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Research Note

Water induces microcracks in the grape berry cuticle

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Introduction: Bunch rots and berry cracking are major limitations in viticulture in humid climate that reduce yield and compromise quality of must and vine (Meneguzzo *et al*. 2008). Microscopic cracks (microcracks) in the cuticular membrane (CM) play a critical role because they impair the barrier function of the CM in pathogen defense and water transport and function as stress concentrators that weaken the exocarp (Considine 1982). Microcracking of the CM occurs in many fruit crops and surface moisture on a strained CM is a critical factor in microcracking (Knoche and Peschel 2006). The CM of grape berries (*Vitis vinifera* L.) is strained (Becker and Knoche 2012), too, and therefore, water on the surface is expected to aggravate microcracking also in grape berries.

The objective of this study was to 1) characterize the distribution of microcracks on the berry and 2) establish the effect of water, temperature, and humidity on formation of microcracks in the cuticle of 'Riesling' berries.

Material and Methods: 'Riesling' grape berries (*Vitis vinifera* L.) were sampled from experimental and commercial orchards at Neustadt an der Weinstrasse, Germany (lat. 49°21'N, long. 8°8'E) and Höhnstedt, Germany (lat. 51°51'N, long. 11°74'E) at commercial maturity and held in cold storage (2 °C, 75 % relative humidity, RH) for up to seven days. Berries of uniform size and maturity without visible defects were selected randomly.

The distribution of microcracks on grape berries was analyzed by staining berries with the fluorescent dye acridine orange (0.1 % w/v) for 10 min. Thereafter, berries were rinsed with deionized water for 10 s and blotted with tissue paper (Kimtech Science; Kimberly-Clark, Surrey, UK). Exocarp segments (ES) consisting of the CM, epidermis, hypodermis, and some adhering parenchyma, were excised using a razor blade and viewed at 100x by microscopy (Ortholux II; Ernst Leitz, Wetzlar, Germany; 390-490 nm excitation wavelength, ≥ 515 nm emission wavelength). The number of microcracks within a 2.1 mm^2 window of the microscope was quantified. Using this procedure the frequency of microcracks in the stylar scar, cheek, and pedicel end regions was determined in six views per region on a total of 20 ES (one ES per berry). The effect of berry orientation was studied by inspecting ten randomly selected views in the stylar scar or cheek region on a total of ten ES (one ES per berry; for cheek and stylar scar region see Fig.1A in Becker and Knoche 2012).

Water induced microcracks were quantified using a laboratory based assay (Knoche and Peschel 2006). Unless specified otherwise a stainless steel washer (inner-diameter 4.3 mm) was mounted on a berry in the cheek or the stylar scar region using a cyanacrylate glue (Loctite 406; Henkel, München, Germany). After curing, ES were prepared by cutting underneath the washer using a razor-blade. The washer preserved the strain of the ES. Subsequently, the dye solution was applied to the surface of the ES exposed in the washer. After 10 min, the solution was removed, the ES transferred to the microscope (BX-60; Olympus, Hamburg, Germany; filter set U-MWU, excitation wavelength: 330-385 nm, emission wavelength: ≥ 420 nm) and the number of microcracks within the washer quantified. The ES with washers attached were then incubated in a Petri dish such that the surface of the ES was either exposed to water or to the ambient atmosphere (22 °C; 41 % RH), while the inner side was always in contact with liquid water. After 72 h, the ES were removed from the Petri dish, stained again and the number of microcracks re-established as described above. This procedure allowed to calculate the change in frequency of microcracks during the incubation period on an individual ES basis. The time course (n = 17-19 ES), the humidity (0 to 100 % RH at 22 °C, n = 12-17 ES), and temperature responses (5 to 35 °C, n = 14-20 ES) of water induced microcracking were established. Defined humidities above the ES were established using dry silica (0 % RH) or salt slurries of $CaCl_2$ (30 % RH), NaCl (75 % RH), KCl (85 % RH) and KNO_3 (93 % RH) and deionized water (100 % RH; Wexler 1995). Data were subjected to analysis of variance or regression analysis (Statistical Analysis System software package, SAS version 9.1.3; SAS Institute Inc., Cary, N.C.) and presented as means ± SE.

Results and Discussion: The frequency of microcracks differed between regions of the berry. It was highest in the stylar scar region (2.31 ± 0.49 No. mm^{-2}), lowest on the cheek (0.05 ± 0.04 No. mm^{-2}), and increased again towards the pedicel end (0.18 ± 0.06 No. mm^{-2}).

Microcracking in the stylar scar, but not in the cheek region, depended on berry orientation. 'Standing' berries in a bunch with the stylar scar facing the sky had fewer microcracks than those facing the ground or oriented towards the side (Table). Within a bunch, areas of contact between neighboring berries had more microcracks than those on the same berry without contact (cheek: 0.63 ± 0.04 vs. 0.22 ± 0.02 No. mm^{-2}).

Exposing the surface of excised ES to water increased the frequency of microcracks in the stylar scar region (Figure A). There was little change in microcracking when the surface of the ES remained dry. Increasing humidity above the ES or increasing temperature increased microcracking (Figure B, C). Qualitatively similar data at a lower frequency of microcracks were obtained for the cheek (Becker 2010, data not shown). Generally, berries from the

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Table

Number of microcracks per unit surface area in the cheek or stylar scar region of 'Riesling' berries as affected by orientation of the berry in the cluster. Orientation was varied by selecting berries with the stylar scar oriented towards the sky ('top'), towards the side ('side') or the ground ('bottom')

	microcracks (No. per mm²)			
	Orientation			
region	top	side	bottom	means
Stylar scar	0.44 (± 0.44) b	1.30 (± 0.92) a	1.35 (± 0.72) a	1.03 (± 0.11)[a]
cheek	0.02 (± 0.04) c	0.04 (± 0.07) c	0.05 (± 0.10) c	0.04 (± 0.01)
means	0.23 (± 0.06)	0.46 (± 0.10)	0.70 (± 0.13)	

[a] Interaction region x orientation significant at a probability level of $P < 0.001$. Mean separation by Duncan's multiple range test at $P = 0.05$.

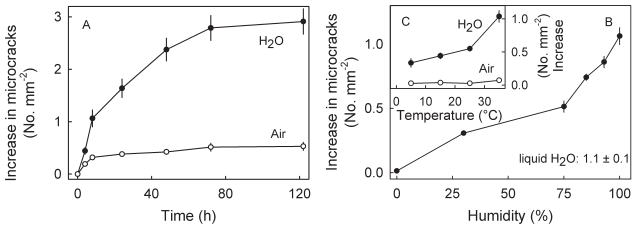

Figure: (**A**) Time course of water induced microcracking of the cuticle of 'Riesling' berries. The surface of excocarp segments (ES) excised from the stylar scar region was exposed to water ('H_2O') or the ambient atmosphere ('Air'). Effect of exposing the surface of ES to different relative humidities (**B**) or to water at different temperatures (**C**) on microcracking. The increase in microcracks was calculated as the difference in microcracks before and after exposure to water or water vapor. Bars represent standard errors of the mean. Where not shown, they were smaller than data symbols.

Neustadt site had more water induced microcracks than those from Höhnstedt (Figure A *vs.* B). Across experiments was the increase in microcracks per unit surface area positively related with the number of microcracks present before experimental exposure to water ($r = 0.51$***).

These data demonstrate that water on or high humidity above the berry surface increased microcracking of the CM. Similar observations were made in sweet cherry where water induced microcracking was attributed to an increased elasticity and susceptibility to fracture of the strained and fully hydrated CM (Knoche and Peschel 2006). These factors may also apply to the grape berry CM. Uptake of water by epidermal cells and bursting of cells was unlikely involved. First, the cut surface of the ES was always in contact with water thereby allowing free uptake regardless of the nature of the donor, *i.e.*, water *vs.* ambient atmosphere. Second, when exposing the outer surface of the ES to a polyethylene glycol 6000 solution (PEG) that was isotonic to the solute potential of expressed berry juice (-1.9 MPa), water induced microcracking was even increased as compared to water (1.19 ± 0.06 *vs.* 0.93 ± 0.02 No. mm^{-2} for PEG vs. water, respectively). Since a driving force for water uptake into the berry from an isotonic PEG solution is absent, water uptake into cells is unlikely to be a factor.

Surface wetness was also a factor in microcracking on berries collected in the field (no experimental exposure to water). For example, the effect of berry orientation on microcracking in the stylar scar region is accounted for by differences in the surface wetness duration. In a "standing" berry, where microcracking was low, surface wetness is shorter as compared to a berry facing the ground where a pending droplet collects at the tip of the berry thereby extending the wetness period and increasing microcracking. Also, areas of contact between neighboring berries have prolonged periods of surface wetness, continuous exposure to high humidity, and, hence, more microcracks.

A possible reason for the higher frequency of microcracks in the stylar scar region compared to the cheek is the greater stiffness of the periderm of the scar relative to the surrounding epidermis, that causes stress concentration and failure in the vicinity of the scar (Brown and Considine 1982).

From a practical point of view these findings are particularly important for cultivars having compact clusters, where the inner surface of the cluster is exposed to high water vapor concentrations and, possibly, extended periods of surface wetness (Vail and Marois 1991). Under these conditions, cultural measures that reduce surface wetness duration and water vapor concentration inside clusters are likely to decrease microcracking and, hence, the susceptibility to bunch rots and berry cracking.

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