CHANGES IN FLAVONOL PROFILE ARE A RELIABLE INDICATOR TO ASSESS THE EXPOSURE OF RED GRAPE BERRIES TO SOLAR RADIATION AND CANOPY ARCHITECTURE

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Abstract:

Context and purpose of the study - Exposure to solar radiation affects berry composition through photomorphogenesis or changes in temperature. Flavonol synthesis is upregulated by UV-B radiation leaving a fingerprint on flavonol profile. This study aimed to test the factors affecting flavonol accumulation and profile and their potential as an indicator to assess the overall exposure of red wine grape berry to solar radiation.

Material and methods - We performed three experiments to study the response of flavonol accumulation and profile to (1) three different solar radiation exclusion treatments (shading nets) during berry development; (2) canopy porosity and leaf area index (LAI); and (3) natural variability of vine vigour and canopy management practices.

Results - Results showed a strong relationship between global radiation, inverse dormant pruning weights or canopy porosity (inversely proportional to LAI) and % kaempferol or % quercetin. Furthermore, the increase in concentration of the above two flavonols was associated with a reduction of % myricetin. Total flavonol content, % kaempferol, % quercetin and % myricetin had significant correlations with inverse dormant pruning weights, but these were less sensitive to over-ripening or water deficits. Flavonol profile was associated to site hydrology (wetness index) through changes in vigour, and to LAI; and responded to shoot thinning or fruit-zone leaf removal. Flavonol profile was also correlated to the maximal temperature reached by the clusters. These results support the reliability of the flavonol profile as an assessment parameter for studies aiming to discuss canopy architecture or the effect of solar radiation on grapevine berries.

Keywords: flavonoids, solar radiation, temperature, fruit ripening, grape composition, precision agriculture, UV-B radiation.

1. Introduction

Flavonols are mainly accumulated in epidermal cells of plant tissues in response to solar radiation, especially UV-B, filtering the most harmful part of the solar spectrum to DNA (Agati and Tattini, 2010). In previous research, Martínez-Lüscher et al., (2014b) reported that flavonol content in grape berry skins increased linearly with the exposure time to an artificial source of UV-B. This response corresponded also to a decrease in the proportion of tri-substituted flavonols (myricetin, laricitin and syringetin glicosides) and an increase in the proportion of disubstituted flavonols (quercetin and isorhamnetin glycosides) and monosubstituted flavonols (kaempferol glycosides).

In grape production, canopy density is controlled either during the dormant period through pruning and trellising, or during the growing season through leaf removal and shoot thinning. These practices result in higher canopy porosity leading to enhanced ripening, air circulation and exposure of grapes to solar radiation. Fruit exposure has been associated to a reduction in humidity and fungal diseases (English et al., 1993), and some desirable effects for specific winemaking targets, such as herbaceous aroma removal (Koch et al., 2012) and higher content of flavonoid compounds in berries (Matus et al., 2009). Conversely, the impact of the excess of solar radiation can be deleterious, resulting in damage to grape

berry and a decrease in berry flavonoid content and acidity (Martínez-Lüscher *et al.*, 2017). Therefore, there is a strong need of assessing canopy porosity and the exposure of red grapes to solar radiation. The aim of this study was to deduce the relationship between flavonol profile and solar radiation under different field conditions and propose the use of flavonol profile in the assessment of parameters associated with canopy architecture and grape berry microclimate.

2. Material and methods

Experiment 1: Response of flavonol profile development under 3 solar radiation exclusion treatments -- An experiment was conducted in 2016 in a vineyard in Oakville, CA. Plants were 7-year-old *V. vinifera* cv. Cabernet Sauvignon clone FPS08 (Foundation Plant Services, University of California Davis, USA) grafted into 110R (*Vitis rupestris x Vitis berlandieri*) spur pruned with a bilateral cordon, shoots vertically positioned and a vine spacing of 2.4 x 2 m (row x vine). A control (uncovered; 0% shade factor) and two polyethylene nets treatments (20% shade factor and 40% shade factor; Ginegar, Kibbutz, Israel) were installed on 27 May (31 days after anthesis). There were 4 treatment replicates consisted of 3 vines each. On dates 20 June, 19 July, 29 July, 9 August, 19 August, 29 August and 9 September, 20-berry samples were collected, weighed and stored at -80°C for later analyses of berry skin flavonols through HPLC-DAD.

Experiment 2: Relationship between variability in canopy porosity, LAI and global radiation and flavonol content and profile -- The experiment was conducted in 2017 in Oakville, CA (38.428° N, 122.409° W, 47m asl) with row orientation NE-SW. Four rows of *V. vinifera* cv. Cabernet Sauvignon clone FPS08 grafted onto 420A (*Vitis riparia x V. berlandieri*) with bilateral cordon and vine spacing of 2 m x 2 m (row x vine). In order to induce some variability cluster exposure, eight rows were pruned as: 1) 2-bud spurs positioning using two sets of wires 0.3 and 0.6m from the cordon to keep shoots growing upwards between the wires in a single plane (i.e. vertical-shoot-positioned trellis); or 2) cane-pruned leaving 0.5 m-long fruiting canes and letting shoots sprawl supported by tendrils on the two sets of wires (i.e. similar to California sprawl). For each of the 8 random grapevines used, 2 clusters facing either east, interior or west (48 clusters in total) were flagged. Images from the cluster perspective were captured at two times (24 of August and 9 September) using a 150° hemispherical lens coupled to a smartphone (Bianchi et al., 2017). The images were processed in R (version 3.2.5-6). Canopy porosity was then calculated as the % of pixels capturing the sky. Leaf area index (LAI) was estimated using the principles of commercial canopy analyzers (Welles and Norman, 1991) adapted to hemispherical images by ter Steege (1997). Global radiation was calculated simulating the trajectory of the sun through the period from beginning of ripening (24 July) to harvest (9 September). Modelled direct radiation values were corrected with the onsite CIMIS meteorological station actual data before summing accumulated radiation. On 9 September, 5-berry samples were collected from the top of each cluster.

Experiment 3: Relationship between flavonol profile and natural and induced variability in commercial vineyards -- A commercial vineyard was monitored during 2016 season in Paso Robles, CA (35.58° N, 120.63° W, 223 asl). The site was 6 ha vineyard of 14-year-old Merlot grafted onto 1103P (*V. berlanderi x V. rupestris*) and a vine spacing of 1.83 x 2.44 m (vine x row). Trellis consisted in a bilateral cordon pruned to 2-bud spurs positioning using two sets of wires 0.3 and 0.6m from the cordon to keep shoots growing upwards between the wires in a single plane. Experimental units, consisting in 5 plants each, were spread in a regularly spaced grid (ca. 40m between the units). Fruit-zone leaf removal on the Northern side, shoot thinning down to 25 shoots per plant (13.6 per meter), and combination of leaf removal and shoot thinning were applied randomly on 27 July to 12 randomly distributed experimental units. At harvest (12 September), 20-berry samples were collected.

Canopy size -- Canopy size was estimated using dormant pruning wood weight. Although dormant pruning wood does not include leaves, it is a quick and reliable indicator of canopy size used in viticulture. In January 2017, previous year's growth was pruned and individually weighed in all the experiments. The transmission of solar radiation through canopies necessarily follows an inverse proportionality relationship as dormant pruning weights increased.

Berry skin HPLC analyses – Berries were peeled and skins were freeze-dried (Cold Trap 7385020, Labconco, Kansas City, MO, USA). Dried tissues were ground with a tissue lyser (MM400, Retsch, Germany). Fifty mg of the powder were extracted with methanol: water: 7M hydrochloric acid (70:29:1,

V:V:V) to determine flavonol concentration and profile using HPLC-DAD as optimized by Martínez-Lüscher et al. (2019). HPLC-DAD system was Agilent 1260 series with a C18 column LiChrospher® 100, 250 × 4mm with a 5 µm particle size and a 4mm guard column. In brief, flow was set to 0.5 ml per minute and the column was at 25°C. Two mobile and gradients were designed to always maintain isocratic 5% of acetic acid throughout the entire run and the following percentages (V/V) of acetonitrile, 0-8min 8%, at 25mins 12.2%, at 35 mins 16.9%, at 70 mins 35.7%, 70-75 mins 65% and 80-90min 8%. Anthocyanins and flavonols were quantified determining the peak area of the absorbance at 520 nm and 365 nm, respectively. Malvidin-3-O glucoside and Quercetin-3-O-glucoside were used as quantitative standards.

Statistical analyses -- Linear regression was performed with Sigmaplot 13.0 (Systat Software, San Jose, USA). Pearson's correlation analyses were performed. Segmented regression was used to determine the breaking point in the relationship between global solar radiation, flavonol content and flavonol profile with 'segmented' 0.5-0.3 R package (Muggeo, 2008). For data arranged by categorical factors (treatments), ANOVA combined with a LSD post hoc was run using 'agricolae' 1.2-8 R package (de Mendiburu, 2016).

3. Results and discussion

3.1. *Flavonol profile is shaped by solar radiation over time.*

Flavonol content per berry increased in relation to berry exposure through throughout ripening (**Fig. 1A**). Regulatory and synthetic genes responsible for flavonol biosynthesis are upregulated by UV-B fraction of solar radiation (Carbonell-Bejerano et al., 2014), and grape flavonol content increases with exposure to UV-B radiation unequivocally (Berli et al., 2011, Martínez-Lüscher et al., 2014b). However, flavonol content decreased especially in exposed grapes (i.e. 0% shading factor) beyond 22°Brix (**Fig. 1A**), reducing the differences between the exposure levels, which may affect the total flavonols at harvest and berry exposure.

The proportion of kaempferol and quercetin were consistently higher (in detriment to myricetin) in grapes under increased solar radiation. This is, no shade nets (**Fig. 1B and C)**, higher canopy porosity or low vigour (**Table 1**). Great similarities can be found in studies reporting kaempferol, quercetin and myricetin percentages (Pastore et al., 2013) and contents (Berli et al., 2011). Contrarily to total flavonol content, flavonol profile maintained the differences between shading factors down to the end of the experiment (**Fig. 1B,C and D**). In addition, the proportion of quercetin and myricetin kept evolving after ripeness (ca. 22°Brix), when net synthesis is presumably not taking place. One plausible reason for profile changing in absence of de novo synthesis is a differential degradation of the flavonols according to their substituents in positions 3' and 5', as the addition of substituents of the flavonoid B-ring may strongly increase the antioxidant capacity of flavonols (Csepregi & Hideg, 2018), and therefore, their lability in senescing grape skins.

Overexposed grapes clearly lost the majority of their flavonols (**Fig. 2**) and other phenolic compounds such as hydroxycynamic acids, gallic acid, flavan-3-ols, anthocyanins and proanthocyanins (data not shown). Flavonol accumulation is one of the main mechanism of defence against high doses of UV radiation (Agati and Tattini, 2010). However, in studies where high doses of UV-B mimicking ozone depletion were tested (Kakani et al., 2003), no decrease in flavonols with increasing doses were reported. In the present study, where flavonols decreased due to over exposure, grapes received different exposures to full spectrum solar radiation and a subsequent increase in temperature, not only UV-B radiation. Efforts to decouple the effects of solar exposure prove that loss of anthocyanins and flavonols is related to temperature gain rather than the radiation itself (Spayd et al., 2002). Thresholds of 550 MJ m^{-2} (11.7 MJ m^{-2} per day) and 11% kaempferol thresholds were found to start observing flavonol degradation (**Fig. 2B and C**). However, degradation thresholds may be quite sensitive to variations in air temperature during ripening. In fact, 2017 was a hot year at the study site of the experiment with a maximum air temperature of 44°C. Solar radiation can increase berry temperature considerably, especially if the clusters are exposed during the warmest part of the day (data not shown). Therefore, certain relationship could be inferred also between flavonol profile and berry maximum temperature also.

3.2. *The use of flavonol profile as a metabolic integrator of the overall radiation received by a grape berry and its implications for canopy architecture.*

The percentage of kaempferol had a strong linear correlation to modelled global radiation (**Fig. 2A**). Previous research indicated that total flavonol content and the proportion of kaempferol, quercetin and myricetin changed with exposure to solar radiation (Martínez-Lüscher et al., 2014a). However, flavonol content was susceptible to degradation due to solar radiation or over-ripening (Martínez-Lüscher et al., 2017), making of relative amounts of each flavonol a better indicator of grape exposure to solar radiation under the conditions and varieties tested. However, it must be noted that large genotypic differences exist in flavonol profile. For instance, baseline levels of % kaempferol can range from 0% in Tannat to 17% in Muscat Rouge, which are very dark and pale red skinned and have high and low apparent F3'5'H activity, respectively (Mattivi et al., 2006). Therefore, cultivar differences in the slope and intercept of the relationship between radiation-flavonol can be expected. For instance, Pastore et al. (2017) reported flavonol profiles of 4 varieties with changes of different magnitude in the proportion of each flavonol in response to defoliation. However, defoliation always corresponded to an increase in the proportion of kaempferol and quercetin in detriment to the proportion of myricetin.

The robust correlation between the flavonol profile with solar radiation was also useful to verify the changes in canopy architecture. In a vineyard with a great variability in vigour (**Table 1**), the amount of solar radiation reaching the grape berry was increased through canopy management practices aiming to increase its exposure to solar radiation. Fruit-zone leaf removal and shoot thinning are the most widely used cultural practices for this purpose, the second being more effective increasing light transmission but potentially reducing yield in the process. Treatments of leaf removal, shoot thinning and their combination, obtained an increase in the proportion of kaempferol and quercetin in detriment of myricetin in the flavonol profile of Merlot due to greater grape berry exposure to solar radiation. Increasing the exposure of grape berry and vegetation vigour control are key factors in the production of quality grapes, such as anthocyanins and tannins accumulation and the removal of herbaceous aromas (Koch et al., 2012, Pastore et al., 2013). In addition, more open canopies are associated with higher wind speed and lower relative humidity at the fruit-zone, reducing the incidence of fungal rot (English et al., 1993).

4. Conclusions

This study aimed to propose flavonol profile, and specially the proportion of kaempferol, as a validation tool for the accumulated solar radiation received by grapes. In our results, flavonol profile was strongly related to canopy porosity and LAI estimations for individual clusters and to dormant pruning weight at the vine level. This reliability was based on the stability of % kaempferol through the progress of ripening. The assessment of berry exposure was exemplified by the response of flavonol profile to vigour groupings and to cultural practices aiming to increase the exposure of the grapes or balancing vine vigour. Given the extended use of HPLC-DAD to determine anthocyanin profile in research, we provided the principles to determine and understand flavonol profile constitution with this method. The flavonol profile is proposed as a fine indicator of the solar radiation intercepted and accumulated by berries and useful to discuss the effect of solar radiation or canopy architecture on grape composition.

5. Acknowledgments

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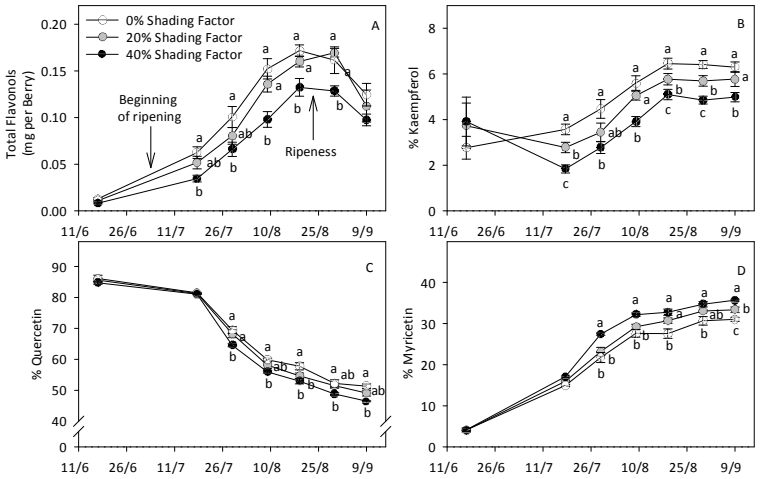

Figure 1. Evolution of flavonol content per berry (A), % kaempferol (B), % quercetin (C) and % myricetin (D) under ambient (0% shading factor) and under two shade nets (20% and 40% shading factor) covering the fruit-zone of cv. Cabernet Sauvignon grapes. Ripening is considered from color change (ca. 12°Bx) to soluble solids of ca. 22°Bx; and over-ripening, from 23°Bx to harvest (25°Bx in this case).

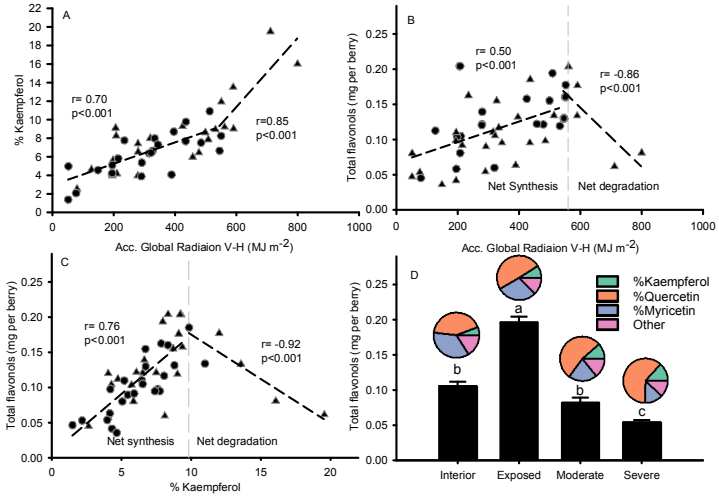

Figure 2. Flavonol profile is intimately related to the accumulated global radiation received by grapes and this response is exacerbated when degradation takes place. Correlation between accumulated global radiation from beginning of ripening to maturity and % kaempferol (A) or total flavonols per berry (B). Correlation between % kaempferol and total flavonols per berry (C). Berry flavonol content (bars) and profile (pies) of grapes from interior, exposed, moderately and severely overexposed clusters (D). Circles for sprawling canopy and triangles for vertical-shoot-positioned trellis. Dashed lines are breaking points determined through segmented regression.

Table 2. Dormant pruning weight and flavonol composition at harvest in a cv. Merlot Paso Robles, CA vineyard from vines homogeneously distributed and grouped by their pruning weights. Effect of the cultural practices leaf removal, shoot thinning and their combination increasing grape exposure.

Vigour groupings (n=15)	Pruning wood (Kg / m)	Total Flavonols (µg per Berry)	% Kaempferol	% Quercetin	% Myricetin
Low	0.39±0.01 c	59±3	3.92±0.18 a	52±1.1 a	36.8±1.2 b
Medium	0.47±0.01 b	50±3	3.19±0.21 b	47.6±0.9 b	40.4±1 a
High	0.59±0.04 a	49±3	2.94±0.23 b	46.6±1.2 b	41.9±1.4 a
p value	<0.001	0.055	0.003	0.009	0.005
Treatments (n=4)					
Control	0.47±0.03a	52±8 c	3.19±0.18 c	48.5±0.8 c	41.2±0.9 a
Leaf removal	0.45±0.06ab	100±4 ab	4.79±0.31 b	51.2±0.4 b	37.3±0.3 b
Shoot thinning	0.34±0.02b	91±6 b	5.36±0.41 ab	52.3±1.4ab	35.4±1.7 b
Combined	0.33±0.03b	135±5 a	6.72±0.23 a	55±0.5 a	32.1±0.6 c
p value	0.047	<0.001	<0.001	0.001	<0.001