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Orange peel disorder in sweet cherry: Mechanism and triggers

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ABSTRACT

Skin shrivelling of sweet cherry (*Prunus avium* L.), referred to here as 'orange-peel' disorder, compromises fruit appearance and thus market value. The objectives were to establish a protocol to describe and quantify orange peel disorder and to identify the mechanism and factors determining its incidence. Fruit was stored for 28 to 33 d at 2 °C and 76% RH and orange peel disorder was quantified as a topographical roughness using an interferometer or by rating the fruit for orange peel using a four-step scoring scheme. Fruit with orange peel disorder had a skin topography similar to that of a citrus fruit − just on a finer scale. Under the conditions of the test, orange peel was first visible after ~7 d and continued to increase in severity thereafter. Orange peel was most severe on the shoulder and in the equatorial and distal regions of the fruit. There was no relationship between the distribution of orange peel and that of stomata or of microcracking. At a microscopic level, the depressions in the fruit surface were markedly larger than the periclinal areas of individual epidermal cells, but similar in size to the mesh formed by the network of minor veins visible just beneath the skin. Susceptibility to orange peel differed among cultivars. Least susceptible were 'Dönissens Gelbe' and, 'Gil Peck', intermediate were 'Sam', 'Kordia', 'Merchant', and the sour cherry 'Ungarische Traubige', and most susceptible were 'Adriana', 'Regina', and 'Hedelfinger'. Incidence of orange peel during storage was negatively related to relative humidity (more at lower humidities) but also developed at 100% RH in the absence of transpiration. Submerging fruit in water for 2 d partly reversed orange peel. There was no significant difference in the permeance of the skins or in turgors of cells of the outer mesocarp between fruit without and with orange peel. There was a significant difference between the osmotic potential of the flesh (more negative) and that of the skin (less negative). During storage, the osmotic potentials of flesh and skin both decreased slightly, but the difference between them remained constant. The results show water loss from the skin is causal in orange peel disorder. The water loss from the skin occurs both by two routes: (1) transpiration to the atmosphere and also (2) by osmotic dehydration to the flesh.

1. Introduction

In the last two decades, sweet cherry has emerged as an economically highly successful fruit crop in cool-temperate climates of the northern and southern hemispheres. The use of dwarfing root stocks, cultivation under rain shelters or in tunnels, and the development of efficient training systems have allowed intensification of production in many growing regions. The market demand for quality fruit has stimulated research on rain cracking, pitting of the fruit during and after harvest and on improving postharvest handling and storage procedures such as hydro-cooling, grading, and modified atmosphere packaging (Christensen, 1996; Kappel et al., 2002; Padilla-Zakour et al., 2004; Toivonen, 2014; Wang et al., 2014). These techniques have now become the 'best-practise' standards in the industry for production of high-quality fruit for export markets.

Achieving premium prices in export markets requires delivery of premium quality, near-perfect fruit (Looney et al., 1996). Three postharvest problems limit fruit quality in high-end markets for sweet cherry. First, stem shrivelling and browning occurs rapidly when the fruit is separated from the tree water supply. This is due in part to transpiration of the stem that is hydraulically connected to the fruit (Manganaris et al., 2007; Linke et al., 2010; Athoo et al., 2015). In addition, the stem is dehydrated by the very negative osmotic potential of the fruit (Knoche et al., 2015). Second, the fruit skin often suffers from pitting caused by impact or compression damage during picking, grading, transit, or storage (Manganaris et al., 2007; Param and Zoffoli, 2016). Finally, during storage the fruit skin can develop a disorder which is variously referred to as 'orange peel', 'lizard skin', 'alligator skin', or 'pebbling' (Zoffoli, personal communication; Zoffoli et al., 2017).

The name of this disorder originates from its similarity to the surface of a citrus fruit. It is characterized by alternating areas of

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depression and elevation giving it a finely 'sculptured' appearance. The altered topography is accompanied by a loss of shine (more diffuse, reflected light), which detracts from the fruit's 'fresh' appearance. The mechanistic basis of this disorder (we will refer to it here as *orange peel*) and the factors affecting its severity are unknown (Zoffoli et al., 2017). A better understanding of the causes and factors contributing to orange peel disorder are prerequisites for developing counter strategies to decrease its incidence and severity and, possibly, for eliminating it.

The purpose of this study was (1) to characterize and quantitatively describe the orange peel disorder and (2) to identify the underlying mechanism(s) and factors affecting its incidence and severity.

2. Materials and methods

2.1. Plant materials

Sweet cherry (*Prunus avium* L. 'Adriana', 'Dönissens Gelbe', 'Gil Peck','Hedelfinger', 'Kordia', 'Merchant', 'Rainier', 'Regina', 'Sam') and sour cherry (*Prunus cerasus* L. 'Ungarische Traubige') grafted on 'Gisela 5′ rootstocks (*Prunus cerasus* L. x *P. canescens* Bois) were cultivated according to current regulations for integrated fruit production in a greenhouse or under a rain shelter in the field at the horticultural research station of the Leibniz University Hannover at Ruthe (52°14′ N, 9°49′ E). No special treatments of foliar feeds (e.g., Ca-sprays) or other products (e.g., anti-cracking agents, etc.) aimed at modifying fruit surface properties were applied.Fruit were picked randomly at commercial maturity from a minimum of three trees in the mornings, placed on a padded surface, covered with a moist paper towel, and brought to the laboratory. In the lab, fruit were selected for uniformity of colour and size. Any fruit not having a perfect, blemish-free surface was discarded. The stem was cut to 2 cm length. This was needed to standardize for stem transpiration between cultivars (Athoo et al., 2015). Unless otherwise specified fruit were held under standard simulated storage conditions at 2 °C for up to 33 days. The RH was adjusted by holding fruit above a salt (NaCl) slurry (~76% RH; Wexler, 1995). Individual fruit mass was determined at the beginning and end of the storage period (ME235P-OCE, Satorius AG, Göttingen, Germany).

2.2. Quantifying orange peel

Orange peel symptoms were quantified using interferometry and a scoring procedure before and after a storage period of 27 to 33 d at 2 °C. Fruit was held above dry silica gel (~7%RH), NaCl (~76% RH), or above water (~100% RH). Following storage, fruit was equilibrated at ambient temperature for a minimum period of 30 min.

Severity of orange peel was evaluated using an interferometer (Contour GT-K, Bruker Nano Surface Division, Tucson, AZ, USA) and image analysis software (Vision 24, 5.41, Bruker). Three-dimensional images of the skin surface were generated by viewing the surface of intact sweet cherry fruit on the shoulder side of the equatorial region using an IX20 lens (Bruker) producing a 20-fold magnification. At this magnification, the area of the fruit surface scanned was $3 \times 3\ \mathrm{mm}^2$. Scans were carried out on a minimum of five fruit per treatment.

Individual scans were then merged ('stitching') to produce an image of $3 \times 3\ \mathrm{mm}^2$ of the fruit surface. The extent of orange peel was quantified as 'areal surface roughness' which is commonly employed in 3D metrology in materials science (root mean square height; Blateyron, 2013). Throughout this paper, we refer to the areal surface roughness as *roughness*. Briefly, a 'virtual mean surface' (height = 0) is calculated and the surface partitioned into regions below ('valleys') and above ('peaks') the virtual mean surface. The areal roughness is then calculated as the root of the mean squared depths of valleys and heights of peaks relative to the virtual mean surface (height = 0). It is important to note that the virtual mean height (height = 0) does not correspond to the surface of the fruit before, during or after formation of orange peel. It simply serves as a virtual base line to which the topography of the surface is related. Preliminary experiments established that rinsing the surface of fruit with deionized water had no effect on roughness (data not shown, H. Schlegel, unpublished data).

In addition to interferometry, a four step scoring scheme was used to quantify the severity of orange peel. The scores 0, 1, 2, and 3 represent fruit: without symptoms or with slight, or intermediate, or severe symptoms, respectively. Unless specified otherwise, orange peel was assessed on the fruit's shoulder in the equatorial plane.

2.3. Experiments

2.3.1. Establishing the protocol

The initial experiments focussed on establishing a protocol for assessing orange peel. A minimum of 10 'Sam' fruit representative of the rating scores 0 to 3 were selected and subsequently subjected to interferometry to calibrate the rating procedure. Calibrated images were taken using a binocular microscope (MZ10; Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) equipped with a digital camera (DP73; Olympus Deutschland GmbH, Hamburg, Germany) and processed by image analysis (cellSens Dimension 1.7.1; Olympus).

2.3.2. Morphological characterization

Frequency distributions for the numbers and volumes of valleys and peaks were established at different mean heights. Roughness was calculated and the relationships between roughness and the volumes of valleys and peaks quantified.

The time course of mass loss and orange peel development was established at 2 °C and 76% RH in 'Sam' sweet cherry. Orange peel was quantified by interferometry and by using the scoring scheme described above. The distribution of orange peel symptoms in different regions of the fruit surface was studied. The fruit was divided into a proximal (stem cavity), equatorial, and a distal (stylar scar) zone and inspected for orange peel in the region of the cheek (front), the two shoulders left and right of the cheek, and the suture (back).

Potential relationships between stomatal density and the occurrence of peak and valley structures of a fruit surface with orange peel symptoms were studied using the images obtained by interferometry. On these images stomata are easily detectable by the ring structure formed by the pair of guard cells and the stomatal aperture in the centre. The height of the stomata was determined as the mean of four measurements taken on the guard cells. A positive height indicates a position on the peak, a negative height a position in the valley. This analysis was carried out in 'Adriana', 'Hedelfinger', 'Kordia', 'Merchant', 'Regina', and 'Sam'. A minimum of five fruit (area $3 \times 3\ \mathrm{mm}^2$) was studied per cultivar.

To be able to relate the size of the depressions of the orange peel skin to morphological features of the fruit, planar periclinal areas of epidermal cells, the size of the isodiametric cells of the outer mesocarp, and the mesh width of the minor vein network in the outer flesh were quantified. Skin sections were prepared by cutting a tangential cap of a sphere from the fruit in the shoulder region using a razor blade. Sections were inspected by light microscopy at × 40 (BX-60; Olympus) and photographed (DP 71 and DP 73; Olympus). The planar periclinal areas of epidermal cells were quantified by image analysis (cellSens Dimension 1.14; Olympus). The size of cells of the outer mesocarp at 200 to 500 µm distance from the surface was quantified following staining with calcofluor white (0.1%). Thereafter, sections were covered with silicone oil and a cover slip, and viewed as described above. Height and width of cells were determined by image analysis and the cross sectional areas and volumes of the cells calculated assuming a spherical shape as a first approximation.

To quantify the mesh width of the minor-vein network, skin sections were prepared as described above and incubated in a solution containing pectinase [$90\ \mathrm{mL\ L^{-1}}$ (Panzym Super E flüssig; Novozymes A/S, Bagsvaerd, Denmark)] and cellulase [$5\ \mathrm{mL\ L^{-1}}$ (Cellubrix L.; Novozymes A/S)] buffered at pH 4.0 using citric acid (50 mM). To this,

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