Einfluss von Fruchtwachstum, Kutikulaentwicklung und Wassertransport auf das Platzen von Weinbeeren als Grundlage für die Verringerung des Befalls durch Traubenfäulen.

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1. Zusammenfassung

Im Weinbau wurde in den vergangenen Jahren verstärkt ein durch Regen verursachtes Aufplatzen von Weinbeeren (*Vitis vinifera* L.) beobachtet, das deutliche Ertragsreduzierungen verursachte. Aufgeplatzte Beeren werden leicht durch Fäulniserreger wie *Botrytis cinerea* oder *Penicillium expansum* besiedelt, welche anschließend die komplette Traube befallen können. Insbesondere die Wasserbalance der Beere und die Beschaffenheit der Fruchthaut beeinflussen das Aufplatzen. Diese beiden Faktoren wurden im Rahmen dieser Arbeit an drei für den deutschen Anbau wichtigen Weißweinsorten (‚Chardonnay', ‚Müller-Thurgau' und ‚Riesling') untersucht.

Gravimetrische Messungen und davon abgeleitete Modellrechnungen an in Wasser untergetauchten Weinbeeren zeigten, dass die Wasseraufnahme über die Oberflächen von Stiel und Haut an einem Regentag bis zu 38% der Gesamtwasseraufnahme in eine Weinbeere ausmachen kann. Messungen an Beeren, bei denen die Wasseraufnahme mittels Silikonkleber auf bestimmte Regionen der Frucht beschränkt wurde ergaben, dass zwischen 55 und 80% der Wasseraufnahme über die Oberfläche des Stiels einer Beere stattfindet.

Messungen der Änderungen der elastischen Dehnung der Kutikula im Verlauf der Entwicklung zeigten, dass diese ab dem Beginn des Weichwerdens bis zur Erntereife der Beeren bei ‚Müller-Thurgau' und ‚Riesling' um 20,7% bzw. 18,4% gedehnt wird. Diese Dehnung resultierte in erster Linie aus einer verlangsamten Cutin-Produktion.

Die Dehnung führte zu einer Zunahme an mikroskopisch kleinen Rissen in der Kutikula der Beeren. Mikrorisse ließen sich durch Wasser bzw. eine hohe relative Luftfeuchtigkeit (>75%) sowie eine Erhöhung der Umgebungs-Temperatur induzieren. Beeren, die in der Traube mit der Narbe Richtung Seite oder zum Boden orientiert waren wiesen eine höhere Mikrorisszahl in der Narbenregion auf als mit der Narbe nach oben orientierte Beeren. Der Grund hierfür ist die Bildung eines Wassertropfens an der Narbe bei den seitlich bzw. nach unten orientierten Beeren und damit eine verlängerte Einwirkdauer von Feuchtigkeit. Wasser das sich zwischen benachbarten Beeren sammelt erhöht ebenfalls die Anzahl an Mikrorissen auf den betroffenen Backenseiten. Diese Seiten trocknen im Vergleich zu Backen, die keinen Kontakt mit benachbarten

Beeren haben langsamer ab. Die mikroskopisch kleinen Risse können sich zu makroskopisch sichtbaren Rissen entwickeln und die Beeren platzen auf.

Diese Ergebnisse lassen darauf schließen, dass Strategien, die darauf abzielen, dass die Fruchtstände schneller abtrocken, wie bsp. das bereits angewandte Entblättern der Traubenzone zur Pathogen-Kontrolle oder die Züchtung weniger kompakter Trauben das größte Potential haben, um die Platzanfälligkeit der Weinbeeren zu reduzieren.

Schlagwörter: Platzen, Wassertransport, Mikrorisse

2. Abstract

Rain induced cracking of grape berries (*Vitis vinifera* L.) increased during the last years leading to essential yield losses. Cracked berries are easily infected by mold producing pathogens like *Botrytis cinerea* or *Penicillium expansum*. Starting from a cracked berry the mold spreads fast throughout the whole bunch. The stability of the fruit skin and the water balance of the berry are important factors in fruit cracking. Therefore these factors were analyzed using three important cultivars (‘Chardonnay’, ‘Müller-Thurgau’, ‘Riesling’) in german viticulture.

Calculations based on gravimetrically determined water uptake of detached, submerged grape berries showed that on a rainy day up to 38% of total uptake occurred through the surfaces of berry and stem. Sealing parts of the berry with silicone rubber, thereby restricting water uptake to certain regions, revealed that 55 to 80% of water uptake through the surface is by stem.

Analyzing the changes in elastic strain of the cuticle throughout development showed that the cuticles of ‘Müller-Thurgau’ and ‘Riesling’ are strained by 20.7 and 18.4%, respectively, between veraison (start of berry softening) and maturity. This strain was mainly due to a reduced cutin production, the synthesis of wax on the other hand remained constant throughout development. The straining caused an increase of microscopic small cracks (microcracks) in the cuticle. Microcracks were also induced by water, a high humidity (>75%) or an increase in temperature. The orientation of the berries in the bunch influenced the frequency of microcracks in the stylar scar region. Berries with their stylar scar orientated towards the side or to the ground had more microcracks due to a pending water droplet than those facing the sky. Also cheek of berries which were in contact with cheeks of neighbouring berries showed more microcracks than cheeks without contact. This was due to prolonged periods of surface moisture in these areas. With time microcracks can extend to macroscopic visible cracks.

Based on these results it is likely that strategies aiming on reducing wetness duration of berries and clusters will reduce cracking of the fruits. These strategies include breeding approaches for loose clusters or cultural measures like defoliation of the cluster-zone.

Keywords: cracking, uptake, microcracks

3. Allgemeine Einleitung

Niederschläge können zu einem Aufplatzen reifender Weinbeeren (*Vitis vinifera* L.) führen (Considine und Kriedemann 1972). In den vergangenen Jahren wurde eine Zunahme dieses Aufplatzens in deutschen Weinbaugebieten beobachtet (Harms 2007, Jörger et al. 2009). Aufgrund des Klimawandels in Europa ist zukünftig häufiger mit Witterungsbedingungen zu rechnen, die zum Aufplatzen von Weinbeeren führen. Modellrechnungen von Stock et al. (2005, 2007) zeigen, dass die Häufigkeit feucht-warmer Witterungsperioden zwischen Reife- und Lesebeginn zunimmt. Ebenso steigt die Wahrscheinlichkeit lokaler Extremwetterereignisse.

Auch das Wachstum von Pilzen wie *Botrytis cinerea* (Graufäule) und *Penicillium expansum* (Grünfäule) wird durch die zu erwartenden Witterungsbedingungen gefördert. Das Mycel benötigt zur Keimung und zum Wachstum hohe Luftfeuchtigkeiten. Temperaturen um 25°C begünstigen ebenfalls die Pilzentwicklung (Kassemeyer und Berkelmann-Löhnertz 2009). Aufgeplatzte Beeren bieten den Pilzen optimale Entwicklungsbedingungen. Die primäre Barriere, die Kutikula, ist zerstört und der austretende kohlenhydratreiche Zellinhalt dient als Substrat für das Pilzwachstum. Von wenigen geplatzten und befallenen Beeren breitet sich die Fäulnis bei entsprechenden Witterungsbedingungen rasant in der Traube, von Traube zu Traube sowie von Rebe zu Rebe aus.

Das Platzen ist nicht auf Weinbeeren beschränkt, sondern ist ein weit verbreitetes Problem bei fleischigen weichen Früchten wie Kirschen (Cline et al. 1995), Pflaumen (Mrozek und Burkhard 1973) und Ribes-Beeren (Khanal et al. 2011). Platzen und anschließender Pilzbefall mindern den Ertrag und die Qualität der Früchte und der daraus gewonnenen Produkte wie Saft, Most und Wein (Meneguzzo et al. 2008, Viret et al. 2004). Hohe ökonomische Verlusten sind die Folge. Im Jahr 2010 gab es beispielsweise im Schnitt 30% (örtlich bis zu 50%) Ertragsverluste im Vergleich zum langjährigen Mittel bei der Weinernte. Dies war unter anderem bedingt durch ein vermehrtes Aufplatzen der Früchte aufgrund einer langen Niederschlagsperiode im August (Deutsches Weininstitut 2010). Ein Fungizideinsatz zur Eindämmung der

Infektion ist kurz vor der Ernte aufgrund der einzuhaltenden Wartezeiten nicht mehr möglich.

Rebsorten unterscheiden sich in ihrer Platzanfälligkeit (z.B. Hill 2007, Lang und Düring 1990, Lustig und Bernstein 1985). Zu den platzanfälligen Sorten zählen die beiden in Deutschland am häufigsten angebauten Rebsorten (über 50% der Anbaufläche für Weißwein) ‚Riesling' (22 601 ha, Thoma 2011) und ‚Müller-Thurgau' (13 554 ha, Thoma 2011). Der weltweit häufig angebaute ‚Chardonnay' ist hingegen platzfest. Aufgrund ihrer ökonomischen Bedeutung und den unterschiedlichen Platzfestigkeiten wurden diese drei Sorten für unsere Experimente ausgewählt.

Voraussetzung zur Entwicklung von Strategien zur Verbesserung der Platzfestigkeit von Weinbeeren ist ein Verständnis der Ursachen und Mechanismen, die zum Platzen führen. Als Hauptursache für das Platzen gilt die Volumenzunahme der Frucht durch die erhöhte Wasseraufnahme infolge des Niederschlages. Die dadurch erzeugte Dehnung kann bis zu einem gewissen Grad durch die viskoelastische Natur der Beerenhaut ausgeglichen werden. Übersteigt die Volumenzunahme jedoch die Dehnbarkeit der Haut, reißt diese auf (Lang und Düring 1990, Lang und Thorpe 1989). Somit sind für die Platzanfälligkeit der Weinbeere zwei unterschiedliche Faktorenkomplexe entscheidend. Zum einen die mechanische Stabilität der Fruchthaut und zum anderen der Wasserhaushalt (Aufnahme + Abgabe von Wasser) der Beere.

Beide Faktorenkomplexe unterliegen Änderungen während der Fruchtentwicklung und sind bislang noch nicht vollständig untersucht.

Im Folgenden werden diese beiden Komplexe detaillierter beschrieben und die sich daraus ergebenden und bearbeiteten Fragestellungen für diese Arbeit abgeleitet.

Die mechanische Beschaffenheit der Fruchthaut spielt eine entscheidende Rolle beim Platzen. Bei Süßkirschen wurde nachgewiesen dass in der letzten Entwicklungsphase des Fruchtwachstum keine Neu-Synthese der Grundbausteine der Kutikula mehr stattfindet (Knoche et al. 2001, Knoche et al. 2004). Da die Oberfläche der Frucht

jedoch weiterhin zunimmt, wird die Kutikula immer stärker gedehnt und verdünnt. Dies führt zur Entstehung von mikroskopisch kleinen Rissen (Mikrorisse), die als Ausgangspunkte für makroskopisch sichtbare Risse dienen (Knoche et al. 2001, Knoche et al. 2004). Für verschiedene Rebsorten wird ebenfalls eine Abnahme der Kutikuladicke ab Beginn des Weichwerdens der Früchte (3. Entwicklungsphase) beschrieben (Alleweldt et al. 1981, Comménil et al. 1997, Considine und Knox 1979). Da zudem auch bei reifenden Weinbeeren das Auftreten von Mikrorissen beobachtet wurde (Blaich et al. 1984, Considine 1982), könnte eine ähnliche Abfolge von Ereignissen wie bei den Süßkirschen zum Platzen der Weinbeeren führen. Bei ‚Chardonnay', ‚Müller-Thurgau' und ‚Riesling' wurden deshalb Fruchtwachstum, Kutikulaentwicklung und die Bildung von Mikrorissen untersucht (Kapitel 4, Becker und Knoche 2012a). Eine weitergehende Literaturübersicht zu diesem Themenkomplex wird in der spezifischen Einleitung des Kapitels gegeben.

Die Bildung von Mikrorissen in der gedehnten Fruchthaut wird auch durch Oberflächenfeuchtigkeit (Niederschlag, hohe Luftfeuchtigkeit) ausgelöst (Knoche und Peschel 2006). Die Anzahl und Verteilung der Mikrorisse wird dabei von verschiedenen Faktoren wie Temperatur, Form der Frucht oder Position auf der Fruchthaut (Narbe, Backe) beeinflusst (Knoche und Peschel 2006). Kapitel 5 (Becker und Knoche 2012b) beschreibt die Ergebnisse der Untersuchungen zur Entstehung und Induzierbarkeit von Mikrorissen bei der Rebsorte ‚Riesling' in Abhängigkeit verschiedener Einflüsse.

Der zweite Faktorenkomplex, der beim Aufplatzen von Weinbeeren eine Rolle spielt, ist der Wasserhaushalt der Früchte. Dieser ist abhängig vom Netto-Wassertransport (Summe aus Wasserabgabe und Wasseraufnahme) in die beziehungsweise aus der Beere. Die Wasserabgabe passiert hauptsächlich über Transpiration über die Beerenhaut. Wasser wird in die Beere einerseits über die Leitbündel (in Rebe, Stielgerüst der Traube und Beere (vaskuläres System)) und andererseits über die Oberflächen von Beerenhaut und –Stiel aufgenommen. Die Wasseraufnahme über das vaskuläre System war bereits Gegenstand einiger Untersuchungen (beispielsweise Düring et al. 1987, Greenspan et al. 1996, Lang und Thorpe 1989, Rogiers et al. 2001,

Tyerman et al. 2004). Im Gegensatz dazu wurde die Wasseraufnahme über die Oberflächen von Haut und Stiel bei Weinbeeren bislang kaum untersucht (Clarke et al. 2010, Rogiers et al. 2004). Wichtige Parameter des Wassertransports über die Haut, wie beispielsweise die Permeabilitäten für die osmotisch getriebene Wasseraufnahme und Transpiration sind deshalb unbekannt.

Die Anteile von Stiel und Haut an der gesamten Wasseraufnahme und –abgabe über die Oberfläche wurden an ‚Chardonnay', ‚Müller-Thurgau' und ‚Riesling' untersucht. Ebenso die treibenden Kräfte und die Permeabilität der Haut für den Wassertransport sowie die Veränderungen dieser Parameter im Verlauf der Entwicklung. Die Ergebnisse werden in Kapitel 6 beschrieben (Becker und Knoche 2011).

In Kapitel 6 wird gezeigt, dass die Stieloberfläche eine entscheidende Rolle bei der Wasseraufnahme spielt. Der Stiel einer Weinbeere lässt sich in mehrere Abschnitte mit potentiell unterschiedlichen Wassertransporteigenschaften unterteilen. Diese Abschnitte sind: 1) Der Stielanteil oberhalb des Blütenbodens (Kissen), 2) das Kissen mit den durch Periderm verschlossenen Ansatzstellen der Blütenorgane und 3) die Verbindung zwischen Stiel und Frucht unterhalb des Kissens. Die Anteile der verschiedenen Stielabschnitte an der Wasseraufnahme sowie die Verteilung des aufgenommen Wassers in der Beere.wird in Kapitel 7 (Becker et al. 2012) behandelt.

Die Einleitungen zu diesen Kapiteln beinhalten jeweils eine weitergehende Literaturübersicht zum Thema Wasserhaushalt der Weinbeeren.

Viele Schadorganismen, wie beispielsweise die Sekundärfäule-Erreger der Grün- oder der Essigfäule, können die Beeren ausschließlich über bereits beschädigte Fruchthäute befallen. Im Gegensatz dazu kann *Botrytis cinerea* mittels Lipasen, Cutinasen und Pektinasen auch intakte Fruchthäute penetrieren (Kassemeyer und Berkelmann-Löhnertz 2009, Tenberge 2007). Durch diese Infektionen wird die Fruchthaut geschwächt und die Platzanfälligkeit der Beeren erhöht. Wie stark sich das Aufplatzen der Weinbeeren durch Feuchtigkeit und der Befall mit Botrytis gegenseitig beeinflussen wurde in Kapitel 8 (Becker et al. 2011) untersucht. Eine Literaturübersicht zu diesem Thema wird in der Einleitung des Kapitels gegeben.

4. Deposition, strain, and microcracking of the cuticle in developing ‘Riesling’ grape berries

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Deposition, strain, and microcracking of the cuticle in developing 'Riesling' grape berries

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Summary

The objectives of this study were to quantify deposition, strain, and microcracking of the cuticular membrane (CM) in developing 'Riesling' (*Vitis vinifera* L.) berries. Mass of the CM, the cutin matrix (DCM), and wax increased pre-veraison (26 to 65 days after anthesis, DAA) on a berry (+ 236, + 211, and + 332 %, respectively) and a surface area basis (+ 11, + 3, and + 43 %, respectively). Post-veraison (65 to 138 DAA), CM and DCM mass per berry remained about constant at 3.4 (± 0.16) and 2.4 (± 0.11) mg per berry, respectively, while wax mass continued to increase from 0.8 (± 0.02) to 1.1 (± 0.02) mg per berry. On an area basis, however, CM and cutin mass decreased from 5.0 (± 0.13) to 4.6 (± 0.04) g·m^{-2} and from 3.5 (± 0.10) to 3.2 (± 0.03) g·m^{-2} between 65 and 138 DAA, respectively, but wax mass remained constant at about 1.5 (± 0.04) g·m^{-2}. The calculated rate of cutin and wax deposition peaked at about 40 DAA, and declined continuously thereafter. There was no strain and no microcracking of the CM up to veraison. Post-veraison strain of the CM and microcracking in the stylar scar region increased linearly with time. The data suggest that the cessation of cutin deposition in post-veraison berries and the ongoing berry expansion resulted in increased strain of the CM which in turn caused microcracking in the CM.

K e y w o r d s : cutin, fracture, skin, splitting, *Vitis vinifera* L., wax.

Introduction

Strain of the cuticular membrane (CM) is an important factor in formation of microscopic cracks (microcracks) in the CM of fleshy fruit. Microcracks impair the barrier function of the CM resulting in an increased incidence of fruit rot, uncontrolled water transport, and, possibly, cracking. In sweet cherry, strain of the cuticle is caused by a mismatch of surface expansion and CM deposition during late development (stage III, final swell; Lilleland and Newsome 1934, Knoche *et al.* 2004, Peschel and Knoche 2005). At this stage CM deposition has essentially ceased, while most growth in surface area still has to occur. Thus, surface expansion distributes an essentially constant amount of CM and its constituents cutin and wax over an enlarging surface. The resulting tangential forces cause strain of and stress in the CM resulting in formation of microcracks. Furthermore, microcracking of the strained CM is aggravated by surface wetness (Knoche and Peschel 2006). Qualitatively similar relationships were identified in other soft and fleshy fruit, e.g. the European plum (Knoche and Peschel 2007) and *Ribes* berries (Khanal *et al.* 2011). In fact, across species strain of the CM at maturity and fruit surface expansion following cessation of CM deposition are closely related (Khanal *et al.* 2011). These data indicate that the balance between CM deposition and relative growth rate in surface area is a critical determinant in strain which, in turn, represents the driving force for microcracking.

The grape berry is expected to be subjected to the same events, because (1) it has a soft fleshy mesocarp (and endocarp) surrounded by a skinny exocarp, (2) berry growth follows a double sigmoidal pattern with time characterized by rapid expansion in post-veraison development (Mullins *et al.* 1992), (3) it has a thin CM that was reported to decrease in thickness post-veraison (Considine and Knox 1979, Alleweldt *et al.* 1981, Comménil *et al.* 1997) and (4) microcracks in the CM represent the first event in the macroscopic cracking of berries (Considine 1982), that often occurs in humid environments. (5) Water uptake through the grape berry surface proceeds along several parallel pathways, i.e., through the surface of its stem and receptacle and the surface of the berry (Becker and Knoche 2011). While uptake through an intact cuticle on the berry surface is by diffusion, uptake through microcracks bypasses the cuticle as a penetration barrier and occurs by viscous flow which is a fast mechanism of transport (Becker and Knoche 2011). Finally, microcracks in the grape berry cuticle facilitate infections by *Penicillium expansum* or *Botrytis cinerea* and these may decrease yield and quality of must and wine (Meneguzzo *et al.* 2008).

To our knowledge, there is no direct evidence for strain of the grape berry CM or any relationship between strain of the CM and formation of microcracks. The objectives of this study therefore were to quantify cuticle deposition, strain, and formation of microcracks in developing grape berry. 'Riesling' was selected because it is an important variety in European viticulture. To broaden the database, 'Chardonnay' and 'Müller-Thurgau' were included in some experiments.

Material and Methods

P l a n t m a t e r i a l : Entire clusters of grape berries (*Vitis vinifera* L. 'Chardonnay', 'Müller-Thurgau', and 'Ries-

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ling') were sampled randomly from experimental vineyards within a 10 km radius (similar climate) around Neustadt an der Weinstrasse, Germany (lat. 49°21'N, long. 8°8'E). All vineyards were cultivated according to current regulations for environmentally sound viticulture (ANONYMOUS 2002). Berries were selected for uniformity of size and color and for freedom from defects by visual inspection.

Berry development: Clusters were collected in the mornings at weekly intervals between 13 d after anthesis (DAA; BBCH 71, LORENZ *et al.* 1995) and maturity and transferred to the laboratory within 30 min. Four samples of 25 berries each were weighed using an analytical balance and the mean mass per berry was calculated.

To establish the relationship between mass and surface area, 25 individual 'Riesling' berries per sampling date were weighed, photographed, and berry diameters determined by digital image analysis (software cell^P; Olympus Soft Imaging Solutions, Münster, Germany). The surface area (A in cm^2) of a berry was calculated from berry diameter (d in cm) assuming a spherical shape as a first approximation ($A = \pi \times d^2$). Plotting the surface area against mass (M in g) and fitting a regression line established the equation $A = 4.4\ (\pm\ 0.01) \times M^{(2/3)}$, n = 425, $r^2 = 1.00$***. This equation was used to calculate berry surface area from berry mass in all subsequent experiments. Since data for 'Chardonnay' and 'Müller-Thurgau' were well within the variation obtained in 'Riesling', the above equation was also used for these cultivars.

Isolation of cuticular membranes and extraction of wax: Cuticles were isolated from four samples of 25 berries per sampling date using standard protocols (ORGELL 1955, YAMADA *et al.* 1964). Briefly, berries were cut in half and incubated in an enzyme solution containing pectinase (90 ml·l^{-1}, Panzym Super E flüssig; Novozymes, Bagsvaerd, Denmark) and cellulase (5 ml·l^{-1}, Cellubrix L; Novozymes) in a 50 mM citric acid buffer at pH 4.0. Sodium azide was added at a final concentration of 30 mM to suppress microbial growth. The enzyme solution was refreshed periodically until CM separated from adhering tissue. When appropriate, the cleaning was supported by carefully removing adhering cellular debris using a fine camel hair brush.

Isolated CMs were rinsed ten times in deionised water, dried at 40 °C, and weighed. Subsequently, wax was extracted by incubating CMs in a chloroform/methanol solution (1:1, v/v) at 38 °C for 30 min. The extraction procedure was repeated ten times. The extracted dewaxed CM (DCM) were dried and weighed and the amount of wax calculated by difference. The DCM reflects primarily the cutin matrix (elsewhere referred to as the polymer matrix or MX; SCHÖNHERR 1982) that remains after solvent extraction of the CM.

Using these procedures, the time courses of cuticle, cutin, and wax deposition were established on an individual berry and a surface area basis in developing 'Riesling' grape berries.

Relationships between berry mass and cuticle, cutin, and wax mass were studied by subjecting post-veraison 'Chardonnay' (99 DAA), 'Müller-Thurgau' (96 DAA) and 'Riesling' (101 DAA) berries to the procedures described above. For these experiments berries were selected for a maximum range in berry mass.

Strain of the cuticle: Strain was established in developing 'Riesling' and, at maturity, in 'Müller-Thurgau' and 'Chardonnay'. Exocarp strips (3.0 mm x 4.2 mm) were excised in vertical (Fig. 1 A, parallel to the stylar scar/pedicel axis) or horizontal (Fig. 1 B, perpendicular to stylar scar/pedicel axis) direction in the equatorial plain of the berries (cheek region) using parallel razor blades (distance between blades 3.0 ± 0.00 mm; KNOCHE *et al.* 2004). Following enzymatic isolation, the CM strips were spread in a water droplet on a glass slide, covered by a cover slip, viewed under a binocular (25x, MZ6 microscope; Leica Mikrosysteme, Bensheim, Germany) and photographed (camera DP71; Olympus, Hamburg, Germany). Width of the strips (w, mm) was determined by image analysis (software cell^P; Olympus Soft Imaging Solutions). Uniaxial strains in vertical (ε_y, %) or horizontal (ε_x, %) direction were calculated according to equation 1, where w represented the width of the strip on the berry equivalent to the distance of the razor blades (Fig. 1 C) and w_0 the width of the relaxed CM strip after isolation (Fig. 1 D).

$$\varepsilon_{x,y} = \frac{w - w_0}{w_0} \times 100 \qquad [1]$$

The biaxial area strain (ε_{xy}, %) was calculated from uniaxial strains according to equation 2 (KNOCHE *et al.* 2004).

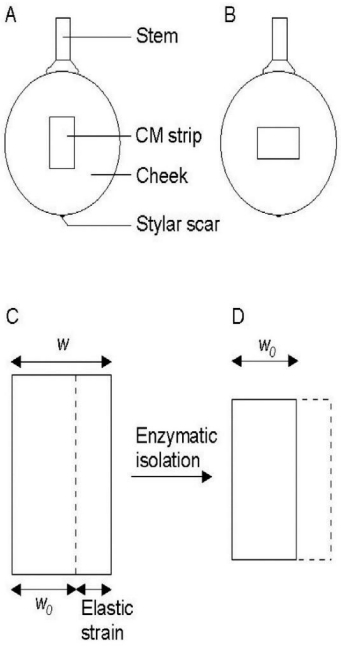

Fig. 1: Schematic drawing illustrating the procedure used for determining strain of the cuticular membrane (CM) on grape berries. **A**, **B**) Epidermal strip cut in vertical (**A**) or horizontal direction (**B**) for assessing uniaxial strain of the CM in equatorial and longitudinal direction of the cheek of a 'Riesling' grape berry. **C**) Epidermal strip of width w on the berry surface (prior to excision). **D**) Same as **C**, but after excision and isolation and subsequent release of (elastic) strain. The width of the relaxed strip has decreased from w to w_0.

$$\varepsilon_{xy} = \left[\left(\frac{\varepsilon_x}{100} + 1 \right) \times \left(\frac{\varepsilon_y}{100} + 1 \right) - 1 \right] \times 100 \quad [2]$$

The number of replications per sampling date and orientation was ten.

Development of microcracks in the CM: The change in the number of microscopic cracks per unit area was monitored from 43 DAA until maturity in the stylar scar and the cheek region of 'Riesling' berries (Fig. 1 A). Preliminary observations established that these regions represented those with the highest and lowest frequency of microcracks on the grape berry surface (Becker 2009, unpublished data). Berries were incubated in an aqueous solution of the fluorescent dye acridine orange (0.1 % w/v). After 10 min, berries were removed from the dye solution, rinsed with deionised water for 10 s, and blotted with tissue paper. Exocarp segments (ES) from the stylar scar or the cheek region were excised by razorblade and viewed at 100x using a fluorescence microscope (Ortholux II; Ernst Leitz, Wetzlar, Germany, 390-490 nm excitation wavelength, ≥ 515 nm emission wavelength). The microcracks within a 2.1 mm² window of the microscope were counted on ten randomly selected areas per ES excised from a total of ten berries.

Data analysis: Unless individual observations are shown (e.g. Fig. 2), data are presented as means ± standard errors. Data were subjected to linear (Proc REG) and nonlinear regression analysis (Proc NLIN) using SAS (version 9.1.3; SAS Institute, Cary, NC). Significance of coefficients of determination (r^2) at the 5, 1 and 0.1 % probability level is indicated by *, ** and ***, respectively.

Results

Berry development and cuticle deposition: Berry growth as indexed by the increase in mass and surface area followed the typical double sigmoidal growth pattern with time. This pattern is characterized by two phases of rapid berry enlargement (stage I and III, Mullins *et al.* 1992) separated by a lag phase with temporarily decreased growth rate (stage II, Fig. 2 A, Tab. 1). Veraison coincided with the onset of stage III development (BBCH 81, Lorenz *et al.* 1995) around 65 DAA for 'Riesling' and 55 DAA for 'Chardonnay' and 'Müller-Thurgau'. Maximum growth rates in mass during stage I and III were 48 and 28 mg·d^{-1} in 'Riesling', 35 and 40 mg·d^{-1} in 'Chardonnay', and 49 and 75 mg·d^{-1} in 'Müller-Thurgau'. The corresponding maximum growth rates in surface area were 17 and 7 mm²·d^{-1}, 13 and 10 mm²·d^{-1}, 17 and 18 mm²·d^{-1} for 'Riesling', 'Chardonnay', and 'Müller-Thurgau', respectively.

Up until about veraison, the growth of the berries was paralleled by an increase in mass of CM, DCM, and wax on a whole berry basis and also, on a surface area basis (Fig. 2 A, B, C, Tab. 1). In post-veraison berries, however, CM and DCM mass per berry remained constant while mass of wax per berry continued to increase albeit at a reduced rate. On a surface area basis, CM and DCM mass even decreased and the amount of wax remained constant

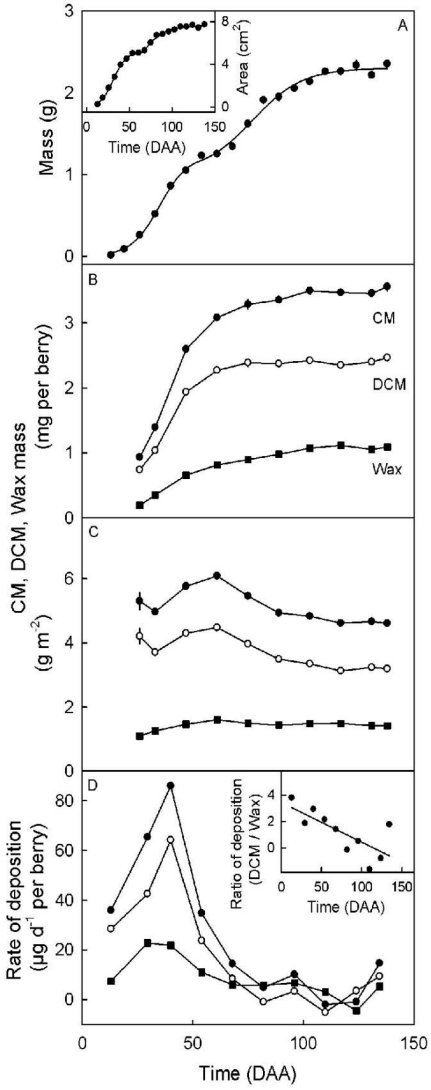

Fig. 2: **A**) Change in berry mass (main graph) and surface area (Inset) with time in developing 'Riesling'. For regression equation see Tab. 1. **B**, **C**) Change in mass of the cuticular membrane (CM), dewaxed CM (DCM), and wax per berry (**B**) and per unit surface area of the berries (**C**). **D**) Rate of deposition of CM, DCM, and wax and the ratio of deposition rates of DCM / wax (inset) in developing berries. The regression equation for the relationship between the ratio of DCM / wax deposition rates and time was: *DCM* / *Wax* (ratio) = 3.45 - 0.03 x *time*, (DAA) r^2 = 0.54*. (DAA = Days after anthesis).

(Fig. 2 B, C). At maturity, CM mass per unit surface area averaged 4.6 (± 0.04), 3.9 (± 0.07), and 3.3 (± 0.03) g·m^{-2} for 'Riesling', 'Chardonnay', and 'Müller-Thurgau', respectively. From the time course depicted in Fig. 2 A, B, C, rates of synthesis and deposition of cutin and wax, the two major constituents of the CM, were calculated for 'Riesling' (Fig. 2 D). Pre-veraison 'Riesling' berries were characterized by an increasing rate of cutin and wax deposition reaching a maximum at about 40 DAA. Thereafter, rates of deposition decreased continuously and approached an asymptote at about 2.0 and 3.3 µg·d^{-1} per berry for the cutin and wax fraction, respectively. The ratio of deposition rates

Table 1

Parameters of regression equations for the relationship between mass (g per berry) and time (DAA = days after anthesis) of 'Chardonnay', 'Müller-Thurgau', and 'Riesling' berries. The segmented regression model was *mass* = a1 / (1 + b1 x exp(-c1 x *time*)) for *time* < x0 and *mass* = (a1 / (1 + b1 x exp(-c1 x x0))) - (a2 / (1 + b2)) + (a2 / (1 + b2 x exp(-c2 x (*time*-x0)))) for *time* > x0

Variety	Parameters							
	a1 (g)	b1 (g)	c1 (DAA^{-1})	a2 (g)	b2 (g)	c2 (DAA^{-1})	x0 (DAA)	r^{2a}
Chardonnay	1.15 (± 0.23)	60.79 (± 52.97)	0.12 (± 0.04)	1.17 (± 0.23)	7.15 (± 5.19)	0.14 (±0.05)	50.35	1.00***
Müller-Th.	1.22 (± 0.11)	172.60 (± 148.90)	0.16 (± 0.03)	1.48 (± 0.11)	21.80 (± 10.00)	0.20 (± 0.04)	49.41	1.00***
Riesling	1.23 (± 0.08)	242.60 (± 185.10)	0.16 (± 0.03)	1.27 (± 0.14)	8.25 (±3.47)	0.09 (± 0.02)	53.19	1.00***

[a)] Significance of coefficient of determination at *P*= 0.05, 0.01, 0.001 indicated by *, **, ***, respectively.

of cutin and wax consistently decreased indicating that cutin deposition decreased more rapidly then wax deposition (Fig. 2 D, inset)

In post-veraison berries, mass of CM, DCM, and wax on a berry and, to a lesser extent, on a surface area basis were positively related to berry mass indicating that larger berries had a higher cuticle mass per area and therefore, slightly thicker cuticles in 'Riesling', 'Chardonnay', and 'Müller-Thurgau' (Fig. 3, Tab. 2).

Strain of the CM: Uniaxial and biaxial elastic strain of the cuticle increased with time in post-veraison 'Riesling' (Fig. 4). There was no difference in uniaxial strains parallel or perpendicular to the stylar scar/pedicel axis (Fig. 4, inset). At maturity, biaxial strain averaged 18.4 (± 1.2) %. Quantitatively similar data were obtained for post-veraison 'Müller-Thurgau' (20.7 ± 0.7 %), but strain of the CM of 'Chardonnay' berries was markedly lower (4.6 ± 0.8 %, data not shown).

Formation of microcracks: The number of microcracks per unit surface area increased linearly with time in the stylar scar region of post-veraison 'Riesling' berries. In the cheek region, the frequency of microcracks was markedly lower and there was no consistent and significant change with time (Fig. 5).

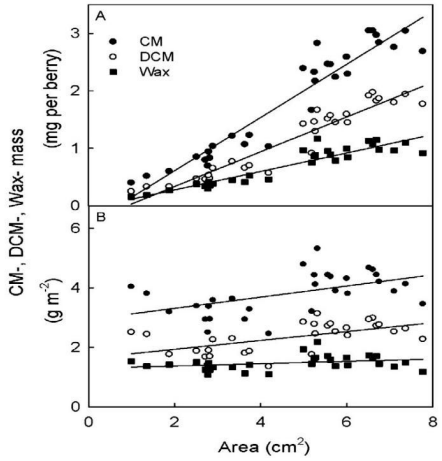

Fig. 3: Relationship between size of post-veraison 'Riesling' berries and mass of the cuticular membrane (CM), dewaxed CM (DCM) and wax on a berry basis (**A**) or a surface area basis (**B**).

Discussion

The data presented herein provide direct evidence for strain of the grape berry CM. Furthermore, the data support the hypothesis of a causal relationship between CM deposition, surface expansion, strain, and microcracking. This conclusion is based on the following arguments.

First, rates of cutin and wax deposition peaked pre-veraison and rapidly declined post-veraison. Growth of the berries followed a double sigmoidal pattern with time characterized by a pre- and post-veraison maximum in surface expansion (Fig. 2). Second, there was no detectable elastic strain in pre-veraison berries when deposition rates of cuticle constituents were at a maximum. However, post-veraison development of (elastic) strain coincided with decreased cutin deposition and increased surface expansion (Figs 2, 3, and 4). Thus, in pre-veraison but not in post-veraison berry CM deposition apparently "fixed" any strain present in older CM layers deposited earlier in develop-

Table 2

Parameters of regression equations for the relationship between berry surface area (cm^2) and mass of the cuticular membrane (CM) on a whole berry basis (mg per berry) and on a surface area basis (g m^{-2}) in mature 'Chardonnay', 'Müller-Thurgau', and 'Riesling' berries. The regression model was *CM mass* = y0 + a x *area*

Variety	Parameters					
	CM mass (mg per berry)			CM mass ($g \cdot m^{-2}$)		
	a ± SE (mg per berry cm^{-2})	y_0 ± SE (mg per berry)	$r^{2\,a}$	a ± SE ($g \cdot m^{-2}$ cm^{-2})	y_0 ± SE ($g \cdot m^{-2}$)	$r^{2\,a}$
Chardonnay	0.40 (± 0.03)	-0.39 (± 0.18)	0.88***	0.13 (± 0.06)	2.51 (± 0.36)	0.16*
Müller-Thurgau	0.35 (± 0.02)	-0.35 (± 0.17)	0.90***	0.07 (± 0.04)	2.51 (± 0.25)	0.12
Riesling	0.46 (± 0.03)	-0.32 (± 0.14)	0.91***	0.19 (± 0.06)	2.94 (± 0.30)	0.26**

[a)] Significance of coefficient of determination at P= 0.05, 0.01, 0.001 indicated by *, **, ***, respectively.

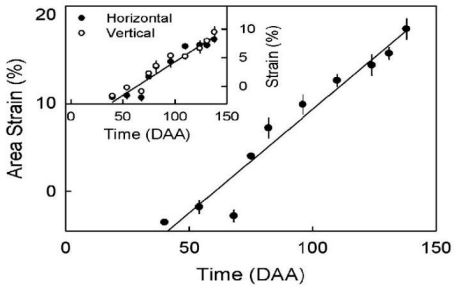

Fig. 4: Biaxial (main graph) and uniaxial elastic strain (inset) of the cuticular membrane in the cheek region of developing 'Riesling' berries. The uniaxial strains in vertical and horizontal directions refer to the strain parallel and perpendicular to the pedicel-stylar scar axis of the grape berry, respectively. (DAA = days after anthesis).

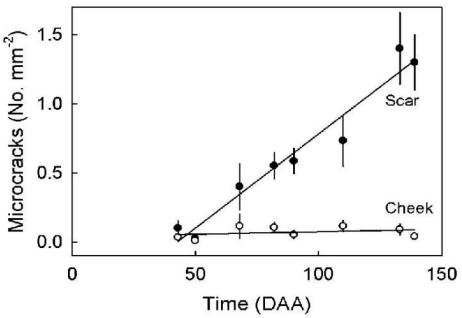

Fig. 5: Frequency of microcracks in the cuticle of developing 'Riesling' berries. The number of microcracks was established in the stylar scar and the cheek region. (DAA = days after anthesis).

ment (Fig. 2 D). In addition, cuticular ridges described on the ovary at anthesis may have served as an additional supply of cuticle for pre-veraison expansion (Rosenquist and Morrison 1988). These ridges disappeared in the course of pre-veraison development as the young cuticle stretched into a film and the “buffering capacity” of the ridges becomes exhausted (Rosenquist and Morrison 1988). Finally, the post-veraison increase in strain was paralleled by a concomitant increase in microcracking in the stylar scar region of the berry.

Quantitatively similar relationships between surface expansion, strain, and microcracking as in 'Riesling' were obtained for 'Müller-Thurgau'. However, for post-veraison 'Chardonnay' elastic strain was much lower than expected based on the higher rate of post-veraison surface expansion as compared to 'Riesling'. Since surface areas of berries at maturity were similar, total strain must have been similar and the lower elastic strain of the CM in 'Chardonnay' implies a higher proportion of plastic (irreversible) strain. The reason for the higher plasticity of the 'Chardonnay' CM as compared to those of 'Riesling' and 'Müller-Thurgau' is not known. Potential explanations include differences in CM composition that possibly result in differing cross-linking of CM constituents.

Cuticle thickness in post-veraison berries as indexed by cuticle mass per unit area was positively correlated with berry size (Fig. 3 B). Hence, larger berries had thicker cuticles. Thus, the effect of berry size on cuticle thickness differed from that of berry development, where the young (and smaller) berry had a thicker CM than the mature larger berry (Fig. 2 C).

Comparing CM thickness in 'Riesling' to other cultivars: The pattern of CM deposition in 'Riesling' as indexed by the change in CM mass of developing berries is in general agreement with published data, particularly those reported for post-veraison development. In the present study CM thickness in post-veraison 'Riesling' decreased by -22 % which is consistent with published data ranging from -20 % in 'Gordo' (Considine and Knox 1979) to -29 % in 'Pinot Noir' (Comménil *et al.* 1997). Also, CM mass per unit area at maturity (4.6 ± 0.04 $g \cdot m^{-2}$ in 'Riesling') was within the range of CM thickness published for other cultivars (4.2 ± 0.2 to 11.9 ± 0.5 g m^{-2} in 'Grenache' to 'Cabernet Sauvignon'; Rosenquist and Morrison 1989). For pre-veraison berries, cuticle deposition was more variable ranging from +11 % in CM thickness in 'Riesling' (Fig. 1 C) to +55 % in 'Gordo' (Considine and Knox 1979). Whether these differences are related to the stage of development at sampling, to genotype or methodology that ranged from microscopical observations to gravimetry of isolated CM, is currently unknown.

Consequences of strain of the CM-Formation of microcracks and water transport: The increase in strain in post-veraison grape berries resulted in increased formation of microcracks in the stylar scar region, but not in the cheek region. The question arises as to why a relationship between strain and failure was limited to the stylar scar region. The 'Riesling' berry is approximately spherical (height (mm) = 0.94 (± 0.01) * diameter (mm) + 0.51 (± 0.06); $r^2 = 0.98$***, n = 426) and, in an homogenous sphere, stress and hence, microcracking is expected to be uniform (Considine and Brown 1981). This, however, was clearly not the case. Microcracks in the stylar scar region occurred more frequently than in the cheek region. Also, in the stylar scar region microcracks developed in a concentric manner around the stylar scar. Several factors may be involved. First, stiffness of the periderm of the stylar scar and the surrounding exocarp is likely to differ. According to Brown and Considine (1982) a rigid plug such as a lenticel or, in analogy, the stylar scar surrounded by an elastic skin acts as a stress concentrator and microcracks will develop preferentially in the immediate vicinity of the plug. If then the berry skin was strained close to the limit of failure, this limit may be exceeded only in the stylar scar region as a result of stress concentration. Second, a higher density of microcracks in the stylar scar region may result from the absence of a network of peripheral vascular bundles that enforces the skin in the cheek, but not the stylar scar region (Chatelet *et al.* 1997). Third, the cuticle and the underlying dermal system in the stylar scar region may be weaker per se as compared to that in the cheek region. For example, the cell walls at the poles of 'Sultana' berries were thinner compared to those of the cheek (Considine 1982). At present, there is no evidence for the latter in 'Riesling'. Finally, surface wetness duration

will differ between the stylar scar region, where a pending droplet collects after precipitation. In the cheek region water droplets are more likely to run-off. In cherries water on the fruit surface of a strained CM induced microcracking (Knoche and Peschel 2006). Whether this applies also to grape berries is currently unknown.

The change in deposition of the cuticular membrane in developing 'Riesling' berries apparently also affected the permeability of the berry surface for transpiration, but not that for water uptake. Recent investigations established that the permeability of the 'Riesling' grape berry surface decreased between 34 and 129 DAA from 5.6 (± 0.2) to 1.6 (± 0.0) $nm \cdot s^{-1}$ in transpiration and from 61.0 (± 13.8) to 4.1 (± 1.2) $nm \cdot s^{-1}$ in water uptake, respectively (Becker and Knoche 2011). Correlating the changes in permeability with cuticle deposition revealed a significant negative relationship between the wax mass per unit area and the permeability of the berry surface for transpiration (r = -0.81*), but not for water uptake (r = -0.55). This observation is consistent with the view that transpiration occurs by diffusion through the berry surface, while water uptake is by diffusion plus some contribution of viscous flow most likely through microcracks that bypass the cuticle as the primary barrier to water uptake (Becker and Knoche 2011).

Conclusion

The data presented herein established that cutin deposition in developing 'Riesling' does not keep pace with post-veraison berry expansion resulting in (1) a continuous increase in elastic strain of the CM and (2) an increase in microcracking of the CM in the stylar scar, but not the cheek region. Furthermore, the change in wax deposition in the course of pre- and post-veraison development was significantly correlated with the change in permeability in transpiration, but not in water uptake. The increase in frequency of microcracks is likely to contribute to an increased incidence of bunch rots and possibly berry cracking in humid environments (Vail and Marois 1990, Pieri and Fermaud 2005).

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References

Alleweldt, G.; Engel, M.; Gebbing, H.; 1981: Histologische Untersuchungen an Weinbeeren. Vitis **20**, 1-7.

Anonymous; 2002: Richtlinien für den kontrolliert umweltschonenden Weinbau in Rheinland-Pfalz. Ministerium für Wirtschaft, Verkehr, Landwirtschaft und Weinbau, Mainz, Germany.

Becker, T.; Knoche, M.; 2011: Water movement through the surfaces of the grape berry and its stem. Am. J. Enol. Vitic. **62**, 340-350.

Brown, K.; Considine, J. A.; 1982: Physical aspects of fruit growth. Stress distribution around lenticels. Plant Physiol. **69**, 585-590.

Chatelet, D. S.; Rost, T. L.; Matthews, M. A.; Shackel, K. A.; 2008: The peripheral xylem of grapevine (*Vitis vinifera*) berries. 2. Anatomy and development. J. Exp. Bot. **59**, 1997-2007.

Comménil, P.; Brunet, L.; Audran, J.-C.; 1997: The development of the grape berry cuticle in relation to susceptibility to bunch rot disease. J. Exp. Bot. **48**, 1599-1607.

Considine, J. A.; 1982: Physical aspects of fruit growth. Cuticular fracture and fracture patterns in relation to fruit structure in *Vitis vinifera*. J. Hort. Sci. **57**, 79-91.

Considine, J. A.; Brown, K.; 1981: Physical aspects of fruit growth. Theoretical analysis of distribution of surface growth forces in fruit in relation to cracking and splitting. Plant Physiol. **68**, 371-376.

Considine, J. A.; Knox, R. B.; 1979: Development and histochemistry of the cells, cell-walls, and cuticle of the dermal system of fruit of the grape, *Vitis vinifera* L. Protoplasma **99**, 347-365.

Khanal, B. P.; Grimm, E.; Knoche, M.; 2011: Fruit growth, cuticle deposition, water uptake, and fruit cracking in jostaberry, gooseberry, and black currant. Sci. Hort. **128**, 289-296.

Knoche, M.; Beyer, M.; Peschel, S.; Oparlakov, B.; Bukovac, M. J.; 2004: Changes in strain and deposition of cuticle in developing sweet cherry fruit. Physiol. Plant. **120**, 667-677.

Knoche, M.; Peschel, S.; 2006: Water on the surface aggravates microscopic cracking of the sweet cherry fruit cuticle. J. Am. Soc. Hort. Sci. **131**, 192-200.

Knoche, M.; Peschel, S.; 2007: Deposition and strain of the cuticle of developing European plum fruit. J. Am. Soc. Hort. Sci. **132**, 597-602.

Lilleland, O.; Newsome, L.; 1934: A growth study of the cherry fruit. Proc. Amer. Soc. Hort. Sci. **32**, 291-299.

Lorenz D. H.; Eichhorn K. W.; Bleiholder H.; Klose R.; Meier U.; Weber E.; 1995: Phenological growth stages of the grapevine, *Vitis vinifera* L. ssp. *vinifera*. Codes and descriptions according to the extended BBCH scale. Aust. J. Grape Wine Res. **1**, 100-103.

Meneguzzo, J.; Miele, A.; Rizzon, L. A.; Ayub, M. A. Z.; 2008: Effect of bunch rot on the sensory characteristics of the Gewürztraminer wine. J. Int. Sci. Vigne Vin **42**, 107-111.

Mullins, M. G.; Bouquet, A.; Williams, L. E.; 1992: Biology of the grapevine. Cambridge Univ. Press, Cambridge.

Orgell, W. H.; 1955: The isolation of plant cuticle with pectic enzymes. Plant Physiol. **30**, 78-80.

Peschel, S.; Knoche, M.; 2005: Characterization of microcracks in the cuticle of developing sweet cherry fruit. J. Am. Soc. Hort. Sci. **130**, 487-495.

Pieri, P.; Fermaud, M.; 2005: Effects of defoliation on temperature and wetness of grapevine berries. In: L. E. Williams (Ed.): Proc. VIIth Int. Symp. Grapevine Physiol. Biotechnol., 109-116. Davis, California, USA, June 21-25, 2004 (International Society for Horticultural Science, Leuven).

Rosenquist, J. K.; Morrison, J. C.; 1988: The development of the cuticle and epicuticular wax of the grape berry. Vitis **27**, 63-70.

Rosenquist, J. K.; Morrison, J. C.; 1989: Some factors affecting cuticle and wax accumulation on grape berries. Am. J. Enol. Vitic. **40**, 241-244.

Schönherr, J.; 1982: Resistance of plant surfaces to water loss. transport properties of cutin, suberin and associated lipids. In: O. L. Lange; P. S. Nobel; C. B. Osmond; H. Ziegler (Eds): Encyclopedia of plant physiology, 153-179. Springer, Berlin.

Vail M. E.; Marois J. J.; 1991: Grape cluster architecture and the susceptibility of berries to *Botrytis cinerea*. Phytopathology **94**, 188-191.

Yamada, Y.; Wittwer, S. H.; Bukovac, M. J.; 1964: Penetration of ions through isolated cuticles. Plant Physiol. **39**, 28-32.

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5. Research Note: Water induces microcracks in the grape berry cuticle

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Research Note

Water induces microcracks in the grape berry cuticle

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Key words: epidermis, exocarp, moisture, splitting, stylar scar, *Vitis vinifera* L.

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 razorblade. 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 $P = 0.05$.

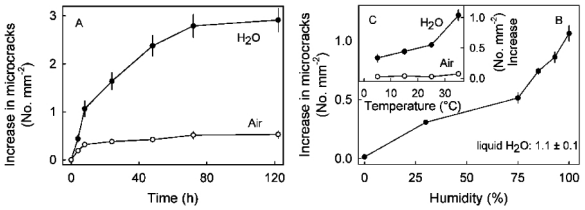

Figure: (**A**) Time course of water induced microcracking of the cuticle of 'Riesling' berries. The surface of excocarp segments (ES) excised from the stylar scar region was exposed to water ('H_2O') or the ambient atmosphere ('Air'). Effect of exposing the surface of ES to different relative humidities (**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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BECKER, T.; KNOCHE, M.; 2012: Deposition, strain, and microcracking of the cuticle in developing 'Riesling' grape berries. Vitis **51**, 1-6.

BROWN, K.; CONSIDINE, J. A.; 1982: Physical aspects of fruit growth. Stress distribution around lenticels. Plant Physiol. **69**, 585-590.

CONSIDINE, J. A.; 1982: Physical aspects of fruit growth. Cuticular fracture and fracture patterns in relation to fruit structure in *Vitis vinifera*. J. Hortic. Sci. **57**, 79-91.

COMMÉNIL, P.; BRUNET, L.; AUDRAN, J. C.; 1997: The development of the grape berry cuticle in relation to susceptibility to bunch rot disease. J. Exp. Bot. **48**, 1599-1607.

KNOCHE, M.; PESCHEL, S.; 2006: Water on the surface aggravates microscopic cracking of the sweet cherry fruit cuticle. J. Am. Soc. Hortic. Sci. **131**, 192-200.

MENEGUZZO, J.; MIELE, A.; RIZZON, L. A.; AYUB, M. A. Z.; 2008: Effect of bunch rot on the sensory characteristics of the Gewürztraminer wine. J. Int. Sci. Vigne Vin **42**, 107-111.

VAIL, M. E.; MAROIS, J. J.; 1991: Grape cluster architecture and the susceptibility of berries to Botrytis cinerea. Phytopathology **81**, 188-191.

WEXLER, A.; 1995. Constant humidity solutions. In: D. R. LIDE (Ed.): Handbook of chemistry and physics, 15-23. CRC Press, Boca Raton, Florida.

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Water Movement through the Surfaces of the Grape Berry and Its Stem

Tobias Becker[1] and Moritz Knoche[2]*

Abstract: Water uptake and transpiration of detached grape berries (*Vitis vinifera* L. cv. Chardonnay, Müller-Thurgau, Riesling) were determined gravimetrically. Water movement was linearly related to time. Abrading the cuticle from the berry surface increased rates of uptake and transpiration 73- and 7-fold, respectively. Restricting water movement to the berry surface by sealing the stem, including the stem/fruit juncture, decreased rates of uptake (-76%) and transpiration (-16%). Rates of uptake were weakly related and those of transpiration were closely related to the surface area of the berry. Transpiration rates were higher in the stylar (+44%) than the cheek region. The water potential of developing Riesling berries (Ψ_{fruit}) was approximately constant between 20 and 76 days after full bloom (DAFB) ranging from -0.52 (±0.18) to -0.58 (±0.15) MPa and decreased thereafter to -1.56 (±0.04) MPa at 131 DAFB when the solute potential was -3.66 (±0.01) MPa. The permeability of the cuticle of Riesling berries to water uptake decreased from 43.3 (±7.0) nm/s to 4.1 (±1.2) nm/s between 28 and 131 DAFB, respectively, and that for transpiration decreased from 7.3 (±0.3) nm/s to 1.6 (±0.0) nm/s. Water uptake was not affected by NaCl, KCl, $CaCl_2$, $FeCl_3$, or $AlCl_3$ (all at 1 to 100 mM). Only $MgCl_2$ slightly increased water uptake. Increasing temperature from 2 to 35°C increased rates of water uptake in Riesling and Müller-Thurgau berries 2.2-fold (equiv. energy of activation 19.6 [±3.3] kJ/mol). Flow rates, fluxes, and permeabilities of stem and berry surfaces in water uptake and transpiration are discussed and a water balance for vascular and surface transport of water in a Riesling berry under hypothetical weather conditions is estimated.

Key words: water uptake, cuticle, pedicel, permeability, solute potential

Cracking is a serious limitation in the production of many fleshy fruit including grapes, where berry cracking is often followed by an increased incidence of fruit rot and a concomitant decrease in fruit yield, must quality, and wine quality (Clarke et al. 2010). Cracking occurs when the fruit volume and, hence, the turgor of the berry increase beyond a critical threshold that exceeds the limits of extensibility of the exocarp (Considine and Kriedemann 1972).

The increase in volume of a grape berry is accounted for by the sum of water uptake through the xylem and phloem vascular systems and through the fruit surface, minus the amount of water lost from the berry either by transpiration or by backflow from the berry to the vine (Greenspan et al. 1996, Keller et al. 2006, Lang and Thorpe 1989). Of these processes, water movement through the vascular system of the grape berry has received the most attention (e.g., Chatelet et al. 2008, Düring et al. 1987, Findlay et al. 1987, Tyerman et al. 2004), but little is known about water movement through the fruit surface. To our knowledge, only a few studies reported on rates of water movement through the grape berry fruit surface (Clarke et al. 2010, Rogiers et al. 2004) and data on the permeability of the grape berry fruit surface are not available. Rates of water movement depend on specific experimental conditions such as driving forces and cross-sectional areas for water movement. These conditions are difficult to standardize and therefore, comparisons of rates of transport are often limited to "head-to-head" comparisons of treatments within an experiment. Permeability constants, on the other hand, are material constants that are independent of specific experimental conditions and, therefore, particularly useful in comparison across systems, species, varieties, seasons, or treatments. From permeability estimates rates of water transport may be calculated for any value of fruit size and driving force.

Primary surfaces of leaves and fruit are covered by a cuticle that represents the rate-limiting barrier in water movement through the fruit surface. Theoretically, water transport across cuticles may occur along a lipophilic and/or polar pathway (for review see Schönherr 2006). The lipophilic pathway is formed by the amorphous wax fraction within the cuticle. It allows diffusion of water molecules independent of each other in a continuum formed by the amorphous wax (solubility membrane; Schönherr 2006). The polar pathway is formed by the orientation, clustering, and hydration of polar functional groups within the cuticle of some species. If these clusters form an aqueous continuum, then they may serve

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as aqueous pores that allow viscous flow of water (porous membrane; Schönherr 2006). Aqueous pores do not represent physical holes in the cuticle that are detectable by microscopy, but are dynamic structures that develop only in the hydrated stage of the cuticle and have average radii between 0.45 and 1.2 nm (Schönherr 2006).

Cuticles isolated from astomatous leaf and fruit surfaces have been used successfully as model systems to characterize water transport. The grape berry surface, however, has stomata and lenticels (Mullins et al. 1992) that exclude the use of isolated cuticles for transport studies. Furthermore, the contribution of structures like the stylar scar, the stem, or the stem/fruit juncture to water movement cannot be assessed using isolated cuticles. Nevertheless, permeability estimates may also be obtained using intact fruit or exocarp segments excised from fruit, provided that the cross-sectional areas for transport and the driving forces are known (Beyer and Knoche 2002, Kerstiens 1996b, Knoche et al. 2000). Such permeabilities represent a weighted mean permeability of all parallel pathways that contribute to water movement through the surface, that is, the cuticle, stomata, lenticels, any cracks in the cuticle, the stylar scar, or the stem/fruit juncture.

Our objective was to identify the pathways for water movement through the grape berry surface, in particular (1) describing water movement quantitatively using permeability constants and its driving forces, (2) identifying any pathways of preferential water movement through the surface of the grape berry, and (3) quantifying the effects of selected factors on such water movement.

Materials and Methods

Plant material. Grape berries (*Vitis vinifera* L. cv. Chardonnay, Huxelrebe, Müller-Thurgau, and Riesling) were sampled in commercial and experimental vineyards within a 10 km radius (similar climate) around Neustadt an der Weinstrasse, Germany (lat. 49°21'N; long. 8°8'E) in the 2008 and 2009 growing seasons. All vineyards were cultivated according to current regulations for environmentally sound viticulture (Ministerium für Wirtschaft 2002). A maximum of one or two clusters per vine was harvested, placed in padded boxes, and transferred to the laboratory for immediate processing or held at 2°C and 75% relative humidity for short-term storage for up to 7 days.

General experimental procedure. Individual berries (<10 per cluster) were excised and inspected for freedom from defects. Unless specified otherwise, stems were cut at the receptacle/pedicel transition using a razor blade so that the receptacle remained attached to the fruit. The stem/fruit juncture, the receptacle, and the cut end were sealed with silicone rubber (3140 RTV Coating; Dow Corning Corp., Midland, MI) (Figure 1). After curing for a minimum of 3 hr at ambient temperature and humidity, experiments were started. Unless specified otherwise, water uptake and transpiration were determined sequentially on an individual fruit basis.

For water uptake, berries were weighed, incubated usually for 2 hr in deionized water, removed, blotted with tissue paper, reweighed, and reincubated for the next incubation interval. Occasionally berries cracked during the course of a water-uptake experiment. These berries were identified during blotting and discontinued. In transpiration experiments, individual berries were incubated in polyethylene beakers filled with dry silica gel such that there was no contact between the silica gel and the fruit surface. Transpiration was established by repeated weighing of individual berries, usually at 1.25 hr intervals. Rates of water movement either in uptake (F_f; g/hr) or in transpiration (F_t; g/hr) were calculated on an individual fruit basis as the slope of a linear regression line fitted through a plot of fruit mass versus time.

Individual experiments. Preliminary experiments were conducted to optimize the experimental procedure. The epicuticular wax layer of grape berries has a delicate crystalline fine structure (Rogiers et al. 2004) that could be easily damaged by the repeated handling and blotting necessary in our water-uptake experiments (Grace and van Gardingen 1996). To establish the effect of handling and blotting on water uptake, rates of uptake were compared in fruit that were weighed repeatedly (at 0, 0.5, 1, 2, and 4 hr) with fruit that were incubated for only one longer interval (0 to 4 hr). There was no significant difference between the two sampling modes (mean rates of uptake across varieties: 0.23 ± 0.03 versus 0.24 ± 0.03 mg/hr for repeated blotting versus blotting once, $p = 0.68$). Therefore, in all subsequent experiments water movement was established on an individual fruit basis by repeated sampling.

Short- (0 to 4 hr) and long-term (0 to 48 hr) time courses of water uptake and transpiration were established in Chardonnay, Müller-Thurgau, and Riesling as described above. The role of the cuticle as the rate-limiting barrier in water uptake and transpiration was investigated by quantifying rates of uptake and transpiration in postveraison Riesling berries having the cuticle abraded with abrasive paper (grit size P600) and in those having an intact cuticle.

Regions of preferential water uptake and transpiration were identified by selective sealing using silicone rubber. In this experiment, the stem was cut to leave a 5 mm length. Water movement in fruit with the pedicel end sealed was compared to fruit with the entire stem, including the stem/fruit juncture, sealed (Figure 1). The latter procedure restricted water movement to the surface of the berry. Rates of water movement

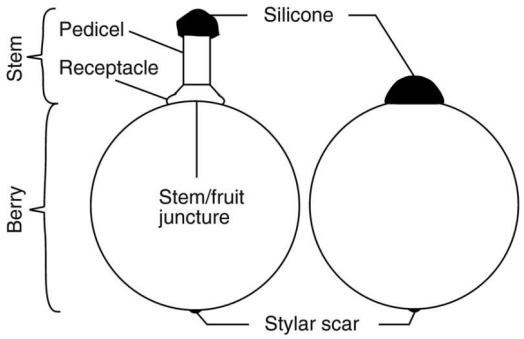

Figure 1 Schematic of a grape berry demonstrating terminology and sealing treatments.

were established and relative contributions of berry surface and stem surface to total water movement were calculated.

Since transpiration occurred largely through the surface of the berry, preferential transpiration through the periderm of the stylar scar, through russeted regions of the berry surface, and through lenticels was studied using diffusion cells and exocarp segments (Knoche et al. 2000). Briefly, the exocarp segments were prepared by cutting a cap of a sphere from the stylar end or from the cheek of the berry using a razor blade. The exocarp segments comprised the cuticle, epidermal, and hypodermal cell layers and some mesocarp tissue. The segments were mounted with silicone grease (Baysilone-Paste, hochviskos; Momentive Performance Materials, Leverkusen, Germany) in stainless-steel diffusion cells such that the cuticle faced the outer side (Geyer and Schönherr 1988, Knoche et al. 2000). The diffusion cells were filled with deionized water and sealed with adhesive tape. Subsequently, the cells were placed upside down above dry silica gel in a polyethylene box. Following equilibration for ~1 hr, transpiration was measured gravimetrically by weighing cells at 0, 1, 2, and 4 hr. To investigate the effect of russeting on transpiration, berries with russeting symptoms were selected for excising exocarp segments. The area covered by russet was quantified by image analysis using a binocular MZ6 microscope (Leica Mikrosysteme GmbH, Bensheim, Germany) and the cell[b] imaging software package (Olympus, Hamburg, Germany). The contribution of lenticels to transpiration was quantified by selecting the exocarp segments for a maximum range in the number of lenticels exposed in the orifice of the diffusion units. The number of lenticels was counted using a binocular (MZ6 microscope; Leica). Subsequently, transpiration was determined as described above.

Relationships between the surface area of berries and rates of water uptake and transpiration were established by selecting postveraison berries for a maximum range in mass. Water uptake was restricted to the fruit surface by selective sealing with silicone rubber as described above. Following the experiment, digital photographs of individual berries (DP71 camera; Olympus) were taken under a binocular (MZ6 microscope; Leica) at 8x. The fruit surface area A (m^2) was calculated from mean diameters determined by digital image analysis (cell[b] software; Olympus) assuming the shape of the berry to resemble a sphere as a first approximation.

The fruit water potential (Ψ_{fruit}, MPa) was determined in pre- and postveraison Riesling berries (20 to 131 DAFB) and in postveraison Chardonnay (101 DAFB) and Müller-Thurgau (98 DAFB) by establishing rates of water uptake in a series of solutions of differing concentrations of polyethylene glycol (PEG; MW 6000 g/mol; Carl Roth GmbH, Karlsruhe, Germany). Water uptake was restricted to the berry surface as described above. The PEG 6000 concentrations were 0, 200, 400, 600, and 700 g/kg H_2O. Solute potentials ($\Psi_{\Pi PEG}$) of PEG solutions were determined by vapor pressure osmometry (VAPRO 5520; Wescor Inc., Logan, UT) and the Ψ_{fruit} calculated. The Ψ_{fruit} corresponded to the x-axis intercept of a linear regression line fitted through a plot of F_f versus the $\Psi_{\Pi PEG}$ of the PEG solutions. Occasionally, relationships between F_f and $\Psi_{\Pi PEG}$ were nonlinear and here regression analysis was limited to a minimum of three data points closely spaced around the x-axis intercept. From the regression equation obtained, the $\Psi_{\Pi PEG}$ of a hypothetical PEG solution that resulted in zero change in fruit mass was calculated. Under this condition there is no driving force for water movement, and the $\Psi_{\Pi PEG}$ of the hypothetical PEG solution can be assumed equal to the Ψ_{fruit}.

To quantify the solute potential ($\Psi_{\Pi fruit}$) of the grape berries, juice was extracted by crushing berries using a garlic press. The juice of five samples of 10 berries each was analyzed by vapor pressure osmometry (VAPRO 5520; Wescor).

Polar pathways through the cuticle can provide a route for rapid water uptake (Schönherr 2006, Weichert and Knoche 2006a). To establish whether such pathways are also present in the grape berry surface, we studied the effect of molecular size of selected osmotica on the apparent fruit water potential (Ψ'_{fruit}). This approach is based on the idea that, in the presence of such size-selective penetration pathways, osmotica of low molecular weight would penetrate through these pathways into the berry thereby causing a decrease (a more negative value) in Ψ'_{fruit}. Conversely, osmotica of high molecular weight that are excluded from penetration would yield a less negative Ψ'_{fruit} (for details see Weichert and Knoche 2006a). In our experiments, water uptake through the surface of Riesling berries was investigated from solutions of varying concentrations of the following osmotica: NaCl (58 g/mol), glycerol (92 g/mol), proline (115 g/mol), sucrose (342 g/mol), PEG 1500 (1500 g/mol), and PEG 6000 (6000 g/mol). Rates of water uptake were determined and the point of zero change in fruit mass was calculated for all osmotica by linear regression analysis as described above.

To obtain additional evidence for the presence of polar pathways in the cuticle, the effects of selected mineral salts were established using a repeated measures design (Beyer et al. 2002, Weichert and Knoche 2006b). During the first incubation interval (0 to 3 hr), fruit were incubated in deionized water. Following weighing, fruit were returned to a salt solution for the second interval (3 to 6 hr). Rates of water uptake (F_f) were calculated as described above. The effects of the salts was indexed by the ratio (F_f^{II}/F_f^{I}) of rates of uptake in salt solution (F_f^{II}) divided by that in deionized water (F_f^{I}). The salts selected were chlorides of mono- (K, Na), di- (Ca, Mg), and trivalent cations (Al, Fe) at 0, 1, 3, 10, 30, and 100 mM.

The effect of temperature on water uptake through the berry surface was established at 2, 10, 15, 24, or 35°C using Riesling and Müller-Thurgau berries and the procedure described above.

Data analysis and terminology. The permeability for water uptake (P_f, m/s) was calculated from F_f, fruit surface areas (A) and Ψ_{fruit} using Eq. 1 (Nobel 1991, modified):

$$P_f = \frac{F_f}{A \cdot \Delta\Psi} \cdot \frac{R \cdot T}{\rho_w \cdot \overline{V_w}} \quad (1)$$

Alternative expressions for P_f used in the literature are the osmotic water permeability coefficient (P_{os}) or filtration permeability (House 1974, Schönherr 1982). In Eq. 1, ρ_w (kg/m^3)

represented the density of water and $RT/\overline{V}_w$ (MPa) the product of the universal gas constant R (m^3 MPa/mol K) and the absolute temperature T (K) divided by the molar volume of water $\overline{V}_w$ (m^3/mol). Again, the fruit surface area A was calculated as described above from berry mass multiplied by berry density obtained from tabulated densities of isopiestic sucrose solutions (Lide 2009).

A permeability estimate for the reverse process of transpiration (P_t, m/s) was derived from F_t, A, and the driving force for transpiration. In analogy to Schönherr (1982), the gradient in water activity (Δa_w) across the grape berry surface was used as the driving force (Eq. 2). As the humidity above dry silica is practically zero, Δa_w equals the water activity of the juice of the grape berries, which is approximately one.

$$P_t = \frac{F_t}{A \cdot \rho_w \cdot \Delta a_w} \quad (2)$$

The P_f and the P_t are directly comparable despite the different driving forces used in their calculation. Water potential and water activity (a_w) are related according to Eq. 3 (Nobel 1991):

$$\frac{RT}{\overline{V}_w} \cdot \ln a_w = \Psi \quad (3)$$

Using Eq. 3, the gradient in water potential $\Delta\Psi$ between the fruit (Ψ_{fruit}) and the incubation solution (Ψ_{donor}) from Eq. 1 may be rewritten as Eq. 4:

$$\Delta\Psi = \Psi_{donor} - \Psi_{fruit} = \frac{RT}{\overline{V}_w} \cdot \left[\ln a_w^{donor} - \ln a_w^{fruit}\right] \quad (4)$$

In our water uptake assays a_w^{donor} equaled 1, since deionized water was used as a donor. Furthermore, hydrostatic pressure and gravitational potential were negligibly small. Also, for the berries in our experiments, the Ψ_{fruit} and hence, a_w^{fruit} were always greater than -2.27 MPa and 0.98, respectively. For $1.0 > a_w > 0.98$, the $1n a_w$ in Eq. 3 approximately equals $a_w - 1$. Upon inserting into Eq. 4, Eq. 5 is obtained that converts the gradient in water potential into a gradient in water activity.

(5)

$$\Delta\Psi = \frac{RT}{\overline{V}_w} \cdot \left[\left(a_w^{donor} - 1\right) - \left(a_w^{fruit} - 1\right)\right] = \frac{RT}{\overline{V}_w} \cdot \left(a_w^{donor} - a_w^{fruit}\right) = \frac{RT}{\overline{V}_w} \cdot \Delta a_w$$

Furthermore, substituting $\Delta\Psi$ in Eq. 1 by Eq. 5 yields a P_f that is now expressed as a function of the water activity gradient in analogy to the P_t (Eq. 6).

$$P_f = \frac{F_f}{A \cdot \rho_w \cdot \Delta a_w} \quad (6)$$

Since the cuticle is the rate-limiting barrier in water transport, we refer to the P_f and P_t as the respective P of the cuticle. It is important to recognize, however, that parallel pathways for water transport through lenticels, the stylar scar, and microscopic cracks may contribute to these estimates. Throughout our study the term "stem" refers to pedicel plus receptacle (Figure 1) and "water transport through the stem surface" to water uptake or transpiration through the stem/fruit juncture and through the surfaces of pedicel and receptacle, but not through the cut end of the pedicel or the berry surface.

Data are presented as means ± standard errors of a minimum of seven berries that remained intact throughout the experiment. Where error bars are not shown, they were smaller than data symbols or the symbols represent individual fruit. Where meaningful, analysis of variance (Proc ANOVA, Proc GLM), correlation (Proc CORR), and regression (Proc REG) were carried out using the SAS software package (version 9.1.3; SAS Institute Inc., Cary, NC). Significance of coefficients of correlation (r) and determination (r^2) at the 5, 1, and 0.1% probability level is indicated by *, **, and ***, respectively, or as ns when not significant.

Results

Water uptake and transpiration rates through the cuticle of mature grape berries were constant with time in the short- (Figure 2) and long-term experiments (Figure 2, insets). The rates of uptake and transpiration were lowest for Riesling, followed by Chardonnay and Müller-Thurgau. Abrading the cuticle on Riesling berries increased rates of uptake and rates of transpiration 73- and 7-fold, respectively, indicating that the cuticle was the primary barrier in uptake and transpiration.

Water movement in transpiration and—even more so—in uptake was significantly affected by the presence of the stem (Figure 3). Rates of transport decreased when water movement was restricted to the berry surface by sealing the stem and the stem/fruit juncture. Uptake through the berry surface

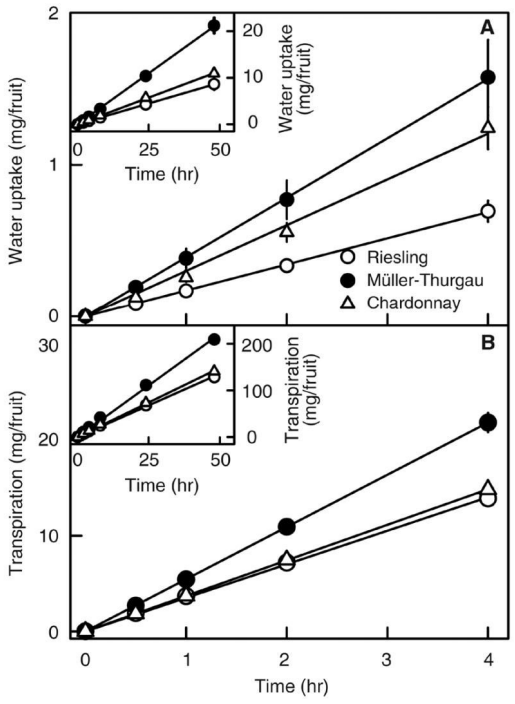

Figure 2 Short- (main graph) and long-term (insets) time courses of water uptake (**A**) and transpiration (**B**) through the berry surface of postveraison Chardonnay (103 days after full bloom; DAFB), Müller-Thurgau (95 DAFB), and Riesling (109 DAFB).

accounted for only 18% of total uptake of a submerged, detached Riesling berry. This contribution remained essentially constant throughout development. For transpiration, however, the relative contribution of the fruit surface was significantly larger and ranged from about 58% between 26 and 59 DAFB to on average 77% between 73 and 129 DAFB. Qualitatively and quantitatively similar data were obtained for Chardonnay and Müller-Thurgau (Table 1). There was no interaction among varieties.

Rates of water uptake were weakly, and those of transpiration were closely, related to berry mass and, hence, to surface area in Chardonnay, Huxelrebe, Müller-Thurgau (Table 2), and Riesling (Figure 4). Furthermore, for three out of four varieties, rates of water uptake and transpiration were positively correlated (r = 0.56**, 0.81***, 0.26 ns, and 0.52** for Chardonnay, Huxelrebe, Müller-Thurgau, and Riesling, respectively).

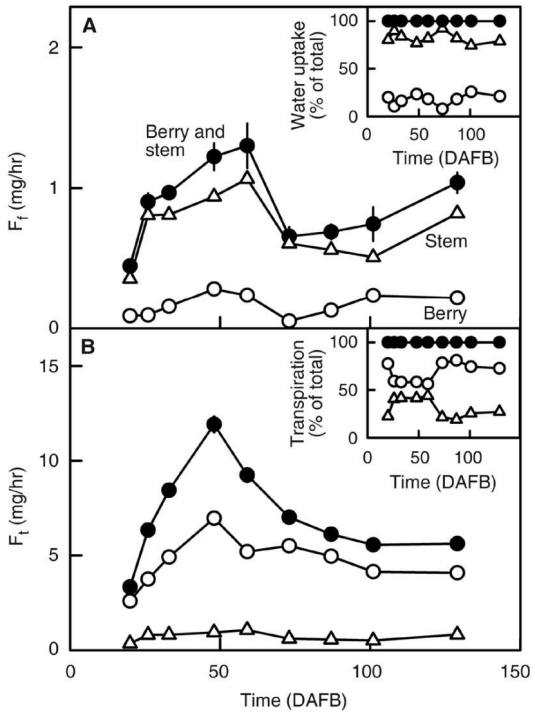

Figure 3 Absolute (main graphs) and relative (insets) rates of water uptake (F_f) (**A**) and transpiration (F_t) (**B**) of developing Riesling berries. Selective sealing of fruit with silicone rubber restricted water movement to the surface of the berry plus stem ('Berry and stem') or to the berry surface only ('Berry'). Water movement through the stem surface including the stem/fruit juncture ('Stem') was calculated by subtracting rates of uptake through the berry surface from rates of uptake through the surface of the berry plus stem. X-axis scale: days after full bloom (DAFB).

Transpiration through the fruit surface occurred at a higher rate at the stylar end (+44%) of the berry as compared to the cheek (Figure 5A). There was no relationship between the stylar scar area and rates of transpiration in mature Riesling berries (Figure 5A, inset). Also, neither the russeted area (Figure 5B) nor the number of lenticels on the berry surface (Figure 5C) had significant effects on rates of transpiration.

Increasing the concentration of PEG 6000 in the incubation solution decreased rates of uptake until fruit lost water to the incubation solution at high PEG concentrations (Figure 6A). The fruit water potential and the solute potential of developing Riesling berries were approximately constant up to ~76 DAFB, but decreased (became more negative) thereafter (Figure 6B). Permeabilities for water uptake (P_f) and for transpiration (P_t) of the berry cuticle of developing Riesling decreased throughout fruit development (Figure 6C, D, Table 3). Similar data were obtained for pre- and postveraison Chardonnay and Müller-Thurgau (Table 3).

Rates of water uptake decreased when mature Riesling berries were incubated in solutions of increasing concentrations of osmotica of differing molecular weights (Figure 7A–F). There was no consistent relationship between the apparent fruit water potentials (Ψ'_{fruit}) in the respective osmotica and the molecular weights of the osmotica (Figure 7G).

Incubating Riesling berries in solutions of various mineral salts generally had no effect on water uptake (data not shown). The only exception was $MgCl_2$, which slightly increased water uptake as the $MgCl_2$ concentration increased [regression equation: $F_f^{II}/F_f^{I} = 1.30$ (±0.09) -0.18 (±0.08) log Concn (mM), $p = 0.03$, $r^2 = 0.11$*].

Rates of water uptake through the berry surface were positively related to temperature in Müller-Thurgau and Riesling (Figure 8). There was no significant interaction between temperature and variety ($p = 0.09$). From the slope of the Arrhenius plot, an energy of activation (E_a) for water uptake was calculated at 19.6 (±3.3) kJ/mol.

Discussion

Sites of preferential water movement. Our results established that water uptake and transpiration proceeded along different pathways. Water uptake occurred mainly through the stem surface including the stem/fruit juncture, while transpiration was essentially limited to the berry surface, which was

Table 1 Rates of water uptake and transpiration through the surfaces of the berry and its stem or the berry surface only in Chardonnay, Müller-Thurgau, and Riesling (2008 season).

	Water uptake (mg/hr)			Transpiration (mg/hr)		
	Berry and stem	Berry	Mean	Berry and stem	Berry	Mean
Chardonnay	0.90 (±0.14)	0.16 (±0.01)	0.53 (±0.11) a[a]	6.15 (±0.25)	4.95 (±0.11)	5.52 (±0.19) b
Müller-Thurgau	0.79 (±0.13)	0.21 (±0.04)	0.50 (±0.10) a	8.13 (±0.31)	7.01 (±0.21)	7.57 (±0.22) a
Riesling	0.87 (±0.07)	0.26 (±0.07)	0.58 (±0.09) a	4.61 (±0.29)	3.85 (±0.30)	4.25 (±0.22) c
Mean	0.85 (±0.07) a[a]	0.20 (±0.03) b		6.36 (±0.32) a	5.37 (±0.28) b	

[a]Mean separation within main effects by Tukey's Studentized range test at $p = 0.05$. Interaction variety x treatment nonsignificant.

Table 2 Parameters of regression equations for the relationship between rates of water movement (F) and fruit surface area (A) of Chardonnay, Huxelrebe, Müller-Thurgau, and Riesling berries. Regression model: F (mg/h) = y_0 + a A (cm²).

	Water uptake			Transpiration		
	a ± SE (mg/hr cm²)	y_0 ± SE (mg/hr)	r^2 [a]	a ± SE (mg/hr cm²)	y_0 ± SE (mg/hr)	r^2 [a]
Chardonnay	0.040 (±0.03)	0.051 (±0.15)	0.09ns	0.554 (±0.09)	0.753 (±0.51)	0.63***
Huxelrebe	0.231 (±0.05)	0.054 (±0.30)	0.41***	0.794 (±0.05)	0.501 (±0.26)	0.91***
Müller-Thurgau	0.009 (±0.01)	0.148 (±0.07)	0.04ns	0.512 (±0.06)	1.222 (±0.42)	0.76***
Riesling	0.019 (±0.01)	0.044 (±0.03)	0.30**	0.463 (±0.02)	0.652 (±0.13)	0.93***

[a]Significance of coefficient of determination: **, ***, and ns indicate significance at p = 0.01, 0.001, and ns, respectively.

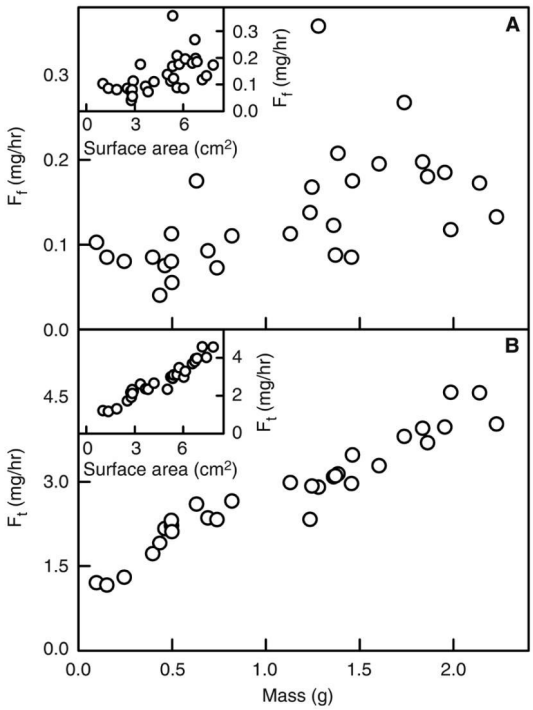

Figure 4 Relationship between rates of water uptake (F_f) (**A**) or transpiration (F_t) (**B**) and mass (main graph) or surface area (inset) of Riesling berries. For regression equations see Table 2.

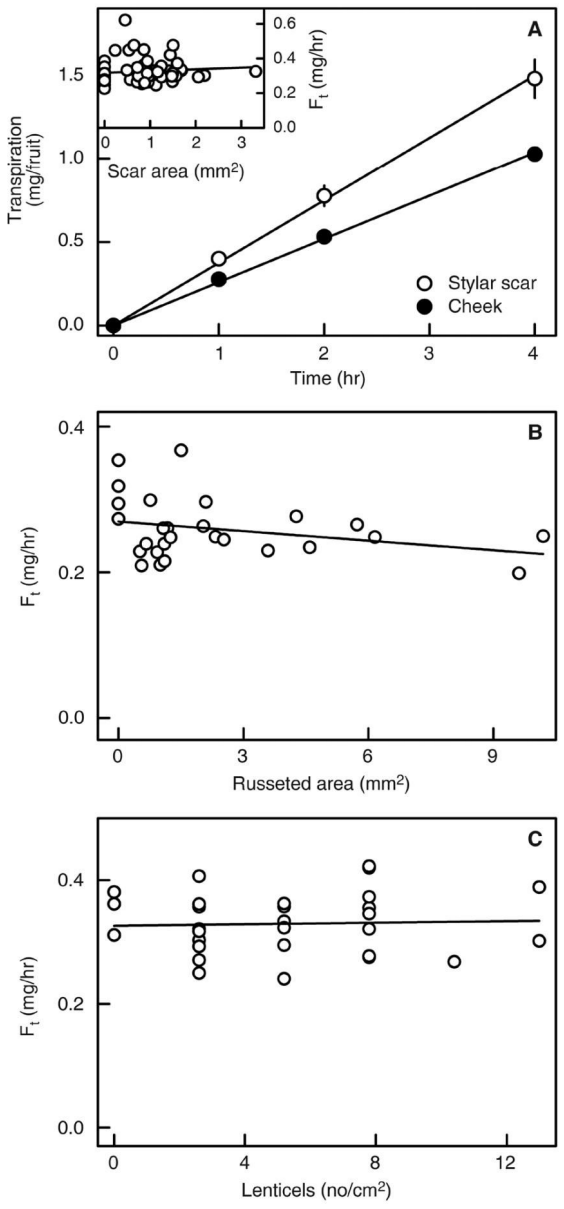

Figure 5 Time course of transpiration in stylar end and cheek region of Riesling berries (**A**). Inset: relationship between stylar scar area and transpiration. Relationship between russeted area of berry surface and rate of transpiration (F_t) (**B**) or between the number of lenticels per cm² and F_t (**C**).

consistent among the three varieties investigated. This finding is new and surprising. We expected water uptake and transpiration to occur primarily through the surface of the berry.

Unfortunately, attempts to identify the exact site of preferential water uptake at the stem region were not successful because the small size of potentially relevant structures prevented unambiguous experimentation using selective sealing with silicone rubber. Theoretically, the following structures may serve as parallel pathways for water uptake through the stem surface: the lenticels on the stem, the periderms covering the rim of the receptacle, the pedicel and/or receptacle surface in between the lenticels, or the juncture between the stem and the berry.

Preliminary experiments indicate that the receptacle and, more specifically, the periderm on the receptacle is a likely candidate for a high-flux pathway. Mechanically, removing this periderm increased rates of water uptake six-fold

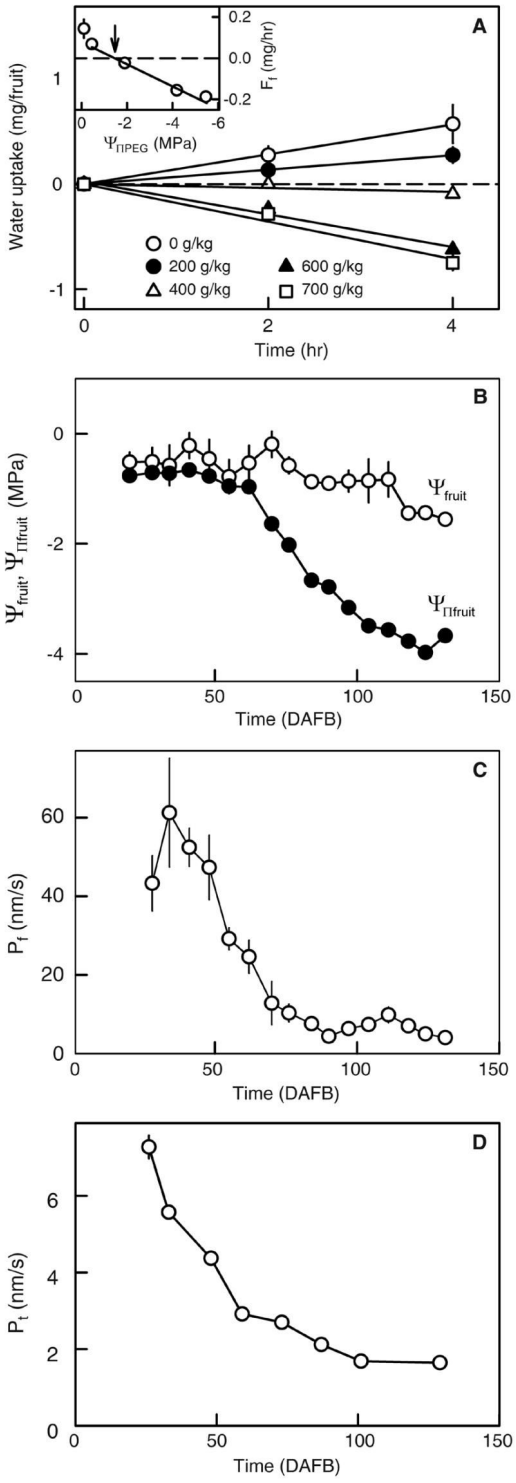

Figure 6 Time course of water uptake of Riesling grape berries (131 DAFB) incubated in solutions containing different concentrations of polyethylene 6000 (PEG) (**A**). Inset: relationship between rates of water uptake (F_f) and the solute potential ($\Psi_{\Pi PEG}$) of the PEG 6000 solution; arrow indicates the x-axis intercept that corresponds to the fruit water potential. Time course of change in the fruit water potential (Ψ_{fruit}) and the solute potential of the fruit juice ($\Psi\Pi_{fruit}$) in developing Riesling berries (**B**). Change in the permeability for osmotic water uptake (P_f) (**C**) and for transpiration (P_t) (**D**) of the berry cuticle of developing Riesling.

(T. Becker, unpublished data, 2008). This periderm represents an abscission layer that is formed prior to shedding of the calyptra during anthesis (Winkler et al. 1974). The drastic increase in water uptake upon removal of the periderm demonstrates that the pathway for penetration beyond this periderm must have a low resistance and a high driving force for water uptake, as would be expected for functional vascular traces connecting sepals, petals, and anthers to the vascular system of the stem (Mullins et al. 1992).

We expect the mechanism of water uptake in the stem region to be viscous flow. This hypothesis is based on the following calculations: Assuming the shape of the grape berry, the receptacle, and the pedicel to resemble a sphere, truncated cone, and cylinder, respectively, as first approximations, the surface areas for a mature 15 mm diameter Riesling berry would be estimated at 707, 22, and 17 mm^2, respectively. At a fruit water potential of -1.94 MPa (means for 2008 and 2009; Table 3) and water uptake rates of 0.24 mg/hr or 0.71 mg/hr for the berry surface or the stem surface including the stem/fruit juncture, respectively (means for 2008 and 2009; Table 1, Figure 3), the permeabilities for water uptake would amount to 6.7 nm/s for the berry surface, but to 376.1 nm/s for the pedicel plus receptacle region. If the receptacle only was involved in water uptake, the permeability would amount to 664.1 nm/s, which is two orders of magnitude higher than the permeability of the berry surface. Our calculation is a conservative estimate, as the cross-sectional areas involved in penetration through the stem surface are likely to be even smaller (and the amplification correspondingly larger). Nevertheless, the permeability estimates for the stem region calculated above are higher than all permeabilities reported for cuticles of terrestrial plants thus far (Beyer et al. 2005, Kerstiens 1996a, Lendzian and Kerstiens 1991). Furthermore, all of the structures lack a continuous penetration barrier such as a cuticle. In fact, when drawing a slight vacuum on submerged berries, gas bubbles form at these sites, indicating that these structures are porous in nature. Therefore, the high water uptake in the stem region is unlikely to be accounted for by a diffusion process through an intact cuticle, but more likely to occur by viscous flow through some, yet unidentified region on the stem.

Permeability of berry cuticle in water uptake and transpiration. The cuticle covering the berry surface is the primary barrier in water uptake and transpiration, and therefore, our permeability estimates represent permeabilities of the grape berry cuticle. These estimates were consistent among the three varieties, but different for water uptake and transpiration. The permeability for water uptake exceeded that for transpiration on average 2.9-fold in grape (range of P_f/P_t within variety and season: 2.1 to 4.5). Comparing our permeability estimates to published data for sweet cherry revealed that the permeability for water uptake of a mature Riesling berry was markedly lower (4.1 nm/s, Table 3; factor: 7- to 33-fold lower) than those of sweet cherry (range 30.7 to 135.3 nm/s depending on variety; Beyer et al. 2005), but permeability in transpiration was similar (1.6 nm/s, Table 3; sweet cherry: range 1.1 to 3.1 nm/s depending on variety; Beyer et al. 2005).

Table 3 Fruit water potential (Ψ_{fruit}), solute potential ($\Psi_{\Pi fruit}$), and permeability of the cuticle on the berry surface for osmotic water uptake (P_f) and transpiration (P_t) in Chardonnay, Müller-Thurgau, and Riesling.

Variety/stage[a]	Ψ_{fruit} (MPa)	$\Psi_{\Pi fruit}$ (MPa)	P_f (nm/s)	P_t (nm/s)
Chardonnay				
Preveraison	-0.31 (±0.36)	-0.66 (±0.00)	54.9 (±3.9)	nd[b]
Postveraison	-1.66 (±0.11)	-3.92 (±0.01)	7.7 (±0.9)	2.2 (±0.1)[c]
Müller-Thurgau				
Preveraison	-0.32 (±0.16)	-0.87 (±0.00)	133.9 (±26.9)	nd[b]
Postveraison	-2.27 (±0.12)	-3.48 (±0.02)	8.9 (±1.5)	2.5 (±0.1)[c]
Riesling				
Preveraison	-0.22 (±0.23)	-0.66 (±0.01)	52.4 (±4.8)	4.4 (±0.1)
Postveraison	-1.56 (±0.04)	-3.66 (±0.01)	4.1 (±1.2)	1.6 (±0.0)

[a]Sampling dates were 43, 37, 44 DAFB for preveraison and 101, 98, 129 DAFB for postveraison Chardonnay, Müller-Thurgau, and Riesling, respectively.
[b]nd: not determined.
[c]P_t for postveraison Chardonnay and Müller-Thurgau was quantified in the 2008 growing season, all other data in the 2009 season. Corresponding postveraison P_f data for the 2008 season Chardonnay (99 DAFB) and Müller-Thurgau (96 DAFB) were 4.6 (±1.1) nm/s and 6.3 (±0.7) nm/s, respectively. For Riesling the corresponding postveraison data for the 2008 season were 2.32 (±0.31) MPa, 7.7 (±2.1) nm/s, and 1.7 (±0.1) for Ψ_{fruit} (115 DAFB), P_f (115 DAFB), and P_t (122 DAFB), respectively.

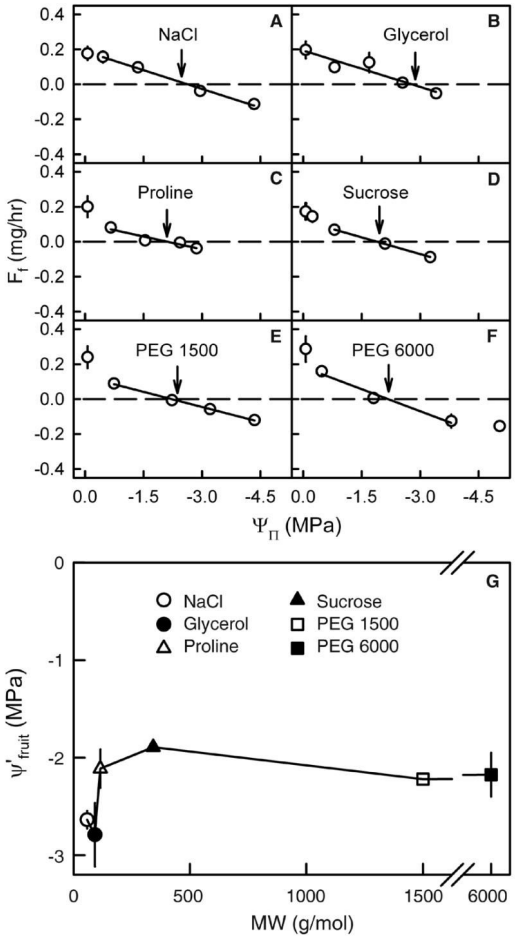

Figure 7 Effect of the solute potential (Ψ_Π) of solutions of osmotica of differing molecular weight on the rate of water uptake through the surface of Riesling berries. The osmotica were NaCl (**A**), glycerol (**B**), proline (**C**), sucrose (**D**), PEG 1500 (**E**), and PEG 6000 (**F**). Arrow indicates the apparent fruit water potential (Ψ'_{fruit}) where the solute potential of the incubation solution equaled the apparent water potential of the fruit as indicated by zero change in fruit mass. Relationship between the molecular weight of the osmoticum and the apparent fruit water potential (Ψ'_{fruit}) of Riesling berries (**G**).

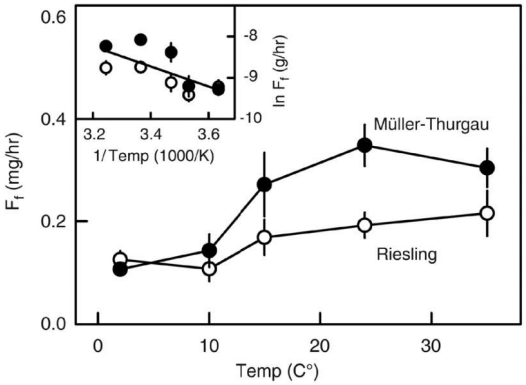

Figure 8 Effect of temperature on the rate of water uptake through the surface of Müller-Thurgau and Riesling berries. Inset: Arrhenius plot. The regression equation was: ln F_f (mg/hr) = 6.1 (±1.4) - 2353.1 (±401.4) [1/T(K)]; r^2 = 0.16***.

The lower permeability of the grape berry cuticle may be attributed to (1) a markedly lower frequency of microcracks as compared to sweet cherry (T. Becker, unpublished data, 2009) and (2) the absence of polar pathways in grape. In sweet cherry, these pathways form an aqueous continuum across the cuticle that accounts for a rapid water uptake by viscous flow (Weichert and Knoche 2006a). The absence of polar pathways in the grape berry cuticle is based on the following experimental evidence. First, fruit water potentials established in osmotica of differing molecular weights were not affected by the molecular weights of the osmotica (Figure 7). In contrast, in sweet cherry apparent fruit water potentials increased, that is, became less negative, as the molecular size of the osmotica increased (Weichert and Knoche 2006a). Second, ferric and alumina chloride had no effect on water uptake into the Riesling grape berry. In contrast, ferric and alumina chloride significantly decreased water uptake (and cracking) in sweet cherry by a precipitation reaction that plugged polar pathways across the cuticle (Weichert and Knoche 2006b). Third, there was no consistent relationship between the viscosity of the incubation solution and the rate

of water uptake through the grape berry surface (T. Becker, unpublished data, 2008). If polar pathways were involved in water uptake, we would expect flow rates to be inversely related to the viscosity of the incubation medium as was observed in sweet cherry (Weichert and Knoche 2006a). That was not the case in grape.

The above arguments are consistent with the hypothesis that diffusion is the primary mechanism for water movement through the berry surface for both water uptake and for transpiration. First, water movement through the grape berry cuticle was constant with time as would be expected for a physical process. Second, significant positive relationships were obtained between water movement in transpiration or water uptake and berry surface area as would be expected for a diffusion process. However, the coefficients of determination were consistently higher for transpiration as compared with water uptake. Third, permeability for water uptake exceeded that of transpiration on average only 2.9-fold. This difference may be caused by a hydration/swelling effect of the grape berry cuticle in the water uptake assay, while in transpiration, swelling of the cuticle is decreased due to exposure of the morphological outer side of the cuticle to dry air (Beyer et al. 2005). Unfortunately, there are no reports on the effect of hydration on the permeability of the grape berry cuticle, and, therefore, the magnitude of the effect of hydration is unknown. For sweet cherry, permeability for transpiration increased 2.1-fold when water activity was increased from 0 to 1 (Beyer et al. 2005) and increases of similar magnitude were reported for leaf cuticles of a range of species (Schreiber et al. 2001, Schönherr and Schmidt 1979). An alternative explanation for $P_f > P_t$ is a minor contribution of viscous flow in addition to diffusion (Beyer et al. 2005, House 1974). Since movement was restricted to the berry surface, potential candidate pathways for this uptake are lenticels or microcracks that are often associated with the stylar scar in grape berries (T. Becker, unpublished data, 2009). The overall contribution of this flow to total water uptake through the berry cuticle, however, must have been small as indexed by the small difference between the permeabilities for water uptake and transpiration.

It may be argued that our P_t overestimated the true P_t of the cuticle because of boundary layer formation between the transpiring berry and the dry silica gel. Using the diffusion coefficient of water vapor in air ($D = 0.26$ cm^2/s; Cussler 1997) and a maximum length of the diffusion path of 10 mm between the transpiring berry and the silica, the boundary layer resistance amounts 385 s/m which is less than 0.0001% of the resistance of the Riesling berry surface ($1/P_t = 1/1.6$ s/nm = 607981315 s/m at 129 DAFB; Table 3). Thus, a boundary layer was not a factor in our study.

Both the P_f and P_t of the Riesling berry surface decreased in the course of development reaching a minimum at maturity. Since P represent material constants, this decrease must be caused by a change in the barrier properties of the cuticle. Unfortunately, little is known about the physicochemical characteristics of the grape berry cuticle. However, the crystalline fraction of the intracuticular wax is considered to be the primary barrier within the cuticle (Reynhardt and Riederer 1994) and an increase in this fraction in the course of development may account for the decrease in P_f and P_t.

Practical implications. Some grape varieties such as Chardonnay and Riesling have compact clusters, where the interior of the cluster represents an essentially closed compartment with a high humidity atmosphere (Pieri and Fermaud 2005). Diurnal fluctuations of temperature that will occur under vineyard conditions are expected to cause condensation of water vapor inside the cluster (Pieri and Fermaud 2005). Because the stem has an easy-to-wet surface, the condensing water droplets are likely to form a liquid film from which rapid water uptake could occur. Since boundary layer resistance will be high due to the absence of air movement within the cluster, extended periods of surface wetness will result (Pieri and Fermaud 2005, Vail and Marois 1991) and, hence, continued water uptake resulting in increased turgor. Furthermore, transpiration that would decrease turgor is essentially limited to that portion of the berry surface that is exposed to the ambient atmosphere on the outer margins of the cluster.

The question arises as to whether this water movement through the surface is an important factor in the grape berry water balance and, possibly, in fruit cracking. A first assessment may be made from the permeabilities, driving forces, and cross-sectional areas for water movement established in our study using Riesling as an example. This assessment is based on the following assumptions. First, the grape berry cluster consists of a single "layer" of berries where one half of the berry is exposed to the inside of the cluster and the other half to the ambient atmosphere. Second, we assume an average Riesling berry having the following characteristics: P_f: 6.7 and 664.1 nm/s for berry and stem surfaces, respectively; P_t for the berry surface 1.6 nm/s, $\Psi_{fruit} = -1.94$ MPa, cross-sectional areas for transport of 707 and 22 mm^2 for berry and stem surfaces, respectively; data represent means of the 2008 and 2009 season. Third, we further assume a 12-hr day/night period at 24°C and an average 50/100% day/night humidity. Under these conditions, a liquid water film would likely cover the pedicels and receptacles inside the cluster and transpiration would be limited to the outer margins of the cluster. In the absence of precipitation events, the transpirational loss of water would be limited to the daytime and the outer half of the berry and amount to -11.9 mg during a 12-hr daytime period, while water uptake through the stem surface is calculated at 18.2 mg/d (day plus night time), yielding a net gain in fruit volume of 6.3 mg/d/berry. This rate could increase 3.8-fold to ~24.1 mg/d/berry, if the entire surface of berry plus stem were wet due to rain or condensation of water vapor. This calculation is conservative, since vascular flow from the vine into the berries is ignored. Unfortunately, vascular transport through xylem and phloem has not been quantified in Riesling berries. However, a maximum net vascular transport into Riesling berries may be estimated as a first approximation from the sum of the daily increase in fruit mass (maximum rate of a stage III Riesling berry + 28 mg/d/berry; T. Becker, unpublished data, 2009) plus the rate of transpirational water loss calculated above

(-11.9 mg/d/berry), yielding a maximum inflow through the vascular system of ~39.9 mg/d/berry. This estimate is lower than the vascular inflow of +152.0 mg/d/berry reported for the larger Italia grape berry (Lang and Thorpe 1989). When downscaling the latter flow rate by a factor of 4 to account for the four-fold larger mass of Italia (8 g; Lang and Thorpe 1989) as compared to the Riesling berry (2 g) in our study, both estimates are in reasonable agreement (+38.0 vs. +39.9 mg/d/berry). From these calculations it can be deduced that (1) the relative contributions of surface (berry plus stem) and vascular transport to total inflow into a Riesling berry (surface plus vascular; 24.1 mg/d/berry + 39.9 mg/d/berry = 64 mg/d/berry) on a rainy day amount to 38 and 62%, respectively; (2) the relative contribution of uptake through surfaces of stem and berry will increase as the growth rate of the berry decreases toward maturity; (3) a net increase in berry volume due to water uptake through the surface may occur in the absence of precipitation, if condensation causes formation of a liquid water film covering the berry stems inside dense clusters; and (4) despite the high permeability of the stem region, the amount of water entering the fruit via the vascular system may still be greater than uptake through berry and stem surfaces.

Conclusion

Our data demonstrate that flow rates and pathways of water movement through the surfaces of the berry and its stem differ between transpiration and water uptake. In mature Riesling, rates of transpiration exceed those of water uptake ~19- and 2-fold for surfaces of berry and stem, respectively. Furthermore, transpiration is mostly limited to the berry surface, but uptake occurs primarily through the stem surface, most likely in the receptacle region. The high permeability of the latter region is unlikely to be accounted for by a diffusion process through an intact cuticle, but is more likely caused by viscous flow through some, yet unidentified, pathway. Because of the potential importance, precise identification of this site of water uptake merits further investigation.

Literature Cited

Beyer, M., and M. Knoche. 2002. Studies on water transport through the sweet cherry fruit surface: V. Conductance for water uptake. J. Am. Soc. Hortic. Sci. 127:325-332.

Beyer, M., S. Lau, and M. Knoche. 2005. Studies on water transport through the sweet cherry fruit surface: IX. Comparing permeability in water uptake and transpiration. Planta 220:474-485.

Beyer, M., S. Peschel, H. Weichert, and M. Knoche. 2002. Studies on water transport through the sweet cherry fruit surface: VII. Fe^{3+} and Al^{3+} reduce conductance for water uptake. J. Agric. Food Chem. 50:7600-7608.

Chatelet, D.S., T.L. Rost, K.A. Shackel, and M.A. Matthews. 2008. The peripheral xylem of grapevine (*Vitis vinifera*). 1. Structural integrity in post-veraison berries. J. Exp. Bot. 59:1987-1996.

Clarke, S.J., W.J. Hardie, and S.Y. Rogiers. 2010. Changes in susceptibility of grape berries to splitting are related to impaired osmotic water uptake associated with losses in cell vitality. Aust. J. Grape Wine Res. 16:469-476.

Considine, J.A., and P.E. Kriedemann. 1972. Fruit splitting in grapes: Determination of the critical turgor pressure. Aust. J. Agric. Res. 23:17-24.

Cussler, E.D. 1997. Diffusion, Mass Transfer in Fluid Systems, p. 102. Cambridge University Press, Cambridge, UK.

Düring, H., A. Lang, and F. Oggionni. 1987. Patterns of water-flow in Riesling berries in relation to developmental-changes in their xylem morphology. Vitis 26:123-131.

Findlay, N., K.J. Oliver, N. Nii, and B.G. Coombe. 1987. Solute accumulation by grape pericarp cells: IV. Perfusion of pericarp apoplast via the pedicel and evidence for xylem malfunction in ripening berries. J. Exp. Bot. 38:668-679.

Geyer, U., and J. Schönherr. 1988. In vitro test for effects of surfactants and formulations on permeability of plant cuticles. *In* Pesticide Formulations. B. Cross and H.B. Scher (eds.), pp. 22-33. ACS Symposium Series. Am. Chemical Society, Washington, DC.

Grace, J., and P.R. van Gardingen. 1996. Plant cuticles under challenge. *In* Plant Cuticles: An Integrated Functional Approach. G. Kerstiens (ed.), pp. 319-329. Environmental Plant Biology Series. BIOS Scientific, Oxford.

Greenspan, M.D., H.R. Schultz, and M.A. Matthews. 1996. Field evaluation of water transport in grape berries during water deficits. Physiol. Plant. 97:55-62.

House, C.R. 1974. Water Transport in Cells and Tissues. Monographs of the Physiological Society, Vol 24. Arnold, London.

Keller, M., J.P. Smith, and B.R. Bondada. 2006. Ripening grape berries remain hydraulically connected to the shoot. J. Exp. Bot. 57:2577-2587.

Kerstiens, G. 1996a. Cuticular water permeability and its physiological significance. J. Exp. Bot. 47:1813-1832.

Kerstiens, G. 1996b. Diffusion of water vapour and gases across cuticles and through stomatal pores presumed closed *In* Plant Cuticles: An Integrated Functional Approach. G. Kerstiens (ed.), pp. 121-134. Environmental Plant Biology Series. BIOS Scientific, Oxford.

Knoche, M., S. Peschel, M. Hinz, and M.J. Bukovac. 2000. Studies on water transport through the sweet cherry fruit surface: Characterizing conductance of the cuticular membrane using pericarp segments. Planta 212:127-135.

Lang, A., and M.R. Thorpe. 1989. Xylem, phloem and transpiration flows in a grape: Application of a technique for measuring the volume of attached fruits to high-resolution using Archimedes' principle. J. Exp. Bot. 40:1069-1078.

Lendzian, K.J., and G. Kerstiens. 1991. Sorption and transport of gases and vapors in plant cuticles. Rev. Environ. Contam. Toxicol. 121:65-128.

Lide, D.R. 2009. CRC Handbook of Chemistry and Physics. CRC Press, Boca Raton, FL.

Ministerium für Wirtschaft, Verkehr, Landwirtschaft und Weinbau. 2002. Richtlinien für den kontrolliert umweltschonenden Weinbau in Rheinland-Pfalz. Mainz, Germany.

Mullins, M.G., A. Bouquet, and L.E. Williams. 1992. Biology of the Grapevine, pp. 76-77. Cambridge University Press, Cambridge, UK.

Nobel, P.S. 1991. Physicochemical and Environmental Plant Physiology. Academic Press, San Diego.

Pieri, P., and M. Fermaud. 2005. Effects of defoliation on temperature and wetness of grapevine berries. *In* Proceedings of the Seventh International Symposium on Grapevine Physiology and Biotechnology. L.E. Williams (ed.). Acta Hort. 689:109-116.

Reynhardt, E.C., and M. Riederer. 1994. Structures and molecular dynamics of plant waxes: II. Cuticular waxes from leaves of *Fagus sylvatica* L. and *Hordeum vulgare* L. Eur. Biophys. J. 23:59-70.

Rogiers, S.Y., J.M. Hatfield, V.G. Jaudzems, R.G. White, and M. Keller. 2004. Grape berry cv. Shiraz epicuticular wax and transpiration during ripening and preharvest weight loss. Am. J. Enol. Vitic. 55:121-127.

Schönherr, J. 1982. Resistance of plant surfaces to water loss: Transport properties of cutin, suberin and associated lipids. *In* Encyclopedia of Plant Physiology, New Series. Vol 12B. O.L. Lange et al. (eds.), pp. 153-179. Springer, Berlin.

Schönherr, J. 2006. Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. J. Exp. Bot. 57:2471-2491.

Schönherr, J., and H.W. Schmidt. 1979. Water permeability of plant cuticles–Dependence of permeability coefficients of cuticular transpiration on vapor-pressure saturation deficit. Planta 144:391-400.

Schreiber, L., M. Skrabs, K.D. Hartmann, P. Diamantopoulos, E. Simanova, and J. Santrucek. 2001. Effect of humidity on cuticular water permeability of isolated cuticular membranes and leaf disks. Planta 214:274-282.

Tyerman, S.D., J. Tilbrook, C. Pardo, L. Kotula, W. Sullivan, and E. Steudle. 2004. Direct measurement of hydraulic properties in developing berries of *Vitis vinifera* L. cv. Shiraz and Chardonnay. Aust. J. Grape Wine Res. 10:170-181.

Vail, M.E., and J.J. Marois. 1991. Grape cluster architecture and the susceptibility of berries to *Botrytis cinerea*. Phytopathology 81:188-191.

Weichert, H., and M. Knoche. 2006a. Studies on water transport through the sweet cherry fruit surface. 10. Evidence for polar pathways across the exocarp. J. Agric. Food Chem. 54:3951-3958.

Weichert, H., and M. Knoche. 2006b. Studies on water transport through the sweet cherry fruit surface. 11. $FeCl_3$ decreases water permeability of polar pathways. J. Agric. Food Chem. 54:6294-6302.

Winkler, A.J., J.A. Cook, W.M. Kliewer, and L.A. Lider. 1974. General Viticulture. University of California Press, Berkeley.

7. Substantial water uptake into detached grape berries occurs through the stem surface

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Substantial water uptake into detached grape berries occurs through the stem surface

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Abstract

Background and Aims: Water uptake through the stem surface contributes to total water uptake of submerged grape berries (*Vitis vinifera* L.). The objective was to identify the site of this uptake.

Methods and Results: Uptake of water and a fluorescent dye was studied using a single berry + stem system. To identify the site of water uptake, the cut end of the stem and selected regions of the stem surface were coated with a silicone sealant. Water uptake was determined gravimetrically. After immersing in dye solution, the stem and the receptacle region of the stem/berry junction fluoresced, but the exocarp did not. Fluorescence of vascular bundles in stem and berry indicated that the dye taken up was transported into the berry. Selective coating of the various stem parts revealed that at steady state (96 to 144 h), the stem/berry junction (0.11 ± 0.04 mg/h), receptacle (0.09 ± 0.03 mg/h) and the remaining stem surface (0.08 ± 0.03 mg/h) contributed approximately equally to total water uptake in the whole stem region (0.24 ± 0.05 mg/h), which in turn accounted for 55% of uptake of a berry + stem (0.43 ± 0.05 mg/h).

Conclusions: Stem/berry junction, and periderms of abscission zones and lenticels represent regions of preferential water uptake into detached grape berries.

Significance of the Study: Extrapolating from the single berry system to an intact bunch on a grapevine demonstrates that water uptake through the stem tissues inside a compact bunch may contribute to berry cracking under vineyard conditions.

Keywords: *fluorescence microscopy*, *lenticel*, *pedicel*, *periderm*, *rachis*, Vitis vinifera *L.*

Introduction

Rain-induced cracking, also referred to as splitting, of grape berries results in significant production losses worldwide. Under warm and moist conditions, cracked berries are commonly infected by bunch rots that reduce both the yield and the quality of the must and wine (Viret et al. 2004, Meneguzzo et al. 2008). Cracking is thought to result from increased turgor due to water uptake by the berry (Considine and Kriedemann 1972, Lang and Thorpe 1989, Clarke et al. 2010). The berry water balance is further upset by reduced rates of water loss through the skin under wet or high-humidity conditions. Excess water uptake by the berry may occur by a number of routes: from the vine via the vascular system, through the surface of the rachis or through the surface of the berry itself.

Whereas vascular transport from the vine into the grape bunch has been studied extensively in recent decades (e.g. Düring et al. 1987, Tyerman et al. 2004, Keller et al. 2006, Chatelet et al. 2008), little is known about water uptake through the berry surface and its stem (Clarke et al. 2010, Becker and Knoche 2011). Recent studies have established that water uptake through surfaces in the bunchstem region including the stem/berry junction account for around 80% of the total water taken up by an immersed grape berry (data for 0 to 4 h; Becker and Knoche 2011). The relative contribution of stem surface uptake to total uptake remains constant throughout berry development. It is not yet known where in the stem region this rapid water uptake occurs. Potential sites of entry include (i) that part of the stem that is proximal to the receptacle that we refer to as the pedicel portion of the stem; (ii) the surface of the receptacle itself that is largely covered by the periderm of the abscission layers of floral organs; and (iii) the junction between the receptacle and the berry. Localising the site(s) of water entry and quantifying fluxes along the different pathways are prerequisites for establishing a complete water balance of the berry and for developing suitable counter measures to decrease the incidence of cracking either through cultural practices or through breeding approaches.

The objective of this study was to identify the main site(s) of water uptake in Riesling grape berries and to quantify the rates and dynamics of water uptake.

Materials and methods

Plant material

Post-veraison grape bunches (*Vitis vinifera* L. cv. Riesling) were sampled from experimental vineyards of the University of Hannover in Ruthe/Sarstedt, Germany (lat. 52°25′N, long. 9°82′E) and the State Education and Research Center for Viticulture and Horticulture Rheinpfalz at Neustadt an der Weinstrasse (lat. 49°21′N, long. 8°8′E) between 79 and 110 days after full bloom (DAFB) in 2010 and 2011. Unless otherwise specified, bunches were cut in the air, and their berries were processed on

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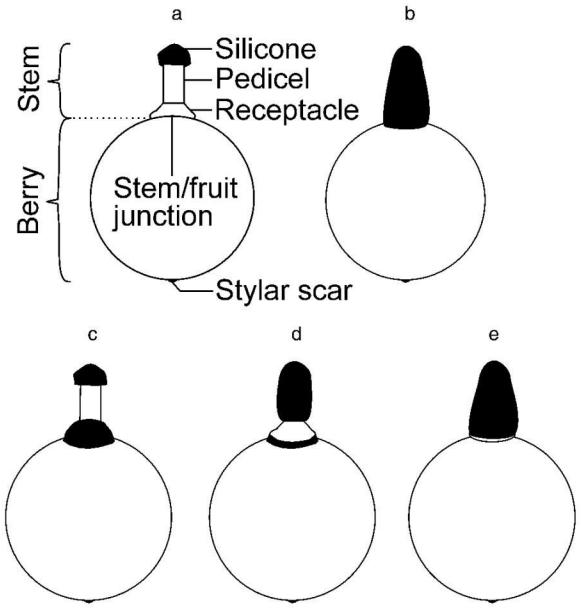

Figure 1. Schematic diagram of a grape berry showing terminology and the sealing treatments used to restrict water uptake to selected regions of the berry and stem surface.

the day of sampling. Berries were selected for uniformity of size and colour and for freedom from visible defects. Unless otherwise specified, pedicels were cut to 5 mm length with a razor blade, and the cut ends were coated with a silicone sealant (Dow Corning 3140 RTV Coating; Dow Corning Corp., Midland, Michigan, USA; Figure 1a) which was allowed to cure at ambient temperature and humidity for a minimum of 4 h.

Experiments

Fluorescence microscopy

Berries were held for 30 h at 2°C and 75% relative humidity, their stems cut at 5 mm, and their cut ends sealed. Following curing overnight, berries were immersed in the aqueous fluorescent dye acridine orange (5% w/w) for 48 h. After staining, berries were removed from the dye, rinsed with deionised water for 30 s to remove surface dye residues and blotted with tissue paper. Berries were then viewed using a fluorescence stereomicroscope (MZ10; Leica Mikrosysteme GmbH, Bensheim, Germany; filter module GFP-plus with 480/40 nm excitation wavelength; ≥510 nm emission wavelength). The dye distribution within the berries and stems was studied in intact berries and also in longitudinal sections cut along the stem/stylar scar axis. The intensity of the fluorescence signal was always higher than any autofluorescence associated with non-treated control berries. The number of repetitions was five.

Water-uptake experiments

Putative regions of preferential water uptake were coated with sealant. Following curing, water uptake was quantified gravimetrically. Berries were weighed, immersed in deionised water at 22°C for selected intervals, removed from the water, carefully blotted with tissue paper and reweighed. Any berries that cracked in the course of the experiment were discarded (on average <10%). Uptake rates were calculated on an individual berry basis as the slope of a regression line of a plot of cumulative water uptake versus time. The coefficients of determination averaged $r^2 = 0.996$ for a total of 138 berries.

The time course of water uptake into the stem + berry systems was established over time periods of 363 h (~15 days). To restrict water uptake to different surfaces of the stem + berry systems, sealant was applied to just the cut end of the stem or to the entire stem including the cut end and the stem/berry junction (Figure 1a,b). For determining uptake into excised stems, stems were cut either immediately before the uptake experiment was started or after berries with stems attached (only cut ends sealed) had already been immersed in deionised water for 240 h. The minimum number of replications per treatment was eight.

To identify whether dehydration that occurs during silicon curing as a result of transpiration affected water-uptake characteristics in our system, stem + berries or berries were sampled, prepared and sealed as described earlier. The time course of water uptake was determined following a 4-h or a 28-h curing period at ambient conditions (22°C and 41% relative humidity).

The site of preferential water uptake on the stem was identified using berries excised underwater from bunches that were also cut underwater. The following sealant treatments were applied: sealing just the cut end of the stem restricted water uptake to the berry and stem surfaces (Figure 1a), coating the entire stem including the stem/berry junction restricted water uptake just to the berry surface (Figure 1b); coating just the distal portion of the stem (receptacle), the cut end of the stem and the stem/berry junction restricted water uptake to the berry surface plus the proximal portion of the stem (pedicel) (Figure 1c). Coating the surface of the pedicel including its cut end and the stem/berry junction restricted water uptake to the berry surface and receptacle (Figure 1d). Coating the entire stem (pedicel plus receptacle plus cut end) limited water uptake to the berry surface and the stem/berry junction (Figure 1e). Water uptake rates were quantified as described earlier for the 0 to 4-h, 24 to 72-h and 96 to 144-h time intervals using 12 to 15 replicates.

The barrier function of the periderm around the rim of the receptacle was investigated using a total of 37 berries that had the entire surface of the berry and the stem including its cut end and the stem/berry junction except for the receptacle coated with sealant. Following a 24-h time period for silicone curing, the periderm on the receptacle of 18 berries was removed using a razor blade, the remaining 19 berries served as the controls. Water uptake was determined earlier.

Terminology and data analysis

Throughout our study, we use the term 'rachis' for the bunch's central axis, branches and stems. The 'stems' connect berries to the central axis or branches. A 'stem' comprises the 'receptacle' at its distal end, and the remaining proximal end is the 'pedicel' (Figure 1a). The 'stem/berry junction' refers to the narrow interface between the berry and the receptacle.

Data in figures and tables are presented as means ± standard errors. Standard errors for differences of means were calculated as the root of the sum of the squared standard errors of the respective means (Sachs 1992). Where error bars in figures are not shown, they were smaller than the data symbols.

Results

After immersing berries in acridine orange solution, red fluorescence on the surface was found to be associated with suberised tissues including the lenticels on the receptacle, the pedicel and the berry, the abscission layers of flower organs and the stylar scar (Figure 2a–c). Yellow and green fluorescent infiltration zones

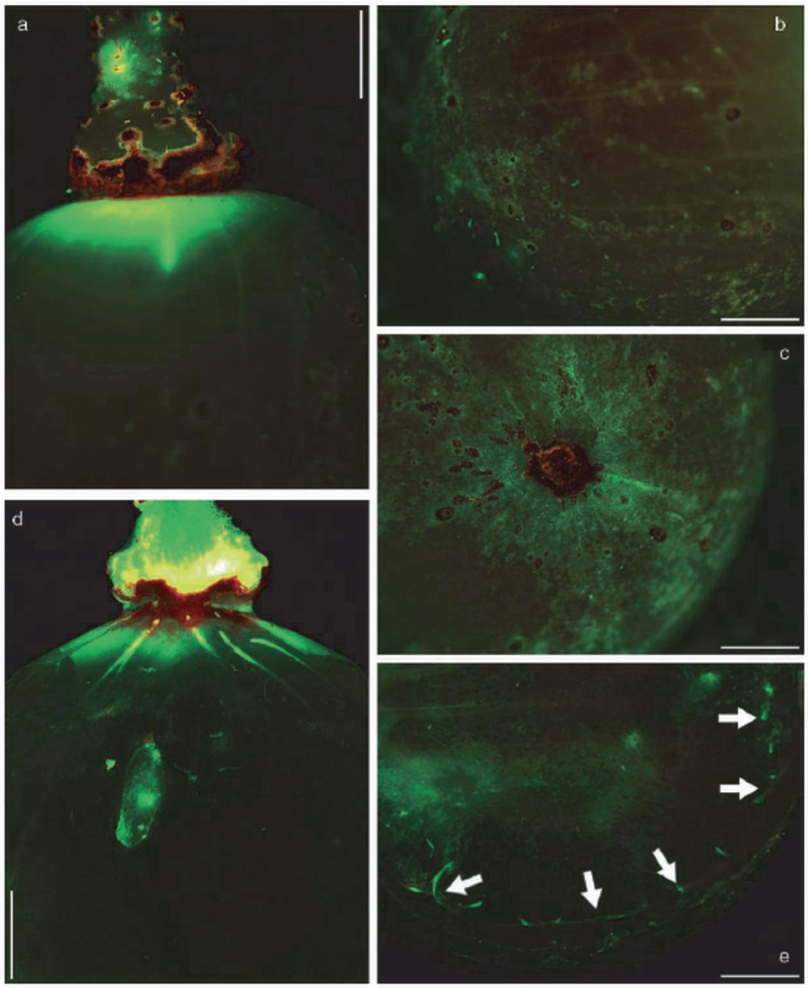

Figure 2. Fluorescence micrographs of a grape berry (98 days after full bloom) after immersion in 5% (w/w) aqueous acridine orange for 48 h. The stem (5 mm in length, cut end sealed with silicone sealant) remained attached to the berry. (a) Berry and stem, (b) berry surface near the cheek and (c) near the stylar scar. Longitudinal section along the stem/stylar scar axis through a berry in the (d) proximal stem region and (e) cheek region. Arrows indicate peripheral vascular bundles. Scale bars 2 mm.

were limited to the lenticels on the receptacle and pedicel, the abscission layers and the region of the stem/berry junction. Generally, fluorescence intensity decreased as the distance from the lenticels or from the stem/berry junction increased (Figure 2a). There were no infiltration zones surrounding lenticels on the exocarp or on the stylar scar (Figure 2b,c).

Cross-sections through the berry revealed red fluorescence at the stem/berry junction and diffuse green fluorescence in the stem-end of the berry, in vascular bundles of the brush region (Figure 2d) and in the peripheral bundles underlying the non-fluorescing exocarp (Figure 2e). In addition, green fluorescence was associated with the seed and, at lower intensity, with the parenchyma of the berry (Figure 2d,e).

Water uptake through the surfaces of the berry + stem or the berry surface increased with time up to 363 h (Figure 3a). Restricting water uptake to the berry surface by coating the stem and the stem/berry junction with sealant decreased water uptake and altered uptake dynamics (Figure 3b, inset). In the berry + stem system, rates of uptake decreased within 48 h and remained approximately constant thereafter, while rates of uptake through the berry surface were approximately constant throughout the experiment (Figure 3b, inset). Assuming that the water-uptake surfaces of the stem and berry can be considered to behave as parallel pathways, the rate of water uptake through the stem surface can be calculated by subtracting the rate of water uptake through the berry surface from that through surfaces of berry plus stem. This calculated uptake through the stem surface occurred at a decreasing rate up to about 48 h that remained constant thereafter as long as the stem was attached to the berry (128.5 µg/h for 48 to 363 h; Figure 3a,b). In contrast, uptake by isolated 5-mm stems (i.e. in the absence of the berry) differed qualitatively and quantitatively from the calculated uptake through stems attached to a berry. The initial rates of uptake into excised stems were six-fold higher than the calculated uptake through stems, they decreased rapidly with time and approached an asymptote at 5.0 µg/h within 100 h. Furthermore, initial rates of uptake by excised stems were even 4.7-times higher than those for berries plus stems. Qualitatively, similar data were obtained when stems were excised from the berries in the course of an uptake experiment after 240 h (Figure 3a, inset). However, initial rates of uptake and total water uptake were lower when stems were excised at 240 h compared with when excised at 0 h (Figure 3a).

The relative contribution of water uptake through the stem surface to that of the berry plus stem decreased from initially more than 80% to about 50% at 300 h (Figure 3c). There was no difference in absolute rates of water uptake through the stem surface or its relative contribution to total uptake between berries that were allowed to cure for 4 h and for 28 h or between berries sampled at 79 and 110 DAFB (both 4-h curing period; Figure 3d).

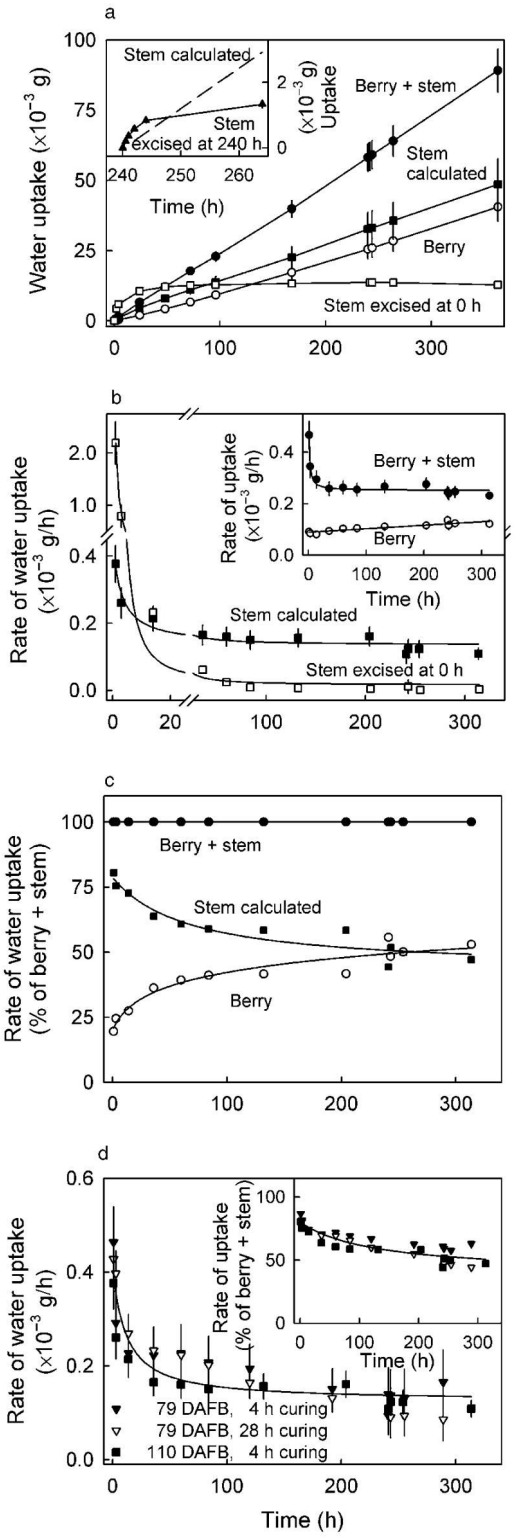

Figure 3. (a) Time course of water uptake into detached grape berries (110 days after full bloom (DAFB), $n = 8–10$). Coating berries with silicone sealant restricted water uptake to the surfaces of the berry plus stem ('Berry + stem') or to the berry surface only ('Berry'). Water uptake through the stem surface including the stem/berry junction was calculated by subtracting the amount of uptake through the berry surface from that taken up through the surface of the berry plus stem ('Stem calculated'). Water uptake into excised stems was measured after excising the stem from a berry ('Stem excised') at 0 h (main graph) or at 240 h after the initiation of the experiment (inset). (b) Rates of water uptake through excised stems ('Stem excised at 0 h'; main graph), through stems that remained attached to the berry ('Stem calculated'; main graph) or through berry + stem (inset) or the berry only (inset). (c) Relative rates of water uptake through berry plus stem ('Berry + stem'), through the berry surface ('Berry') or through a stem attached to a berry ('Stem calculated'). (d) Absolute (main graph) and relative rates of water uptake (inset) through stems attached to berries. Berries were sampled at 79 DAFB and allowed to transpire water for a 4- or 28-h period for silicone curing. For comparison, data for berries sampled at 110 DAFB (4 h curing) were redrawn from (b) and (c).

Selective coating of the stem surface with sealant established that stem uptake (including stem/berry junction) occurred in approximately equal amounts through the stem/berry junction, through the receptacle and through the pedicel surface (Table 1). Furthermore, the decrease in flow rates through the stem surface was accounted for by a decrease in flow rates through all three pathways.

When removing the periderm at the rim of the receptacle, rates of water uptake increased about six-fold (Figure 4).

Discussion

Our results establish: (i) that the substantial water uptake in the stem region of a detached grape berry occurs at approximately equal rates through the stem/berry junction, through the receptacle and through the pedicel surface (Figures 2,3; Table 1); and (ii) that the water taken up in these regions enters the vascular system of the stem and is transported into the berry (Figures 2,3).

Primary epidermal and secondary peridermal tissues make up the surfaces of the berry and its stem. In the berry, the periderms arise from the abscission layers associated with the various flower organs shed from the rim of the receptacle (Winkler et al. 1974). They also arise from the gradual differentiation of stomata into lenticels (Comménil et al. 1997). Cuticle and suberised cork tissue represent the respective barriers to water entry (Figure 4). In the grape berry, the barrier functions of these dermal tissues are impaired at the stem/berry junction and by any cracks that occur within the periderm and at the interface of the epidermal and peridermal tissues. These cracks probably result from the different elasticities of the epidermis and periderm that cause stress concentrations and failure, as has been described for lenticels on the berry exocarp (Brown and Considine 1982). Evidence for rapid dye, and hence, water uptake at the junction and in these regions comes from the gradient in fluorescence that changes from red to yellow to green as the acridine orange concentration decreases (Scheibe and Zanker 1962). Thus, the red fluorescence of the stem/berry junction and the periderms is indicative of a high uptake rate at the site of penetration, and the gradient from red to yellow to green is evidence for its subsequent transport into the surrounding tissues (Figure 2a,d). The fluorescing seed and the gradient in fluorescence within the mesocarp is consistent with dye transport into the berry, most likely in vascular bundles (Figure 2d,e).

Further evidence for vascular transport between the stem and the berry comes from the water-uptake experiment where the calculated stem uptake differed both in rate and total amount from that determined directly using excised stems (Figure 3). This discrepancy is accounted for by a two-compartment model

Table 1. Rates of water uptake along different pathways into a grape berry. Water uptake was restricted to the respective pathway by coating other surfaces with a silicone sealant.

Uptake through surfaces of	Rate of water uptake ($\times 10^{-3}$ g/h)			Relative contribution (% of rate of berry + stem)		
	Time interval			Time interval		
	0–4 h	24–72 h	96–144 h	0–4 h	24–72 h	96–144 h
Berry + stem	0.95 (±0.12)	0.53 (±0.05)	0.43 (±0.05)	100.0	100.0	100.0
Berry	0.18 (±0.03)	0.24 (±0.04)	0.19 (±0.02)	19.2	46.0	44.6
Stem	0.77 (±0.13)	0.29 (±0.06)	0.24 (±0.05)	80.8	54.0	55.4
Pedicel	0.21 (±0.06)	0.07 (±0.05)	0.08 (±0.03)	22.0	12.6	18.2
Receptacle	0.30 (±0.06)	0.12 (±0.06)	0.09 (±0.03)	31.3	22.1	21.3
Stem/berry junction	0.28 (±0.04)	0.09 (±0.05)	0.11 (±0.04)	29.4	17.6	24.9

Rates of water uptake were calculated for the intervals 0 to 4 h, 24 to 72 h and 96 to 144 h after initiation of the experiment. Data represent means and standard errors of the means of two independent experiments at 95 and 97 days after full bloom. The stem comprises a length of pedicel, the receptacle and the stem/berry junction.

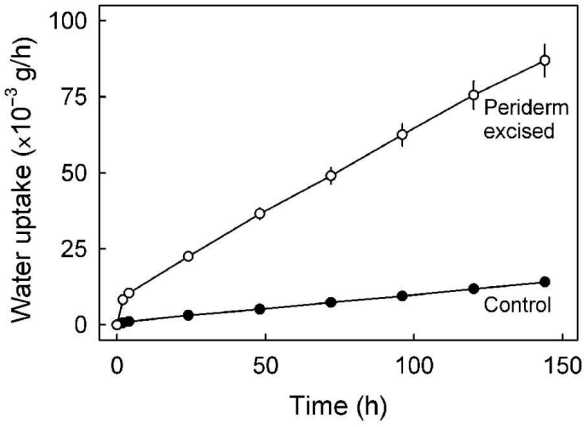

Figure 4. Effect of removal of the periderm of the receptacle on water uptake into grape berries (99 days after full bloom). Water uptake was restricted to the receptacle by sealing surfaces of the berry and the stem except for the receptacle. The periderm was either excised by razor blade ('Periderm excised') or left intact ('Control').

where the berry and its stem represent two compartments that we assume to differ in the following characteristics: compared with the berry compartment, the stem compartment has a small but highly permeable surface and a small volume. Furthermore, we assume the stem tissue compartment to have a higher modulus of elasticity (i.e. its tissues are stiffer) than the berry compartment. The water potential of the stem is assumed to be equal to or less negative than that of the berry. When submerging a berry with its stem, water uptake will occur primarily through the stem surface. Because of the low stem volume and its higher modulus of elasticity, the water potential will increase relative to that of the berry. The gradient in water potential between the berry and its stem will cause subsequent transport via the vascular system from the stem into the berry, thereby allaying a decrease in driving force into the stem. In contrast, water uptake by excised stems rapidly increases the stem's water potential, thereby causing the driving force to decrease, and hence, the water uptake rate falls off. This model accounts for: (i) the saturation kinetics of water uptake in isolated stems where the rate of uptake decreases to essentially zero within 100 h of incubation (mean rate 5.0 ± 3.2 µg/h for 100 to 363 h), while for stems that remain attached to a berry, rates of uptake average 129.8 ± 26.9 µg/h in the same time interval; (ii) a calculated stem uptake that beyond 100 h of immersion period exceeds the uptake of excised stems; and (iii) the staining of the stem and berry vascular systems when berries with stems attached were immersed in a fluorescent dye solution.

We exclude as an explanation of these results, the idea that the high uptake through stems is solely because of rehydration, as a consequence of water loss by transpiration during silicone curing. First, the calculated rates of water uptake through stems attached to berries decrease only during the first 48 h of incubation and remain constant and well above zero thereafter (Figure 3b). Second, when repeating the experiment using the short and extended 'dehydration' periods of 4 and 28 h for silicone sealant curing, the rates of water uptake were qualitatively and quantitatively similar (Figure 3d). If rehydration were a significant factor, we would expect the longer curing period to be associated with more severe dehydration, and so, higher rates and amounts of water uptake by the stems. Third, the calculated uptake through the stem exceeded that in excised stems from 100 h onwards after the beginning of the experiment. At this time, uptake into excised stems had long since reached an asymptote at approximately zero, indicating that its uptake capacity was fully saturated, most likely because sufficient driving force was lacking.

Also, cavitation of the vascular system is unlikely to account for the decrease in flow rates through the stem surfaces during the first 48 h of an experiment. When bunches and berries were cut underwater and sealed with silicone within 15 min of excision, cavitation would not be a factor, but flow rates still decreased with time (Table 1). Also, stems that were excised from berries after 240 h of incubation revealed the same uptake kinetic as those excised prior to the experiment. At 240 h, the berry + stem system was at steady state and hence, any cavitated vessels would have been filled (Figure 3). Finally, microscopy and image analysis of vascular bundles in stem cross-sections revealed that the cumulative lumen of the vascular system of a stem at 5 mm length would be only one-eighth of the amount of water taken up within 48 h by excised stems (Tobias Becker, unpublished data).

It may be argued that the data obtained are biased because berries that cracked were excluded from data analysis. However, there are several arguments why this is unlikely the case. First,

cracked berries represented less than 10% of the berry population, and thus, our data are representative for the majority of the berries. Second, there was no difference in mass between cracked and non-cracked berries. Cracked berries, depending on sealing treatment, sometimes had significantly higher rates of water uptake than non-cracked berries (e.g. 0 to 4 h time interval: 0.68 ± 0.23 vs 0.58 ± 0.04 mg/h for cracked and non-cracked berries with stem ends sealed, respectively, $P = 0.576$; 0.46 ± 0.10 vs 0.12 ± 0.01 mg/h for cracked and non-cracked berries having the entire stem including the receptacle sealed, respectively, $P < 0.0001$). However, in these cases, it is not clear whether the crack caused the high rate of water uptake or the high rate of water uptake caused cracking.

Our data are consistent with a functional vascular connection between stem and berry in post-veraison grape berries that is neither physically blocked by tylosis nor interrupted by ruptured vascular bundles as a result of berry growth (Bondada et al. 2005, Keller et al. 2006). Furthermore, the accumulation of fluorescent tracer in the peripheral vascular system of the berry is evidence for long-distance transport in the vascular system between the site of entry at the stem and the berry.

To relate the data obtained in the berry + stem system to an intact grape berry bunch, surface areas of berries and rachis were determined in Riesling. Surface areas averaged 681 cm^2 per bunch for the berries and 69 cm^2 per bunch for the entire rachis (mean of three Riesling bunches with 96 ± 7 berries per bunch; Tobias Becker, unpublished data). Assuming the pedicel uptake per unit surface area to be representative for the entire rachis, water uptake through berry and rachis surfaces (including stem/berry junction) was calculated at 17.6 and 94.4 mg/h per bunch equivalent to 15.7 and 84.3% of total uptake per bunch, respectively, which is close to the relative contribution of berry (19.2%) and stem (80.8%, including stem/berry junction) calculated from the berry + stem system (all calculations for 0 to 4 h; see Table 1). This calculation is only a first approximation and likely an overestimation of the relative uptake through the rachis because the rachis was assumed to have the same permeability and driving force as the pedicel in the berry + stem system and its hydraulic resistance to be negligible. Also, our time-course studies demonstrate that the relative contribution of uptake through the stem surface to total water uptake of berry + stem will decrease as the wetness duration, and hence, the length of a precipitation event increases (Figure 3d). Nevertheless, the calculation clearly demonstrates that uptake through the surface of the rachis can contribute significantly to the bunch water balance.

In summary, our data provide evidence for preferential water uptake into grape berries across the stem/berry junction and also the surfaces of the receptacle and of the pedicel. The sum of the water uptakes of these regions can even exceed uptake through the berry surface up to 4.9-fold. These findings are relevant under vineyard conditions where the surface of the rachis inside compact grape bunches is subjected to extended periods of surface wetness (Vail and Marois 1991, Pieri and Fermaud 2005), and hence, water uptake, and possibly, subsequent cracking of the berries.

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References

Becker, T. and Knoche, M. (2011) Water movement through the surfaces of the grape berry and its stem. American Journal of Enology and Viticulture **62**, 340–350.

Bondada, B.R., Matthews, M.A. and Shackel, K.A. (2005) Functional xylem in the post-veraison grape berry. Journal of Experimental Botany **56**, 2949–2957.

Brown, K. and Considine, J. (1982) Physical aspects of fruit growth: stress distribution around lenticels. Plant Physiology **69**, 585–590.

Chatelet, D.S., Rost, T.L., Shackel, K.A. and Matthews, M.A. (2008) The peripheral xylem of grapevine (*Vitis vinifera*). 1. Structural integrity in post-veraison berries. Journal of Experimental Botany **59**, 1987–1996.

Clarke, S.J., Hardie, W.J. and Rogiers, S.Y. (2010) Changes in susceptibility of grape berries to splitting are related to impaired osmotic water uptake associated with losses in cell vitality. Australian Journal of Grape and Wine Research **16**, 469–476.

Comménil, P., Brunet, L. and Audran, J. (1997) The development of the grape berry cuticle in relation to susceptibility to bunch rot disease. Journal of Experimental Botany **48**, 1599–1607.

Considine, J.A. and Kriedemann, P.E. (1972) Fruit splitting in grapes. Determination of critical turgor pressure. Australian Journal of Agricultural Research **23**, 17–24.

Düring, H., Lang, A. and Oggionni, F. (1987) Patterns of water-flow in Riesling berries in relation to developmental-changes in their xylem morphology. Vitis **26**, 123–131.

Keller, M., Smith, J.P. and Bondada, B.R. (2006) Ripening grape berries remain hydraulically connected to the shoot. Journal of Experimental Botany **57**, 2577–2587.

Lang, A. and Thorpe, M.R. (1989) Xylem, phloem and transpiration flows in a grape. Application of a technique for measuring the volume of attached fruits to high-resolution using Archimedes' principle. Journal of Experimental Botany **40**, 1069–1078.

Meneguzzo, J., Miele, A., Rizzon, L.A. and Ayub, M.A.Z. (2008) Effect of bunch rot on the sensory characteristics of the Gewürztraminer wine. Journal International des Sciences de la Vigne et du Vin **42**, 107–111.

Pieri, P. and Fermaud, M. (2005) Effects of defoliation on temperature and wetness of grapevine berries. Proceedings of the VIIth International Symposium on Grapevine Physiology and Biotechnology, Davis, California, USA, pp. 109–116.

Sachs, L. (1992) Angewandte statistik (Springer Verlag: Berlin) p. 161.

Scheibe, G. and Zanker, V. (1962) Über die Metachromasie der Absorption und Fluoreszenz von Acridinfarbstoffen. Histochemie **3**, 122–126.

Tyerman, S.D., Tilbrook, J., Pardo, C., Kotula, L., Sullivan, W. and Steudle, E. (2004) Direct measurement of hydraulic properties in developing berries of *Vitis vinifera* L. cv Shiraz and Chardonnay. Australian Journal of Grape and Wine Research **10**, 170–181.

Vail, M.E. and Marois, J.J. (1991) Grape cluster architecture and the susceptibility of berries to *Botrytis cinerea*. Phytopathology **81**, 188–191.

Viret, O., Keller, M., Jaudzems, V.G. and Cole, F.M. (2004) *Botrytis cinerea* infection of grape flowers. Light and electron microscopical studies of infection sites. Phytopathology **94**, 850–857.

Winkler, A.J., Cook, J.A., Kliewer, W.M. and Lider, L.A. (1974) General viticulture (University of California Press: Berkeley).

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8. Das Platzen von Weinbeeren (Vitis vinifera) bei Befall mit Grauschimmel (Botrytis cinerea)

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Zusammenfassung

Das Platzen reifer Beeren der Weinrebe (Vitis vinifera L.) nach Niederschlägen verursacht Verluste und Qualitätseinbußen im Weinbau. Häufig ist dies in der Spätphase der Fruchtentwicklung mit einem Befall der Trauben mit Fäulnispilzen verbunden. In der vorliegenden Studie wird die Rolle des Grauschimmels (Botrytis cinerea Pers.:Fr.), der als dominierender Fäulniserreger auftritt, beim Platzen von Beeren der Sorte Riesling untersucht. Hierzu wurden Weinbeeren mit charakteristischen Befallssymptomen (braun-violette Verfärbung der Fruchthaut) des Pilzes nach natürlicher Infektion am Standort ausgewählt. Nach Niederschlag platzten diese ausschließlich in einem Bereich, in dem Befallssymptome vorlagen. Die Risse waren mehrheitlich, d.h. bei 55% der Früchte, quer zur Fruchtachse zwischen Stiel- und Griffelansatz orientiert. Auf der Fruchthaut von befallenen Weinbeeren traten gehäuft in der Griffelregion und z.T. an Lentizellen mikroskopische Risse auf, die als Mikrorisse bezeichnet werden. Diese Risse zeigten nach Inkubation mit Acridinorange Infiltrationshöfe unterschiedlicher Größe. Die infiltrierte Fläche korrelierte positiv mit der Risslänge ($r2 = 0,43^*$). Die Wasseraufnahme von abgeschnittenen Beeren stieg mit der Zeit an. Die Wasseraufnahmerate variierte stark, war für infizierte Beeren höher als für solche ohne Symptome und korrelierte bei infizierten Beeren mit der kumulativen Risslänge pro Beere (Produkt aus Anzahl der Risse pro Beere und mittlerer Risslänge; $r2 = 0,45^*$). Durch Anwesenheit von Wasser auf der gespannten Fruchthaut wurden Mikrorisse induziert, deren Zahl in befallenen Beeren gegenüber solchen ohne Symptome um 55% anstieg. Die Ergebnisse belegen, dass Botrytis-Befall die Platzempfindlichkeit von Weinbeeren der Sorte Riesling erhöht, insbesondere durch Schwächung der Fruchthaut, einen Anstieg der Risslänge pro Beere, wodurch sich eine erhöhte Wasseraufnahme ergibt

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9. Allgemeine Diskussion

Mit der vorliegenden Arbeit wurde gezeigt, dass

1) eine verringerte Kutikula Deposition ab Beginn des Weichwerden der Beeren zur Dehnung der Kutikula führt, diese infolge dessen mikroskopisch kleine Risse (Microcracks = MC) bildet, welche wiederum das Aufplatzen der Beeren begünstigen,

2) MCs zusätzlich durch Feuchtigkeit und hohe Temperaturen in der Narbenregion induziert werden können,

3) die Wasseraufnahme über die Oberflächen der Frucht hauptsächlich über die Stieloberfläche erfolgt und

4) Beschädigungen der Fruchthaut und Infektion mit *Botrytis cinerea* zu einem vermehrten Aufplatzen von Weinbeeren führen können.

Das Aufplatzen von Weinbeeren ist somit die Folge einer Schwächung der Stabilität der Fruchthaut und einer Volumenzunahme der Beeren durch eine erhöhte Wasseraufnahme. Dieser Zusammenhang ist ebenfalls für weiche fleischige Früchte anderer Arten beschrieben (Khanal et al. 2011, Knoche und Peschel 2007, Peschel und Knoche 2005).

Vergleich mit anderen Arten

Ein wichtiger Faktor für das Platzen von Früchten ist die Stabilität der Haut.

Die Flächendehnung der Kutikula von reifen Rieslingbeeren ist vergleichbar mit der von Stachel- und Jostabeeren. Süßkirschen haben im Vergleich dazu eine 2,5 – 3fach höhere Flächendehnung (Tabelle 9.1). Die Epidermiszellen sind mit im Mittel 691 μm^2 (eigene, unveröffentlichte Daten) kleiner als die der anderen Früchte und die Platzrate ist im Vergleich mit den genannten Arten und Sorten am niedrigsten.

Das Zusammenspiel von Kutikula und Epidermiszellen wurde von Khanal et al. (2011) an *Ribes*-Beeren untersucht. Die geringste Platzanfälligkeit der drei untersuchten *Ribes*-Sorten zeigten die Schwarzen Johannisbeeren, die gleichzeitig auch die geringste Flächendehnung der Kutikula (Tabelle 9.1) aufwiesen. Die Flächendehnungen der

Kutikulas von Stachelbeeren und Jostabeeren glichen sich und waren deutlich höher. Die beiden Sorten unterschieden sich jedoch beträchtlich in ihrem Platzverhalten. Während die Platzrate innerhalb von 24 Stunden von Stachelbeeren (32,2%) der der schwarzen Johannisbeeren (25,3%) ähnelte, war die Platzrate der Jostabeeren (55,5%) fast doppelt so hoch. Khanal et al. (2011) führen dies auf deutliche Unterschiede in der durchschnittlichen Größe der Epidermiszellen zurück. Die Epidermiszellen von schwarzer Johannisbeere (1528 ± 119 μm^2) und Jostabeere (2054 ± 77 μm^2) sind größer als die der Stachelbeeren (852 ± 25 μm^2). Khanal et al. (2011) vermuten, dass die kleineren Zellen der Stachelbeeren eine höhere mechanische Unterstützung gegen das Platzen bieten als die große Zellen der anderen Arten. Die Kombination von höherer Flächendehnung und größeren Epidermiszellen führte dementsprechend bei Jostabeeren zu höheren Platzraten.

Für Kirschen gibt es in der Literatur keinen kompletten Datensatz mit Platzrate, Flächendehnung und Größe von Epidermiszellen. Die veröffentlichten Daten für die Größe von Epidermiszellen liegen mit 1423 ± 65 μm^2 für die Sorte ‚Burlat' und 2144 ± 224 μm^2 für die Sorte ‚Sam' in derselben Größenordnung wie die Epidermiszellen von schwarzen Johannisbeeren und Jostabeeren. ‚Sam' und ‚Hedelfinger' zeigen eine starke Flächendehnung der Kutikula (Tabelle 9.1) und sowohl bei ‚Burlat' als auch bei ‚Hedelfinger' platzten 100% der Früchte innerhalb von 10 Stunden Inkubation in Wasser auf (Knoche et al. 2004, Peschel und Knoche 2005). Der von Khanal et al. (2011) beobachtete Zusammenhang von Flächendehnung, Zellgröße der Epidermiszellen und Platzrate scheint dementsprechend allgemeingültig für weiche Früchte zu sein.

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Tabelle 9.1: Vergleich von Gesamtwasserpotentialen der Frucht (Ψ_{fruit}), osmotischen Potentialen des Fruchtsaftes (Ψ_{π}), osmotischen Permeabilitäten für Wasseraufnahme (P_f) und Transpiration (P_t) durch die Fruchthaut, maximalen Raten der Oberflächenzunahme der Fruchthaut (AWR_{max}), Flächengewichten der Kutikula (CM Masse) sowie den Flächendehnungen der Kutikula bei Reife ($\varepsilon_{x,y}$) bei verschiedenen Sorten von Weinbeere, Süßkirschen und Johannis-, Stachel-, und Jostabeeren.

	Kennwerte Wassertransport						
Sorte (Frucht)	Ψ_{fruit} (MPa)	Ψ_{π} (MPa)	P_f (nm/s)	P_t (nm/s)	AWR_{max} (cm^2/d)	CM Masse (g/m^2)	$\varepsilon_{x,y}$ (%)
Chardonnay (Wein)	- 1,7 (± 0,1)[a]	- 3,9 (± 0,0)[a]	7,7 (± 0,9)[a]	2,2 (± 0,1)[a]	0,1[b]	3,3 (± 0,0)[b]	4,6 (± 0,8)[b]
Müller – Thurgau (Wein)	- 2,3 (± 0,1)[a]	- 3,5 (± 0,0)[a]	8,9 (± 1,5)[a]	2,5 (± 0,1)[a]	0,2[b]	3,9 (± 0,1)[b]	20,7 (± 0,7)[b]
Riesling (Wein)	- 1,6 (± 0,0)[a]	- 3,7 (± 0,0)[a]	4,1 (± 1,2)[a]	1,6 (± 0,0)[a]	0,2[b]	4,6 (± 0,0)[b]	18,4 (± 1,2)[b]
Adriana (Süßkirsche)	- 2,5[f]		30,7 (± 2,8)[c]	2,2 (± 0,6)[c]			
Hedelfinger (Süßkirsche)	- 2,8[f]		135,3 (± 3,0)[c]	3,1 (± 1,1)[c]		1,1 (± 0,0)[e]	51,3 (± 1,4)[e]
Sam (Süßkirsche)	- 2,7[f]	- 3,4[e]	24,8 (± 4,4)[c]	1,8 (± 0,1)[c]	0,6[e]	1,1 (± 0,0)[e]	63,8 (± 1,3)[e]
Regina (Süßkirsche)	- 2,0 (± 0,1)[e]	- 2,7 (± 0,0)[e]					
Burlat (Süßkirsche)	- 2,4[c]		110,1 (± 12,7)[c]	2,7 (± 0,1)[c]			
Zema (Schwarze Johannisbeere)	- 2,1 (± 0,2)[d]	- 2,7 (± 0,0)[d]	77,0 (± 4,0)[d]		0,1[d]	5,2[d]	8,2[d]
Rote Triumph (Stachelbeere)	- 1,2 (± 0,0)[d]	- 1,9 (± 0,0)[d]	52,0 (± 1,0)[d]		0,3[d]	5,8[d]	23,8[d]
Jostine (Jostabeere)	- 1,9 (± 0,2)[d]	- 2,5 (± 0,0)[d]	33,0 (± 3,0)[d]		0,2[d]	5,0[d]	19,5[d]

[a] (Becker und Knoche 2011), [b] (Becker und Knoche 2012a), [c] (Beyer et al. 2005), [d] (Khanal et al. 2011), [e] (Knoche et al. 2004), [f] (Weichert und Knoche 2006a)

Ein weiterer Unterschied zwischen Weinbeeren sowie schwarzen Johannis-, Stachel-, und Jostabeeren einerseits und Süßkirschen andererseits ist die Dicke der Kutikula (Tabelle 9.1, CM Masse per Oberfläche). Diese ist bei Weinbeeren zwischen 3- bis 4fach und bei schwarzen Johannis-, Stachel-, und Jostabeeren etwa 5fach dicker als bei Süßkirschen. Dementsprechend sind die Platzraten der Weinbeeren mit 3% für Rieslingbeeren (Hill 2007) und 25 – 55% bei *Ribes*-Beeren (Khanal et al. 2011)) innerhalb von 24 h im Vergleich zu den Süßkirschen mit 100% in 10 h (Weichert et al. 2004) deutlich niedriger.

Die maximale Wachstumsrate pro Tag war bei den Weinbeeren sowie den *Ribes*-Beeren geringer als bei Süßkirschen (Tabelle 9.1). Die schnellere Größenzunahme der Süßkirschen spiegelt sich in den höheren Flächendehnungen und niedrigeren Kutikelmassen im Vergleich zu den anderen weichen Früchten wieder.

Der zweite wichtige Faktorenkompex für das Platzen der Früchte ist ihr Wasserhaushalt. Die Wasserabgabe findet nahezu ausschließlich über die Oberflächen von Haut und Stiel statt. Auch ein Teil der Gesamtwasseraufnahme geschieht über diese Oberflächen. Entsprechend den Modellrechnungen (Kapitel 6) beträgt die Aufnahme über die Fruchtoberfläche bis zu 40% an einem Regentag bei ‚Riesling', die verbleibenden 60% werden über das Leitbündelsystem des Stiels in die Beere transportiert. Die Aufnahme und Abgabe von Wasser über die Fruchtoberfläche wird durch die Permeabilitäten der Haut und die treibenden Kräfte für den Wassertransport bestimmt.

Die (osmotische) Permeabilität der Haut für die Wasseraufnahme ist bei Weinbeeren zwischen 2,8 und 33mal niedriger als bei Süßkirschen (Tabelle 9.1). Eine mögliche Erklärung hierfür sind die in der Kutikula der Süßkirschen nachgewiesenen „polaren pathways" (Weichert und Knoche 2006a). Im Gegensatz dazu sind bei ‚Chardonnay', ‚Müller-Thurgau' und ‚Riesling' keine „polaren pathways" an der Wasseraufnahme beteiligt (Kapitel 6). Diese poren-ähnliche Strukturen können sich dynamisch in der Kutikula bei Anwesenheit von Wasser durch Hydratation und (neu)Ausrichtung einiger der funktionellen polaren Gruppen der die Kutikula bildenden Polymere formen. Dadurch entstehen zeitweise hydrophile Bereiche in der ansonsten hydrophoben Kutikula, über die ein schneller Massefluss von Wasser stattfinden kann (Schönherr

2006). Die Fruchthaut von Johannis-, Stachel- und Jostabeeren ist ähnlich permeabel für die Wasseraufnahme wie die der Süßkirschen (Tabelle 9.1), es ist aber nicht bekannt, ob bei den *Ribes*-Beeren ebenfalls „polare pathways“ eine Rolle bei der Wasseraufnahme spielen.

Im Gegensatz zu den hohen Differenzen in den Permeabilitäten der Haut für die Aufnahme bei den verschiedenen Früchten und Sorten gibt es nur geringe Unterschiede in der Höhe der Permeabilität für die Transpiration, diese ist zudem um einen Faktor zwischen 2,6 (bei ‚Riesling‘ und 43,6 (bei ‚Hedelfinger‘) niedriger als die jeweilige Permeabilität für die Wasseraufnahme (Tabelle 9.1).

Die unterschiedlich hohen osmotischen Permeabilitäten für die Wasseraufnahme wirken sich offensichtlich auch auf das Platzverhalten von Früchten aus. Innerhalb von 24 Stunden platzen nur 1 bis 3 % der Rieslingbeeren (Hill 2007) aber etwa 100% der Süßkirschen (‚Burlat‘ (Knoche und Peschel 2006) und ‚Hedelfinger‘ (Weichert et al. 2004)). Die Werte für schwarzen Johannisbeeren, Stachelbeeren und Jostabeeren betrugen 25,3 %, 32,2 % und 55,5% (Khanal et al. 2011). Die entsprechenden Permeabilitäten lagen bei 4, 77, 52, 33, 110 und 135 nm/s für ‚Riesling‘, Schwarze Johannis-, Stachel-, Jostabeere, ‚Burlat‘ und ‚Hedelfinger‘ (Tabelle 9.1).

Die treibende Kraft für die osmotische Wasseraufnahme, das Gesamt-Wasserpotential der Frucht (Ψ_{fruit}), ist bei den Weinbeeren und den *Ribes*-Beeren absolut betrachtet niedriger (weniger negativ = geringerer Potentialunterschied) als bei den meisten untersuchten Sorten von Süßkirschen. Die niedrigere Platzrate von Weinbeeren im Vergleich zu den Süßkirschen ist dementsprechend das Resultat einer niedrigeren Permeabiltität für die Wasseraufnahme und einer schwächeren treibenden Kraft in Form eines weniger negativen Gesamtwasserpotentials.

Das osmotische Potential des Fruchtsaftes (Ψ_{π}), eine Teilkomponente des Gesamt-Wasserpotentials, ist hingegen bei den Weinbeeren am stärksten negativ (der Potentialunterschied am größten), gefolgt von den Süßkirschen. Schwarze Johannis-, Stachel- und Jostabeeren haben im Vergleich zu den anderen Arten das am wenigsten negative osmotische Potential des Fruchtsaftes (Tabelle 9.1). Der Unterschied im osmotischen Potential des Fruchtsaftes der verschiedenen Arten erklärt sich durch eine unterschiedlich hohe Akkumulation von Kohlenhydraten.

Praktische Anwendungen zur Reduktion des Platzens

Im Gegensatz zu den frei hängenden Einzelfrüchten bei Kirschen wird das Aufplatzen bei Weinbeeren zusätzlich durch die Morphologie des Fruchtstandes beeinflusst. Der Grad der Kompaktheit beeinflusst die benötigte Zeit für das Abtrocknen der Fruchtstände (Vail und Marois 1991). Bei kompakten Trauben dauert das Abtrocknen länger als bei lockeren Fruchtständen, da weniger Oberflächen direkt der Sonneneinstrahlung exponiert sind. Im inneren kompakter Trauben sind zusätzlich höhere Grenzschichtwiderstände zu erwarten, da hier im Gegensatz zu lockeren Trauben keine Durchmischung der Luftschichten durch Wind stattfindet. Die höheren Grenzschichtwiderstände verlangsamen das Abtrocknen der Beeren und ihrer Stiele zusätzlich. Somit können die Beeren länger Wasser über ihre Oberflächen aufnehmen.

Zudem gibt es in kompakten Trauben deutlich mehr Kontaktstellen zwischen den Beeren. An diesen Kontaktstellen ist die Kutikuladeposition verringert sowie die Anfälligkeit für *Botrytis cinerea* erhöht (Marois et al. 1986, Percival et al. 1993). Ein weiterer Faktor ist das gegenseitige Abdrücken der Beeren in kompakten Fruchtständen. Es kommt also zum inneren Druck durch die Wasseraufnahme ein äußerer Druck auf die Haut hinzu. Dieser Faktor wurde in der vorliegenden Arbeit nicht untersucht. Auch in anderen Fachpublikationen finden sich keine Daten zum gegenseitigen Abdrücken von Beeren, es ist somit keine Aussage zu dessen Anteil am Gesamtprozess des Platzens möglich.

Strategien, die auf eine Auflockerung der Traubenstruktur abzielen, sollten eine effiziente Möglichkeit darstellen, das Aufplatzen von Weinbeeren im Feld zu verringern. Langfristig könnte dies durch entsprechende Züchtung erreicht werden. Ein Ansatzpunkt ist hierbei das Stielgerüst der Traube. Insbesondere die mittlere Länge der Internodien des Stielgerüstes beeinflusst nach Shavrukov et al. (2004) die Kompaktheit einer Traube. Das Längenwachstum dieser Internodien beruht hauptsächlich auf Zelldehnung und ist zu Beginn der Blüte abgeschlossen (Shavrukov et al. 2004).

Mechanische und chemische Behandlungen zur Ausdünnung des Blüten- oder jungen Fruchtstandes haben ebenfalls eine aufgelockerte Traubenstruktur zur Folge. Insbesondere die mechanischen Methoden wie das Traubenteilen (halbieren der

Trauben) oder das Entfernen einzelner Blüten bzw. Beeren aus der Traube sind jedoch sehr arbeitsintensiv (Hanni 2009, Petgen 2005). Alternativen wie das Ausdünnen mittels eines pneumatischen Gebläses oder der Einsatz der Rüttelmechanik des Vollernters sind weniger arbeitsintensiv. Allerdings erfolgt hierbei die Ausdünnung unpräzise und nicht an die einzelne Traube angepasst, so dass die Ertragsreduzierung stärker als geplant ausfallen kann (Hanni 2009, Walg 2011). Als Besonderheit tritt bei der Ausdünnung per Vollernter ein vorübergehender Wachstumsstillstand (vermutlich aufgrund von Embolien im Xylem) auf, der kleinere, dickhäutigere Beeren zur Folge hat (Walg 2011). Im Gegensatz dazu nimmt die Beerengröße nach einer Ausdünnung per Hand zu (Walg 2011).

Für die chemische Ausdünnung werden vor allem Phytohormone (Gibbereline, Auxine, Cytokinine) oder deren Antagonisten zur Wuchsförderung oder Hemmung eingesetzt. In Deutschland kommen Gibb3 (bislang nicht generell zugelassen, Sondergenehmigung notwendig) und der Gibberlin- Antagonist Prohexadione-Calcium (Regalis, bislang für einzelne Sorten bis 2014 zugelassen) während der Blüte zum Einsatz. Eine positive Wirkung ist aber jeweils nur bei bestimmten Sorten zu beobachten und scheint hauptsächlich vom sortenspezifischen endogenen Hormonspiegel abzuhängen (Böll et al. 2009, Petgen 2009). ‚Riesling' sowie einige weitere Sorten reagieren auf eine Gibberelin-Behandlung zunächst positiv haben aber im Folgejahr eine stark verminderte Blütenstandsbildung sowie einen schwachen Austrieb während andere Sorten dieses Verhalten nicht zeigen (Bleyer und Kast 2010, Kast et al. 2005, Petgen 2009).

Neben der Erniedrigung der Infektionsanfälligkeit und Erhöhung der Platzfestigkeit beeinflusst eine Ausdünnung der Trauben auch die Inhaltstoffe der Beeren und resultiert oftmals in einem erhöhten Zucker (und damit Alkoholgehalt) und Phenolgehalt. Dadurch wird bei einigen Sorten die sensorische Wahrnehmung der aus diesen Trauben gekelterten Weine beeinflusst (Diago et al. 2010).

Eine bessere Durchlüftung und damit ein schnelleres Abtrocknen der Fruchtstände wird zudem durch eine teilweise Entblätterung der Traubenzone (per Hand oder maschinell mittels Druckluft oder ansaugen und schneiden bzw. abzupfen der Blätter, Strauß 2005) erreicht. Diese verbessert auch die Effizienz bei der Applikation von Pflanzenschutzmitteln. Bei einer frühen Entblätterung zwischen den

Entwicklungstadien BBCH 63 (Blüte, Lorenz et al. 1995) und BBCH 77 (Beginn des Traubenschlusses, Lorenz et al. 1995) kommt es zusätzlich zu einer Auflockerung der Traubenstruktur. Dadurch wird die aktive Assimilationsfläche verringert und deshalb können nicht alle Beeren einer Traube ausreichend mit Assimilaten versorgt werden. Es fallen vermehrt Beeren ab und die in der Traube verbleibenden Beeren bleiben kleiner (Molitor et al. 2011a). Durch die verbesserte Belichtung entwickeln die Beeren zudem dickere Schalen und sind dadurch resistenter gegenüber Pathogenen und dem Aufplatzen (Fox 2006).

Eine Kombination von chemischer Ausdünnung (Regalis) und früher Teilentblätterung erzeugte eine stärkere Auflockerung der Trauben als eine der beiden Behandlungen alleine, da ein verstärktes Dickenwachstum der Beeren zum Ausgleich für die Reduktion des Fruchtansatzes (vermutlich aufgrund der verringerten Assimilatversorgung) unterblieb (Molitor et al. 2011b).

Sowohl Ausdünnungs- als auch Entblätterungsmaßnahmen werden bereits im Weinbau zur Verbesserung der Beerengesundheit eingesetzt. Kurz- und mittelfristig ist der Einsatz dieser Methoden auch zur Verringerung des Beerenplatzens ratsam, insbesondere da hierfür auf bereits vorhandene Erfahrungen und Maschinen zurück gegriffen werden kann. Langfristig ist die Züchtung von Trauben mit verlängertem Stielgerüst eine interessante Option um lockerbeerige Fruchtstände zu erhalten, ohne dabei die charakteristischen geschmacklichen Merkmale der Sorten zu stark zu verändern.

10. Literaturverzeichnis

Alleweldt, G., Engel, M. und Gebbing, H. (1981). Histologische Untersuchungen an Weinbeeren. Vitis 20, 1–7.

Becker, T. und Knoche, M. (2011). Water movement through the surfaces of the grape berry and its stem. American Journal of Enology and Viticulture 62, 340–350.

Becker, T. und Knoche, M. (2012a). Deposition, strain, and microcracking of the cuticle in developing 'Riesling' grape berries. Vitis 51, 1–6.

Becker, T. und Knoche, M. (2012b). Research Note: Water induces microcracks in the grape berry cuticle. Vitis 51.

Becker, T., Grimm, E. und Knoche, M. (2012). Substantial water uptake into detached grape berries occurs through the stem surface. Australian Journal of Grape and Wine Research 18, 109–114.

Becker, T., Walter, R., Kortekamp, A., Grimm, E. und Knoche, M. (2011). Das Platzen von Weinbeeren (Vitis vinifera) bei Befall mit Grauschimmel (Botrytis cinerea). Erwerbs-Obstbau 53, 85–92.

Beyer, M. und Knoche, M. (2002). Studies on water transport through the sweet cherry fruit surface. V. Conductance for water uptake. Journal of the American Society for Horticultural Science 127, 325–332.

Beyer, M., Lau, S. und Knoche, M. (2005). Studies on water transport through the sweet cherry fruit surface. IX. Comparing permeability in water uptake and transpiration. Planta 220, 474–485.

Beyer, M., Peschel, S., Weichert, H. und Knoche, M. (2002). Studies on water transport through the sweet cherry fruit surface. 7. Fe3+ and Al3+ reduce conductance for water uptake. Journal of agricultural and food chemistry 50, 7600–7608.

Blaich, R., Stein, U. und Wind, R. (1984). Perforationen in der Cuticula von Weinbeeren als morphologischer Faktor der Botrytisresistenz. Vitis 23, 242–256.

Bleyer, K. und Kast, W. (2010). Vorteile und Risiken beim Einsatz von Bioregulatoren. Rebe&Wein 63, 13–16.

Böll, S., Lange, T., Hofmann, H. und Schwapbach Peter (2009). Correspondence between gibberellin-sensitivity and pollen tube abundance in different seeded vine varieties. Mitteilungen Klosterneuburg 59, 129–133.

Bondada, B.R., Matthews, M.A. und Shackel, K.A. (2005). Functional xylem in the post-veraison grape berry. Journal of Experimental Botany 56, 2949–2957.

Brown, K. und Considine, J.A. (1982). Physical aspects of fruit growth. Stress distribution around lenticels. Plant Physiology 69, 585–590.

Chatelet, D.S., Rost, T.L., Matthews, M.A. und Shackel, K.A. (2008a). The peripheral xylem of grapevine (Vitis vinifera) berries. 2. Anatomy and development. Journal of Experimental Botany 59, 1997–2007.

Chatelet, D.S., Rost, T.L., Shackel, K.A. und Matthews, M.A. (2008b). The peripheral xylem of grapevine (Vitis vinifera). 1. Structural integrity in post-veraison berries. Journal of Experimental Botany 59, 1987–1996.

Clarke, S.J., Hardie, W.J. und Rogiers, S.Y. (2010). Changes in susceptibility of grape berries to splitting are related to impaired osmotic water uptake associated with losses in cell vitality. Australian Journal of Grape and Wine Research 16, 469–476.

Cline, J., Sekse, L., Meland, M. und Webster, A. (1995). Rain-induced Fruit Cracking of Sweet Cherries: I. Influence of Cultivar and Rootstock on Fruit Water Absorption, Cracking and Quality. Acta Agriculturae Scandinavica, Section B - Plant Soil Science 45, 213–223.

Comménil, P., Brunet, L. und Audran, J.-C. (1997). The development of the grape berry cuticle in relation to susceptibility to bunch rot disease. Journal of Experimental Botany 48, 1599–1607.

Considine, J.A. (1982). Physical aspects of fruit growth. Cuticular fracture and fracture patterns in relation to fruit structure in Vitis vinifera. The Journal of Horticular Science 57, 79–91.

Considine, J.A. und Brown, K. (1981). Physical aspects of fruit growth. Theoretical analysis of distribution of surface growth forces in fruit in relation to cracking and splitting. Plant Physiology 68, 371–376.

Considine, J.A. und Knox, R.B. (1979). Development and histochemistry of the cells, cell-walls, and cuticle of the dermal system of fruit of the grape, Vitis vinifera L. Protoplasma 99, 347–365.

Considine, J.A. und Kriedemann, P.E. (1972). Fruit splitting in grapes. Determination of critical turgor pressure. Australian Journal of Agricultural Research 23, 17–24.

Cussler, E.L. (1997). Diffusion. Mass transfer in fluid systems (Cambridge University Press: Cambridge, New York).

Deutsches Weininstitut (**2010).** Der Weinjahrgang 2010 – Ein Blick in die 13 deutschen Anbaugebiete (Mainz).

Diago, M.P., Vilanova, M., Blanco, J.A. und Tardaguila, J. (2010). Effects of mechanical thinning on fruit and wine composition and sensory attributes of Grenache and Tempranillo varieties (Vitis vinifera L.). Australian Journal of Grape and Wine Research 16, 314–326.

Düring, H., Lang, A. und Oggionni, F. (1987). Patterns of water-flow in riesling berries in relation to developmental-changes in their xylem morphology. Vitis 26, 123–131.

Elmer, P.A.G. und Michailides, T.J. (2007). Epidemiology of Botrytis cinerea in orchard and vine crops. *In* Botrytis. Biology, pathology and control. Eds. Y. Elad, B. Williamson, P. Tudzynski and N. Delen (Springer: Dordrecht), pp. 243–272.

Findlay, N., Oliver, K.J., Nii, N. und Coombe, B.G. (1987). Solute accumulation by grape pericarp cells. IV. Perfusion of pericarp apoplast via the pedicel and evidence for xylem malfunction in ripening berries. Journal of Experimental Botany 38, 668–679.

Fox, R. (2006). Physiologische Aspekte der Traubenzonen-Entlaubung. Schweizer Zeitschrift für Obst- und Weinbau 142, 6-8

Geyer U. und Schönherr, J. (1988). In vitro test for effects of surfactants and formulations on permeability of plant cuticles. *In* Pesticide formulations. Eds. Cross Barrington and Scher Herbert B. (American Chemical Society: Washington, DC), pp. 22–33.

Grace, J. und Gardingen, P.R. van (1996). Plant cuticles under challenge. *In* Plant cuticles. An integrated functional approach. Ed. G. Kerstiens (BIOS Scientific Publishers: Oxford), pp. 319–329.

Greenspan, M.D., Schultz, H.R. und Matthews, M.A. (1996). Field evaluation of water transport in grape berries during water deficits. Physiologia Plantarum 97, 55–62.

Harms, M. (2007). Fäulnis - Erfolg nur im Gesamtkonzept. Der Deutsche Weinbau 7, 66–71.

Hanni, E. (2009). Mechanische Ausdünnung im Weinbau. Obstbau, Weinbau 46, 85–88.

Hill, G.K. (2007). Lässt sich ein Aufplatzen der Beeren vermeiden? Der Deutsche Weinbau 16, 50–53.

Holz, G., Coertze, S. und Williamson, B. (2007). The ecology of botrytis on plant surfaces. *In* Botrytis. Biology, pathology and control. Eds. Y. Elad, B. Williamson, P. Tudzynski and N. Delen (Springer: Dordrecht), pp. 9–27.

House, C.R. (1974). Water transport in cells and tissues. Monographs of the Physiological Society, Vol. 24 (Arnold: London).

Jörger, V., Schreieck Patrick, Littek, T. und Doye, E. (2010). Kulturführung: Einfluss auf Botrytis- und Essigbefall. Das Deutsche Weinmagazin 7, 24–29.

Kassemeyer, H.-H. und Berkelmann-Löhnertz, B. (2009). Fungi of Grapes. *In* Biology of Microorganisms on Grapes, in Must and in Wine. Eds. H. König, G. Unden and J. Fröhlich (Springer: Berlin, Heidelberg), pp. 61–87.

Kast, W., Fox, R. und Schiefer, H.-C. (2005). Bio-Wachstumsregulatoren. Chancen und Risiken des Einsatzes von Gibb3. Rebe&Wein 58, 16–19.

Keller, M., Smith, J.P. und Bondada, B.R. (2006). Ripening grape berries remain hydraulically connected to the shoot. Journal of Experimental Botany 57, 2577–2587.

Kerstiens, G. (1996a). Cuticular water permeability and its physiological significance. Journal of Experimental Botany 47, 1813–1832.

Kerstiens, G. (1996b). Diffusion of water vapour and gases across cuticles and through stomatal pores presumed closed. *In* Plant cuticles. An integrated functional approach. Ed. G. Kerstiens (BIOS Scientific Publishers: Oxford), pp. 121–134.

Khanal, B.P., Grimm, E. und Knoche, M. (2011). Fruit growth, cuticle deposition, water uptake, and fruit cracking in jostaberry, gooseberry, and black currant. Scientia Horticulturae 128, 289–296.

Knoche, M. und Peschel, S. (2006). Water on the surface aggravates microscopic cracking of the sweet cherry fruit cuticle. Journal of the American Society for Horticultural Science 131, 192–200.

Knoche, M. und Peschel, S. (2007). Deposition and strain of the cuticle of developing European plum fruit. Journal of the American Society for Horticultural Science 132, 597–602.

Knoche, M., Beyer, M., Peschel, S., Oparlakov, B. und Bukovac, M.J. (2004). Changes in strain and deposition of cuticle in developing sweet cherry fruit. Physiologia Plantarum 120, 667–677.

Knoche, M., Peschel, S., Hinz, M. und Bukovac, M.J. (2000). Studies on water transport through the sweet cherry fruit surface. characterizing conductance of the cuticular membrane using pericarp segments. Planta 212, 127–135.

Knoche, M., Peschel, S., Hinz, M. und Bukovac, M.J. (2001). Studies on water transport through the sweet cherry fruit surface. II. Conductance of the cuticle in relation to fruit development. Planta 213, 927–936.

Lang, A. und Düring, H. (1990). Grape berry splitting and some mechanical properties of the skin. Vitis 29, 61–70.

Lang, A. und Thorpe, M.R. (1989). Xylem, phloem and transpiration flows in a grape. Application of a technique for measuring the volume of attached fruits to high-resolution using Archimedes' principle. Journal of Experimental Botany 40, 1069–1078.

Lendzian, K.J. und Kerstiens, G. (1991). Sorption and transport of gases and vapors in plant cuticles. Reviews of Environmental Contamination and Toxicology 121, 65–128.

Lide D.R. (Hrsg.) (2009). CRC handbook of chemistry and physics. A ready-reference book of chemical and physical data (CRC Press: Boca Raton, FLa.).

Lilleland, O. und Newsome, L. (1934). A growth study of the cherry fruit. Proceedings of the American Society for Horticultural Science 32, 291–299.

Lorenz, D., Eichhorn, K., Bleiholder, H., Klose, R., Meier, U. und Weber, E. (1995). Phenological growth stages of the grapevine, Vitis vinifera L. ssp. vinifera. Codes and descriptions according to the extended BBCH scale. Australian Journal of Grape and Wine Research 1, 100–103.

Lustig, I. und Bernstein, Z. (1985). Determination of the mechanical properties of the grape berry skin by hydraulic measurements. Scientia Horticulturae 25, 279–285.

Marois, J.J., Nelson, J.K., Morrison, J.C., Lile, L.S. und Bledsoe, A.M. (1986). The influence of berry contact within grape clusters on the development of Botrytis cinerea and epicuticular wax. American Journal of Enology and Viticulture 37, 293–296.

Meneguzzo, J., Miele, A., Rizzon, L.A. und Ayub, M.A.Z. (2008). Effect of bunch rot on the sensory characteristics of the Gewürztraminer wine. Journal International des Sciences de la Vigne et du Vin 42, 107–111.

Ministerium für Wirtschaft, Verkehr, Landwirtschaft und Weinbau (**2002).** Richtlinien für den kontrolliert umweltschonenden Weinbau in Rheinland-Pfalz (Mainz, Germany).

Molitor, D., Behr, M., Evers, D. und Fischer, S. (2011a). Entblätterung der Traubenzone. Der richtige Termin entscheidet! Das Deutsche Weinmagazin 11, 32–35.

Molitor, D., Behr, M., Rothmeier, M. und Evers, D. (2011b). Fäulnisvermeidung. Gesundes Lesegut im Fokus. Das Deutsche Weinmagazin 7, 34–37.

Mrozek, R.F. und Burkhard, T.H. (1973). Factors causing prune side cracking. Transactions of the American Society of Agricultural Engineers 16, 686–692.

Mullins, M.G., Bouquet, A. und Williams, L.E. (1992). Biology of the grapevine (Cambridge Univ. Press: Cambridge).

Nelson, K.E. (1956). The effect of botrytis infection on the tissue of tokay grapes. Phytopathology 46, 223–229.

Nobel, P.S. (1991). Physicochemical and environmental plant physiology (Academic Press Inc: San Diego).

Orgell, W.H. (1955). The isolation of plant cuticle with pectic enzymes. Plant Physiology 30, 78–80.

Percival, D.C., Sullivan, J.A. und Fisher, K.H. (1993). Effect of cluster exposure, berry contact and cultivar on cuticular membrane formation and occurrence of bunch rot (Botrytis cinerea Pers.: Fr.) with 3 Vitis vinifera L. cultivars. Vitis 32, 87–97.

Peschel, S. und Knoche, M. (2005). Characterization of microcracks in the cuticle of developing sweet cherry fruit. Journal of the American Society for Horticultural Science 130, 487–495.

Petgen, M. (2005). Was bringen alternative Ausdünnmöglichkeiten? Der Deutsche Weinbau 9, 14–18.

Petgen, M. (2009). Einsatz von Bioregulatoren. Meilensteine in der Fäulnisbekämpfung? Das Deutsche Weinmagazin 4, 26–30.

Pieri, P. und Fermaud, M. (2005). Effects of defoliation on temperature and wetness of grapevine berries. *In* Proceedings of the VIIth International Symposium on Grapevine Physiology and Biotechnology. Davis, California, USA, June 21 - 25, 2004. Ed. L.E. Williams (International Society for Horticultural Science: Leuven), Acta Horticulturae 689, 109–116.

Reynhardt, E.C. und Riederer, M. (1994). Structures and molecular dynamics of plant waxes. II. Cuticular waxes from leaves of Fagus sylvatica L. and Hordeum vulgare L. European Biophysics Journal with Biophysics Letters 23, 59–70.

Ribéreau-Gayon, P. (1983). Alterations of wine quality caused by Botrytis damages. Vignevini 10, 48–52.

Rogiers, S.Y., Hatfield, J.M., Jaudzems, V.G., White, R.G. und Keller, M. (2004). Grape berry cv. Shiraz epicuticular wax and transpiration during ripening and preharvest weight loss. American Journal of Enology and Viticulture 55, 121–127.

Rogiers, S.Y., Smith, J.A., White, R.G., Keller, M., Holzapfel, B.P. und Virgona, J.M. (2001). Vascular function in berries of Vitis vinifera (L) cv. Shiraz. Australian Journal of Grape and Wine Research 7, 47–51.

Rosenquist, J.K. und Morrison, J.C. (1988). The development of the cuticle and epicuticular wax of the grape berry. Vitis 27, 63–70.

Rosenquist, J.K. und Morrison, J.C. (1989). Some factors affecting cuticle and wax accumulation on grape berries. American Journal of Enology and Viticulture 40, 241–244.

Sachs, L. (1992). Angewandte Statistik. Anwendung statistischer Methoden ; 291 Tabellen (Springer: Berlin [u.a.]).

Scheibe, G. und Zanker, V. (1962). Über die Metachromasie der Absorption und Fluoreszenz von Acridinfarbstoffen. Histochemie 3, 122–126.

Schönherr, J. (1982). Resistance of plant surfaces to water loss. transport properties of cutin, suberin and associated lipids. *In* Encyclopedia of plant physiology. Eds. O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler (Springer: Berlin), pp. 153–179.

Schönherr, J. (2006). Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. Journal of Experimental Botany 57, 2471–2491.

Schönherr, J. und Schmidt, H.W. (1979). Water permeability of plant cuticles - dependence of permeability coefficients of cuticular transpiration on vapor-pressure saturation deficit. Planta 144, 391–400.

Schreiber, L., Skrabs, M., Hartmann, K.D., Diamantopoulos, P., Simanova, E. und Santrucek, J. (2001). Effect of humidity on cuticular water permeability of isolated cuticular membranes and leaf disks. Planta 214, 274–282.

Shavrukov, Y.N., Dry, I.B. und Thomas, M.R. (2004). Inflorescence and bunch architecture development in Vitis vinifera L. Australian Journal of Grape and Wine Research 10, 116–124.

Stock, M., Badeck, F., Gerstengarbe, F.-W., Hoppmann, D., Kartschall, T., Österle, H., Werner, P.C. und Wodinski, M. (2007). Perspektiven der Klimaänderung bis 2050 für den Weinbau in Deutschland (Klima 2050). Schlußbericht zum FDW-Vorhaben: Klima 2050. http://www.pik-potsdam.de/research/publications/pikreports/.files/pr106.pdf (29 September 2011).

Stock, M., Gerstengarbe, F.-W., Kartschall, T. und Werner, P.C. (2005). Reliability of climate change impact assessments for viticulture. *In* Proceedings of the VIIth International Symposium on Grapevine Physiology and Biotechnology. Davis, California, USA, June 21 - 25, 2004. Ed. L.E. Williams (International Society for Horticultural Science: Leuven), Acta Horticulturae 689, 29–39.

Strauß, M. (2005). Entblätterungstechnik im Test. Weniger Blätter und Ertrag auf einen Schlag. Rebe&Wein 58, 16–19.

Tenberge, K.B. (2007). Morphology and cellular organisation in Botrytis interactions with plants. *In* Botrytis. Biology, pathology and control. Eds. Y. Elad, B. Williamson, P. Tudzynski and N. Delen (Springer: Dordrecht), pp. 67–84.

Thoma, K. (2011). Keltertraubensorten in Deutschland. *In* Deutsches Weinbaujahrbuch 2012. Eds. H.R. Schultz and M. Stoll (Ulmer, E: Stuttgart), pp. 229–232.

Tyerman, S.D., Tilbrook, J., Pardo, C., Kotula, L., Sullivan, W. und Steudle, E. (2004). Direct measurement of hydraulic properties in developing berries of Vitis vinifera L. cv Shiraz and Chardonnay. Australian Journal of Grape and Wine Research 10, 170–181.

Vail, M.E. und Marois, J.J. (1991). Grape cluster architecture and the susceptibility of berries to Botrytis cinerea. Phytopathology 81, 188–191.

Viret, O., Keller, M., Jaudzems, V.G. und Cole, F.M. (2004). Botrytis cinerea infection of grape flowers. Light and electron microscopical studies of infection sites. Phytopathology 94, 850–857.

Walg, O. (2011). Der Vollernter als "Ausdünnmaschine". *In* Deutsches Weinbaujahrbuch 2012. Eds. H.R. Schultz and M. Stoll (Ulmer, E: Stuttgart), pp. 191–200.

Weichert, H. und Knoche, M. (2006a). Studies on water transport through the sweet cherry fruit surface. 10. Evidence for polar pathways across the exocarp. Journal of agricultural and food chemistry 54, 3951–3958.

Weichert, H. und Knoche, M. (2006b). Studies on water transport through the sweet cherry fruit surface. 11. FeCl3 decreases water permeability of polar pathways. Journal of agricultural and food chemistry 54, 6294–6302.

Weichert, H., Jagemann, C. von, Peschel, S., Knoche, M., Neumann, D. und Erfurth, W. (2004). Studies on water transport through the sweet cherry fruit surface. VIII. Effect of selected cations on water uptake and fruit cracking. Journal of the American Society for Horticultural Science 129, 781–788.

Wexler, A. (1995). Constant humidity solutions. *In* CRC handbook of chemistry and physics. Ed. D.R. Lide (CRC Press: Boca Raton, Fla), pp. 15–23.

Winkler, A.J., Cook, J.A., Kliewer, W.M. und Lider, L.A. (1974). General viticulture (University of California Press: Berkeley).

Yamada, Y., Wittwer, S.H. und Bukovac, M.J. (1964). Penetration of ions through isolated cuticles. Plant Physiology 39, 28–32.

11. Abbkürzungsverzeichnis

A	surface area	Flächeninhalt
$AlCl_3$	aluminium chloride	Aluminiumchlorid
a_w	water activity	Wasseraktivität
AWR_{max}	maximum growth rate in surface area	maximale Rate der Oberflächenzunahme
$CaCl_2$	calcium chloride	Kalziumchlorid
CM	cuticular membrane	Kutikula
D	diffusion coefficient	Diffusionskoeffizient
DAA	days after anthesis	Tage nach (Voll)Blüte
DAFB	days after full bloom	Tage nach Vollblüte
DCM	dewaxed cuticular membrane	entwachste Kutikula
E_a	energy of activation	Aktivierungsenergie
ε	strain	Dehnung
Eq	Equation	Gleichung
equiv	equivalent	Äquivalent
ES	exocarp segment	Exocarp - Segment

$FeCl_3$	ferric chloride	Eisen(III)-chlorid
Fig	figure	Abbildung
F_f	rate of water uptake	Wasseraufnahmerate
F_t	rate of transpiration	Transpirationsrate
KCl	potassium chloride	Kaliumchlorid
KNO_3	potassium nitrate	Kaliumnitrat
M	mass	Masse
MC	microcrack	Mikroriss
$MgCl_2$	magnesium chloride	Magnesiumchlorid
n	number (of specimen)	Anzahl
NaCl	sodium chloride	Natriumchlorid
nd	not determined	nicht bestimmt/erfasst
ns (statistics)	not significant	nicht signifikant
P (statistics)	probability level	Signifikanzwert
P_f	permeability for water uptake	Permeabilität für Wasseraufnahme
P_t	permeability for transpiration	Permeabilität für Transpiration
PEG	polyethylene glycol	Polyethylenglycol

Ψ_{donor}	solute potential of incubation solution	Osmotisches Potential Inkubationslösung
Ψ_{fruit}	water potential of the fruit	Gesamt-Wasserpotential
$\Psi_{\Pi fruit}$	solute potential of fruit juice	Osmotisches Potential des Fruchtsaftes
$\Psi_{\Pi PEG}$	solute potential of a PEG solution	Osmotisches Potential einer PEG Lösung
R	universal gas constant	allgemeine Gaskonstante
r (statistics)	coefficient of correlation	Korrelationskoeffizient
r^2 (statistics)	coefficient of determination	Bestimmtheitsmaß
RH	relative humidity	Relative Luftfeuchtigkeit
ρ_w	density of water	Dichte von Wasser
SE (statistics)	standard error	Standardfehler
T	temperature	Temperatur
Tab	table	Tabelle
$\bar{V}_w$	molar volume of water	Molvolumen von Wasser
w	width	Breite

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Publikationsliste

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Becker, T. und Knoche, M. (2011). Water movement through the surfaces of the grape berry and its stem. American Journal of Enology and Viticulture 62, 340–350.

Becker, T. und Knoche, M. (2012). Deposition, strain, and microcracking of the cuticle in developing 'Riesling' grape berries. Vitis 51, 1–6.

Becker, T. und Knoche, M. (2012). Research Note: Water induces microcracks in the grape berry cuticle. Vitis 51, 141-142.

Becker, T., Kortekamp, A., Louis, F., Grimm, E. und Knoche, M. (2011). Rain induced grape berry splitting: a problem of surface Water transport? *In* 47. Gartenbauwissenschaftliche Jahrestagung. Kurzfassung der Vorträge und Poster. Ed. B.u.D.G.G.e. Bundesverband der Hochschulabsolventen/Ingenieure Gartenbau und Landschaftsarchitektur e.V.

Becker, T., Kortekamp, A., Louis, F. und Knoche, M. (2011). Rain-induced grape berry splitting: A problem of surface water transport? *In* Patholux - First luxembourgian conference on the impact of plant pathogens on food quality of acriculturals crops and wine. Abstracts and selected articles of presentations and posters (Edizioni ETS: Pisa).

Becker, T., Walter, R., Kortekamp, A., Grimm, E. und Knoche, M. (2011). Das Platzen von Weinbeeren (Vitis vinifera) bei Befall mit Grauschimmel (Botrytis cinerea). Erwerbs-Obstbau 53, 85–92.

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Erklärung zur Dissertation

gemäß §6(1) der Promotionsordnung der Naturwissenschaftlichen Fakultät der Gottfried Wilhelm Leibniz Universität Hannover für die Promotion zum Dr. rer. hort. erkläre ich, hiermit, dass ich meine Dissertation mit dem Titel:

„Einfluss von Fruchtwachstum, Kutikulaentwicklung und Wassertransport auf das Platzen von Weinbeeren als Grundlage für die Verringerung des Befalls durch Traubenfäulen.“

selbständig verfasst und die benutzten Hilfsmittel und Quellen sowie gegebenenfalls die zu Hilfeleistungen herangezogenen Institutionen vollständig angegeben habe.

Die Dissertation wurde nicht schon als Masterarbeit, Diplomarbeit oder andere Prüfungsarbeit verwendet.

Tobias Becker