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Structural and physiological changes associated with the skin spot disorder in apple

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ABSTRACT

Skin spot is an important physiological disorder of 'Elstar' apples (*Malus* × *domestica* Borkh.) that occurs after fruit have been removed from controlled atmosphere storage. Skin spots are irregular patches of small, round, brown blemishes. Cross-sections reveal a browning of protoplasts (coagulated) and of cell walls that extends into the hypodermis. Skin spots are always associated with linear, gaping and non-gaping microcracks in the cuticle. Staining of apple skin with calcofluor white usually results in white fluorescence of cell walls but, within a skin spot, the white fluorescence is weak or absent. Cell walls within, and in the immediate vicinity of skin spots stain with phloroglucin/HCl indicating the presence of lignin. The area of skin affected by skin spots was positively and linearly correlated with the area of the non-blush fruit surface infiltrated by acridine orange. In general, skin spots were limited to the non-blush fruit surface and occurred more frequently near the stem-end than the calyx region of the fruit. Skin spot areas were correlated with a 2.5-fold increase in water vapour permeability compared with unaffected areas ($23.8 \pm 4.0\ m\ s^{-1}$ with skin spots, $9.6 \pm 2.1 \times 10^{-5}\ m\ s^{-1}$ without skin spots). Strips of the fruit skin from regions with skin spots had an increased maximum force and modulus of elasticity. Dipping fruit in ascorbic acid (0.1 or 0.3 mM for 10 min) before storage decreased the area affected by skin spots. There was no effect of dipping in ethanol/water (70%, v/v, 15 min) or in solutions of captan ($1.5\ g\ L^{-1}$, 10 min) or trifloxystrobin ($0.1\ g\ L^{-1}$, 10 min). In contrast, prestorage treatment with 1-methylcyclopropene ($630\ nL\ L^{-1}$ for 24 h) or poststorage incubation in H_2O_2 (10% for 2, 6, 10 and 16 h) increased skin spots. Our data are consistent with a typical cell response to an oxidative burst that seems to be focussed on particular regions of the 'Elstar' fruit surface by concentrations of cuticular microcracks, and that is possibly caused by reoxygenation injury upon removal from CA storage.

1. Introduction

Skin appearance is an important quality criterion in most table fruit species and visible surface defects usually reduce their commercial value. Russeting in 'Golden Delicious' fruit is a well known example of this. Skin spot is a less well known, yet commercially important disorder that occurs in 'Elstar' apples (Hampson and Kemp, 2003), but occasionally is also found in 'Golden Delicious' fruit (Zanella, unpublished data). The physiological mechanisms underlying the formation of skin spots are largely unknown and effective counter-measures to control or reduce skin spots without compromising storability are not yet available.

Such information as is available is based largely on anecdote, is at times contradictory, and with few exceptions, has not been adequately documented in the scientific literature. Based on the reports available, the skin spot disorder of 'Elstar' is characterized by irregular, 'dot matrix' patches of small, round, brown individual skin spots that appear within two or three weeks of removal of fruit from controlled atmosphere storage (Köpcke et al., 2002; Veltman et al., 2003). The term skin spot is commonly used to refer to both the surface disorder as well as to the individual spots that form in these patches. The occurrence of skin spot is highly variable both on temporal and spatial scales. Skin spot incidence varies, within a fruit, from fruit to fruit within a tree, from tree to tree within an orchard and between orchards and seasons (Hampson and Kemp, 2003; Veltman et al., 2003; Köpcke, personal communication). Fruit from coastal sites in The Netherlands and Germany are believed to be more susceptible than those from inland sites (Köpcke, personal communication). Postharvest factors associated with increased skin spot incidence are higher storage temperatures (2 °C vs. 0.5 °C; Van Schaik, 1989), higher humidities (Van der Valk and Tomassen, 1999), higher CO_2-concentrations (0% vs. 1–4%; Van Schaik, 1989), 1-MCP treatment (Quast, 2004), and prolonged storage duration (Köpcke et al., 2002). Hennecke et al. (2008) reported

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that skin spot susceptibility decreased after a critical storage duration was exceeded. Dynamic controlled atmosphere storage (DCA) decreased skin spot when compared with controlled atmosphere storage (CA; Veltman et al., 2003; Hennecke et al., 2008). The antioxidant diphenylamine may decrease skin spot (Roelofs, 1997).

Preharvest factors also appear to affect skin spot. Fruit that is poorly colored and is harvested late in the season from the inner canopy of vigorous trees is considered especially susceptible (Van Schaik, 1995; Van der Valk and Tomassen, 1999; Quast, 2003; Hampson and Kemp, 2003). In addition, a high nitrogen supply to the trees and a high dry matter content of the fruit are thought to be associated with increased incidence (Van Schaik, 1995). Skin spot bears some similarities with stem end browning in 'Cox's Orange Pippin' and 'Topaz' fruit (McCormick and Streif, 2008; Lallu et al., 2010) and diffuse skin browning of 'Golden Delicious' apples (Gamrasni et al., 2010). As with skin spot, both disorders are accompanied by browning of the fruit surface and are reported to increase after 1-MCP treatment (McCormick and Streif, 2008; Gamrasni et al., 2010).

Based on the above, it is not possible to identify any simple physiological basis for the skin spot disorder and a better understanding is needed if we are to develop strategies to mitigate or eliminate commercial losses due to this and related disorders. The objectives of this study were: (1) to characterize 'Elstar' skin spot at a macroscopic and microscopic scale, (2) to identify correlated physical properties of the fruit surface such as water vapour permeability and skin mechanical characteristics, and (3) to study the effects of selected postharvest treatments on skin spot incidence.

2. Materials and methods

2.1. Plant materials

Mature 'Elstar' apple (*Malus* × *domestica* Borkh.) fruit from trees grafted on Malling 9 rootstocks were obtained from experimental or commercial orchards or experimental or commercial storage facilities in a 40 km radius around Jork (N: 53°31′, E: 9°40′), Altes Land, Germany. All fruit was grown according to current European regulations for integrated fruit production.

Unless specified otherwise, to increase the likelihood of finding skin spot we focussed on poorly colored fruit from second or third pickings that were subjected to various pretreatments before storage (see Section 2.2.5) or where conditions after CA storage were in the range 1.7–2.3 °C, 1.3–1.5% O_2 and 2.1–2.7% CO_2. Following removal from CA storage, fruit was held in refrigerated storage at 3–5 °C for a minimum period of 14 d. These conditions are representative of normal handling of 'Elstar' apples in commercial operations.

2.2. Experiments

2.2.1. Macroscopic and microscopic characteristics of skin spots

Fruit with typical skin spot symptoms were selected for macroscopic and microscopic investigations. Epidermal segments (ES) approximately 7 mm in diameter were excised by a tangential cut beneath a patch of skin spots using a sharp razor blade. The ES obtained comprise cuticle, epidermis and hypodermis and some parenchyma tissue. Cross-sections of the dermal system in the skin spot affected area were prepared by hand. Specimens were inspected in white, blue, and UV light using a fluorescence microscope (model BX-60; Olympus Europa Holding GmbH, Hamburg, Germany) or a dissecting microscope (MZ10F; Leica Mikrosysteme GmbH, Wetzlar, Germany), appropriate filter combinations (BX-60, filters U-MWU 330–385 nm excitation, ≥420 nm emission, U-MWB 450–480 nm excitation, ≥520 nm emission; Olympus; MZ10F, filters GFP-plus 480–440 nm excitation, ≥510 nm emission; UV 360–440 mm excitation, ≥420 emission; Leica), digital cameras (DP 71; Olympus), and image analysis software (cell^P; Olympus).

To identify microcracks in the fruit surface and modifications of the cell wall underlying skin spots, whole fruit were incubated for 10 min in 0.1% (w/w) acridine orange (Carl Roth GmbH, Karlsruhe, Germany) or 0.1% (w/w) calcofluor white made up in 0.03 M phosphate–citrate buffer at pH 8.0 (fluorescent brightener 28; Sigma–Aldrich Chemie GmbH, Munich, Germany). The infiltration was facilitated by application of a gentle vacuum. Thereafter, fruit was rinsed with deionized water (30 s) and blotted dry using soft tissue paper. ES were excised from the equatorial plane as described above, transferred to the microscope and inspected in incident white and UV light (MZ10F with filter GFP plus; Leica) and in transmitted white and incident blue light (BX-60 with filter U-MWB; Olympus).

Cross-sections through the fruit surface were hand-cut from regions with and without skin spot using a razor blade. Sections were viewed for tissue browning in transmitted white light (40×, BX-60; Olympus). Changes in cell walls within skin spots were investigated by staining with calcofluor white for 10 min as described above. After rinsing in deionized water, segments were viewed in transmitted white and incident UV light at 20× (BX-60, U-MWU filter; Olympus). Autofluorescence was studied using the U-MWB filter (Olympus). Lignin impregnation of cell walls was identified by incubating segments for 10 min in 2% (w/w) phloroglucin prepared in 95% (v/v) aqueous ethanol. After adding a droplet of 35% (v/v) HCl, specimens were viewed in transmitted white light (40×, BX-60; Olympus).

Relationships between microcracks and skin spots were established following infiltration of intact fruit with acridine orange as described above. Subsequently, ES with varying incidences of skin spot were excised from non-blush fruit surfaces using a razor blade. The areas of the ES infiltrated by fluorescent dye and the areas affected by skin spot were quantified (epifluorescence, 1.25×, MZ10F, GFP-plus filter; Leica; software cell^P, Olympus).

2.2.2. Distribution of skin spots on the fruit surface

The relationship between skin color and skin spot was studied using fruit selected postharvest but prestorage having a maximum range in blush. After CA and subsequent cold storage, fruit were cut along the stem/calyx axis to create eight similar sectors, the sectors were peeled and the boat-shaped areas of skin were flattened onto a glass plate. We refer to the colored site of the fruit presented to the sun during growth as the blushed cheek or area, and to the green/yellow background colored areas less exposed to direct sunlight as the non-blush surface. The blushed areas and those affected by skin spot were painted using acrylic paint. A calibration scale was included and digital photographs were taken (Kodak EasyShare P880; Eastman Kodak Company, Rochester, NY, USA). Total fruit surface area, the blush and non-blush areas with and without skin spot were quantified on a total of 141 fruit (software cell^P; Olympus). Preliminary experiments established that the optical contrast between areas with and without skin spot was insufficient for unambiguous automatic detection and quantification.

For studying the distribution of skin spot along the stem/calyx axis, fruit were selected poststorage having skin spot incidence ranging from <5% to >50%. The distribution of skin spot along the stem/calyx axis was evaluated by slicing fruit perpendicularly to the stem/calyx axis into four pieces of equal thickness. The areas affected by skin spot and the non-blush surface areas were quantified by image analysis as described above. The four regions along the stem/calyx axis are referred to as stem, stem center, calyx center and calyx region. The analysis was performed on a total of 38 fruit.