ITALUS HORTUS
Vol. 29 (2022), Pages 1-13
doi: 10.26353/j.itahort/2022.1.C1
Review

The unzipping of sweet cherry fruit skin and strategies to prevent it

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Received: 31 January 2022; Accepted: 04 March 2022; Available online: 22 April 2022

Abstract: Rain cracking in sweet cherry (*Prunus avium* L.) imposes a severe limitation on commercial producers of this high value fruit. In recent years, considerable progress has been made in unraveling the mechanistic basis of fruit cracking in sweet cherry. In this mini review we discuss the so-called Zipper hypothesis that explains the events leading up to the 'unzipping' of the fruit skin and ultimately to cracking. Using the Zipper model, we then explore the reasons why fruits may sometimes crack under rain shelters or postharvest in the box, in transit, and why the measured response of fruit cracking to Ca sprays is so frustratingly inconsistent.
Based on the Zipper hypothesis, cracking is the result of a series (causal chain) of events that ultimately 'unzip' the fruit. Tension (stress) develops in the skin during stage III growth. Stress results in microcracks in the cuticle. Microcracking is exacerbated by surface wetness and high humidity. Microcracks focus subsequent water uptake into particular regions on the fruit surface. Here, they allow water to bypass the cuticle and to penetrate through to the cells of the skin and flesh. Ultimately, water causes the large, thin-walled parenchyma cells to burst. This allows their cell contents (including malic acid) to leak into the apoplast. The malic acid causes the neighboring cells also to leak and it also extracts Ca from the cell walls. The cell walls then swell and cell-to-cell adhesion decreases. In this way a crack forms and propagates, 'unzipping' the skin in a way somewhat analogous to that in which a 'ladder' will propagate from a small area of damage in a knitted fabric.

Keywords: cuticle; *Prunus avium*; microcrack; calcium; rain shelter; review; xylem; water uptake.

1. Introduction

Rain cracking of sweet cherry reduces both the quality and the marketable yield of this high value fruit in all production areas where rainfall occurs during the ripening season (Balbontín et al., 2013; Børve et al., 2000; Peschel and Knoche, 2005). Depending on harvest costs, an upper threshold of 25% of cracked fruit on a tree is said to render the harvest operation uneconomic (Looney, 1985). Lower percentages of cracking still result in significant levels of fruit rots, reduced fruit quality and decreased storage and shelf life.

The explanation for fruit cracking still prevalent in horticultural textbooks is that it results from excessive fruit turgor, arising from water uptake by wet fruit (Considine and Kriedemann, 1972). While this idea seems both intuitive and logical, there is actually no satisfactory evidence to support it.

After the maturing sweet cherry fruit changes color from yellow to red, the low fruit turgor is quite insignificant relative to the very negative osmotic potential of its juice (Schumann et al., 2014). Furthermore, there are no detectable changes in fruit turgor in response either to fruit transpiration or to fruit water uptake, even up to the point where fruit cracking occurs (Knoche et al., 2014). These and other observations have led to the development of the 'Zipper' hypothesis that is consistent with all

published experimental findings to date (Winkler et al., 2016a; Brüggenwirth and Knoche, 2017; Knoche and Winkler, 2017; Schumann et al., 2019). The Zipper hypothesis explains cracking as being the result of a complex series of events (causal chain) that ultimately lead to the well-known 'unzipping' (i.e., cracking) of the fruit skin.

In this review, the Zipper hypothesis is discussed in detail and then used, together with literature findings, to explain previously inexplicable observations such as fruit cracking under rain shelters, postharvest fruit cracking in a box in a cool store, the inconsistent performance of Ca sprays, and the lucrative opportunities fruit cracking creates for purveyors of a range of 'snake oils' and 'magic bullets'.

2. The Zipper Hypothesis

We use a previously published sketch to illustrate the sequence of events that comprise the Zipper hypothesis (Figure 1).

Tension (stress) develops in the fruit skin during stage III growth and particularly in the cuticle due to the down-regulation of genes involved in cutin and wax synthesis (Alkio et al., 2012, 2014). During stage III (also referred to as final swell) the fruit surface area increases rapidly (at rates up to 1 cm^2 d^{-1}) but with no new cuticle deposition. This results in a now fixed amount of cuticle material being distributed over an ever increasing area of fruit surface (Knoche et al., 2004; Grimm et al., 2012; Lai et al., 2016). The stretching and thinning of the cuticle are inevitable.

The rising stress and strain results in the formation of microscopic cracks in the cuticle - the so-called microcracks (Peschel and Knoche, 2005). Surface wetness resulting from dew, condensation of fog, or rain or even exposure to high humidity triggers and exacerbates microcracking (Knoche and Peschel, 2006). Those areas of the fruit skin suffering longer durations of surface wetness are particularly prone to microcracking. These are the stem cavity and the stylar end of the fruit. A puddle of water forms in the stem cavity during rain and it remains there long after the rain has stopped. Droplets coalesce and run down the fruit surface to form a hanging drop in the stylar scar region, at the lower extremity of the fruit. This drop also remains in place long after rain has stopped. In sweet cherries, cuticular microcracks are not caused by water uptake but more likely by the altered mechanical properties of the strained cuticle (Knoche and Peschel, 2006).

The presence of microcracking impairs the cuticle's barrier properties, focusing the osmotic uptake of water into the microcracked regions of the fruit surface. This water can now substantially bypass the cuticle, and thus penetrate through to the cellular layers beneath. Here it migrates by diffusion to sites with the most negative water potential. These sites are the large, thin-walled parenchyma cells of the outer mesocarp. These have a more negative osmotic potential than the smaller, thick-walled epidermal and hypodermal cells (Grimm and Knoche, 2015). Their more negative water potential probably results from the reticulum of minor veins that distribute the incoming sugars of the phloem supply.

In late developmental stages, water import into the fruit through the pedicel occurs mainly via the phloem. This is because the xylem ruptures physically during stage III growth, so the xylem contribution to the total water inflow from the tree decreases steadily (Brüggenwirth et al., 2016; Winkler et al., 2016a; Grimm et al., 2017). Thus, by maturity, xylem sap inflow through the pedicel has ceased and phloem sap inflow is the only source of vascular water.

Recent results indicate that osmotic potential is not uniform within a fruit. The stylar end of the fruit has a more negative osmotic potential than the stem end (Grimm et al., 2020), and the outer flesh has a more negative osmotic potential than the skin (Grimm and Knoche, 2015). In addition, it is likely that the cells close to the veins have a more negative osmotic potential than those between the veins (Grimm et al., 2020). There may also be an underlying random pattern of osmotic heterogeneity among otherwise similarly disposed cells of the fruit.

As a consequence of water uptake via the fruit surface, or of water inflow via the phloem, or of water redistribution along gradients of water potential within the fruit, the more osmotically concentrated parenchyma cells begin to burst. Cell content leaks into the apoplast (Simon, 1977) and the already-

low fruit turgor will decrease even further. Malic acid is included in the leaking cell contents, now in the apoplast. This extracts Ca bound to the cell wall and increases the permeability of the plasma membranes of the neighboring (still intact) cells. This causes the neighboring cells also to leak (Winkler et al., 2015, 2016a). As a result of these processes, the local cell walls begin to swell (Brüggenwirth and Knoche, 2017). This causal chain of processes will most likely start in those regions of the fruit where osmotic potential is most negative, i.e., in the outer flesh and towards the stylar end of the fruit (Grimm et al., 2020). The outer flesh is of course the region bordering the skin: the epidermal and hypodermal cells which form the structural 'backbone' of the sweet cherry fruit (Brüggenwirth et al., 2014).

The swelling of the middle lamella decreases skin stiffness, fracture tension and cell adhesion, resulting in the separation of neighboring cells along their cell walls (Brüggenwirth and Knoche, 2017). This process weakens the skin. The low tension already present in the skin, previously caused by growth straining, is now sufficient to cause the cells to separate along their swollen walls and thus for the skin to fail (tear) (Schumann et al., 2019).

The above processes continue, especially at the crack tip where stresses naturally concentrate and thus cause the crack to extend or propagate (Schumann et al., 2019). Thus, the fruit skin 'unzips' in a way somewhat analogous to the way a piece of knitted fabric fails from an initiating minor failure to form a 'ladder'. The primary mode of cracking is the separation of adjacent cells along the middle lamellae (Brüggenwirth and Knoche, 2017; Schumann et al., 2019).

The Zipper model is consistent with all published experimental results. It also explains unexpected phenomena such as cracking in the absence of a net water uptake (Winkler et al., 2016a), the postharvest cracking of fruit in a box without contact with moisture (Knoche M., personal observations), the cracking of fruit under a rain shelter without contact with free water (Cline et al., 1995) etc.

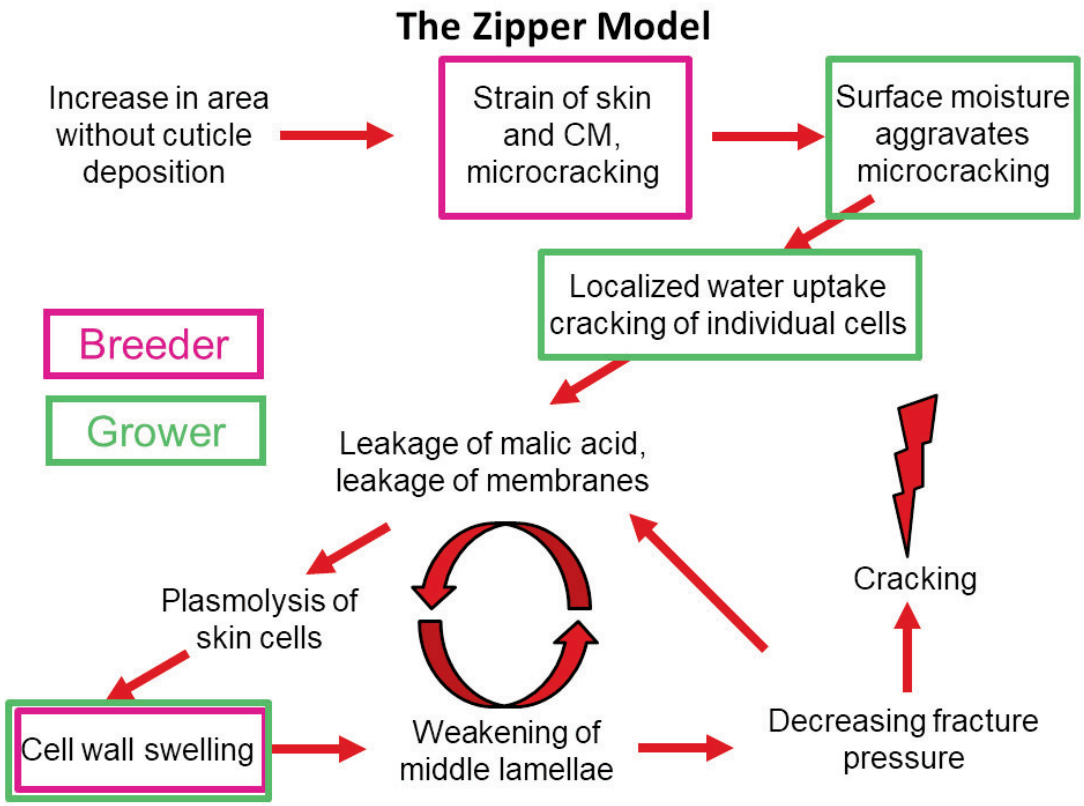


Figure 1. Sketch of the Zipper model that explains processes involved in rain cracking of sweet cherry fruit. Processes in green boxes are those that may be manipulated by growers, those in pink boxes by breeders. CM, cuticular membrane. For details see text. The figure is reproduced from Knoche and Winkler, 2019, Italus Hortus 26, 59-65.

3. Explaining the unexplainable and the hope for miracles

In the following section we discuss unexpected observations such as cracking under a rain shelter, postharvest cracking of fruit in the box, the inconsistent performance of Ca sprays and potential opportunities for 'snake oils' and 'magic bullets' that are difficult to explain and whose effects are sometimes counterintuitive. We use the Zipper model and/or literature results to provide confirming explanations consistent with current knowledge.

3.1. Cracking under rain shelters

Rain shelters and plastic tunnels have long been used by growers to protect sweet cherry fruit from rain. They have become standard for newly-established plantations in many production areas of the world where rainfall occurs during the ripening season.

Rain shelters are the most effective strategy for reducing cracking. It seems obvious that maintaining a dry fruit surface will protect fruit from cracking. However, some fruit cracking also occurs under a rain shelter (Cline et al., 1995). Occasionally, disastrous incidences of fruit cracking have been observed under rain shelters, especially after extended periods of rain. The distribution of cracked fruit within the canopy excludes the possibility that the cracked fruits were somehow wetted by the rainfall. The question arises why this fruit cracked despite protection from rain. Two explanations are offered.

3.1.1. Explanation 1 - Water uptake via the root system and vascular inflow

Typical rain shelters protect the trees from precipitation by 'funneling' rainwater into the alley between rows. Thus, while the water is kept away from the canopy, it remains in the orchard. Depending on soil properties, this water either stays in the root zone (where it is accessible to the tree) or it infiltrates below the rootzone (where it is inaccessible to the tree).

Measham et al. (2010) suggested that it is increased xylem vascular flow that induces cracking of sweet cherry fruit. Studies on xylem inflow into developing fruit reveal that up to the end of stage II growth, the xylem accounts for more than 80% of the vascular inflow into the fruit. At the onset of stage III growth, the xylem begins to progressively lose its functionality. At maturity, only about 5% of the total inflow into the sweet cherry is accounted for by the xylem, the remaining 95% is via the phloem (Brüggenwirth et al., 2017). This means that inflow into the fruit via the xylem is negligible in the last weeks of the fruits' development. Interestingly, there is a component of xylem inflow that occurs by osmotic pull even in the absence of transpiration (Winkler et al., 2016b). Unfortunately, unlike water transport in the xylem, there is no close link between the water availability in the soil, the air relative humidity (i.e., the water vapor pressure deficit in the orchard) and the rate of phloem transport. Instead, physiological factors such as the strength of the source:sink signals determine the rate of phloem transport. However, it is conceivable that the continuing inflow of water via the phloem causes cell bursting thereby initiating the Zipper cascade and eventually whole fruit bursting during stage III growth.

If water uptake by the fruit via the phloem were a causal factor in rain cracking, current shelter constructions may have to be modified to remove the excess water from the orchard. This would involve a large volume of water to carry away (10,000 L ha^{-1} mm^{-1} of rainfall) so would be logistically difficult, especially in a violent downpour. We are unaware of any shelter constructions that do this. An easy to install system could include a strip of plastic film placed in a shallow, leveled ditch having a y-shaped cross section possibly with a drainage pipe located in its center (Figure 2). Any rain that is funneled into the tractor alley would be collected and removed from the orchard. This aspect merits further research.

3.1.2. Explanation 2 - Increased humidity and increased temperature

The increased humidity and increased temperature under a rain shelter (reduced ventilation) could also increase the likelihood of fruit cracking for several reasons:

Small fluctuations in temperature could cause condensation on the fruit surface and so extended periods of surface wetness - particularly after a cold night when fruit temperature is low. Condensation

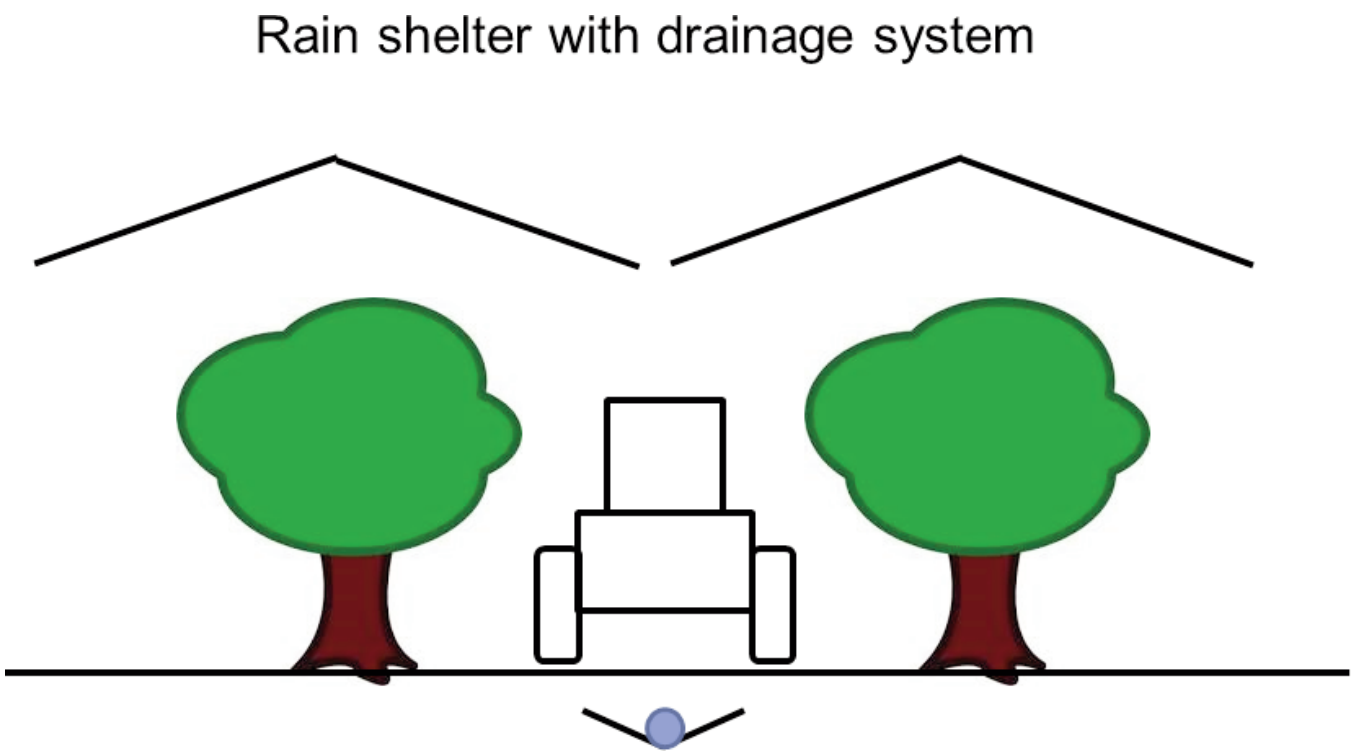


Figure 2. Sketch of the rain shelter with drainage system to remove excess water from the interrow.

on the fruit surface would have the same effects as exposure to rain – i.e., cuticular microcracking and localized water uptake.

It is a possibility that fruit water uptake could occur from the vapor phase but there are at least two reasons why this pathway is unlikely to be significant. A ripe sweet cherry fruit has an equilibrium humidity of about 98.4% RH (Beyer et al., 2005). Fruit exposed to this humidity neither loses nor gains mass. Only at humidity levels exceeding this threshold, can water uptake occur from the vapor phase. Thus, the driving force for vapor uptake is small. In addition, the permeability of the fruit skin to uptake from the vapor phase is much lower than that for uptake from the liquid phase (Beyer et al., 2005). Both factors make vapor phase water uptake an unlikely contributor to fruit cracking.

The reduced transpiration in a high humidity environment presumably maintains skin strain at a high level. Indeed, allowing fruits to transpire before carrying out an *in vitro* cracking assay slightly delays cracking and thus (for a given duration of incubation) decreases cracking. This behavior was described in studies by Winkler et al. (2016a) who reported that transpiration (before incubation) increased the time necessary for 50% of the fruit to crack and increased the amount of water required for 50% of the fruit to crack.

Transpiration is the predominant driver for xylem transport. Hence, under rain covers, low fruit transpiration throughout the fruit development period will reduce fruit Ca accumulation. This effect will be additive to the natural reduction in xylem functionality with development (Winkler et al., 2020).

Increased temperature increases cracking susceptibility of sweet cherries (Bullock, 1952; Richardson 1998). This effect is due to a decrease in fracture force of the fruit skin as temperature increased (Brüggenwirth and Knoche, 2016).

These observations indicate that the consequences of reduced transpiration, vascular dysfunction and inflow via the phloem and a mechanically weaker skin are likely to be additive. Unfortunately, options for increasing fruit transpiration by ventilation (to decrease humidity and temperature) under a rain shelter are limited, especially when nets are installed to protect against *Drosophila suzukii*. Indirect measures to lower humidity include maintaining an open canopy structure and more frequent mowing of the in-row and inter-row understorey grass.

3.2. Postharvest cracking of fruit in a box

Distributers sometimes report cracking in sweet cherry fruit at market. This follows grading, bagging, packing, cool storage and cool transit. It would seem the fruit was in good condition when packed, with no free water (from the hydrocooler) included in the bag and with the fruit resting on a water-absorbent pad to remove any tiny adherent residues. Later, when the fruit is unboxed at market, it is found to suffer extensive microcracking and some 10% of it can be severely cracked. There may or may not also be free water evident in the bag. (1) One possible explanation for this is the failure to properly initiate the cool chain (i.e., fruit is inadequately cooled before packing) and another (2) is the failure to ensure continuity of the cool chain while in transit (i.e., cartons are allowed to warm slightly at some stage). (1) Failure to initiate the cool chain can occur in the packhouse if, for example, a large amount of warm fruit arrives for packing over a short period of time – maybe the packhouse refrigeration lacks the capacity to cope. (2) Failure to maintain the cool chain can occur in transit if, for example, there is an excessive delay in transferring the packed product, between, say, a cool store and a cool container.

In either case, fruit respiration in a palleted stack of densely packed cartons can allow 'runaway' warming i.e., respiratory heat is generated by the fruit faster than it can escape, so the fruit warms up but later sweats when it is re-cooled. In either case the cause can be established by packing small temperature loggers in marked cartons (to allow later retrieval and downloading). These temperature logs will establish if cool chain failure has occurred and, if so, the point of failure. Cool chain failure can result in very large claims being made against packhouses and freighting companies.

3.2.1. Temperature stability

The relative humidity in a sealed polyethylene bag of fruit is always close to 100% as fruit do transpire and such a bag is substantially proof against vapor phase water. This high humidity can cause cuticular microcracking in an already-strained sweet cherry skin, and to an extent similar to that from exposure to liquid-phase water (Knoche and Peschel, 2006). Although, as established above, water uptake from the vapor phase is negligibly low (Beyer et al., 2005). Nevertheless, even minor oscillations of temperature will induce cycles of condensation and evaporation. Under these conditions, the fruit becomes wet and uptake from the liquid phase will occur. With unstable temperatures, the cycles of condensation and evaporation will be both more extreme and more numerous with the result that the accumulation duration of exposure to liquid phase water will be lengthened.

3.2.2. Gradients in water potential within a sweet cherry

The traditional view of a cherry (and of many other 'soft' fruits) is that in physical terms they behave as thin-walled pressure vessels (a term used by engineers to simplify the mathematics) and that they are filled with a semifluid solution (the pulp) which is of reasonably high osmotic concentration – commonly high in sugar (Grimm et al., 2020). Because the cuticle forms a substantial barrier to water movement relative to the plasma membranes (Schönherr and Riederer, 1989), the tissues of the fruit are expected to be largely in water potential equilibrium.

Recent evidence, however, sheds doubt on the above assumption. We observed considerable gradients in water potential and its components within a mature sweet cherry (Grimm and Knoche, 2015; Grimm et al., 2020). The osmotic potential becomes more negative along the longitudinal axis from the stem end to the stylar end of the fruit (Grimm et al., 2020). Also, there is a latitudinal gradient of decreasing osmotic potential (more negative) from the pit to the outer flesh (Grimm et al., 2020) and an increase in osmotic potential (less negative) again in the skin (Grimm and Knoche, 2015). The outer flesh below the fruit skin has the most negative osmotic potential. The outer flesh harbors an extensive reticulum of minor veins (Grimm et al., 2020). Minor veins represent the unloading phloem where osmolytes are unloaded into the surrounding tissue. Furthermore, there is evidence for preferential partitioning of water along radial veins (Grimm et al., 2020). A likely explanation is a more negative osmotic potential along the radial veins.

These observations suggest that the sweet cherry is highly heterogeneous from a water potential point of view and is more likely in a transient steady state rather than in a stationary equilibrium. The transient steady state results from a high rate of loading with osmolytes and a lagging behind of the distribution of these osmolytes within the fruit.

When the inflow of osmolytes to the fruit is 'interrupted' by harvesting, these internal gradients are expected to equilibrate over time. Thus, water will move from regions of less negative water potential to ones of more negative water potential, and osmolytes will move from regions of high osmotic concentration (more negative osmotic potential) to ones of lower osmotic concentration (less negative osmotic potential).

We have found that the fruit portion with the most negative osmotic potential is in the outer flesh at the stylar end of the fruit (Grimm et al., 2020). Here, incidentally, the Ca concentration is also least (see discussion below) and the cross linking of cell walls is least (Winkler et al., 2020; Schumann and Knoche, 2020). Osmotic water uptake by the flesh cells results in cell bursting. The Zipper cascade is initiated, and the skin unzips without the need for water uptake or exposure to rain. The lack of transpiration in the high humidity, low radiation, stable temperature environment of a fruit box makes this more likely.

3.3. Limited performance of foliar Ca sprays

The literature on the effects of Ca on sweet cherry cracking was recently summarized in a review (Winkler and Knoche, 2019).

Ca is often, but not always, reported to be effective in decreasing fruit cracking. Ca consistently decreases fruit cracking in incubation assays even at very low concentrations, when fruit is incubated in solutions containing Ca salts. In contrast, mixed results are reported following spray application of Ca salts in the field. Unfortunately, fruit Ca content has often not been quantified and, hence, it is not known whether the foliar applications of Ca salts have increased fruit Ca content.

The question arises as to what is the reason for the lack of consistent performance?

3.3.1. Physiology of Ca in sweet cherry fruit

The total amount of Ca in the fruit increases continuously through to maturity, whereas the amount of Ca per unit dry mass (DM) (mg Ca per g DM) decreases continuously (Winkler et al., 2020). The reason for this pattern is the loss in xylem functionality and, hence, the decrease in xylem inflow (Brüggenwirth et al., 2016). Ca transport is limited to the xylem (White, 2012). Fruit Ca is highly variable, both between fruit but also within fruit. Within a batch of fruit, the Ca content per fruit can differ by a factor of two. Within a particular fruit, the Ca content of the stem end can be two- or three-times higher than that of the stylar scar end (Winkler et al., 2020). Fruit grown under low relative humidity (26.2%) contained more Ca per fruit and had a higher amount of Ca per unit dry mass compared with fruit grown under high humidity (91.6%). There was a positive relationship between Ca mass per fruit and cumulative transpiration (Winkler et al., 2020). These results indicate that xylem functionality and transpiration are the primary determinants of fruit Ca.

3.3.2. How to increase fruit Ca?

Due to the loss of xylem functionality during stage III growth, fertilizing the soil using Ca is ineffective in increasing the fruits' Ca content. Anyway, most soils are Ca-sufficient. Foliar Ca applications remain the only option. Unfortunately, Ca is a poor penetrant of the cuticle of sweet cherry fruit, but also of many other fruit crops. Ca-salts mostly dissociate in aqueous solution into the Ca-cation and one or several anions depending on the salt. The Ca-cation carries two positive charges. Since the cuticle is a polyelectrolyte, ions are subject to Donnan exclusion that severely hinders cuticular penetration. In addition, to maintain electrical neutrality, penetration of a Ca cation must be matched by a stoichiometric penetration of corresponding anions.

Recently, the penetration of Ca through the sweet cherry fruit skin has been studied in greater detail (Winkler et al., 2021a,b). Experiments using $^{45}CaCl_2$ revealed that (1) the stem fruit junction is a site of preferential Ca uptake, (2) penetration increases with increasing temperature and (3) increasing relative humidity (RH) from 0, 28, 75 to 100% increases penetration of $CaCl_2$, but penetration of Ca-formate is restricted to 100% RH (Winkler et al., 2021a). There is little difference in permeance of the fruit skin to $CaCl_2$, $Ca(NO_3)_2$, Ca-formate, Ca-acetate, Ca-lactate or Ca-propionate. Only Ca-heptagluconate penetrates at a slower rate.

The effect of surfactants on Ca uptake depends on the surfactant. Spraying Ca salts in combination with Glucopon CSUP or the silicone surfactant Break-Thru S 240 showed no consistent increase in fruit Ca uptake (Winkler et al., 2021b). Occasionally, Ca uptake was actually decreased by additions of surfactant, compared with the no-surfactant controls. The most likely explanation for the surfactant effect is a decrease in the volume of Ca solution retained on the sprayed fruit surface due to more complete surface drainage with Break-Thru S 240.

Penetration studies demonstrate that Ca-penetration is limited by the mobility of the Ca ion in the dried-down droplet residue when the point of deliquescence of the applied salt or the applied formulation exceeds the ambient RH (Winkler et al., 2021a,b). Multiple applications are needed to achieve significant increases in fruit Ca.

3.3.3. Can Ca ‘jam’ the Zipper?

The Zipper hypothesis offers several reasonable explanations for the consistent effects of Ca on fruit cracking in an immersion assay but no explanations for the inconsistent of null responses following Ca spray application in the field. How can this inconsistency be explained?

There is no effect of Ca on water uptake rates. At concentrations used in immersion assays or in spray applications Ca salts have no effect on water uptake (Weichert *et al.*, 2004). The osmotic potentials of these solutions are too high (osmotic concentrations too low) relative to the water potential of a sweet cherry fruit to significantly decrease the driving force for water uptake (Winkler and Knoche, 2019).

Ca has no effect on cuticle mass (Morales and Winkler, unpublished data). Hence, cuticular stress and strain and microcracking are unlikely to be affected.

Ca markedly decreases the extension of microcracks into macrocracks (Winkler, unpublished data). Microcracks impair the barrier functions of the cuticle. Thus, in an immersion assay the Ca-ion has free access to the epidermal cell walls exposed by a cuticular microcrack. In contrast, in a field spray application microcracks are only hit by chance by the Ca containing spray solution. The overall increase in cell wall bound Ca following penetration of an intact cuticle is probably too low to reproducibly decrease fruit cracking. This argument also explains why even very low concentrations of Ca down to 1 mM are effective in immersion assays (Christensen, 1972) but six consecutive foliar applications of Ca solutions (50 mM) led to a 50% increase in fruit Ca content but without an effect on cracking (Winkler, unpublished data).

Ca decreases swelling of cell walls (Brüggenwirth and Knoche, 2017). This is due to the role of Ca in cross linking cell walls. According to the familiar ‘egg-box model’ of cell walls, the Ca cation (the egg) crosslinks between two adjacent homogalacturonan chains (the top and bottom of the eggbox). Homogalacturonans are an important constituent of the pectin middle lamella and hence, are responsible for cell to cell adhesion. This explains why Ca has positive effects on the mechanical properties of the fruit skin, provided it gets there in sufficiently high amounts (Brüggenwirth and Knoche, 2017). Incubating sweet cherry fruit skin segments in a Ca solution increases the stiffness and the fracture force but decreases fracture strain.

Thus, the effect of Ca on cracking is the result of a decrease in the extension of microcracks in the fruit skin and an increase in the crosslinking of cell walls in the cellular layers of the fruit skin. The most

likely reason for the lack of consistent effects following spray applications in the field is the low penetration of Ca through an intact cuticle. However, following microcracking Ca penetration is no longer so hindered. Ca will bind immediately to the sites of action and thus help prevent further extension of the microcracks.

3.4. Magic bullets and snake oils

We are not aware of any detailed studies documenting significant and reproducible decreases in fruit cracking following spray applications of 'magic bullet' products that also identify their modes of action. Based on what we know, various strategies could possibly work.

3.4.1. Stimulating cuticle deposition

The stimulation of cuticle synthesis by foliar applications of cutin monomers or precursors could be a viable strategy. The sweet cherry fruit cuticle has a mixed C16/C18 type cutin (Peschel et al., 2007) and, therefore, the application of oleic and palmitic acids has potential merit. Both fatty acids are likely to be incorporated into the cutin polymer (Si et al., 2021). Based on published results for apple fruit (Khanal et al., 2014), increased deposition of cutin is expected to decrease elastic strain in the cuticle. Accumulated elastic strain is in turn the main driver of microcracking, particularly during stage III growth (Knoche et al., 2004; Peschel and Knoche, 2005).

Unfortunately, cuticle deposition is limited to stages I and II (Knoche et al., 2004, Peschel et al., 2007). The cessation of cuticle deposition during stage III growth results from downregulation of the genes involved in cutin monomer and wax synthesis (Alkio *et al*., 2012, 2014). Also, a comparison of 32 sweet cherry cultivars did not reveal any variations in the pattern of cuticle formation (Peschel and Knoche, 2012). These findings make it unlikely that precursors applied to the fruit surface would, after penetration through to the epidermis, actually be incorporated into the cuticle. It should also be noted that any uptake and incorporation that did occur would likely be a 'contact response' i.e., one that is limited to the site of the monomer deposit (see below).

3.4.2. Film forming agents

In discussions among growers, consultants and researchers, applications of film forming agents (e.g., liquid cuticle formulations) are suggested as a potential remedy for fruit cracking. The mode of action would be to seal the microcracks and so reducing water uptake via the fruit surface. While this idea has a certain attraction, it still lacks sound scientific support.

Decreased rain-cracking has been demonstrated following applications of coatings in several studies (Davenport et al., 1972; Meland et al., 2014; Torres et al., 2014; Dumitru et al., 2015; Measham et al., 2020; Gutiérrez-Jara et al., 2021). Unfortunately, the mode of action of these coatings has not been identified. To serve as an effective coating, the entire fruit surface must be covered with a continuous film. Moreover, the film must remain on the fruit surface and it must decrease the cuticle's permeability to water uptake. Experiments in our laboratory using a simulated spray solution containing a fluorescent tracer revealed that even when sprayed to run off, not more than 18% of the fruit surface was covered with the spray deposit. Because film formation is a contact response, the remaining 82% of the surface would not be covered and so would remain 'unprotected'.

Clearly, microcracks would be an ideal target for coating strategies but it is not clear how preferential deposition at microcracks could be achieved. In addition, to be effective, the film must have a low permeability to water uptake relative to the cuticle. There are few substances that can achieve this level of exclusion at reasonable thickness. Finally, during spray application, leaves will also be coated. This inevitable coating of leaves must not compromise gas exchange required for photosynthesis and transpiration. We are not aware of any results documenting commercially available substances that fulfill these conditions.

3.4.3. Protocols for unbiased assessment

Due to the economic importance of fruit cracking in sweet cherries, new treatments, new products and reformulations of older products will always trigger industry interest and thus field trial investigations. However, conducting such trials is not as straightforward as some would seem to think.

Carrying out cracking assessments in the field is the most realistic assessment. It is, however, logistically challenging, since potted trees in sufficient numbers and rain-on-shelters for standardized exposure to artificial rain are often not available. This leaves laboratory based cracking assays as the second method of choice to simulate a standardized rain event.

Cracking susceptibility varies within a population of fruit on a tree and between different trees. Differences in maturity have significant effects on the cracking susceptibility of fruit. Such differences are not readily identified by visual inspection. Therefore, the following protocol may be helpful in conducting experiments while minimizing bias by confounding factors.

Not all fruit are the same. For cracking assays, one carefully selects fruit that are as near perfect and identical in color (maturity) and size as the eye can see and the balance can record. Ideally, the entire population of fruit from several trees ($n \geq 3$) is sampled. The fruit is graded for maturity based on size and color.

Cracking susceptibility is then quantified within one or several grades of maturity to allow unbiased head-to-head comparisons.

Cracking susceptibility is quantified with replicates to allow statistical comparisons. The traditional so called 'Christensen test' lacks replications (Christensen, 1972).

Fruit of the different treatment groups are used to quantify the osmotic potential of the juice. This can be done after conducting the cracking assay. The water uptake that occurs during incubation is too low to significantly affect the osmotic potential of the juice. Fruit should be held at low temperature in a closed container to prevent changes in mass until analysis of the juice. If an osmometer is not readily available, the juice may also be analyzed by refractometer. The latter yields essentially the same information, since osmotic potential and the refractive index of sweet cherry juice are closely related (Winkler and Knoche, 2018).

The absence of significant differences in fruit size, color and osmotic potential between the treatment groups should be documented using appropriate statistical analyses.

The cracking susceptibility of cultivars is particularly difficult to compare. First, due to variation in ripening time the exposure to rain is likely to differ causing differential predisposition and – possibly - microcracking. Second, size, color and osmotic potentials are all likely to differ in addition to the "intrinsic susceptibility" of a particular cultivar. Here, repeated assessments in the course of ripening are needed for a sound comparison.

These guidelines will help to conduct unbiased experiments.

4. Conclusions

The Zipper hypothesis provides a conclusive unifying explanation for rain cracking of sweet cherry fruit that is consistent with all experimental results of which we are aware. It also explains observations made in horticultural practice that at first sight seem less intuitive such as the occurrence of significant fruit cracking under a rain shelter or the postharvest fruit cracking in a box. The Zipper hypothesis and recent research on the physiology of Ca also provide a reasonable explanation for the inconsistent responses of cracking to Ca treatments. It seems likely that Ca can 'jam' the Zipper by binding to and crosslinking the cell walls, provided of course that it is available in sufficient concentration to do this. Such is the case when Ca solutions come into direct contact with a microcracked fruit surface.

Funding: This research was funded by the Deutsche Forschungsgemeinshaft (DFG), grant number KN 402/10-1.

Acknowledgments: We thank Simon Sitzenstock, Peter Grimm-Wetzel, Hana Weiß and Marcel Pastwa for technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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