‘Cabernet Sauvignon’ (*Vitis vinifera* L.) Berry Skin Flavonol and Anthocyanin Composition is Affected by Trellis Systems and Applied Water Amounts

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Keywords: anthocyanins, flavonols, trellis systems, water deficits, viticulture

Abstract

Trellis systems are selected in wine grape vineyards to mainly maximize vineyard yield and maintain berry quality. This study was conducted in 2020 and 2021 to evaluate six commonly utilized trellis systems including a vertical shoot positioning (VSP), two relaxed VSPs (VSP60 and VSP80), a single high wire (SH), a high quadrilateral (HQ), and a guyot (GY), combined with three levels of irrigation regimes based on different crop evapotranspiration (ET_c) replacements, including a 25% ET_c, 50% ET_c, and 100% ET_c. The results indicated SH yielded the most fruits and accumulated the most total soluble solids (TSS) at harvest in 2020, however, it showed the lowest TSS in the second season. In 2020, SH and HQ showed higher concentrations in most of the anthocyanin derivatives compared to the VSPs. Similar comparisons were noticed in 2021 as well. SH and HQ also accumulated more flavonols in both years compared to other trellis systems. Overall, this study provides information on the efficacy of trellis systems on grapevine yield and berry flavonoid accumulation in a currently warming climate.

Introduction

Global warming has been increasingly jeopardizing the productivity and sustainability of wine grape vineyards (Rienth et al., 2021; Venios et al., 2020). Due to the significance of berry flavonoids on berry and wine quality, the negative influences (*i.e.* degradation) on the flavonoid concentrations in grape berries from frequent heatwaves, excessive solar radiation, and other environmental stresses have been more noticeable, which often lower berry quality at harvest (Gambetta & Kurtural, 2021; Torres et al., 2020; Torres, Martínez-Lüscher, et al., 2021). Within flavonoids, anthocyanin is one family of coumponds that determine grape berry/wine color. Flavonol is another family that forms co-pigmentation with anthocyanins during wine aging to stabilize color. These two families have shown to be sensitive to solar radiation and heat, and in some most recent research, overexposure will lead to degradation in both families (Johann Martínez-Lüscher et al., 2017; Torres, Martínez-Lüscher, et al., 2021).
Trellis system selection in vineyards plays an important role in determining canopy microclimate, hence influences berry development (Sanchez-Rodriguez & Spósito, 2020; Torres, Martínez-Lüscher, et al., 2021). As mentioned, the current warming trend is making some of the commonly utilized trellis systems more prone to berry cluster overexposure, resulting in over-ripening conditions and color and flavor reductions, and shortened wine aging potential (Torres, Martínez-Lüscher, et al., 2021). There are many relatively new trellis systems that need to be evaluated under the current warming climate trend to understand their performance and the potential effect on cluster exposure to compare with the traditional trellises. Water is another critical factor in wine grape vineyard management. Different levels of water deficits achieved by replacing different amount of crop evapotranspiration (ET_c) alter grapevine physiological development and berry chemisty. Usually, some degree of water deficits promote berry sugar/flavonoid accumulation with reduced grapevine water status and

photosynthetic capacity (Torres, Yu, Martínez-Lüscher, et al., 2021b, 2021a; Torres et al., 2022). However, trellis system selection might also influence the water demand and/or interact with applied water amounts due to canopy architecture and size as a direct function of trellis system.
Thus, the goal of this study was to compare different trellis systems combined with various irrigation regimes to investigate their contributions to grapevine physiological development and berry composition in the context of a warming climatic trend.

Materials and Methods

Vineyard site, Plant Materials, and Weather Conditions

The experiment was conducted on a 'Cabernet Sauvignon' vineyard grafted on 3309C rootstock (*V. riparia* × *V. rupestris*) in 2020 and 2021. The vineyard was in Oakville, Napa County, CA, USA. Grapevines were spaced at 1.52 m × 2.13 m (vine × row). The rows were oriented NE-SW.
Experimental Design: This study was conducted in a split-plot factorial design, including a main factor (6 trellis systems) combined with three different applied water amounts with four replications. There were 72 experimental units in total composed of five vines in each for measurements and berry sampling.
The six trellising systems included a traditional vertical shoot positioned (VSP) trellis, as well as two additional VSP designs that were modified with more opened canopies (with ~60° and ~80° shoot orientation: VSP60 and VSP80), a high quadrilateral (HQ) trellis, a single high wire (SH) trellis, and a guyot (GY) trellis design .
Irrigation treatments applied to the grapevines were based on calculated ET_c using the following equation: $ET_c = ET_o \times K_c$, where ET_o was the reference evapotranspiration and K_c is the crop coefficient. ET_o was assessed weekly from the California Irrigation Management Information System (CIMIS) station located on site and close to the experiment, and K_c was assessed by using the shade cast method (Williams & Ayars, 2005). The irrigation regimes included 100% ET_c, 50% ET_c and 25% ET_c.

Yield Components, Berry Sampling, Primary and Secondary Metabolite Assessment

In both seasons, all fruit in each treatment were harvested when berry must total soluble solids (TSS) reached around 25 °Brix in VSP trellis system. Yield components were recorded for berry weight and total yield. 50 berries in total were sampled from each experimental unit at harvest in both seasons to assess berry primary and secondary metabolites. 30 berries were used for primary metabolite analysis. Berry must TSS was assessed by a refractometer (Atago PR-32, Bellevue, WA, USA) in the unit of °Brix. Berry must pH and titratable acidity (TA) were measured by using an auto-titrator (862 Compact TitroSampler, Metrohm, Switzerland) and were recorded as g L^{-1} of tartaric acid at the titration end point of pH 8.2.
The other 20 berries were used for secondary metabolite analysis in berry skins. Skins were manually removed and lyophilized (Centrivap Benchtop Centrifugal Vacuum Concentrator 7810014 equipped with Centrivap -105 °C Cold Trap 7385020, Labconco, Kansas City, MO, USA). Then, the dried skin masses were ground into fine powder using a mixing mill (MM400, Retsch, Mammelzen, Germany). 50 mg of powder was mixed with 1 mL of methanol:water:7M hydrochloric acid overnight in a refrigerator at 4°C to extract flavonoids. Then, the supernatants were separated from the solids by using a centrifuge at 16,500 rpm for 15 mins and transferred into HPLC vials after filtration by PTFE membrane filters (diameter: 13 mm, pore size: 0.45 µm, VWR, Seattle, WA, USA). Finally, the samples were injected for HPLC chromatographic analysis.
The concentrations of skin anthocyanins and flavonols were analyzed using a reversed-phase HPLC (Model 1260, Agilent, Santa Clara, CA, USA). The method was reported previously (Johann Martínez-Lüscher et al., 2019), including two mobile phases: (A) 5.5% formic acid in water and (B) 5.5% formic acid in acetonitrile. The flow rate of the mobile phase was 0.5 mL min^{-1} and the 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 min, 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 90 min. A C18 reversed-phase HPLC column was used for this method as the solid phase (LiChrosphere 100 RP-18, 4 × 520 mm^2, 5 mm particle size, Agilent Technologies, Santa Clara, CA, United States). The column temperature was maintained at 25°C on both the left and right sides. Detection of anthocyanins and flavonols was recorded by a diode array detector (DAD) at 520nm and 365nm, respectively. A chromatographic workstation was used for quantification with Agilent OpenLAB software (Chemstation edition, version A.02.10).
Statistical Analysis: A two-way ANOVA with trellis systems and irrigation regimes as two independent factors was used for the analysis with RStudio (RStudio, Inc., Boston, MA, USA). Normal distribution was performed

for all included datasets by using a Shapiro-Wilkinson test prior to the ANOVA analysis. A Duncan's Multiple Range *post-hoc* test was conducted to investigate the ranks of different treatments. And p value £ 0.10 from ANOVA was considered for the Duncan's *post-hoc* tests.

Results and Discussion:

Components of Yield and Berry Primary Metabolites

Components of yield and berry primary metabolites were assessed at harvest in 2020 and 2021 (**Table 1**). In the first season, SH and HQ showed the lightest grape berry mass compared to the other trellis system. The highest yield and TSS was observed with SH, while the VSPs had the lowest observed TSS at harvest. These observations might be attributed to the warming trend in air temperature observed in recent decades, VSPs were prone to experience overexposure inside the canopies, resulting in yield loss and color degradation (Johann Martínez-Lüscher et al., 2017; Torres et al., 2020). Also, higher solar exposure might have led to greater berry dehydration, resulting in higher TSS concentrations (Torres et al., 2017). In 2021, VSP60 and VSP80 along with GY had the heaviest berries compared to the other trellises, while HQ had the lightest berries again. Contrary to 2020, there was no difference observed in yield in 2021. SH and HQ showed the lowest TSS accumulation among all the trellis systems in 2021. Yield was not constantly determined by the trellis systems in both seasons. This might be attributed to different leaf area distribution characteristics of the canopies. SH and HQ had more open space compared to the other trellis systems to place leaf areas exposed to solar radiation (Bettiga et al., 2003).

As for applied water amounts, in 2020, the highest berry weights were observed with 100% ET_c. The relationships between applied water amounts, berry weight and yield were mostly linear, where more water applied resulted in more berry weight and yield although 50% and 100% ET_c did not differentiate in yield. This observation agreed with many previous studies investigating the relationships between grapevine water status, berry weight and yield (Torres, Yu, & Kurtural, 2021; Torres, Yu, Martínez-Lüscher, et al., 2021a). Moreover, applying 25% ET_c resulted in the highest TSS while applying100% ET_c resulted in the lowest TSS at harvest. This might be due to berry dehydration from low water status (Torres et al., 2017) and potential promotion in sugar accumulation by the water deficits implemented to the grapevines (Zarrouk et al., 2016). In 2021, 100% ET_c showed the heaviest berries and the most yield among the three irrigation regimes. However, there was no difference in TSS at harvest. This might be due to applying 25% ET_c promoted the development of relatively smaller canopies with less fruits, which would have maintained a similar level of source-sink balance among all the irrigation regimes, causing the TSS accumulation to be unaltered.

Berry Secondary Metabolites

Berry skin anthocyanins and flavonols were assessed in both season at harvest (**Table 1**). These two families of flavonoids have shown to be highly responsive to environmental factors (Arrizabalaga-Arriazu et al., 2020; de Rosas et al., 2017; J Martínez-Lüscher et al., 2014; Yu et al., 2020). As for the trellis systems, in 2020, SH had the highest anthocyanin content per berry among the six trellis systems, with all other trellis systems having lower values. The same pattern was observed with flavonol content as well, where SH had the most flavonols content per berry compared to the other trellises. There was no difference in tri- to di-hydroxylated anthocyanin ratio among all the trellis systems. However, SH showed the highest tri- to d-hydroxylated flavonol ratio. In this season, SH might have had greater advancement in berry development due to increased temperature in 2020 resulting in a greater degree of berry dehydration and solar/heat exposure in other more exposed trellises (Gambetta & Kurtural, 2021; Torres et al., 2017). SH produced berries with greater anthocyanin and flavonol content. Berry flavonoid degradation might not be the reason SH produced the higher flavonoid content compared to the other trellises due to the relatively synchronized accumulation in berry TSS and flavonoids. In 2021, SH again showed the highest total anthocyanin content per berry, followed by HQ. VSP, VSP60, VSP80, and GY all had lower values compared to SH. VSP80, SH, HQ, and GY had higher flavonol content per berry. In this year, however, the decoupling of TSS and berry flavonoids was noticeable, which suggests SH and HQ performed better than the VSPs and GY due to their relatively higher canopy porosity (Johann Martínez-Lüscher et al., 2019). Similar to 2020, there was no difference in tri- to di-hydroxylated anthocyanin ratio among the six trellis systems. HQ had the lowest tri- to di-hydroxylated flavonols compared to all the other trellises. This might be attributed to the less exposed canopies of SH and HQ compared to relatively open VSPs and GY. Tri-hydroxylated flavonoids are more chemically stable against oxidation and degradation (Heim et al., 2002).

As for applied water amount, in 2020, there was higher tri- to di-hydroxylated anthocyanin ratio with 25% ET_c compared to 50% and 100% ET_c. However, in 2021, there was no difference observed in all these variables among the three applied water amounts. Previous studies validated that higher water deficit would promote flavonoid accumulation as well as tri-hydroxylation (Brillante et al., 2017; Yu et al., 2020), this might explain the difference in anthocyanin hydroxylation observed in this study in 2020. In 2021 however, there was no difference in flavonoids among the three applied water amounts presumably attributed to the relatively uniform berry development without extremely dry and hot conditions in that growing season, leaving all the treatments to have similar degrees of flavonoid development in berry skins (Ferri et al., 2011).

Conclusion

Overall, this study provides evidence of how the six trellis systems combined with the three applied water amount affected grapevines' yield components and berry flavonoid contents. As shown in this study, SH and HQ were more efficient in advancing berry maturity and grapevine production. In addition, these 2 trellis showed greater flavonoid content in berry skins with relatively more chemically stable flavonols compared to the other trellises.

Table 1. Effects of Trellis Systems and Applied Water Amounts on Yield Components and Berry Composition of Cabernet Sauvignon in Oakville, CA, USA in 2020 and 2021 [a,b]

		Trellis							Irrigation				Trellis ×
		VSP	VSP 60	VSP 80	SH	HQ	GY	*p* value	25% ET_c	50% ET_c	100% ET_c	*p* value	Irrigation
2020	50 Berry Weight (g)	48.5 a	50.0 a	50.5 a	43.0 b	43.5 b	48.5 a	**	41.5 c	48.0 b	52.5 a	***	ns
	Yield (tons ha^{-1})	13.19 ab	13.41 ab	11.00 b	14.55 a	11.00 b	11.95 b	*	10.47 b	12.94 a	14.15 a	**	ns
	TSS (°Brix)	23.5 b	23.7 b	23.7 b	24.6 a	24.1 ab	24.2 ab	*	24.8 a	24.1 b	22.9 c	***	ns
	pH	3.47 a	3.49 a	3.46 ab	3.40 c	3.42 bc	3.48 a	**	3.47	3.45	3.44	ns	ns
	TA (g L^{-1})	7.74	7.36	7.69	7.53	7.78	7.71	ns	7.50	7.70	7.80	ns	ns
	Tri-/Di-OH Anthocyanins	10.17	10.24	10.17	10.90	8.89	9.48	ns	11.39 a	9.67 b	8.86 b	***	ns
	Total Anthocyanin (mg $berry^{-1}$)	1.89 b	2.07 b	2.19 b	2.63 a	2.07 b	2.01 b	***	2.11	2.19	2.12	ns	ns
	Tri-/Di-OH Flavonols	0.74 a	0.83 a	0.80 a	0.77 a	0.61 b	0.75 a	***	0.73	0.77	0.75	ns	ns
	Total Flavonols (mg $berry^{-1}$)	1.89 b	2.07 b	2.19 b	2.63 a	2.07 b	2.01 b	***	2.11	2.19	2.12	ns	ns
2021	50 Berry Weight (g)	50 ab	51.5 a	51.5 a	44.0 ab	41.5 b	51.5 a	**	44.0 b	47.0 b	53.5 a	***	ns
	Yield (tons ha^{-1})	21.10	23.07	20.26	25.33	32.34	20.72	ns	18.94 b	26.81 a	25.36 a	.	ns
	TSS (°Brix)	23.1 a	23.2 a	23.3 a	21.7 b	21.9 b	22.7 ab	*	22.6	22.5	22.9	ns	ns
	pH	3.62 a	3.59 ab	3.57 ab	3.55 b	3.53 b	3.58 ab	.	3.59	3.56	3.57	ns	ns
	TA (g L^{-1})	5.98	5.96	5.89	5.63	5.71	8.43	ns	6.81	5.82	6.18	ns	ns
	Tri-/Di-OH Anthocyanins	15.55	16.69	16.07	14.22	15.56	16.17	ns	15.57	16.08	15.49	ns	ns
	Total Anthocyanin (mg $berry^{-1}$)	2.03 c	2.24 bc	2.28 bc	2.74 a	2.49 ab	2.06 c	***	2.33	2.36	2.23	ns	ns
	Tri-/Di-OH Flavonols	0.74 a	0.83 a	0.80 a	0.77 a	0.61 b	0.75 a	***	0.73	0.77	0.75	ns	ns
	Total Flavonols (mg $berry^{-1}$)	0.12 b	0.12 b	0.13 ab	0.15 ab	0.16 a	0.14 ab	*	0.13	0.14	0.14	ns	ns

[a] ANOVA to compare data (*p* value indicated); Letters within columns indicate significant mean separation according to Duncan's multiple range test at where ".": *p* value < 0.1; where "*": *p* value < 0.05; "**": *p* value < 0.001, "***": *p* value < 0.0001. [b] Abbreviations: VSP: vertical shoot positioned; VSP 68: vertical shoot positioned 68° ; VSP 78: vertical shoot positioned 78°; SH: single high wire; HQ: high quadralateral; GY: guyot; TSS: total soluble solids; TA: titratable acidity; ET_c: crop evapotranspiration; OH: hydroxylated; ns: not significant.Berry skin flavonoids were expressed in the unit of mg per berry.

References

Arrizabalaga-Arriazu, M., Gomès, E., Morales, F., Irigoyen, J. J., Pascual, I., & Hilbert, G. (2020). High temperature and elevated carbon dioxide modify berry composition of different clones of grapevine (Vitis vinifera L.) cv. tempranillo. *Frontiers in Plant Science*, *11*, 1888. https://doi.org/10.3389/fpls.2020.603687.

Bettiga, L. J., Golino, D. A., McGourty, G., Smith, R. J., Verdegaal, P. S., & Weber, E. (2003). *Wine grape varieties in California* (Vol. 3419). UCANR Publications.

Brillante, L., Martínez-Lüscher, J., Yu, R., Plank, C. M., Sanchez, L., Bates, T. L., Brenneman, C., Oberholster, A., & Kurtural, S. K. (2017). Assessing spatial variability of grape skin flavonoids at the vineyard scale based on plant water status mapping. *Journal of Agricultural and Food Chemistry*, *65*(26), 5255–5265. https://doi.org/10.1021/acs.jafc.7b01749

de Rosas, I., Ponce, M. T., Malovini, E., Deis, L., Cavagnaro, B., & Cavagnaro, P. (2017). Loss of anthocyanins and modification of the anthocyanin profiles in grape berries of Malbec and Bonarda grown under high temperature conditions. *Plant Science*, *258*, 137–145. https://doi.org/10.1016/j.plantsci.2017.01.015

Ferri, M., Righetti, L., & Tassoni, A. (2011). Increasing sucrose concentrations promote phenylpropanoid biosynthesis in grapevine cell cultures. *Journal of Plant Physiology*, *168*(3), 189–195. https://doi.org/ 10.1016/j.jplph.2010.06.027

Gambetta, G., & Kurtural, S. K. (2021). Global warming and wine quality: are we close to the tipping point? *OENO One*, *55*(3), 353–361. https://doi.org/10.20870/oeno-one.2021.55.3.4774

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry*, *13*(10), 572–584. https://doi.org/https://doi.org/10.1016/S0955-2863(02)00208-5

Martínez-Lüscher, J, Torres, N., Hilbert, G., Richard, T., Sánchez-Díaz, M., Delrot, S., Aguirreolea, J., Pascual, I., & Gomès, E. (2014). Ultraviolet-B radiation modifies the quantitative and qualitative profile of flavonoids and amino acids in grape berries. *Phytochemistry*, *102*, 106–114. https://doi.org/https://doi.org/10.1016/j.phytochem.2014.03.014

Martínez-Lüscher, Johann, Brillante, L., & Kurtural, S. K. (2019). Flavonol profile is a reliable indicator to assess canopy architecture and the exposure of red wine grapes to solar radiation. *Frontiers in Plant Science*, *10*, 10. https://doi.org/10.3389/fpls.2019.00010

Martínez-Lüscher, Johann, Chen, C. C. L., Brillante, L., & Kurtural, S. K. (2017). Partial solar radiation exclusion with color shade nets reduces the degradation of organic acids and flavonoids of grape berry (Vitis vinifera L.). *Journal of Agricultural and Food Chemistry*, *65*(49), 10693–10702. https://doi.org/10.1021/acs.jafc.7b04163

Rienth, M., Vigneron, N., Darriet, P., Sweetman, C., Burbidge, C., Bonghi, C., Walker, R. P., Famiani, F., & Castellarin, S. D. (2021). Grape Berry Secondary Metabolites and Their Modulation by Abiotic Factors in a Climate Change Scenario–A Review. *Frontiers in Plant Science*, *12*, 262. https://doi.org/10.3389/fpls.2021.643258

Sanchez-Rodriguez, L. A., & Spósito, M. B. (2020). Influence of the trellis/training system on the physiology and production of Vitis labrusca cv. Niagara Rosada in Brazil. *Scientia Horticulturae*, *261*, 109043. https://doi.org/10.1016/j.scienta.2019.109043

Torres, N., Hilbert, G., Luquin, J., Goicoechea, N., & Antolín, M. C. (2017). Flavonoid and amino acid profiling on Vitis vinifera L. cv Tempranillo subjected to deficit irrigation under elevated temperatures. *Journal of Food Composition and Analysis*, *62*, 51–62. https://doi.org/10.1016/j.jfca.2017.05.001

Torres, N., Martínez-Lüscher, J., Porte, E., & Kurtural, S. K. (2020). Optimal Ranges and Thresholds of Grape Berry Solar Radiation for Flavonoid Biosynthesis in Warm Climates. *Frontiers in Plant Science*, *11*, 931. https://doi.org/10.3389/fpls.2020.00931

Torres, N., Martínez-Lüscher, J., Porte, E., Yu, R., & Kaan Kurtural, S. (2021). Impacts of leaf removal and shoot thinning on cumulative daily light intensity and thermal time and their cascading effects of grapevine (Vitis vinifera L.) berry and wine chemistry in warm climates. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2020.128447

Torres, N., Yu, R., & Kurtural, S. K. (2021). Arbuscular Mycrorrhizal Fungi Inoculation and Applied Water Amounts Modulate the Response of Young Grapevines to Mild Water Stress in a Hyper-Arid Season. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.622209

Torres, N., Yu, R., Martinez-Luscher, J., Girardello, R. C., Kostaki, E., Oberholster, A., & Kaan Kurtural, S. (2022). Shifts in the phenolic composition and aromatic profiles of Cabernet Sauvignon (Vitis vinifera L.) wines are driven by different irrigation amounts in a hot climate. *Food Chemistry*, *371*, 131163. https://doi.org/10.1016/j.foodchem.2021.131163

Torres, N., Yu, R., Martínez-Lüscher, J., Kostaki, E., & Kurtural, S. K. (2021a). Application of Fractions of Crop Evapotranspiration Affects Carbon Partitioning of Grapevine Differentially in a Hot Climate. *Frontiers in Plant Science*, *12*, 75. https://doi.org/10.3389/fpls.2021.633600

Torres, N., Yu, R., Martínez-Lüscher, J., Kostaki, E., & Kurtural, S. K. (2021b). Effects of Irrigation at Different Fractions of Crop Evapotranspiration on Water Productivity and Flavonoid Composition of Cabernet Sauvignon Grapevine. *Frontiers in Plant Science*, *12*, 1858. https://doi.org/10.3389/fpls.2021.712622

Venios, X., Korkas, E., Nisiotou, A., & Banilas, G. (2020). Grapevine Responses to Heat Stress and Global Warming. *Plants*, *9*(12). https://doi.org/10.3390/plants9121754

Williams, L. E., & Ayars, J. E. (2005). Grapevine water use and the crop coefficient are linear functions of the shaded area measured beneath the canopy. *Agricultural and Forest Meteorology*, *132*(3–4), 201–211. https://doi.org/10.1016/j.agrformet.2005.07.010

Yu, R., Brillante, L., Martínez-Lüscher, J., & Kurtural, S. K. (2020). Spatial variability of soil and plant water status and their cascading effects on grapevine physiology are linked to berry and wine chemistry. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00790

Zarrouk, O., Brunetti, C., Egipto, R., Pinheiro, C., Genebra, T., Gori, A., Lopes, C. M., Tattini, M., & Chaves, M. M. (2016). Grape ripening is regulated by deficit irrigation/elevated temperatures according to cluster position in the canopy. *Frontiers in Plant Science*, *7*, 1640. https://doi.org/https://doi.org/10.3389/fpls.2016.01640