

# Photoselective shade films mitigate heat wave damage by reducing anthocyanin and flavonol degradation in grapevine (*Vitis vinifera* L.) berries

Lauren E. Marigliano<sup>1</sup>, Runze Yu<sup>1,2</sup>, Nazareth Torres<sup>1,3</sup>, Justin D. Tanner<sup>1</sup>, Mark Battany<sup>4</sup> and S. Kaan Kurtural<sup>1,\*</sup>

<sup>1</sup> Department of Viticulture and Enology, University of California Davis, 1 Shields Avenue, Davis, California, 95616, United States

<sup>2</sup> Former post-doctoral scholar Current address: Department of Viticulture and Enology 2360 E. Barstow Ave M/S VR89 California State University Fresno, Fresno CA 93704 USA

<sup>3</sup> Former post-doctoral scholar Current address: Departamento de Agronomía, Biotecnología y Alimentación, Universidad Pública de Navarra, Pamplona, Navarra, Spain

<sup>4</sup> University of California Cooperative Extension San Luis Obispo County 2156 Sierra Way # C, San Luis Obispo, CA 93401

\*Corresponding author: [skkurtural@ucdavis.edu](mailto:skkurtural@ucdavis.edu)

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## Abstract

Wine grape production is challenged by forecasted increases in air temperature and droughts due to climate change. This study aimed to evaluate the vulnerability of ‘Cabernet Sauvignon’ grape berries to solar radiation overexposure and optimize shade film use for preserving berry composition. An experiment was conducted for two years with four shade films (D1, D3, D4, D5) with differing solar radiation spectra transmittance and compared to an uncovered control (C0). Integrals for leaf gas exchange and mid-day stem water potential were unaffected by shade films in both years. At harvest, berry primary metabolites were unaffected by treatments applied in either year. Despite precipitation exclusion during the dormant seasons in shaded treatments, yield was unaffected. In 2020, total skin anthocyanins (mg.berry<sup>-1</sup>) in the shaded treatments were 27% greater than C0 during berry ripening and at harvest. Conversely, flavonol content in 2020 decreased in partially shaded grapevines compared to C0. Berry flavonoid content in 2021 increased until harvest while flavonoid degradation was apparent from veraison to harvest in 2020 across all treatments. Our results highlighted the need for new approaches in warm viticulture areas given the impact that composition of solar radiation had on berry chemistry in the context of climate change.

## Introduction

Grapevine (*Vitis vinifera* L.) is a resilient and lucrative crop with a vast global distribution (Kurtural & Gambetta, 2021). Historically, climate and cultivar associations have developed regional wine identities that are commercially and culturally valued. However, steady increases in air temperature across the world’s most famous growing regions have been observed since 1980, threatening to shift appropriate climatic growing conditions to regions located in higher latitudes and altitudes in search for cooler climates (Kurtural & Gambetta, 2021). Concern for shifting regional climates is based in the understanding that certain grape cultivars thrive in specific optimum air temperature regimes where wine quality is optimized. Consequently, for a region to adapt to ever-warming air temperatures without detrimental decreases in wine composition, mitigation strategies need to be developed.

Among grape berry secondary metabolites, flavonoids play important roles in berry and wine composition. Anthocyanins are responsible for berry and wine color (Torres et al., 2022), while flavonols act as photoprotectants in plants, scavenging free oxygen radicals and preventing enzymatic reactive oxygen formation, while also contributing to wine color through co-pigmentation with anthocyanins (Torres et al., 2020). Flavonoids are produced through the phenylpropanoid pathway (Castellarin et al., 2007), which is

responsive to environmental conditions, including solar radiation. In warm climates, net accumulation of flavonols might be impeded by flavonol temperature sensitivity (Spayd et al., 2002). Anthocyanins are also susceptible to chemical or enzymatic degradation at high temperatures while sugar accumulation is unaffected, resulting in a decoupling of flavonoid and sugar development in the grape berry (Spayd et al., 2002). The effect of sugar and anthocyanin decoupling on berry and wine composition was investigated where ‘Cabernet Sauvignon’ berries subject to leaf removal and shoot removal treatments were harvested at 24°Brix and vinified (Torres et al., 2020; Torres, Martínez-Lüscher, et al., 2021). Compared to an untreated control, wines from leaf and shoot removal treatments had reduced color stability due to less anthocyanin hydroxylation as a function of higher temperatures and solar radiation exposure.

Efforts to reduce berry heat gain and through solar radiation exposure in vineyards through overhead and partial shading have been attempted but remain controversial in commercial wine grape vineyards. Martínez-Lüscher et al. partially excluded solar radiation with colored polyethylene shade nets (Martínez-Lüscher et al., 2017). They concluded that partial shading the canopy produced quantifiable differences in berry microclimate by reducing canopy temperature by 4°C on the SW-facing side of the canopy. The authors attributed the highest anthocyanin content in the Black-40% shade net lessened anthocyanin degradation from lower canopy temperature. However, partial shading in this experiment failed to selectively omit harmful solar radiation from the fruit, but rather reduced total solar radiation exposure by a fraction of the total radiation. The objective of this study was to selectively remove portions of solar radiation spectrum using overhead shade films in the vineyard, to mitigate the vulnerability of ‘Cabernet Sauvignon’ grape berry to solar radiation overexposure and optimize berry composition at harvest with desirable sugar accumulation and minimized flavonoid degradation.

## Materials and methods:

### *Experimental Site, Plant Materials and Experimental Design*

The study was conducted at the University of California Davis, Oakville Experimental Vineyard using “Cabernet Sauvignon” (*Vitis vinifera* L.) clone FPS08 grafted onto 110 Richter rootstock. The grapevines were trained to bilateral cordons, vertically shoot positioned, and pruned to 30-single bud spurs. The grapevines were planted at 2.0 m × 2.4m (vine × row) and oriented NW to SE. Irrigation was applied uniformly from fruit set to harvest at 25% evapotranspiration ( $ET_c$ ) as described elsewhere (Torres, Yu, & Kurtural, 2021). The experiment was arranged in a randomized complete block with four replications. Four photosensitive shade films with varying transmission spectra (Table 1) (Daios S.a. Naousa, Greece) and an untreated control were installed in 3 adjacent rows on 12 September 2019. The shade films remained suspended over the vineyard until 20 October 2021. The shade films were 2 m wide and 11m long and were secured on trellising approximately 2.5 m above the vineyard floor. Each experimental unit consisted of 15 grapevines in 3 adjacent rows. Measurements were taken in the middle row, from three adjacent grapevines, leaving the distal plants as borders.

### *Leaf Gas Exchange and Plant Water Status*

Leaf net carbon assimilation ( $A_{net}$ ), stomatal conductance ( $g_s$ ) and intrinsic water use efficiency ( $WUE_i$ ) were measured bi-weekly from anthesis to harvest using a portable infrared gas analyzer CIRAS-3 (PP Systems, Amesbury, MA, USA) as previously described (Torres, Yu, Martínez-Lüscher, et al., 2021a). Plant water status was measured as mid-day stem water potential ( $\Psi_{stem}$ ) bi-weekly from anthesis until harvest each year. The  $\Psi_{stem}$  was measured at solar noon from 13:00 to 14:00 h following previously reported procedures (Torres et al., 2021). Measurements were taken using a pressure chamber (Model 615, PMS Instrument Company, Albany, OR, USA). The integrals for  $A_{net}$ ,  $g_s$ ,  $WUE_i$  and  $\Psi_{stem}$  were calculated by natural cubic splines for each parameter and calculating the area. The area divided by the number of days elapsed between the first measurement date and the last measurement date is the resulting integral values as previously reported (Torres, Yu, Martínez-Lüscher, et al., 2021a).

### *Fruit Sample Collection and Preparation*

Seventy berries were collected each year at the following developmental stages: green berry, veraison, mid-ripening and at harvest and processed the same day. Twenty berries were set aside and skinned by hand as previously reported (Martínez-Lüscher et al., 2019). Grape skins were freeze-dried (Centrivap, Labconco, Kansas City, MO, USA) and ground into powder. 50 g of powder was extracted overnight at 4°C with methanol:

water: 7M hydrochloric acid (70:29:1) for anthocyanin and flavonol quantification. Samples were centrifuged for 10 mins at 4000RPM. Supernatants were filtered (0.45 $\mu$ m; VWR, Seattle, WA, USA) and transferred to HPLC vials.

### *HPLC Procedures*

Skin anthocyanins and flavonols were analyzed using a reversed-phase HPLC (Agilent model 1260, Agilent Technologies, Santa Clara, CA, USA). A C18 reversed-phase column (LiChrosphere 100 RP-18, 4 x 520 mm<sup>2</sup>, 5 $\mu$ m particle size, Agilent Technologies, Santa Clara, CA, USA) was utilized for flavonoid analysis as well. The mobile phase flow rate was 0.5 mL min<sup>-1</sup>, and two mobile phases were used, which included solvent A = 5.5% aqueous formic acid; solvent B = 5.5% formic acid in acetonitrile. The HPLC flow gradient started with 91.5% A with 8.5% B, 87% A with 13% B at 25 min, 82% A with 18% B at 35 min, 62% A with 38% B at 70 mins, 50% A with 50% B at 70.01 min, 30% A with 70% B at 75 min, 91.5% A with 8.5% B from 75.01 min to 91 min. The column temperature was maintained at 25°C. This elution allowed for avoiding co-elution of anthocyanins and flavonols as previously reported (Martínez-Lüscher et al., 2019). Flavonols and anthocyanins were detected by the diode array detector at 365 nm and 520 nm, respectively. A computer workstation with Agilent OpenLAB (Chemstation edition, version A.02.10) was used for chromatographic analysis.

### *Statistical Analysis*

Statistical analyses were conducted with R studio version 4.0.5 (RStudio: Integrated Development for R., Boston, MA, United States) for Windows. Seasonal integrals of  $\Psi_{\text{stem}}$  and gas exchange variables for each growing season and for both seasons were calculated by using the same software. All data were subjected to Shapiro–Wilk’s normality test. Data were normally distributed and subsequently submitted to an analysis of variance (ANOVA) to assess the statistical differences between the overhead shade film treatments. For all data, means  $\pm$  standard errors (SE) were calculated, and when the F value was significant ( $p \leq 0.05$ ), Duncan’s new multiple range *post hoc* test was executed using “agricolae” 1.2-8 R package (de Mendiburu, 2016).

## **Results and Discussion**

### *Primary Metabolism*

The integrals for gas exchange and mid-day stem water potential were calculated (Table 2). In either year, there was no effect of overhead shade films on  $A_{\text{net}}$ ,  $g_s$  or  $WUE_i$  or  $\Psi_{\text{stem}}$  integrals. This may be attributed to the transmission spectra of the polyethylene shade films. Each shade film reduced PAR transmission by approximately 20% from full transmission. The photosynthetic capacity of grapevines is optimized between 800 and 1200  $\mu\text{mol}\cdot\text{m}^{-2}\text{s}^{-1}$  of solar radiation (Carvalho et al., 2016), despite 2000  $\mu\text{mol}\cdot\text{m}^{-2}\text{s}^{-1}$  of solar radiation provided under control conditions. Since leaf area was maintained across treatments and PAR was only reduced by 20%, the photosynthetic capacity of the grapevines was unaltered under the shade films. Negligible differences in canopy size and the replacement of 25%  $ET_c$  resulted in no significant effect on  $\Psi_{\text{stem}}$  or  $g_s$  integrals between treatments within a given year. However, C0 and D4 in both years were trending towards more negative  $\Psi_{\text{stem}}$  values, which may be due to larger transmittance of NIR radiation and increased evaporative demand. By maintaining aspects such as canopy size and plant water status required for adequate ripening across the treatments (Bergqvist et al., 2001), the effects of shading on berry composition were most likely related to the fruit zone microclimate, specifically reduction of temperature.

### *Skin Flavonoid Content*

Compared to the control, grape berries grown under shade film had higher skin anthocyanins at both mid-ripening and harvest (Figure 1A) in 2020. In all treatments, total skin anthocyanin content peaked at mid-ripening and then decreased from mid-ripening to harvest, with D5 showing the smallest decrease in total skin anthocyanin content (Figure 1A). However, the shade treatment films resulted in 27% greater anthocyanin content than C0 at harvest. In 2021, differences in total skin anthocyanin content were evident at veraison and mid-ripening (Figure 2A). At veraison, total skin anthocyanin content was higher in D5 compared to D1. Shade films C0, D3, and D4 had similar total skin anthocyanin content to D1 and D5 at veraison. At mid-ripening, D5 has significantly higher total skin anthocyanin content to C0, with D1, D3 and D4 having similar anthocyanin content. At harvest, overhead shade films did not have an impact on total skin anthocyanin content. However,

anthocyanin content increased from mid-ripening to harvest in D1, D3 and D4, while they appeared to reach a plateau in anthocyanin content in D5 and C0.

Optimum temperature thresholds were established for anthocyanin accumulation in grape berries. It was identified that anthocyanin accumulation was maximized at 875 GDD and a daily mean light intensity of 220klm·m<sup>-2</sup> after which anthocyanin content decreased in Cabernet Sauvignon (Torres et al., 2020). Previous works that used partial shading that transmitted 60% of solar radiation had also resulted in increased anthocyanin content compared to unshaded fruit in under similar growing season climatic conditions as what was observed in 2020 in our study (Martínez-Lüscher et al., 2017; Martínez-Lüscher et al., 2020). In 2021, shade films did not affect the anthocyanin content in berry skins at harvest, due to the cooler growing season limiting anthocyanin degradation post-veraison. The reduction in anthocyanin content observed in 2020 may result from repressed anthocyanin biosynthesis at hot temperatures via the MYB4 repressor (Mori et al., 2007). However, it is also highly likely that elevated temperatures in 2020 resulted in increased anthocyanin degradation in exposed fruit compared to shaded fruit, leading to shaded fruit having greater anthocyanin content.

In 2020, total skin flavonol content increased in both shaded treatments (D1, D3, D4, D5) and the unshaded control (C0) until the veraison (Figure 1B). However, C0 consistently had higher flavonol content compared to shaded treatments. Between the shaded treatments, D4 and D5 produced fruits with significantly more flavonol content per berry compared to D1 and D3 at each sampling time point, except at immediate pre-veraison, where flavonol content in D4 was not significantly different compared to D1 and D3. At mid-ripening flavonol content decreased in both shaded and unshaded fruits. At harvest, there was no significant difference in flavonol content between C0, D4, and D5. Shade films D1 and D3 had less total skin flavonols than C0, D4 and D5, containing approximately 0.06 mg/berry. In 2021, the accumulation trend of skin flavonol content differed compared to that of 2020. At the first sampling point, total skin flavonols were the highest in C0 while D1 had the lowest flavonol content (Figure 2B). The flavonol content continued to increase as ripening progressed. From mid-ripening to harvest, C0, D5 and D4 had the highest flavonol content compared to D1 and D3. In 2021, total skin flavonols did not decrease prior to harvest.

Thresholds for optimal sunlight exposure have been elucidated in previous solar radiation exclusion experiments, where Martínez-Lüscher et al. tracked flavonol development over the growing season under 20% and 40% shading conditions (Martínez-Lüscher et al., 2019). It was determined that net flavonol biosynthesis occurs until approximately 570 MJ m<sup>-2</sup> of accumulated global radiation which corresponds with 7.6% molar abundance of kaempferol in grape skins (Martínez-Lüscher et al., 2019). Beyond these thresholds, flavonols started to be degraded in the grape berries. Our study showed a similar trend for flavonol content in hot years like 2020. In cooler years like 2021, flavonol degradation was not observed at the global radiation threshold as a result of the cooler growing season. Rather, biosynthesis continued to increase flavonol content until harvest in 2021. Shade films effectively lengthened the period of flavonol biosynthesis and reduced the amount of time during ripening where clusters are under flavonol degrading conditions.

**Table 1.** Percent Solar Radiation Transmitted by Overhead Shade Films

Treatment	UVA	UVB	UVC	NIR	PAR
C0	100	100	100	100	100
D1	23.3	0	0	81.2	87.8
D3	25.9	1	1	81.9	87.1
D4	66.7	53.6	16.7	82.5	86.9
D5	48.2	30.8	9.7	81.2	73.2

## Conclusion

In the context of climate change, more frequent heat wave events may be deleterious on grape and wine quality. This study aimed to elucidate the optimal solar spectrum to avoid deleterious impacts on grapevine physiology and berry composition associated with increased temperatures. Overhead shade film D5 effectively reduced cluster temperature by blocking near infrared radiation resulting in 27% greater anthocyanin content. Grapevine water status, leaf gas exchange and berry primary chemistry were maintained underneath overhead shade films. Anthocyanin content was increased under shade films in warmer than average years, ultimately due to reduced



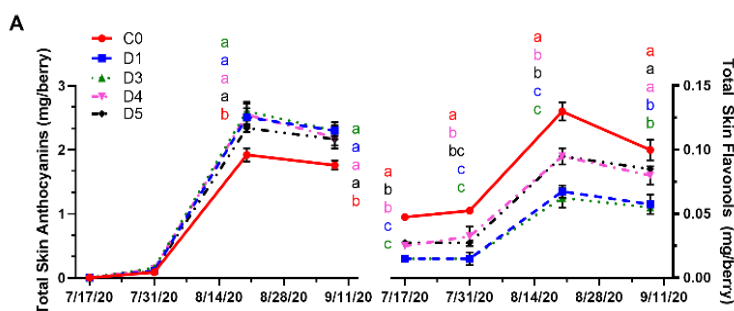
degradation from excessive cluster temperatures. Shade film D5 produced temperature and solar radiation conditions which optimized berry flavonoid content. Overhead shade films are a novel solution for grape producers in hot climate viticultural regions, as more frequent heat wave events are forecasted with climate change.

**Table 2.** Effects of Photo-selective Overhead Shade Films on Integrals of Leaf Gas Exchange and Mid-day Stem Water Potential integrals on Cabernet Sauvignon/110R in Oakville, CA USA<sup>ab</sup>

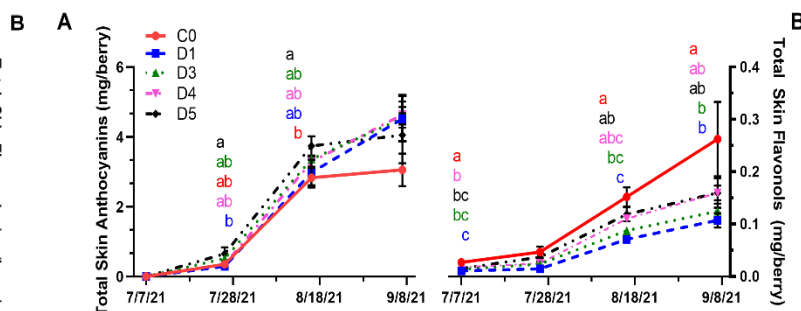
Treatment	$A_{net}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2}\cdot\text{s}^{-1}$ )	$g_s$ ( $\text{mmol H}_2\text{O m}^{-2}\cdot\text{s}^{-1}$ )	$\text{WUE}_i$ ( $\mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$ )	$\Psi_{stem}$ (MPa)
2020				
Control	10.56 ± 1.44	151 ± 22	0.073 ± 0.006	-1.10 ± 0.84
D1	8.78 ± 0.71	173 ± 24	0.058 ± 0.006	-1.03 ± 0.74
D3	9.56 ± 1.33	188 ± 29	0.055 ± 0.005	-1.04 ± 0.83
D4	9.28 ± 1.12	185 ± 36	0.058 ± 0.007	-1.11 ± 0.91
D5	9.47 ± 0.87	187 ± 36	0.061 ± 0.006	-1.05 ± 0.70
<i>p</i> value	n.s.	n.s.	n.s.	n.s.
2021				
Control	14.68 ± 1.37	182 ± 27	0.087 ± 0.010	-1.21 ± 0.11
D1	12.79 ± 0.84	187 ± 23	0.076 ± 0.009	-1.18 ± 0.11
D3	13.58 ± 1.00	196 ± 25	0.076 ± 0.010	-1.16 ± 0.10
D4	13.13 ± 1.01	185 ± 28	0.079 ± 0.010	-1.25 ± 0.11
D5	13.96 ± 0.91	202 ± 27	0.077 ± 0.010	-1.16 ± 0.09
<i>p</i> value	n.s.	n.s.	n.s.	n.s.

<sup>a</sup> Values in each column are reported as mean ± standard error of the mean

<sup>b</sup> n.s. indicates a *p* value ≥ 0.05



**Figure 1.** Total skin anthocyanin (A) and flavonol (B) content throughout berry development in untreated (C0) and shade film treatments (D1, D3, D4, D5) in 2020. Points are means ± standard error (n = 4). Means with no letters in common are significantly different ( $p \leq 0.05$ ).



**Figure 2.** Total skin anthocyanin (A) and flavonol (B) content throughout berry development in untreated (C0) and shade film treatments (D1, D3, D4, D5) in 2021. Points are means ± standard error (n = 4). Means with no letters in common are significantly different ( $p \leq 0.05$ ).

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