Review n. 37 – ***Italus Hortus*** **26 (1), 2019:** 59-65 doi: 10.26353/j.itahort/2019.1.5965

The mechanism of rain cracking of sweet cherry fruit

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Nuovi approcci nella difesa delle spaccature dei frutti nel ciliegio

Riassunto. Le spaccature che si formano nelle ciliegie (*Prunus avium* L.) rappresentano un problema molto serio per la produzione mondiale. Si pensa che esse siano causate da un eccessivo assorbimento di acqua e da un successivo aumento del turgore cellulare, tale per cui quando viene superata una soglia critica ('turgore critico') il frutto si spacca. Tuttavia, mancano evidenze sperimentali a supporto di questo concetto ampiamente diffuso mentre i dati pubblicati mettono in dubbio l'ipotesi del "turgore critico" ed una spiegazione alternativa deve ancora essere confermata. Questo articolo riassume le ricerche sperimentali che, negli ultimi due decenni, hanno portato allo sviluppo di un'ipotesi alternativa delle cause dello spacco nel ciliegio: la così detta ipotesi della "cerniera lampo". Secondo quest'ultima, la spaccatura del frutto è il risultato di una serie di eventi che portano alla propagazione di una fessurazione attraverso la buccia e la polpa "aprendo" il frutto. Questa ipotesi si basa sulla seguente sequenza di eventi: nella buccia, ed in particolare nella cuticola, si sviluppa una tensione (stress) durante la fase III di crescita, a causa di una regolazione negativa dei geni coinvolti nella sintesi della cutina e delle cere. Lo stress nella buccia determina tensioni e microfessurazioni nella cuticola. Inoltre, l'umidità della superficie e della cuticola in tensione aggrava le micro-spaccature, le quali a loro volta compromettono le funzioni di barriera della cuticola e concentrano l'assorbimento dell'acqua in una particolare regione della superficie del frutto. L'acqua passa la cuticola, penetra nel frutto e si muove in zone dove il potenziale idrico è più negativo, ossia nelle grandi cellule del parenchima del mesocarpo, dotate di pareti cellulari sottili, che hanno un potenziale osmotico più negativo delle piccole cellule dell'epidermide e dell'ipoderma, aventi una parete cellulare più spessa. L'assorbimento di acqua da parte di queste cellule ne causa la rottura. Di conseguenza, il contenuto cellulare fuoriesce nell'apoplasto. I principali costituenti del ciliegio dolce, come il glucosio, il fruttosio e l'acido malico si riversano nell'apoplasto in concentrazioni comparabili a quelle del simplasto e le conseguenze sono molteplici: i) il turgore cellulare diminuisce ed è interamente perso quando le cellule dell'epidermide sono soggette a plasmolisi; ii) l'acido malico estrae il Calcio legato alle pareti cellulari, le indebolisce ed aumenta la permeabilità delle membrane plasmatiche causando una reazione a catena di perdita di adesione tra cellule adiacenti. Il distacco tra le cellule ed il crollo del (già basso) turgore cellulare porta al rigonfiamento delle pareti cellulari, in particolare delle pectine della lamella mediana. Le pareti cellulari gonfie hanno una rigidità inferiore ed una maggior tensione alle fratture ed adesione cellulare che portano alla separazione delle cellule adiacenti lungo la parete cellulare. La tensione generata dallo sforzo dell'epidermide è ora sufficiente per causare la separazione delle cellule lungo le loro pareti rigonfiate e rompere la buccia. Questo processo continua agli estremi della spaccatura dove si concentra lo stress, causando l'estensione della spaccatura stessa. L'epidermide si rompe nello stesso modo con cui una cerniera o una smagliatura si apre e si propaga in un pezzo di tessuto lavorato a maglia.

Parole chiave: spaccature del frutto, ciliegio, *Prunus avium*, relazioni idriche del frutto.

Introduction

Cracking of sweet cherry fruit is an important limitation in all production areas where rain fall occurs during the ripening and harvesting season. Exposure of sweet cherry to rain compromises fruit quality even before macroscopic fruit cracking is observed (Borve *et al*., 2000; Peschel and Knoche, 2005). A threshold of 25% cracked fruit on a tree is said to render the harvest uneconomical (Looney, 1985). Even lower percentages result in impaired quality and fruit rots.

A frequently listed explanation for cracking is the critical turgor hypothesis originally proposed for grapes, but also used for sweet cherry (Considine and Kriedemann, 1972). Cracking is believed to be caused by water uptake through fruit surface and vasculature. As a consequence, the flesh of the cherry is thought to generate a pressure that subjects the skin to tensional

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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).

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 hypothesis dominated the literature in the last 50 years. It is referred to as the critical turgor hypothesis (Considine and Kriedemann, 1972; Andersen and Richardson, 1982; Measham *et al*., 2009), but may equally be called the critical strain hypothesis. The hypothesis provides an easy to understand explanation and hence, is widely accepted. In fact, it is the explanation provided in students text books.

In the last decade, however, experimental evidence accumulated that questioned the validity of the critical turgor hypothesis and that led to an alternative explanation, the so called Zipper model (Winkler *et al*., 2016). Based on this hypothesis, the skin ruptures in a manner similar like a zipper or ladder separating a piece of fabric.

In this review we summarize experimental evidence that led to the Zipper hypothesis. For comprehensive detailed reviews the reader is referred to Balbontin *et al*. (2013) and Knoche and Winkler (2017).

The need for a new hypothesis - limitations of the critical turgor concept

According to the critical turgor hypothesis, cracking occurs when fruit turgor exceeds a critical threshold as a result of osmotic water uptake. Unfortunately, supporting experimental evidence for this intuitive concept is lacking.

First, turgor in mature sweet cherry is consistently low and not affected by water uptake. In fact, when incubating sweet cherry in deionized water until cracking began there was no detectable increase in turgor (Knoche *et al*., 2014). Second, there is no effect of interrupting the skin on turgor indicating that the sweet cherry is not an inflated balloon where a taut skin holds the flesh under pressure. Third, cracking can be induced experimentally despite a net loss of water. In fact, placing a fruit on a test tube filled with water induced cracking although the entire fruit lost mass via transpiration (Winkler *et al*., 2016). Fourth, the amount of water required to induce cracking differs depending on whether fruit is incubated in water (49 µl for 50% cracking), perfused by injecting water using a syringe (1840 µl; Winkler *et al*., 2016) or when irrigated on a tree by overhead sprinkling (Winkler, unpublished data). Fifth, attempts to calculate the critical turgor from osmotic potentials (down to – 4 MPa; Knoche *et al*., 2014) and assumed predawn water potentials (-0.1 MPa) exceeded the pressure of a car tire (ca. 0.2 MPa) up to 20-fold. A sweet cherry having this pressure should feel like steel. Sixth, excised fruit skins have a much higher strain at fracture in biaxial tensile tests (4.1 to 10.9 %) than the skin on a fruit induced to crack by incubation in deionized water (0.08%; Brüggenwirth and Knoche, 2016a).

These arguments question the critical turgor hypothesis and an alternative hypothesis must be thought of.

Background information

In the following section, key findings from published literature are reviewed that provide essential components to the Zipper hypothesis (Winkler *et al*., 2016).

Skin of sweet cherry fruit

The fruit skin of the sweet cherry drupe is a material composite comprising a polymeric cuticular membrane (CM) and the cellular layers of epidermis and hypodermis.

The CM is a lipophilic polyester consisting of a cutin matrix (0.95 g m^{-2} equiv. to 74.2%), wax (0.33 g m^{-2} equiv. to 25.8%), and polysaccharides. The amount of the latter in the sweet cherry CM is unknown. Compared to fruit of other species, the CM of sweet cherry is very thin (mean of 31 cultivars 1.07 µm, range 0.71-1.32 µm). The CM fulfils important functions (Peschel and Knoche, 2012). It is the primary barrier in water transport (uptake and transpiration), penetration of gas (CO_2, O_2), and in infection with fruit rot pathogens. Due to its limited thickness it plays no role in the mechanical properties of the skin.

The sweet cherry epidermis is formed by a single layer of small collenchyma-type cells with thick cell walls. The length to width ratio of the cells changes from isodiametric to tangentially elongated as the fruit approaches maturity. The sweet cherry fruit surface is stomatous carrying a cultivar specific number of stomata, but there are no trichomes or hairs. Stomata are fixed in a practically open state and non-functional at maturity (Peschel *et al*., 2003).

The hypodermis is formed by several layers of collenchymatous cells that gradually increase in size as distance from the surface increases. Epidermal and

hypodermal cells form the structural backbone of the sweet cherry fruit skin and hence, are responsible for the mechanical properties of the skin (Brüggenwirth *et al*., 2014).

It is important to note that the skin of mature sweet cherries has a markedly less negative osmotic potential as compared to the flesh of the same fruit. In fact, exposing the fruit skin to juice from the same fruit induced plasmolysis (Grimm and Knoche, 2015). Because a significant turgor is absent (Knoche *et al*., 2014), the difference in osmotic potentials represented the difference in water potential. Averaged across different cultivars this difference amounted to 1.1 MPa. At present, the basis of this internal gradient is not understood.

Stress and strain of the cuticle

Sweet cherry growth follows a double-sigmoidal pattern characterized by stages I, II and III typical for stone fruit. During stage I, cell division in the pericarp accounts for a small increase in mass to 1.5 to 2.5 g per fruit. In stage II, mass remains essentially constant, the endocarp lignifies and the embryo develops. Stage III represents the final phase of rapid growth as a result of cell enlargement in the flesh. Generally, cracking susceptibility increases during stage III where fruit growth is most rapid and fruit surface area increases rapidly with peak rates up to 1 cm^2 d^{-1}.

During stage III, considerable strain of the fruit skin develops as indexed by the following observations: (i) cutting into the fruit results in 'gaping' of the cut (Grimm *et al*., 2012); (ii) exocarp segments excised by cutting tangentially underneath the surface rapidly decrease in area (Grimm *et al*., 2012); (iii) the fruit surface develops a mottled appearance due to tensional failure of the hypodermis in a manner analogous to 'stretch marks' in human skin in puberty, obesity and pregnancy (Grimm *et al*., 2013); (iv) microcracks develop in the cuticle. These are orientated perpendicular to the longest dimension of the underlying epidermal cell, suggesting a cause-and-effect relationship (Peschel and Knoche, 2005); and (v) the length-to-width ratio of epidermal and hypodermal cells changes increases and the shape of the cells changes from portrait to landscape between stage II and maturity, which is indicative of strain.

There is also considerable elastic strain in the CM. This increases from close to zero at the end of stage II to 80% at maturity. Recent evidence indicates that the total elastic strain (following wax extraction) may be as high as 150% (Lai *et al*., 2016). At the same stage of development, the elastic strain of the complete skin composite (cuticle, epidermis, hypodermis) is only about 40% (Grimm *et al*., 2012). It is interesting to note that the cuticle on a strained sweet cherry cannot sustain the enormous strain without the support by underlying cellular layers (Peschel and Knoche, 2005).

The enormous strain of the cuticle results from a cessation of cuticle and wax deposition during stage II about 2 to 3 weeks after full bloom. From there on, cutin and wax mass remain constant and fruit growth distributes a constant amount of cuticle over an increasing surface area (Knoche *et al*., 2004; Peschel and Knoche, 2005; Peschel *et al*., 2007). This deposition pattern is common to all 32 cultivars investigated (Peschel and Knoche, 2012). The cessation of cuticle deposition results from a downregulation of genes involved in cutin monomer and wax synthesis (Alkio *et al*., 2012, 2014).

Formation of microcracks

Microscopic cracks are cracks that are limited to the cuticle and do not extend into epidermal and hypodermal cell layers. Microcracks result from the stress and strain of the fruit skin. They are highly oriented perpendicular to the direction of major strain. Cracks occur most frequently in the stylar scar and the stem cavity region in essentially all fruit grown in the open field (Peschel and Knoche, 2005). Formation of microcracks is aggravated by surface wetness or exposure to high humidity (Knoche and Peschel, 2006). Microcracks impair the barrier function of the cuticle thereby allowing rapid water uptake into and transpiration out of the fruit. Microcracks also account for the high susceptibility of sweet cherries to fruit rot pathogens (Borve *et al*., 2000).

Pathways and mechanism of water uptake

Water uptake may occur through the vascular system of fruit and pedicel or through the fruit surface.

Information on vascular flows in sweet cherry was derived using heat pulse techniques (Measham *et al*., 2014) and linear variable displacement transducers that quantify fruit diameter changes on the tree, in the field, non-destructively (Brüggenwirth *et al*. 2016). Manipulating the pedicel using steam girdling or by detaching fruit allows to separate flows through xylem, phloem and the fruit skin (for details see procedure developed by Lang, 1990). The data demonstrate that during stage III development, total inflow via the xylem into the fruit decreased from an initial 85% (equivalent to 11.6 µl h^{-1}) of total sap (12.4 µl h^{-1}) inflow, to essentially zero at maturity (0.6 µl h^{-1} of a total of 11.9 µl h^{-1}). In the same interval, phloem flow increased from 0.8 to 11.3 µl h^{-1} at maturity

(Brüggenwirth *et al*., 2016). Thus, there is no xylem inflow into mature fruit. Magnetic resonance imaging and dye infiltration studies further revealed a sequential loss of xylem functionality beginning in the stylar scar region and progressing towards the stem cavity. The reason for the loss of functionality is physical rupture (Grimm *et al*., 2017).

Water transfer through the fruit surface occurs along a number of parallel pathways. These are the cuticle, the stem/fruit junction and microcracks. There is no evidence for stomata playing a role in water transfer unless a silicone surfactant or hydrostatic pressure is used (Peschel *et al*., 2003; Peschel and Knoche, 2012). Also, the periderm of the stylar scar has no role in water transport (Beyer *et al*., 2002).

The cuticle represents the primary barrier in water transport. Abrading the cuticle increased water uptake 33-fold and transpiration 5-fold (Knoche and Winkler, 2017). Water uptake through the cuticle occurs along a continuum of polar domains in the cutin matrix, which results from the hydration and orientation of polar functional groups. These polar domains are referred to as aqueous pores or polar pathways (Schönherr, 2006; Weichert and Knoche, 2006). They account for the high permeability of the sweet cherry fruit cuticle in osmotic water uptake.

The stem/fruit junction represents a site of preferential water uptake into the fruit (Beyer *et al*., 2002). Uptake along the junction amounted to up to 70% of total uptake of a submerged mature fruit (Beyer *et al*., 2002). Since water collects in the stem cavity during rain, the stem/fruit juncture has a long wetness duration leading to continuing water uptake after rain.

Microcracks impair the barrier function of the cuticle and hence increase the permeability of the fruit skin, particularly to water uptake and, to a lesser extent, to transpiration (Knoche and Peschel, 2006). It has been suggested that microcracks propagate to form macrocracks (Glenn and Poovaiah, 1989).

Mechanical properties of the fruit skin

The mechanical properties were the subject of interest in a number of recently published papers (Brüggenwirth *et al*., 2014; Brüggenwirth and Knoche, 2016a-c, 2017). The authors employed a biaxial tensile test 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 (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 (Brüggenwirth *et al*. 2014; Brüggenwirth and Knoche, 2016a). 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, 2016a). The less cracking-susceptible 'Regina' has a stiffer skin and a higher pressure at fracture than the cracking-susceptible 'Burlat'. Differences between 'Regina' and 'Burlat' are most likely accounted for by differences in physical and chemical properties of the epidermal/hypodermal cell walls (Brüggenwirth and Knoche, 2016c).

Microscopy of fracture surfaces revealed that natural cracking and cracking of excised skins in biaxial tensile tests (at low strain rates) occurred by separation of neighboring cells along the middle lamella. Further, cell separation was preceded by a loss of vitality of epidermal cells and the concurrent swelling of cell walls. Varying cell wall swelling in excised skins subjected to biaxial tensile tests established a negative linear relationship between the pressure at fracture and the extent of cell wall swelling. Hence, swollen cell walls offered little resistance to cell separation (Brüggenwirth and Knoche, 2017).

The Zipper hypothesis

In light of lacking experimental support and the unrealistic predictions of the critical turgor concept, a new hypothesis was developed that accounts for rain cracking of fruit. This hypothesis is referred to as the Zipper hypothesis (fig. 1). It is consistent with all experimental findings up to date.

The zipper hypothesis comprises a series of events that ultimately lead to propagation of a crack through the skin and flesh and consequently to a cracked fruit. It is based on the following sequence of events: tension (stress) develops in the skin during stage III growth and particularly in the cuticle due to a down regulation of genes involved in cutin and wax synthesis during stage III growth (Alkio *et al*., 2012, 2014). Stress results in strain and microcracks in the cuticle (Knoche *et al*., 2004; Peschel and Knoche, 2005). Furthermore, surface wetness on and high humidity above a strained cuticle aggravates microcracking (Knoche and Peschel, 2006). Microcracking impairs

the cuticle's barrier function and focuses water uptake in a particular region of the fruit surface like the aperture in an optical instrument. Water now bypasses the cuticle, penetrates into the fruit and moves to sites with the most negative water potential. These are the large thin-walled parenchyma cells of the outer mesocarp that have a more negative osmotic potential than the small thick walled epidermal and hypodermal cells (Grimm and Knoche, 2015). Water uptake causes individual cells to burst. As a consequence, cell content leaks into the apoplast. Major constituents of sweet cherry such as glucose, fructose and malic acid acid now occur in the apoplast at comparable concentrations as in the symplast. The consequences are several fold: First, cell turgor decreases and is entirely lost when epidermal cells are plasmolyzed by juice from the flesh. Second, malic acid extracts cell wall bound Ca, weakens cell walls and increases the permeability of plasma membranes causing a chain reaction of leakage of adjacent cells (Winkler *et al*., 2015, 2016). The leakage of cells and the loss of the (low) turgor will result in swelling of cell walls, in particular of the pectin middle lamella. Swollen cell walls have decreased stiffness, fracture tension and cell adhesion resulting in the separation of neighbouring cells along their cell walls (Brüggenwirth and Knoche, 2017). The tension (low) generated by the strain of the skin is now sufficient to cause the cells to separate along their swollen walls and to rupture the skin. This process continues at the crack tip where the stress concentrates and causes the crack to elongate. The skin 'unzips' in the same way like a 'zipper' or a 'ladder' that propagates in a piece of knitted fabric.

Potential countermeasures

The Zipper model allows to assess potential countermeasures to reduce cracking. These include the following. First, differences in cracking susceptibility among cultivars do exist. These must be taken into account when selecting cultivars for open field production without rain shelters in regions with summer rains. Based on our experience, fruit of all cultivars cracks. Second, rain shelters are an effective, but expensive means to prevent/reduce surface wetness of fruit. This eliminates osmotic water uptake, reduces microcracking and markedly reduces fruit cracking. Third, the Ca supply of the fruit is an important factor in swelling of cell walls which in turn affects the mechanical properties of the fruit skin. Increasing Ca concentration requires frequent applications of high doses of Ca-salts during development. Fourth, attempts to decrease the driving force for osmotic water uptake by applications of solutions containing

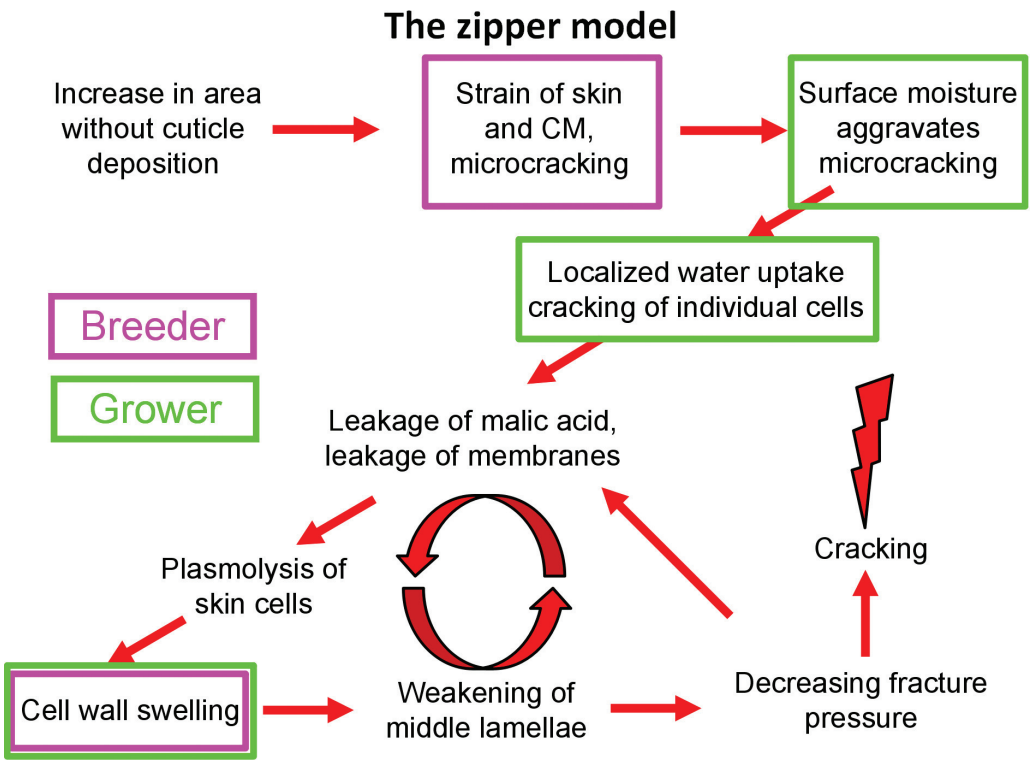

Fig. 1 - Sketch of zipper model that explains processes involved in rain cracking of sweet cherry fruit. Processes in green boxes are those that may be manipulated by growers, those in pink boxes by breeders. For details see text.

Fig. 1 - Schizzo del modello cerniera lampo che spiega i processi coinvolti nel meccanismo delle spaccature dei frutti di ciliegio. I processi nel riquadro verde sono quelli che potrebbero essere manipolati dai frutticoltori, quelli in rosa dai genetisti. Per dettagli si rimanda al testo.

osmolytes are unlikely to be successful. Such osmolytes must be water soluble for spray application, but at the same time water insoluble to provide rain fastness. These conditions are mutually exclusive. Also, the osmolyte concentration must be high to balance water potentials down to -4 MPa. Lastly, it should be pointed out that "magic bullets" that will reduce cracking are not on the market up to now. Film forming agents - unless selectively applied to fruit only - will hinder gas exchange of leaves thereby compromising photosynthesis and respiration. Further, the resistance of such films to water penetration is usually not sufficiently low. Thus, thick spray deposits would be needed on the fruit surface, which are unacceptable from a consumer's perspective. In addition, coverage is incomplete and formation of a film requires a contact mode of action.

In the long run, synchronizing cuticle deposition and surface expansion during development may have merit in breeding. In addition, modifications of cell wall characteristics to improve cell-to-cell adhesion are likely to contribute to reduced cracking. Further research is necessary on the latter aspect to identify the limiting factor.

Conclusions

The zipper hypothesis offers a plausible explanation for fruit cracking that is consistent with essentially all experimental findings reported to date. The view of a sweet cherry as a balloon containing a sugary solution surrounded by a taut skin is an unrealistic oversimplification. Instead cracking must be viewed as a localized phenomenon. This hypothesis also explains why relationships between water uptake and cracking are highly variable and why the application of film forming agents ('coating strategies') aiming at reduced water uptake will be of limited success in reducing cracking.

Abstract

Sweet cherry (*Prunus avium* L.) cracking is a severe limitation in production worldwide. It is thought to be caused by excessive water uptake and a subsequent increase in turgor. When a critical threshold is exceeded ('critical turgor') the fruit is believed to crack. Experimental evidence supporting this wide spread concept is lacking. Instead, published data question the critical turgor hypothesis and an alternative explanation must be thought of. This mini review summarizes experimental research published in the last two decades that resulted in an alternative explanation of sweet cherry fruit cracking, the so called Zipper hypothesis. According to this hypothesis, cracking is the result of a series of events that ultimately propagate a crack through skin and flesh and 'unzip' the fruit. It is based on the following sequence of events: Tension (stress) develops in the skin during stage III growth and particularly in the cuticle due to a downregulation of genes involved in cutin and wax synthesis. Stress in the skin results in strain and microcracks in the cuticle. Furthermore, surface wetness on and high humidity above the strained cuticle aggravates microcracking. Microcracking impairs the cuticle's barrier function and focuses water uptake in a particular region of the fruit surface. Water bypasses the cuticle, penetrates into the fruit and moves to sites where water potential is most negative. These are the large thin-walled parenchyma cells of the outer mesocarp that have a more negative osmotic potential than the small thick walled epidermal and hypodermal cells. Water uptake causes individual cells to burst. As a consequence, cell content leaks into the apoplast. Major constituents of sweet cherry such as glucose, fructose and malic acid now occur in the apoplast at comparable concentrations as in the symplast. The consequences are several fold: First, cell turgor decreases and is entirely lost when epidermal cells plasmolyse in the juice from the flesh. Second, malic acid extracts cell wall bound Ca, weakens cell walls and increases the permeability of plasma membranes causing a chain reaction of leakage of adjacent cells. The leakage of cells and the loss of the (low) turgor results in swelling of cell walls, in particular of the pectin middle lamella. Swollen cell walls have decreased stiffness, fracture tension and cell adhesion resulting in the separation of neighbouring cells along their cell walls. The tension generated by the strain of the skin is now sufficient to cause the cells to separate along their swollen walls and to rupture the skin. This process continues at the crack tip where the stress concentrates and causes the crack to elongate. The skin 'unzips' in the same way like a 'zipper' or a 'ladder' that propagates in a piece of knitted fabric.

Keywords: cracking, fruit growth, cherry, Prunus avium, fruit water relations

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