Deposition and Strain of the Cuticle of Developing European Plum Fruit

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Abstract. Time courses of change in 1) fruit mass and surface area, 2) deposition of the cuticular membrane (CM), 3) strain of the CM, and 4) formation of microcracks in the CM of developing fruit of European plum (Prunus domestica L. ssp. domestica) were established. Fruit mass, fruit surface area, and CM mass per fruit increased between 50 and 133 days after full bloom (DAFB). Rates of CM deposition were higher during early stage III (50–71 DAFB) when amounts of wax and cutin per fruit increased, resulting in an increase in CM thickness from 3.1 to 5.9 g m⁻². Thereafter, cutin deposition ceased and CM thickness decreased to 4.7 g m⁻² at 133 DAFB. Percentage strain, determined on enzymatically isolated CM disks using image analysis, slightly decreased from 12.0% at 50 DAFB to 4.55% at 71 DAFB, but increased thereafter, averaging about 40% at 133 DAFB. The breakpoint in the time course of strain at 71 DAFB corresponded to the change in rate of cutin deposition. Frequency of microscopic cracks in the CM was closely related to strain of the CM across different developmental stages within a cultivar (pedicel end and cheek region) and across different cultivars at maturity. There was little change in microscopic cracking up to ~20% strain. However, microcracks markedly increased when strain exceeded 20%. Most microcracks (91.0% ± 3.7% at 133 DAFB) were associated with stomata. These data indicate that a mismatch between surface area expansion of the growing fruit and cutin deposition caused strain and subsequent microcracking of the CM of developing plum.

The cuticular membrane (CM) covers all aboveground, primary surfaces of terrestrial plants. It serves as a protective barrier against water loss, infection with pathogens, and mechanical damage. Maintaining these functions throughout development requires an intact CM. This is a particular challenge for the CM on fruit that often are characterized by continuous surface expansion until maturity. Recent investigations in sweet cherry (Prunus avium L.) established that the CM is markedly strained during stage III [“final swell” (Littleland and Newsome, 1934)] of fruit development, which is characterized by a rapid increase in fruit mass and, hence, surface area in the absence of CM deposition (Knoche et al., 2004). Furthermore, the resulting strain of the CM is closely and positively related to frequency and severity of microscopic cracks in the CM (Knoche and Peschel, 2006). Cracks impair the barrier function of the CM and serve as entry ports for fruit rot pathogens, including Botrytis cinerea (Pers. and Exocarp segments (Aderh. & Ruhland) Honey 1945 (Borve et al., 2000). Cracks increase the barrier against water loss, infection with pathogens, and mechanical damage. Maintaining these functions throughout development requires an intact CM. This is a particular challenge for the CM on fruit that often are characterized by continuous surface expansion until maturity. Recent investigations in sweet cherry (Prunus avium L.) established that the CM is markedly strained during stage III [“final swell” (Littleland and Newsome, 1934)] of fruit development, which is characterized by a rapid increase in fruit mass and, hence, surface area in the absence of CM deposition (Knoche et al., 2004). Furthermore, the resulting strain of the CM is closely and positively related to frequency and severity of microscopic cracks in the CM (Knoche and Peschel, 2006). Cracks impair the barrier function of the CM and serve as entry ports for fruit rot pathogens, including Botrytis cinerea (Pers. and Exocarp segments (Aderh. & Ruhland) Honey 1945 (Borve et al., 2000). Cracks increase the barrier against water loss, infection with pathogens, and mechanical damage. Maintaining these functions throughout development requires an intact CM. This is a particular challenge for the CM on fruit that often are characterized by continuous surface expansion until maturity. Recent investigations in sweet cherry (Prunus avium L.) established that the CM is markedly strained during stage III [“final swell” (Littleland and Newsome, 1934)] of fruit development, which is characterized by a rapid increase in fruit mass and, hence, surface area in the absence of CM deposition (Knoche et al., 2004). Furthermore, the resulting strain of the CM is closely and positively related to frequency and severity of microscopic cracks in the CM (Knoche and Peschel, 2006). Cracks impair the barrier function of the CM and serve as entry ports for fruit rot pathogens, including Botrytis cinerea (Pers. and Exocarp segments (Aderh. & Ruhland) Honey 1945 (Borve et al., 2000). Cracks increase the barrier against water loss, infection with pathogens, and mechanical damage. Maintaining these functions throughout development requires an intact CM. This is a particular challenge for the CM on fruit that often are characterized by continuous surface expansion until maturity. Recent investigations in sweet cherry (Prunus avium L.) established that the CM is markedly strained during stage III [“final swell” (Littleland and Newsome, 1934)] of fruit development, which is characterized by a rapid increase in fruit mass and, hence, surface area in the absence of CM deposition (Knoche et al., 2004). Furthermore, the resulting strain of the CM is closely and positively related to frequency and severity of microscopic cracks in the CM (Knoche and Peschel, 2006). Cracks impair the barrier function of the CM and serve as entry ports for fruit rot pathogens, including Botrytis cinerea (Pers. and

Materials and Methods

Plant material. Fruit of European plum cultivars Auerbacher, Cacaks Fruchtbar, Elena, President, Valjevka, and Verity (‘Auerbacher’ grafted on ‘Myrobalane’ rootstocks; all others grafted on ‘GF 655’) were obtained from commercial orchards located near Eisleben, Germany (lat. 51°31’N, long. 11°44’E). Fruit were harvested at commercial maturity except for ‘Valjevka’, which was sampled weekly or at 2-week intervals between 50 and 133 d after full bloom (DAFB). Fruit were selected for uniformity of development (based on color and size) and freedom from visual defects and were processed fresh.

Fruit development. Fruit mass and surface area were monitored in developing ‘Valjevka’ fruit. Surface area ($A_{fruit}$) was calculated from the polar radius ($a$, equivalent to fruit height divided by two) and equatorial radius ($b$), assuming the shape of a prolate ellipsoid:

$$A_{fruit} = 2\pi b \left( b + \frac{a^2}{\sqrt{a^2 - b^2}} \right) \cdot \frac{ar \sin(\sqrt{a^2 - b^2})}{a}$$

[1]

Fruit height and equatorial diameter were determined on 20 fruit using an electronic caliper. Osmotic potential of fruit juice extracted from the mesocarp was determined by vapor pressure osmometry (model 5520; Wescor, Logan, UT). Juice samples from five individual fruit were pooled for a single observation, and determinations were carried out on five independent replicates.

Cuticular membrane isolation. Exocarp segments comprising cuticle, epidermal and hypodermal cell layers, and...
adhering mesocarp tissue were excised from the cheek region of plum using a cork borer (i.d., 8.9 mm). Cuticular membranes were isolated from exocarp segments as described previously (Knoche and Peschel, 2006). Briefly, exocarp segments were incubated in 50 mM citric acid buffer solution (pH, 4.0) containing pectinase [90 mL·L⁻¹ (Panzym Super E flüssig; Novozymes AG, Dittingen, Switzerland)] and cellulase [5 mL·L⁻¹ (Celluclast; Novozymes, Bagsvaerd, Denmark)]. NaN₃ was added at a final concentration of 30 mM to prevent microbial activity. After separation and isolation, CM were desorbed in deionized water and dried. Soluble cuticular lipids were quantified by batch extracting CMs with 10 consecutive changes of 0.5 h each in CHCl₃/methanol (1:1, v/v) at 80 °C. Extracted CMs are referred to as dewaxed CM (DCM). Mass of CM or DCM was determined gravimetrically on a minimum of 15 CM/DCM disks per replicate, with a total of five replications.

Strain of CM disks was quantified as described previously using image analysis (Knoche et al., 2004). Digitized calibrated images of individual CM disks were prepared at ×4 (MZ6 microscope; Leica Mikrosysteme GmbH, Bensheim, Germany) using a video camera (Hitachi Denshi Europa GmbH, Rodgau, Germany), the perimeter of the CM disk traced, and the CM area quantified (analySIS 3.0 software package; Soft Imaging System GmbH, Münster, Germany). Strain [ε (%)] was calculated according to Eq. 2, where A represents the surface area of the CM covered on the fruit before excision and A₀ is the area of the relaxed CM after isolation:

\[ ε = \frac{A - A₀}{A₀} \cdot 100 \]  

Because the diameter of the cork borer was small relative to the size of the fruit, the cross-sectional area of the cork borer was used as an estimate for A. Numbers of replications were 5 and 10 for quantifying CM mass per unit area and CM strain respectively.

**Monitoring microscopic cracks in the cuticle of developing fruit.** Formation of microscopic cracks, subsequently referred to as microcracks, was monitored in developing ‘Valjevka’ fruit and in mature fruit of selected plum cultivars using the procedure by Peschel and Knoche (2005). These microcracks are limited to the CM, do not extend into the epi- and hypodermal cell layers or the mesocarp of the fruit, and are only detected by microscopy (Glenn and Poovaiah, 1989; Peschel and Knoche, 2005). Uniformly developed fruit free of microbial activity. After separation and isolation, CM were transferred to the stage of a fluorescence microscope (model BX-60; Olympus, Hamburg, Germany) and viewed at ×100 (filter module U-MWU, 330–385 nm excitation wavelength, 420 nm emission wavelength; Olympus). Five randomly selected areas (621 × 468 μm²) per exocarp segment of a total of 20 fruit per sampling date, region, and cultivar were inspected for microcracks in the CM. Microcracking was quantified using the rating scheme previously adopted for sweet cherry fruit (see Fig. 1 in Peschel and Knoche, 2005), where 0 is surface without cracks, 1 is a single microcrack shorter than the longest dimension of adjacent epidermal cells, 2 is more than one microcrack shorter than the longest dimension of adjacent epidermal cells or single microcrack longer than the longest dimension of adjacent epidermal cells, 3 is less than eight microcracks longer than the longest dimension of adjacent epidermal cells, and 4 is eight or more microcracks longer than the longest dimension of adjacent epidermal cells. Arithmetic means and s.e. of means were calculated.

**Data analysis and presentation.** Data are presented as means and s.e. of means. Linear and nonlinear regression and correlation analyses were carried out on treatment means using SAS (version 9.1; SAS Institute, Cary, NC).

**Results**

Fruit mass increased and osmotic potential (Ψₛ) of the mesocarp decreased with time between 50 and 133 DAFB (Fig. 1A). The observed change in fruit mass with time corresponded to stage III of stone fruit development. Fruit mass and Ψₛ were closely related (r = −0.98, P ≤ 0.0001). Fruit surface area increased nearly linearly with time, indicating that the rate of surface area expansion remained constant between 50 and 133 DAFB (0.39 ± 0.01 cm²·d⁻¹; r² = 0.99, P ≤ 0.0001; Fig. 1B).

The mass of CM per unit fruit surface area changed in a biphasic pattern with time (Fig. 2A). Between 50 and 71 DAFB,
CM mass per unit area increased from 3.1 to 5.9 g m\(^{-2}\) (equivalent to about 138 mg m\(^{-2}\) d\(^{-1}\); \(r^2 = 0.99, P \leq 0.0021\)). Thereafter, however, CM mass per unit area continuously decreased (−20 mg m\(^{-2}\) d\(^{-1}\); \(r^2 = 0.87, P \leq 0.0007\)), averaging 4.7 g m\(^{-2}\) at 133 DAFB (Fig. 2A). The initial increase (50 to 71 DAFB) was accounted for by both an increase in wax (50 mg m\(^{-2}\) d\(^{-1}\); \(r^2 = 0.96, P \leq 0.0188\)) and cutin deposition (88 mg m\(^{-2}\) d\(^{-1}\); \(r^2 = 0.99, P \leq 0.0048\)), and the net decrease thereafter (71 to 133 DAFB) by a continuing increase in wax deposition (10 mg m\(^{-2}\) d\(^{-1}\); \(r^2 = 0.83, P \leq 0.0015\)) and a simultaneous decrease in cutin deposition per unit area (−30 mg m\(^{-2}\) d\(^{-1}\); \(r^2 = 0.95, P \leq 0.0001\)).

There was little difference in CM mass per unit area between different regions of the fruit surface (Table 1). Assuming the cheek region to be representative of the entire fruit surface, the amount of CM on a whole-fruit basis was calculated from CM mass in the cheek region and fruit surface area (Fig. 2B). These calculations revealed that CM mass per fruit increased rapidly initially (up to 71 DAFB), but thereafter at a decreased rate. Up to 71 DAFB, the increase in CM mass was accounted for by both increased cutin and increased wax deposition. From 71 DAFB to maturity, the amount of cutin per fruit remained about constant, but wax deposition continued to increase.

Percentage strain of the CM decreased from 12.0% to 4.5% between 50 and 71 DAFB, but increased thereafter to about 40% at maturity (Fig. 3A). Qualitatively similar relationships were obtained when expressing CM strain as a function of surface area (Fig. 3A, inset). Plotting CM strain versus CM mass per unit area also revealed a biphasic relationship. The initial increase in CM mass per unit area (50 to 71 DAFB) was accompanied by decreasing strain of the CM. However, as CM mass per unit area decreased thereafter (71 and 133 DAFB), strain of CM increased (Fig. 3B). The breakpoint in this

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**Table 1.** Mass of the cuticular membrane (CM) per unit fruit surface area in selected regions of mature ‘Valjevka’ European plum.

<table>
<thead>
<tr>
<th>Region</th>
<th>CM mass [mean ± SE (g m(^{-2})]</th>
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</thead>
<tbody>
<tr>
<td>Pedicel end</td>
<td>4.67 ± 0.04 c†</td>
</tr>
<tr>
<td>Cheek</td>
<td>4.86 ± 0.03 bc</td>
</tr>
<tr>
<td>Stylar end</td>
<td>5.21 ± 0.10 a</td>
</tr>
<tr>
<td>Suture</td>
<td>5.09 ± 0.09 ab</td>
</tr>
<tr>
<td>Grand mean</td>
<td>4.96 ± 0.06</td>
</tr>
</tbody>
</table>

*Mean separation by Tukey’s studentized range test at \(P \leq 0.05\).
relationship corresponded to the change in rate of cutin deposition at 71 DAFB (Fig. 2).

There was little microcracking of the CM up to 92 and 106 DAFB in pedicel end and cheek region respectively. Thereafter, however, microcracking markedly increased. Rating scores were always higher for the pedicel end than the cheek region (Fig. 4A). Plotting rating scores for microcracking in the cheek region versus strain of the CM in that region yielded a sigmoidal relationship across different developmental stages of ‘Valjevka’ fruit, but also across selected cultivars at maturity respectively (Knoche and Peschel, 2006). Third, in both species the extent of microcracking increased toward maturity, when strain of the CMs was high. In European plum, the bimodal relationship between rating scores for microcracking and strain of the CM indicated that beyond a threshold of critical strain ($\varepsilon_{\text{crit}}$) of about 20%, microcracking markedly increased. Performing the same analysis in sweet cherry yielded a similar threshold for the relationship between microcracking and elastic strain of the CM in the cheek region (M. Knoche, unpublished data). Quantitatively, there was more microcracking (i.e., a higher frequency of fruit with microcracks or more severe microcracking in European plum

**Discussion**

Our data established that cuticle deposition did not keep pace with fruit surface expansion in developing European plum. Furthermore, deposition of the two major CM constituents—wax and cutin—differed. Up to about 71 DAFB, deposition of cutin and wax occurred concurrently, but thereafter deposition of cutin nearly ceased, resulting in a thinning of the cutin matrix on the enlarging fruit surface. In contrast, wax deposition continued at a constant rate until maturity. Because the cutin matrix is cross-linked and, therefore, largely responsible for the mechanical properties of the CM (Petracek and Bukovac, 1995), a close relationship between cutin deposition, fruit surface expansion, and strain was obtained. During early stage III (up to about 71 DAFB), cutin deposition was sufficiently high to prevent thinning and strain of the CM on the enlarging surface. However, when cutin deposition ceased around 71 DAFB, CM thickness decreased and strain of the CM increased as the fruit surface expanded. The increase in strain resulted in microcracking of the CM. Currently, it is not known why cutin deposition ceased, but wax deposition continued to occur throughout development.

CM deposition in developing European plum resembled that in sweet cherry in several aspects. First, CM deposition in both species did not keep pace with surface expansion. However, the pattern of cutin and wax deposition slightly differed. In Plum, cutin deposition ceased not before mid stage III (>71 DAFB), but ceased in sweet cherry as early as stage II (Peschel et al., 2007). Also in plum, wax deposition occurred throughout development, resulting in an increase in wax content from 20.9% to 48.7% between early stage III and maturity, whereas in sweet cherry wax content of the CM remained essentially constant throughout stage III [29.7% vs. 30.5% for early stage III vs. maturity respectively (Knoche et al., 2001)]. To our knowledge, chemical analyses of wax and cutin fractions of the plum CM have not been published. For the sweet cherry CM, such analyses revealed that the change in amount of wax per fruit was the net effect of opposing processes (i.e., increasing alcohols and alkanes, but little change in triterpenes)(Peschel et al., 2007). Second, CMs of mature plum and sweet cherry fruit are markedly strained (Knoche et al., 2004). Percentage strain in European plum was lower than that reported for sweet cherry [40% vs. up to 100% and more for ‘Valjevka’ plum vs. ‘Summit’ sweet cherry respectively (Knoche and Peschel, 2006)]. Third, in both species the extent of microcracking increased toward maturity, when strain of the CMs was high. In European plum, the bimodal relationship between rating scores for microcracking and strain of the CM indicated that beyond a threshold of critical strain ($\varepsilon_{\text{crit}}$) of about 20%, microcracking markedly increased. Performing the same analysis in sweet cherry yielded a similar threshold for the relationship between microcracking and elastic strain of the CM in the cheek region (M. Knoche, unpublished data). Quantitatively, there was more microcracking (i.e., a higher frequency of fruit with microcracks or more severe microcracking in European plum
compared with sweet cherry) (Peschel and Knoche, 2005). The reason for these differences are unknown. In both species, more microcracking occurred in the pedicel end than in the cheek region (Peschel and Knoche, 2005). This would be expected because 1) the pedicel end region has a higher curvature than the cheek and 2) the strongest structure for a given "shell" morphology is the one with the least curvature (i.e., a perfect sphere) (Considine and Brown, 1981). Interestingly, in plum, essentially all microcracks were associated with stomata (Knoche and Peschel, 2005), confirming earlier observations by Mrozek and Burkhardt (1973) and Storey and Price (1999). In sweet cherry, however, microcracks were rarely associated with stomata and occurred mostly above periclinal cell walls of epidermal cells (Peschel and Knoche, 2005). Preferential formation of microcracks at lenticels was reported for grape [Vitis vinifera L. (Brown and Considine, 1982)]. Here, lenticels represented areas of limited extensibility in the extensible epidermis causing stress concentration and hence, crack formation in the vicinity of lenticels. In European plum, cell-to-cell adhesion of guard cells represented the weakest link, because microcracks commenced at and extended along the length axis of the stomatal aperture (Fig. 5).

From a practical point of view, two questions arise: Are microcracks in the CM of developing fruit of European plum important? And, if so, what can be done to avoid microcracking? Microcracks impair the barrier function of the CM. In sweet cherry fruit, microcracks facilitate infection with fruit rot pathogens such as B. cinerea and M. laxa (Borve et al., 2000), allow rapid uptake of water and subsequent fruit cracking (Glenn and Poovaiah, 1989; Peschel and Knoche, 2005), and increase transpiration (Knoche and Peschel, 2007). We expect similar relationships for European plum, for which Monilinia fruit rot and fruit cracking pose serious limitations to crop production, particularly during wet growing seasons. Also, russeting and russet scab of plum are likely to be related to microcracking of the CM, possibly as a result of exposure of the fruit surface to liquid water or high concentrations of water vapor (Knoche and Peschel, 2006; Michailides, 1991). Furthermore, increased transpiration as a result of strain cracking of the CM may be involved in shriveling of the pedicel region of European plum, a disorder that regularly occurs in southern Germany. These arguments demonstrate that microcracking of the CM is important in European plum.

The data presented herein demonstrate that strain of the CM represents the driving force for microcracking and strain, in turn, results from a mismatch of fruit surface expansion and CM deposition. Strategies to avoid microcracking of the CM should therefore focus on synchronizing CM deposition and fruit surface expansion. Whether this can be achieved by breeding approaches or by cultural means (e.g., spray application of gibberellins on developing fruit as recently demonstrated in tomato (Lycopersicon esculentum Mill.) (Knoche and Peschel, 2007)) merits investigation.

Table 2. Properties of the fruit cuticular membrane (CM) of selected cultivars of European plum at commercial maturity.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Mass [mean ± SE (g per fruit)]</th>
<th>CM mass [mean ± SE (g m⁻²)]</th>
<th>Strain [mean ± SE (%)]</th>
<th>Microcracks [mean ± SE (rating)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auerbacher</td>
<td>24.1 ± 0.6 d*</td>
<td>5.59 ± 0.05 b</td>
<td>22.9 ± 2.4 c</td>
<td>2.5 ± 0.1 b</td>
</tr>
<tr>
<td>Cacaks Fruchtbare</td>
<td>30.6 ± 0.9 c</td>
<td>4.44 ± 0.07 d</td>
<td>36.8 ± 1.5 b</td>
<td>3.4 ± 0.1 a</td>
</tr>
<tr>
<td>Elena</td>
<td>26.7 ± 0.9 cd</td>
<td>7.78 ± 0.04 a</td>
<td>23.9 ± 0.8 c</td>
<td>0.9 ± 0.2 c</td>
</tr>
<tr>
<td>President</td>
<td>65.8 ± 1.7 a</td>
<td>4.39 ± 0.05 d</td>
<td>34.5 ± 1.9 b</td>
<td>2.7 ± 0.2 b</td>
</tr>
<tr>
<td>Valjevka</td>
<td>38.7 ± 0.9 b</td>
<td>4.84 ± 0.05 c</td>
<td>42.3 ± 4.4 ab</td>
<td>3.5 ± 0.1 a</td>
</tr>
<tr>
<td>Verity</td>
<td>41.3 ± 1.1 b</td>
<td>4.13 ± 0.05 e</td>
<td>49.6 ± 1.8 a</td>
<td>2.4 ± 0.2 b</td>
</tr>
</tbody>
</table>

*Mean separation within columns by Tukey’s studentized range test at P ≤ 0.05.

Cuticular mass (CM), strain, and microcracking were determined in the cheek region. For details on the rating scheme of microcracking, see Materials and Methods.

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Fig. 5. (A–D) Fluorescence light micrographs of epidermal segments showing microcracks in the cuticle of 'Valjevka' European plum fruit. (A) Initiation of microcrack above common anticlinal wall of a pair of guard cells. Extending microcracks (B, C) that merge into larger, branched cracks involving several stomata (D). Magnification: ×200 (A–C), ×100 (D).

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Literature Cited


