysis. Pairwise comparisons of treatment means with the nontreated controls were carried out using Dunnett's test or Tukey's Studentised range test at $P \leq 0.05$ (package multcomp 1.3-1, procedure glht, R version 3.0.2; R Foundation for Statistical Computing, Vienna, Austria). Regression analysis was conducted using R version 3.0.2 and SigmaPlot (version 12.5; Systat Software, San Jose, CA). The significances of coefficients of correlation (r) and of determination (r^2) at $P \leq 0.05$, 0.01, and 0.001 are indicated by *, **, and ***, respectively.

Results

Incubating sweet cherry fruit in water resulted in rapid fruit cracking. The time to 50% cracking (T_{50}) averaged 7.5 ± 1.3 h for 19 different cultivars with considerable variability between cultivars (range 1.5 to 18.6 h T_{50}). By 48 h of incubation, most fruit had cracked (Fig. 1A). Expressing cracking as a function of water uptake slightly reduced the variability ($r^2 = 0.74$*** vs. $r^2 = 0.68$***) for the relationships with water uptake and time (Fig. 1A and 1B). There was no genotype that stood out as

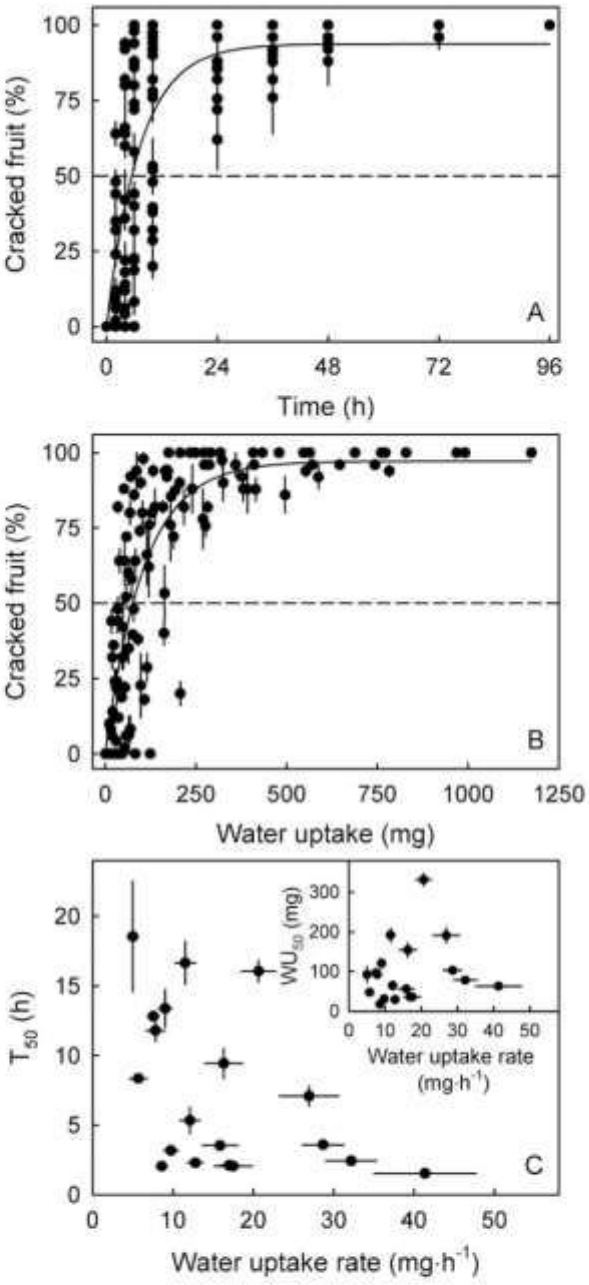

Fig. 1. (**A**) Time course of cracking of sweet cherry fruit. Data points represent fruit from 19 cultivars. The regression equation was: cracked fruit (%) = −29173.3 + [29267.1/(1 + exp{−[time (h) −40.9)/7.1]})], $r^2 = 0.68$***. (**B**) Cracking of sweet cherry fruit expressed as a function of water uptake. The regression equation was: cracked fruit (%) = −21685.4 + [21782.6/(1 + exp{−[uptake (mg)+572.3]/105.7})], $r^2 = 0.74$***. (**C**) Relationship between the time to 50% cracking [T_{50} (main graph)] or the amount of water taken up at 50% cracking [WU_{50} (inset)], and the rate of water uptake. Data symbols represent individual sweet cherry cultivars.

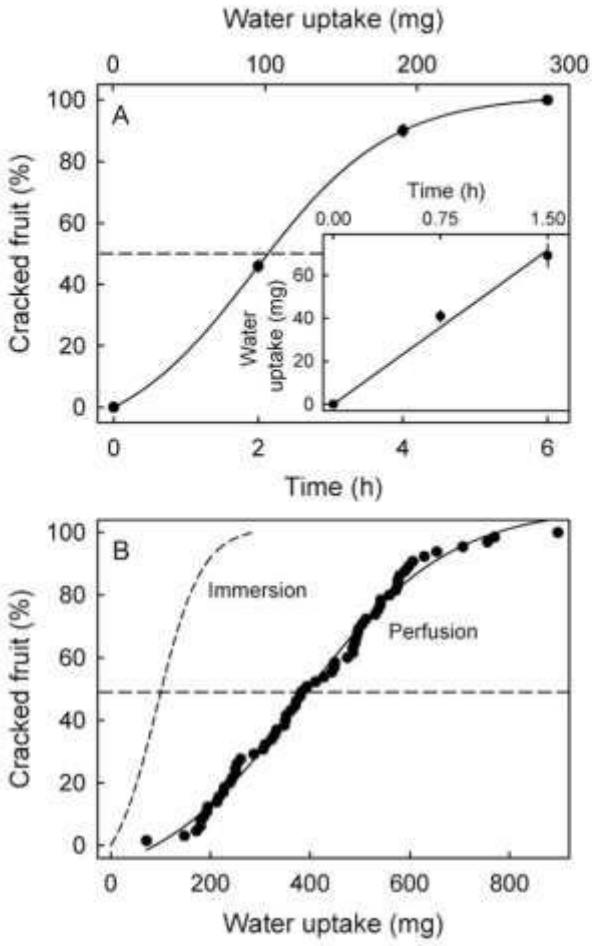

Fig. 2. (**A**) Time course of water uptake (inset) and cracking when incubating sweet cherry fruit in deionized water (main graph). (**B**) Cracking as a function of the amount of water perfused into fruit of the same batch. The dashed horizontal lines indicate 50% cracking. The *x* coordinates of the intercept between the sigmoid regression line and the 50% line represent the time to 50% cracking [T_{50} (**A**)] or the water uptake at 50% cracking [WU_{50} (**A** and **B**)]. Dashed line in **B** represents the sigmoidal regression line from **A** redrawn on the *x* axis scale of **B**.

having fruit exhibiting a low percentage of cracking despite a long incubation and/or a high water uptake rate. Across cultivars, the amount of water taken up at 50% cracking (WU_{50}) averaged 96.5 ± 17.6 mg (range from 17.7 to 331.5 mg among cultivars). When expressed as the percentage increase in mass the WU_{50} averaged 1.0% ± 0.2% (range from 0.1% to 4.1%). There was no correlation between either the T_{50}, the WU_{50}, or the percentage increase in mass and the rate of water uptake (Fig. 1C). Also, there was no significant relationship between the T_{50} (r = 0.58) and only a weak relationship between the WU_{50} (r = 0.80*) for 8 of the 19 cultivars that were tested in two consecutive seasons (data not shown).

For an individual genotype, cracking often followed a sigmoidal pattern with time and (at a constant rate of uptake) also with the amount of water taken up (Fig. 2A). For 'Burlat' the T_{50} and WU_{50} were 2.1 ± 0.1 h and 102.6 ± 4.0 mg, respectively. After 6 h and 287 mg of water uptake all fruit had cracked (Fig. 2A). When fruit from the same batch was perfused with water, the percentage of cracked fruit also increased in a sigmoidal pattern with water uptake. The WU_{50} on perfusion, however, was 397.6 mg and thus, was 3.9 times higher than in the immersion assay (Fig. 2B).

Pretreating fruit by incubation in isotonic PEG 6000 for 24 h resulted in an increased rate of water uptake during subsequent incubation in water as compared with fruit without such pretreatment (Table 1). The T_{50} was lower for fruit incubated in PEG 6000, but the WU_{50} remained constant. However, perfusing fruit from the same batch yielded a WU_{50} ≈38 times higher than in the incubation assays. There was only a marginal effect on the WU_{50} in perfusion when fruit were pretreated by incubation in isotonic PEG 6000 (Table 1).

The difference between the WU_{50} determined in the incubation and perfusion assays was not related to the presence of water on the surface (Table 2). When the fruit surface was wetted during perfusion, the WU_{50} was almost identical to that when dry fruit were perfused (Table 2). Again, the WU_{50} was markedly higher in perfusion than in immersion assays.

Water uptake differed markedly between cultivars (Table 3). Sealing the pedicel/fruit junction consistently decreased the rate of water uptake in all cultivars. Sealing had no effect on the T_{50}, but decreased the WU_{50}.

Simulated skin defects increased the rate of water uptake compared with the intact control. The increase was positively related to the defect size; i.e., depth and width of the cut. The

Table 1. Effect of water uptake on cracking of sweet cherry fruit. Water uptake occurred via the surface during immersion of whole fruit or by perfusion through a hypodermic needle inserted into the fruit. To assess the role of microcracks, fruits were preincubated for 24 h in isotonic polyethylene glycol (PEG) 6000 solution before immersion. Fruit without preincubation served as control. Cracking was indexed by the time to 50% cracking (T_{50}) and the amount of water taken up for 50% fruit to crack (WU_{50}).

		Water uptake rate	Cracking		
Treatment	Pretreatment	[mean ± SE (mg·h^{-1})]	T_{50} [mean ± SE (h)]	WU_{50} [mean ± SE (mg)]	WU_{50} (% of immersion control)
Immersion	Control	3.5 ± 0.4[z]	13.9 ± 0.1	48.8 ± 0.4	100
	24 h PEG 6000	5.1 ± 0.5*	8.7 ± 1.2	44.7 ± 6.0	94
	$Mean_{Immersion}$	4.3 ± 0.3	11.1 ± 1.5	46.7 ± 2.7	
Perfusion	Control		0.7[y]	1,840.4	3,772
	24 h PEG 6000		0.6[y]	1,706.5	3,497
	$Mean_{Perfusion}$		0.7[y]	1,773.5	

[z]Significance of difference of water uptake rate compared with the control indicated by *, Student's t test at $P \leq 0.05$.
[y]Calculated by dividing the WU_{50} by the rate of perfusion of 44.9 mg·min^{-1}.

Table 2. Effect of surface wetness during perfusion on cracking of 'Summit' sweet cherry. Water uptake occurred by perfusion through a hypodermic needle inserted into the fruit. To assess the effect of surface wetness during perfusion, fruit was held with a dry surface in the ambient atmosphere or incubated in isotonic polyethylene glycol (PEG) 6000 solution. Cracking was indexed by the time to 50% cracking (T_{50}) and by the amount of water taken up for 50% of the fruit to crack (WU_{50}). Fruit incubated in deionized water in a classical immersion assay served as control.

Treatment	Incubation	Water uptake rate [mean ± SE (mg·h^{-1})]	Cracking		
			T_{50} [mean ± SE (h)]	WU_{50} [mean ± SE (mg)]	WU_{50} (% of immersion control)
Immersion	Water	9.8 ± 1.8	7.0 ± 1.7	68.2 ± 16.2	100
Perfusion	Air		0.6[z]	1,576.1	2,310
	PEG 6000		0.5[z]	1,299.9	1,601
	$Mean_{Perfusion}$		0.5[z]	1,438.0	

[z]Calculated by dividing the WU_{50} by the rate of perfusion of 44.9 mg·min^{-1}.

Table 3. Effect of sealing the pedicel/fruit junction on the rate of water uptake and on cracking of sweet cherry fruit. Sealing the pedicel/fruit junction restricted water uptake to the fruit surface. Cracking was indexed by the time to 50% cracking (T_{50}) and by the amount of water uptake at 50% fruit cracking (WU_{50}).

Cultivar	Pedicel/fruit junction	Water uptake rate [mean ± SE (mg·h^{-1})]	Cracking	
			T_{50} [mean ± SE (h)]	WU_{50} [mean ± SE (mg)]
Burlat	Open	53.7 ± 5.8[z]	6.3 ± 0.3	337.8 ± 17.2
	Sealed	10.5 ± 1.1*	6.9 ± 1.2	72.7 ± 12.7
Regina	Open	15.3 ± 0.7	18.4 ± 3.0	281.3 ± 46.2
	Sealed	7.8 ± 0.5*	21.3 ± 6.6	165.3 ± 50.9
Sam	Open	13.0 ± 1.3	1.7 ± 0.5	22.7 ± 6.6
	Sealed	5.2 ± 0.8*	1.3 ± 0.1	6.6 ± 0.5
Samba	Open	20.4 ± 3.9	3.4 ± 0.1	69.6 ± 1.7
	Sealed	7.8 ± 0.5*	2.7 ± 0.2	20.9 ± 1.7
Grand mean	Open	25.9 ± 2.8	7.5 ± 2.5	177.8 ± 51.6
	Sealed	7.3 ± 0.4*	8.0 ± 3.3	66.4 ± 25.5

[z]Significance of difference of water uptake rate compared with the open control indicated by *, Student's t test at $P \leq 0.05$.

Table 4. Effect of simulated skin defects on the rate of water uptake and cracking of sweet cherry fruit. Skin defects were simulated by puncturing the skin in the cheek region using a double-edged blade. Cracking was indexed by the time to 50% cracking (T_{50}) or by the amount of water taken up at 50% fruit cracking (WU_{50}).

Depth × width of puncture (mm)	Water uptake rate [mean ± SE (mg·h^{-1})]	Cracking	
		T_{50} [mean ± SE (h)]	WU_{50} [mean ± SE (mg)]
Control	8.0 ± 1.2[z]	1.8 ± 0.1	14.4 ± 0.6
0.8 × 0.8	12.4 ± 1.9*	1.6 ± 0.1	20.4 ± 0.8
2.1 × 1.7	13.1 ± 1.4*	1.7 ± 0.1	22.0 ± 1.8
3.6 × 2.6	14.0 ± 1.6*	2.3 ± 0.6	32.3 ± 8.8

[z]Significance of difference of water uptake rates compared with the control indicated by *, Dunnett test at $P \leq 0.05$.

skin defects had only a small effect on the T_{50}, but increased the WU_{50} relative to the control (Table 4). Furthermore, in only two out of 150 fruit was the site of cracking related to the site of the defect; i.e., the crack only rarely passed through or even close to the defect. In all other cases, the crack location was unrelated to that of the defect. In general, the crack would appear close to the stylar scar (data not shown).

Abrading the cuticle increased the rate of water uptake as compared with the control. It made little difference whether the cuticle was abraded on the cheek or in the stylar scar region (Fig. 3A). Abrading the cuticle on the cheek had little effect on the time course of cracking, compared with the control (T_{50}: 1.2 ± 0.1 h vs. 1.5 ± 0.3 h), but increased the water uptake, compared with the control (WU_{50}: 50.4 ± 6.5 mg vs. 17.6 ± 3.2 mg). Performing the same treatment in the stylar scar region resulted in a 3-fold increase in the T_{50} (3.6 ± 0.0 h) and a nearly 10-fold increase of the WU_{50} (154.8 ± 1.3 mg). Also, the site of cracking changed from cracking primarily in the stylar scar region (control) to cracking in the transition zone between abraded and nonabraded skin, in the shoulder region, or in the cheek region when the stylar scar was abraded.

Manipulating the fruit's water balance by allowing fruit to transpire at different RHs resulted in mass loss of up to 646.7 ± 26.2 mg per fruit (Table 5). When fruits were subsequently incubated in deionized water, the T_{50} and WU_{50} increased as the water loss due to transpiration increased. Interestingly, the WU_{50} remained markedly lower than the amount of water previously transpired (Table 5).

Immersing the stylar end of the fruit in water, while exposing the remaining surface to air of 0%, 28%, or 75% RH resulted in a net loss of water (Table 6). At 100% RH, the mass of the fruit remained nearly constant. Despite a net loss of water, fruit cracked at the stylar end in all treatments. The T_{50} was highest after incubation above dry silica gel (0% RH) and decreased markedly when RH was increased to 28% or higher. The negative WU_{50} values at 28% RH and higher indicate fruit cracked despite a net loss of water (Table 6).

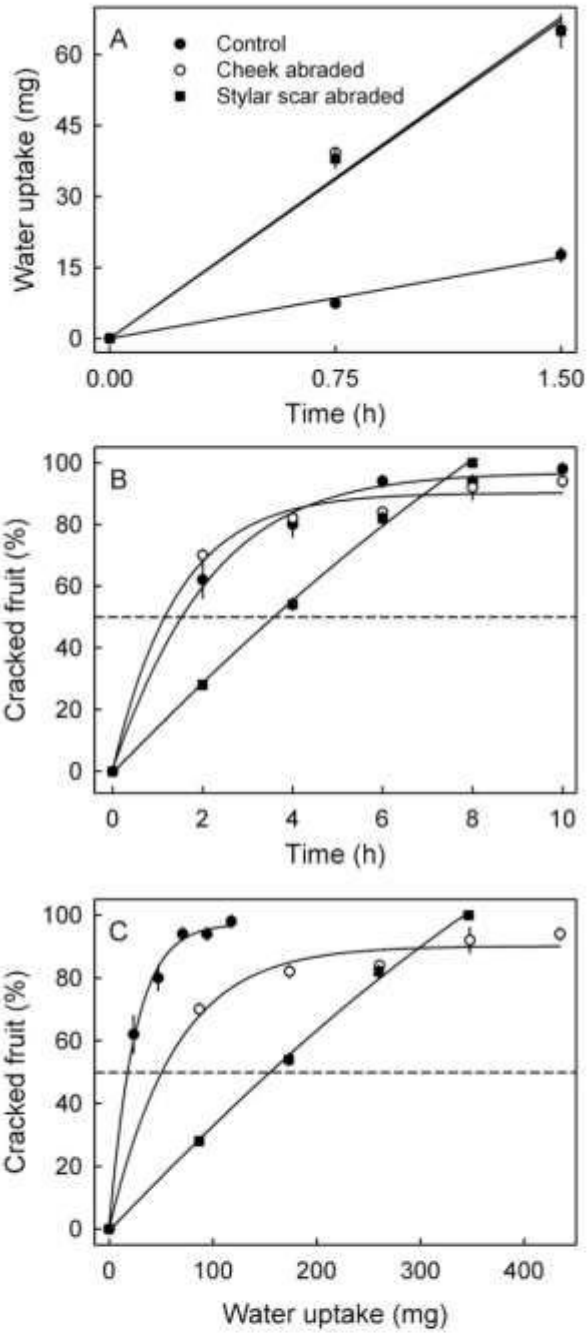

Fig. 3. Effect of abrading the cuticle in the cheek or stylar scar regions on the time course of (**A**) water uptake and (**B**) cracking of sweet cherry fruit incubated in deionized water. (**C**) Percentage of cracked fruit as a function of water uptake. Dashed horizontal line indicates 50% cracking (**B** and **C**).

Discussion

The results demonstrate that 1) cracking is not a simple function of the amount or of the rate of water uptake and, consequently, 2) the simplistic conceptual model of a sweet cherry being a balloon filled with sugary water held under pressure by a taut skin must be revised.

These conclusions are based on the following arguments. First, the amount of water needed for 50% cracking (WU_{50}) is 3.9- to 38-times larger in the perfusion, compared with in the immersion assays. This difference is not due to the formation of microcracks in the cuticle, nor to the presence of water on the surface during the perfusion period (Tables 1 and 2). Second, although significant water uptake occurred along the pedicel/fruit junction in this and also in our earlier study [Table 3 (Beyer et al., 2002; Weichert et al., 2004)], this uptake had no effect on the T_{50}. Apparently, the fraction of water taken up along the junction does not contribute to cracking. Thus, the increase in the WU_{50} must have been an artefact arising from there being a proportion of the water taken up into the fruit that does not contribute to cracking. Third, increasing water uptake by puncturing the fruit surface increased water uptake, but had a surprisingly small effect on the T_{50} and the WU_{50} (Table 4). Only in two out of 150 fruit was the site of cracking related to the site of the puncture on the cheek. Similarly, abrading the stylar scar area markedly increased the rate of water uptake, but also the WU_{50} (Fig. 3). Fourth, water loss due to transpiration did not result in a matching increase in the WU_{50} (Table 5). In fact, the amount of water lost, exceeded the amount that was subsequently required to cause cracking up to 5-fold. This behavior is quite counterintuitive with the critical turgor hypothesis in mind. Fifth, fruit cracking occurred due to local exposure of the fruit surface to water in this, and also in our earlier study, even when the net water uptake rate is negative [Table 6 (Knoche and Peschel, 2006)]. These data suggest cracking is not a simple function of the amount of water taken up, as would be expected based on the critical turgor model.

The above conclusion is also consistent with a number of puzzling findings reported in the literature. First, the low coefficients of correlation or the lack of correlation between cracking susceptibility and determinants of water uptake such as the fruit size over a range of cultivars (Christensen, 1975; Tucker, 1934; Yamaguchi et al., 2002), the driving force as indexed by the ψ_S of the fruit juice (Christensen, 1972; Moing et al., 2004; Tucker, 1934; Verner and Blodgett, 1931), or the permeance of the fruit surface (Peschel and Knoche, 2012) is in line with the above. Second, the cracking susceptibilities of the same genotypes but assessed in different countries or in different years are often poorly or not significantly correlated [data from Christensen (1996) reanalyzed (M. Knoche, unpublished data)]. Third, water uptake up to the onset of fruit cracking had no detectable effect on turgor (Knoche et al., 2014). Conversely, transpiration had only a small effect on turgor (Knoche et al., 2014). Fourth, water uptake had no effect on fracture strains of the skin in biaxial tensile tests, whereas—based on the critical turgor model—fracture strains would be expected to decrease (Brüggenwirth and Knoche, 2016a). Fifth, there is a more than 20-fold difference between the critical turgor values reported in the literature [range 2.0 to 3.8 MPa (Measham et al., 2009)] and the turgors obtained by direct measurement at maturity [< 100 kPa (Knoche et al., 2014; Schumann et al., 2014)]. Sixth, biaxial tensile tests of the fruit skin yield fracture strains in the range from 11% to 38% (Brüggenwirth and Knoche, 2016a, 2016b; Brüggenwirth et al., 2014). These are markedly higher than those calculated from incubation assays where fruit is immersed in water. Interestingly, the WU_{50} in the perfusion assay in the present study was

Table 5. Effect of transpiration on subsequent cracking of sweet cherry fruit when immersed in deionized water. For transpiration, fruit were in air above saturated slurries of $CaCl_2$ [28% relative humidity (RH) (Wexler, 1995)] or NaCl [75% RH (Wexler, 1995)], or above water (≈100% RH) for 48 h at 22 °C. Cracking was indexed by the time to 50% cracking (T_{50}) and by the amount of water taken up at 50% cracking (WU_{50}).

			Cracking	
RH (%)	Transpiration (mg)	Water uptake rate [mean ± SE (mg·h^{-1})]	T_{50} [mean ± SE (h)]	WU_{50} [mean ± SE (mg)]
100	11.4 ± 2.1 c[z]	6.0 ± 0.7 a	5.9 ± 0.2	35.5 ± 1.3
75	317.5 ± 18.7 b	5.9 ± 0.8 a	17.9 ± 1.6	105.7 ± 9.3
28	646.7 ± 26.2 a	5.6 ± 0.8 a	23.5 ± 3.9	130.3 ± 21.9

[z]Mean separation within columns by Tukey's Studentised range test at $P \leq 0.05$.

Table 6. Effect on cracking of partially wetting the surface of sweet cherry fruit. Fruit were positioned such that their stylar ends were in contact with water whereas the remaining surfaces were in air above dry silica gel [≈0% relative humidity (RH)] or saturated slurries of $CaCl_2$ [28% RH (Wexler, 1995)] or NaCl [75% RH (Wexler, 1995)], or above water (100% RH) at 22 °C. Cracking was indexed by the time required for 50% fruit to crack (T_{50}) or by the amount of water uptake at 50% fruit cracking (WU_{50}). A negative rate of water uptake implies a net water loss.

	Water uptake rate	Cracking	
RH (%)	[mean ± SE (mg·h^{-1})]	T_{50} (h)	WU_{50} (mg)
0	−5.9 ± 0.7 b[z]	24.6	−144.9
28	−5.3 ± 1.0 b	2.3	−12.1
75	−1.2 ± 0.2 a	2.1	−2.6
100	0.7 ± 0.3 a	2.1	1.5

[z]Mean separation by Tukey's Studentised range test at $P \leq 0.05$.

up to 38-fold higher than that in the immersion assay. Calculating the strain at fracture for an average 9.6 ± 0.1 g fruit yields a fracture strain of 9.1% ± 1.8% for the perfusion assay, but of only 0.5% ± 0.1% for the immersion assay. Thus, comparing the skin area increases in the elastometer test and in the perfusion test with those in the immersion test, those in the elastometer and perfusion tests were roughly similar to one another but were more than an order of magnitude larger than those in the immersion test (Brüggenwirth and Knoche, 2016c). Part of this wide discrepancy may be attributable to the higher strain rates occurring in the elastometer and perfusion tests, than in the immersion test (Brüggenwirth and Knoche, 2016c). However, a second factor may be the absence of cell wall swelling when cracking is the result of the mechanical straining of an excised skin or the perfusion of an intact fruit (M. Brüggenwirth, unpublished data). Importantly, if the fruit behaved as predicted by the critical turgor model, we would not expect major differences between fracture strains and fracture pressures determined in biaxial tensile tests, the WU_{50} measured in perfusion assays and those determined in classical incubation assays. But this is clearly not the case. Thus, we must seek an alternate explanation for rain cracking that accounts for the localized nature of the cracking event.

Unzipping the skin—the zipper model. In the following section, we propose an alternate explanation to account for the localized nature of rain cracking in sweet cherry fruit (and perhaps in some other rain cracking-susceptible species too). We will refer to this model as the zipper model. First, rain cracking is initiated by localized water uptake that occurs through microcracks in the cuticle. Microcracks focus water uptake to a particular region of the epidermis. Second, water is taken up osmotically into the symplast of skin cells and—even more so—that of flesh cells which have a more negative ψ_S (Grimm and Knoche, 2015; Simon, 1977). Unlike the collenchyma-type epidermal and hypodermal cells, flesh cells are parenchyma cells that are structurally weak and begin to crack. Third, the bursting of individual flesh cells causes their symplast contents to leak into the apoplast (Herrmann, 2001; Winkler et al., 2015). These contents include malic acid as a major osmolyte. Malic acid is known to disrupt plasma membranes and weaken the cell walls of the adjacent cells causing the bursting to spread (Winkler et al., 2015). Fourth, the continuing loss of osmolytes from symplast to apoplast will cause nearby skin cells to plasmolyse. In turn, plasmolysis is associated with cell wall swelling (Grimm and Knoche, 2015). Also, malic acid is expected to extract Ca ions from the cell wall which would weaken cross-linking between cell wall constituents and increase swelling (Demarty et al., 1984; Glenn and Poovaiah, 1989; Hepler, 2005). Swelling of cell walls markedly decreases the modulus of elasticity and the fracture pressure (M. Brüggenwirth, unpublished data). In consequence, resistance to strain diminishes and the water uptake continues. The tangential propagation of these processes causes the skin to "unzip", so a cuticular microcrack soon extends into a skin macrocrack in much the same way as a "ladder" will propagate in a piece of fine, knitted fabric. Cuticle and skin are strained (Grimm et al., 2012; Knoche et al., 2004). Upon cracking they will contract causing the crack to gape and to become visible macroscopically.

The zipper model is consistent with the higher frequency of cracking in stem cavity and stylar scar region as compared with the cheek or suture region (Glenn and Poovaiah, 1989; Measham et al., 2009; Sekse, 1998b; Verner and Blodgett, 1931). These regions have a higher frequency of microcracks (Peschel and Knoche, 2005). Microcracks result from a mismatch between surface area expansion and cuticular deposition (Knoche et al., 2004), the small radii of curvature of the fruit surface in these regions (Considine and Brown, 1981), extended periods of surface wetness (Knoche and Peschel, 2006), and—possibly—the mechanical stiffness transition between stylar scar and pedicel vs. skin (A. Lang, personal communication).

The zipper model is also consistent with the well-known engineering phenomenon of cracks focusing stresses at the ends of the crack thereby stimulating crack extension in tangential direction. Extension in radial direction; i.e., toward the pit, would occur by the same mechanism, a stress concentration on the cell walls of epidermal and hypodermal cells that causes the cells to separate and the microcrack to become visible as a macrocrack. Due its low modulus of elasticity (Brüggenwirth et al., 2014), the flesh offers little structural resistance to cracking.

Clearly, some of the links in the causal chain sequence of the zipper model outlined above are more hypothetical than others.

However, there is sound experimental evidence for most of the links making the zipper model both an attractive and also a logically plausible explanation for the random, unpredictable and local nature of sweet cherry fruit cracking. The zipper model also explains why cracking may still occur despite a fruit suffering an overall net loss of water. This and other recorded behaviors and measured properties do not find ready explanation in the critical turgor hypothesis.

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6. General discussion

New and important results of this work are:

1) Water uptake into the sweet cherry fruit is a complex phenomenon and the sweet cherry fruit is not a simple osmometer. There is evidence for the concurrent transport of osmolytes that occurs in gravimetrically detectable amounts. This transport may cause artefacts when determining water potentials by incubating fruit in osmolyte solutions. These curves are displaced or change in slope yielding different apparent water potentials due to the inflow or outflow of osmolytes that accompanies water flow (chapter 2).
2) Xylem flow through the pedicel of detached fruit occurs when the fruit is allowed to transpire, but also when transpiration is completely inhibited. The xylem flow of sweet cherry fruit is at maximum at the transition stage II/III and decreases towards maturity. Flow into transpiring fruit exceeds that into non-transpiring fruit. This difference, however, decreases from early stage III on to maturity. Xylem flow cannot be stopped by 'feeding' hypertonic sucrose solutions of osmotic potential down to -10 MPa. An effect of xylem flow on cracking is unlikely (chapter 3 (Winkler et al., 2016a)).
3) Malic acid, a major osmolyte of the sweet cherry symplast, markedly increases cracking when brought into contact with an intact fruit. The effect of malic acid on cracking is a simple function of the pH of the incubation solution and can be reproduced with other acids. Malic acid weakens cell walls and increases permeability of membranes as indicated by the leakage of anthocyanins even in hypertonic incubation solutions (chapter 4 (Winkler et al., 2015)).
4) Cracking of sweet cherry fruit is a local phenomenon that is not accounted for by the "critical turgor pressure model" of Considine and Kriedemann (1972) that at present is almost universally used to explain cracking. Hence, a revised model is needed that accounts for cracking also when local exposure leads to cracking. This new model is proposed here and referred to as the zipper model (chapter 5 (Winkler et al., 2016b)).

These findings are discussed in detail together with the relevant literature in the respective chapter. The general discussion here will now concentrate on (1) some puzzling aspects relating to the mechanism of water uptake via the skin and the xylem of the pedicel and (2) an alternative view of the mechanism of cracking.

6.1 Mechanisms of water uptake via the skin and the xylem of the pedicel of sweet cherry fruit

The mechanisms and pathways of water uptake via the skin have been examined in many publications (Christensen, 1972a; Beyer and Knoche, 2002; Beyer et al., 2002a,b,2005; Weichert et al., 2004; Weichert and Knoche, 2006a,b). The results about the mechanism of water uptake in the literature are described in chapter 1.6. However, there are several findings that have been difficult to explain till now when plausible explanations can be offered using the results from this dissertation. These findings include (i) the “water uptake” of fruit that is incubated in its own juice and (ii) the unrealistic high (less negative) “water potential” (and thus an extremely high turgor) identified in a concentration series of PEG 6000 solutions.

This work demonstrates for the first time the outflow of osmolytes when incubating sweet cherry fruit in water. It is likely this outflow will also occur when fruit is incubated in solutions of osmolytes that do not occur in sweet cherry fruit. Thus, the gravimetric determination of water uptake yields data that reflects the net change in mass due to water uptake and osmolyte inflow and outflow. As pointed out in chapter 2, the error in assuming the mass changes observed were due to water uptake alone is about 14%. This error is small and for most practical purposes acceptable, considering the ease of the procedure relative to the effort and instrumentation required to use radioactive or stable isotopic tracers.

However, some points remain to be clarified in further research:

1) The permeances of the fruit skin for the typical osmolyte constituents of sweet cherry juice are unknown. Knowing just those for glucose, fructose, and sorbitol would provide sufficient information on which to base corrections accounting for osmolytes outflow- and/or inflow-rates with roughly 80% accuracy, since these comprise about 80% of the fruit’s osmolytes. Permeances can be determined using ^{14}C labeled sugars and the infinite dose system (Bukovac and Petracek, 1993; Weichert and Knoche, 2006).

2) At this stage, there are two possible explanations for the consistent observation of mass uptake from its own juice: First, the presence of an active transport mechanism for osmolytes into the symplast. This hypothesis may be tested using uptake experiments of ^{14}C labelled osmolytes applied with and without respiratory inhibitors, e.g. sodium azide and sugar transport inhibitors, i.e., p-chloromercuriphenylsulphonic

acid or phloretin. Second, the presence of swollen cells walls that represent a significant boundary layer for diffusion processes (unpublished data). Experiments investigating plasmolysis of the skin upon exposure to juice from the same fruit revealed considerable variability indicative for heterogeneity of the cells osmotic water potentials (Grimm and Knoche, 2015). When incubating fruit in its own juice, cells with higher (less negative) osmotic potential plasmolyze, causing their cell walls to swell. As a consequence, a significant boundary layer is formed that increases diffusive resistance and hence, decreased water transport (uptake and loss). In contrast, cells having a more negative (lower) osmotic potential will remain turgescent with non-swollen cell walls and hence, no or little change in boundary layer resistance. This may result in a net uptake of water under seemingly isotonic conditions. This hypothesis may be tested using tritiated water (^3H_2O) fed to skin segments mounted in infinite dose diffusion cells. Swelling of cell walls may be varied by inducing plasmolysis.

3) Flow and tension when feeding hypertonic sucrose solutions (up to osmotic potentials down to about -10 MPa) via the pedicel have been observed (chapter 3 (Winkler et al., 2016a)). The driving force responsible for this effect is unknown. Several hypothetical explanations may be offered: First, cell wall swelling may be involved. Incubating tissue in hypertonic solutions results in plasmolysis of cells and – as a consequence – in swelling of cell walls. If the water partitioning into the cell wall generated a tension in the apoplast, this could result in water uptake beyond that expected from the osmolarity of the juice. Second, a chemical gradient between the feeding solution and the apoplast may cause diffusion of osmolytes into the apoplast. Any active component that loaded these osmolytes into the symplast would generate an osmotic gradient at the ends of the xylem strands for the subsequent inflow of water. This could account for the apparent flow and water uptake from hypertonic solutions.

Whether one of these theories accounts for this tension and flow, is unknown and further studies are required to evaluate these hypotheses.

6.2 Mechanism of cracking of sweet cherry fruit – the zipper model

The results of this work demonstrate that cracking of sweet cherry fruit is not a simple function of water uptake as postulated in the “critical turgor pressure model”. It is more complex than implied by the analogy of the bursting of an over-inflated balloon.

In chapter 5 (Winkler et al., 2016b) a detailed list of arguments based on own data and those in the literature is provided for why the “critical turgor pressure model” cannot be applied to sweet cherry fruit. Also, an alternative hypothesis – the zipper model – was presented that is consistent my own data and also that in the literature. In the following section a more detailed account of the cracking process is given.

Sequence of cracking in detail

1) Cracking is initiated by localized water uptake through microcracks in the cuticle. These microcracks are primarily located in the pedicel cavity and at the stylar end of the fruit (Peschel and Knoche, 2005). These regions of the fruit surface are characterized by suffering the longest durations of wetness after rainfall. This is, after rainfall, a puddle remains in the pedicel cavity and a hanging droplet attaches to the stylar scar region. We know the formation of microcracks is triggered by surface wetness (Knoche and Peschel, 2006). Microcracks impair the barrier function of the cuticle. Water uptake is thus focused through the microcracks onto the few epidermal cells immediately underlying the crack. Water is taken up osmotically into the symplast of these skin cells. Due to a more negative osmotic potential of flesh cells compared to epidermal and hypodermal cells (Grimm and Knoche, 2015), water would penetrate into the symplast of the flesh. In contrast with the epidermal and hypodermal cells, flesh cells are large and thin-walled and consequently, structurally weak and prone to bursting. As a consequence, water uptake leads to the bursting of individual cells as indexed by the leakage of anthocyanins in this (chapter 4 (Winkler et al., 2015)) and earlier studies (Simon, 1977).
2) The bursting of individual cells leads to the leakage of cell content into the apoplast. The five major osmolytes of the sweet cherry fruit are glucose, fructose, sorbitol, malic acid, and potassium malate. These account for about 98% of the osmolarity of the fruit (Herrmann, 2001). Malic acid weakens cell walls and increases membrane permeability even at a concentration of 1 mM which is nearly two orders of magnitude lower than that of the expressed juice of the fruit (70 mM; Herrmann 2001). Even very low concentrations (1 mM) show this effect. The effect is not specific for malic acid but can be reproduced by other organic acids at the same pH. While low pH stimulates cracking,

cracking is reduced at high pH (chapter 4; Winkler et al., 2015). The leakage of cell contents from the symplast into the apoplast decreases the osmotic potential of the apoplast and eventually causes cells of the skin to plasmolyze. Plasmolysis is associated with the swelling of cell walls (Grimm and Knoche, 2015). Additionally, malic acid extracts Ca ions from the cell wall. These Ca ions are responsible for cross-linking of cell wall constituents and for inhibiting cell-wall swelling (Demarty et al., 1984; Glenn and Poovaiah, 1989; Hepler, 2005; Schumann, personal communication). Incubating epidermal segments in $CaCl_2$ solutions results in thinner cell walls as compared to with water or malic acid (Brüggenwirth and Knoche, 2016a). Alternatively, incubating fruit in the presence of the chelate EGTA, which has a high affinity for Ca, markedly increases cracking susceptibility (Glenn and Poovaiah, 1989). This suggests an important role for Ca in cell-wall swelling and cracking. Consistent with this theory, and with the stylar scar as a site of preferential cracking, is that a gradient in Ca concentration exists in the fruit that decreases in the direction from the pedicel to the stylar end (unpublished data).

3) Swollen cell walls are mechanically weaker than non-swollen cell walls. The fracture pressure and the modulus of elasticity are negatively correlated with the extent of swelling as indexed by the thickness of the anticlinal cell walls (Brüggenwirth and Knoche, 2016a). As a consequence, stress increases, while the resistance to strain decreases. If these processes continue, the main structural elements of the fruit skin, the epidermal and hypodermal cells, (Brüggenwirth et al., 2014) cannot withstand the strain (Grimm et al., 2012; Knoche et al., 2004) and the skin begins to “unzip”. Thereafter, the skin will contract, the swollen cell walls will separate, presumably along their middle lamellae allowing more water to penetrate, more cells to burst, and so on. The force of the contracting skin will “unzip” itself to a depth of several layers into the flesh and along a considerable distance (Brüggenwirth and Knoche, 2016a). As a consequence, the crack gapes and so a microcrack develops into a macrocrack.

This zipper hypothesis is consistent with the literature. It provides a logical and plausible explanation for sweet cherry fruit cracking. Microcracking, localized water uptake, and the effect of malic acid on the cell wall mechanics are identified as critical

steps in the process of cracking. What remains to be clarified is the mechanism by which the swollen cell walls separate and what biochemical changes in the cell wall precede swelling. Presumably, separation is along the middle lamellae and pectin solubilization is a plausible candidate for the biochemical change. However, direct experimental evidence for this is still lacking.

From a practical point of view, decreasing the cracking susceptibility of fruit is highly desirable. In principle, two approaches may be considered: 1) by breeding and 2) by cultural practices. In both cases, cell-wall swelling would offer a new and interesting target.

Breeding efforts could focus on manipulating biochemical changes in the cell wall and the pectin middle lamella that precede swelling. There are numerous reports on the molecular and biochemical background to fruit softening (Brummel, 2006). Efficient use of such techniques requires a better understanding of the process of cell-wall swelling.

Among the cultural practices, an increase in Ca content of the cell wall is a promising target. Indirect evidence suggests Ca plays a critical role in the swelling of cell walls (Demarty et al., 1984; Tibbits et al., 1998). Furthermore, Ca has been reported to reduce cracking of sweet cherry fruit (Verner, 1937; Glenn and Poovaiah, 1989). Little is known about the Ca content of fruit in the course of development. Calcium is translocated solely in the xylem (Bukovac and Wittwer, 1957; Clarkson, 1984). Unfortunately, the hydraulic conductance of the xylem of sweet cherry fruit decreases from the beginning of stage III to maturity (chapter 3 (Winkler et al., 2016a); Brüggenwirth et al., 2016). In the mature fruit, the xylem is essentially non-functional. Thus, the Ca import into the fruit does not keep pace with the increase in fruit mass causing the Ca concentration ($mg \cdot g^{-1}$ dry matter) to decrease (unpublished data). At the same time, the swelling of cell walls increases as development proceeds (Redgwell et al., 1997).

The inconsistent response of Ca spray applications at the whole tree level on cracking (chapter 1, Table 1) may be due in part to lack of uptake of Ca into the fruit. Low penetration of Ca through fruit cuticles and skins is not unique for sweet cherry, but has also been documented for apple (Schlegel and Schönherr, 2002).

Further research in the field of cracking should focus on the cell wall. In particular, the following questions should be addressed: Which cell wall component is responsible for swelling? What are the biochemical processes that precede swelling? How can these processes be manipulated in order to reduce cell-wall swelling? Is spray application of Ca salts the only possibility for reducing cell-wall swelling and, if so, how can we maximize Ca import via the vasculature of developing fruit and uptake through the fruit surface following spray application. These subjects merit further study.

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