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ORIGINAL RESEARCH ARTICLE

Impacts of photoselective bunch zone shading on the volatile composition and sensory attributes for *Vitis vinifera* L. cv. Riesling

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ABSTRACT

Photoselective shading is a process that modulates the radiation intensity in specific regions of the electromagnetic spectrum. It is a common practice in horticulture to manipulate specific plant physiological responses, but to date has only received minimal attention in viticulture. The potent odorant 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) is of particular relevance for aged Riesling wine, which are also known to be impacted by the magnitude of bunch zone light exposure during berry development. Hence, in this study, the effect of photoselective bunch zone shading on the formation of TDN in wine was investigated across two consecutive growing seasons. Applying red, black or green shade cloth (SC) to the bunch zone provided unique bunch zone light environments and yielded distinct differences in grape and wine composition compared with the unshaded control. Overall, bunch zone shading through shade cloth was effective in reducing overall photosynthetically active radiation compared to the control and the photoselectivity of the SC treatments differently affected a number of grape and wine measures. Fruit yield was somewhat but not significantly lower under black SC treatments, while juice pH was increased in grapes grown under green and black SC across both vintages compared to the control. Both grape sugar accumulation ($P = 0.035$) and ammonia nitrogen ($P = 0.043$) showed evidence of treatment effects, although with low F-statistics (4 and 3, respectively). Measures of hydrolytically released TDN in juice and free TDN concentrations in wine were lower in SC treatments. Unexpectedly, sensory descriptive analysis of the wines demonstrated that increased 'kerosene-like' aroma was not consistently associated with free TDN concentrations in wine. In summary, photoselective bunch shading was demonstrated to be an effective method for manipulating grape and wine outcomes and may aid in overcoming viticultural obstacles and quality impacts associated with climate change.

KEYWORDS: Riesling, TDN, light manipulation, fruit composition, aroma, wine sensory

INTRODUCTION

For centuries, viticultural techniques that maximise bunch exposure have been promoted to reduce bunch rot and improve Riesling wine quality (Percival *et al*., 1994; Poni *et al*., 2006; Smart and Robinson, 1991; Zoecklein *et al*., 1992; Zoecklein *et al*., 1998). Additionally, increasing shading during grape growing has usually been regarded as detrimental due to maturity delays via a decrease in sugar accumulation (Kliewer *et al*., 1967; Reynolds *et al*., 1986; Rojas-Lara and Morrison, 1989), increases in juice pH (Smart *et al*., 1985) and the potential for enhancing unripe, herbaceous flavour and aroma characteristics in resulting wines (Allen and Lacey, 1993).

However, in the case of Riesling, increased sun exposure during grape ripening may lead to the development of a premature 'aged' character in the finished wine (Marais, 2001; Marais *et al*., 1992b; Winterhalter, 1991), linked to increased concentrations of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN). A potent odorant, TDN is characteristic of aged Riesling wine through its association with the kerosene-like aroma (Simpson, 1978; Simpson and Miller, 1983). It is thought to evolve from non-volatile precursors formed through the biodegradation of carotenoids (Baumes *et al*., 2002; Mendes-Pinto, 2009) which are involved in light protective mechanisms during ripening (Britton *et al*., 2008; Eskling *et al*., 1997). Ultimately, TDN precursors accumulate in grapes as a response to increased light exposure during berry development (Kwasniewski *et al*., 2010; Mendes-Pinto, 2009).

Given the close metabolic relationship between vineyard light environments, carotenoid metabolism and subsequent TDN formation, amongst other odorants, studies on TDN modulation have focused on altering the amount of incident light at the bunch zone. Generally, this has been achieved by either exposing grapes to more sunlight via leaf removal or canopy manipulation (Kwasniewski *et al*., 2010), by accessing naturally shaded and exposed grapes (Marais *et al*., 1992b) or by increasing shading via natural (Linsenmeier and Lohnertz, 2007) or artificial shading (Gerdes *et al*., 2002; Hixson *et al*., 2018). Across these studies, increasing bunch zone shading was associated with reductions in TDN concentrations in grapes and the resulting wines. However, these studies have only considered the magnitude or overall quantity of intercepted light, while both quantity and quality (wavelength range) of incident light are crucial environmental factors that can influence carotenoid accumulation and degradation in plant tissues (Frede *et al*., 2019). Thus, it was of interest to investigate the effect of both light quality and quantity on TDN concentration in Riesling wines, which can be achieved by altering the wavelength ranges encountered at the bunch zone via photoselective shading.

The simplest form of photoselective shading to achieve different wavelengths of light is via coloured netting, which is a common practice in horticulture (Shahak, 2014; Stamps, 2009). However, this practice to date has only received limited attention in viticulture, which might in part be due to early canopy management research having been performed in cool climates where increased shading was associated with unripe characteristics of the finished wines (Smart, 1987). Given the emerging effects of climate change, there has been renewed interest in reconsidering traditional vineyard management practices (Santos *et al*., 2020; Schultz, 2010), including approaches for reducing bunch zone sun exposure.

As such, this study evaluates the use of three different coloured shade cloth (SC) treatments applied to the bunch zone of Riesling vines across two growing seasons compared with a non-shaded control treatment. The applied photoselective shading was selected to provide changes in light quantity as well as quality, intended to impact TDN concentrations via modulation of carotenoid absorbing wavelength regions. The grapes and wines were monitored via chemical and sensory analysis to highlight the effects of the photoselective shading treatments. The aims were to understand the ability of photoselective shading to reduce TDN concentrations in wine while maintaining the canopy and overall photosynthetic capacity of the vines, with the overall objective of producing Riesling wines that can be selectively lower in TDN without sacrificing overall wine flavour.

MATERIALS AND METHODS

1. Experimental field design

Over two consecutive growing seasons (2018 and 2019), shade cloth (SC) trials were conducted in a commercial vineyard in Eden Valley, South Australia containing Riesling vines (own-rooted GM 110) grown on a vertical shoot position (VSP) trellis system. Shade cloth (green, red and black; all inert, loose weave of nominal 70 % transmission, 1.8 m wide rolls; Rally Shade cloth – Premium, Sunrise Marketing Pty Ltd, Wingfield, SA, Australia) were cut lengthwise to give 90 cm wide strips, which were secured on VSP training wires to provide full coverage of the bunch zone in randomised block design in triplicate with three panels per treatment, each covering approximately five vines per panel (see Supplementary Data, Figures S1 and S2). For both vintages, SC treatments were applied prior to veraison (20/12/2017 and 19/12/2018, E-L stages 31-32) to ensure shading aligned with key stages previously associated with light-related TDN modulation (Kwasniewski *et al*., 2010). Due to vine availability, different rows within the same block were allocated for each season. No other treatments (i.e. defoliation) were applied to the control or shaded vines.

Prior to the first SC application, spectral measurements of the light passing through each type of SC were collected under controlled conditions at midday on a cloudless summer day using a Lighting PassportTM spectrometer (AsenseTek Inc., Taipei, Taiwan) interfaced with Spectrum Genius mobile phone application. For the entirety of the shading experiment, daily irradiance was measured inside the canopy in duplicate using handmade ceptometers 'PARbars' that were assembled as described by Salter *et al*. (2018). Data

collection was made using CR10 data loggers (Campbell Scientific Ltd., Leicester, UK). Air temperature under the canopy was measured with Tinytag Transit 2 temperature loggers (Hastings Data Loggers distributor, Port Macquarie, NSW, Australia) inside shaded covers, performed in duplicate per treatment with data processed using Tinytag Explorer (Gemini Data Loggers Ltd., West Sussex, UK). Both solar radiation and temperature were measured automatically at one-hour or 15-minute intervals, respectively. Maturity samples (total soluble solids) were collected at fortnightly intervals in the period leading up until harvest. In the 2018 vintage, all grapes were harvested simultaneously on 14/03/2018, while the following year harvest dates for each treatment were chosen according to ripening stage (TSS), with control and red treatments harvested on 08/03/2019 and green and black treatments five days later (13/03/2019) to achieve more consistent grape maturity between the control and shaded treatments.

General meteorological measures (mean January temperature, annual rainfall, growing season rainfall and growing degree days) for each vintage can be found in Supplementary Data, Table S1.

2. Winemaking

All fruit was hand-picked and stored at 0 ºC on arrival at the winery prior to processing, where vineyard replicates were processed separately. Grapes were de-stemmed, crushed and immediately pressed (membrane press, DIEMME Enologia S.p. A, Lugo RA, Italy) to yield approximately 700 L/t. After settling (0 °C), clarified juices were adjusted to 45 mg/L of total sulfur dioxide (10 % potassium metabisulfite) and then transferred into 25 L stainless steel vessels. After coming to ambient temperature, they were inoculated with approximately 50 mg/L of Maurivin PDM yeast (AB Mauri, Sydney, NSW, Australia) and fermented at 20–22 °C. Once fermentation was complete, as determined by residual sugar levels below 2.0 g/L (glucose + fructose), the wines were racked off gross lees, total sulfur dioxide adjusted to 30 mg/L and cold stabilised at 0 °C for 21 days. The wines were racked to remove fine lees, total sulfur dioxide was adjusted as required to at least 30 mg/L and filtered through a Z6 pad membrane prior to bottling into screwcap sealed 375 mL bottles. The wines were stored at 15 °C until analysis.

Prior to bottling the 2018 wines, a 5 L aliquot was taken from each treatment (vineyard replicates combined into a single wine) and the pH was adjusted to 3.0 by the addition of tartaric acid. The pH-adjusted wines were bottled and stored at 15 °C until undergoing chemical analysis for free volatile compounds after 10 and 18 months.

3. Juice analysis

3.1. General winemaking analysis

Juice samples were collected immediately following pressing. The basic chemical composition was determined by the AWRI Commercial Services using methods as detailed in Iland *et al*., 2013. The titratable acidity was measured using FTIR WineScan (FOSS, Hillerød, Denmark). Maturity analysis (TSS, as °Baumé) of grape juice was performed manually using a digital refractometer (Atago WM-7, USA) with temperature correction on the day.

3.2. Post-hydrolysis 'total' aroma composition

The hydrolysis and subsequent analysis of released volatiles from juice were performed as described in our previous work (Grebneva *et al*., 2019). Briefly, a 10 mL aliquot of juice was spiked with a deuterated standard mix solution (d_7-linalool, d_7-α-terpineol, d_8-naphthalene and d_4-β-damascenone at 40.0 mg/L in EtOH), the pH was adjusted to 1.7 using 2 M HCl; and the resulting mixture was heated in a sealed pressure vessel (25 mL; 26 mm thick walled tube with PTFE cap, Emerald Scientific Glassblowing, Morphett Vale, SA, Australia) for 60 min at 75 °C. Post-heating, the vessel was immediately cooled in ice water. Samples were loaded onto preconditioned Bond Elute C18 cartridges, which were then rinsed with water prior to elution with *n*-pentane/ethyl acetate (2:1, v/v). The eluent was concentrated under nitrogen gas using a Zymark TurboVap® LV evaporator (Biotage, UK) at 40 °C to a final volume of approximately 100 µL. Volatiles were analysed by GC-MS as described below.

4. Wine analysis

4.1. General winemaking analysis

The basic chemical composition of all Riesling wines was determined at bottling by AWRI Commercial Services, with titratable acidity, volatile acidity as acetic acid, pH, glucose and fructose, and alcohol measured using FTIR WineScan (FOSS, Hillerød, Denmark). The analysis of malic acid was performed using the discrete analyser method (Gallery Discrete Analyzer, Thermo Fisher Scientific Inc.).

4.2. Free aroma composition

Analysis of free aroma compounds was performed at the time of sensory analysis for each wine. Prior to the analysis of free C_{13}-norisoprenoids and monoterpenes in wine, a liquid-liquid extraction was performed using a procedure adapted from Pedersen *et al*. (2003). Briefly, a 10 mL aliquot of wine spiked with Internal Standard solution including the labelled standards (as described above, 40.0 mg/L in EtOH) was extracted with *n*-pentane/ethyl acetate (2:1 v/v, 3 mL). The resultant organic layer was analysed by GC-MS as described below.

4.3. Sensory analysis

Quantitative descriptive analysis (QDA) was conducted on both of the wine sets, with each vintage assessed separately (either 12 months post-bottling or additionally 24 months post-bottling for the 2018 trial). A panel of not less than eight assessors were convened for each QDA, with all panellists having extensive training and experience in wine sensory descriptive analysis and were part of the AWRI external descriptive analysis panel. The panel assessed the wines for appearance, aroma and palate. The panel formally assessed the wines, using attributes they generated during the training days, in duplicate (30 mL) in isolated booths under daylight lighting, with randomised presentation order within each tray

of samples across judges. The intensity of each attribute was rated using an unstructured 15 cm line scale (numericised 0 to 10), with indented anchor points of 'low' and 'high' placed at 10 % and 90 %, respectively.

For attribute definitions and reference standards used, see Supplementary Data, Table S2. Data were acquired using Compusense Cloud sensory evaluation software (Compusense Inc., Guelph, Canada). Panel performance was assessed using Compusense software and RStudio with the SensomineR and FactomineR packages (Lê and Husson, 2008; Lê *et al*., 2008). All judges were found to be performing to an acceptable standard.

5. GC-MS analysis

Quantitation of both free and post-hydrolysis (total) aroma compounds in juice and wine samples was performed on an Agilent 7890 series gas chromatograph with Agilent 5975C mass spectrometer based on the method by Daniel *et al*. (2009), with minor modifications. Separation was achieved on an approximately 60 m × 0.250 mm × 0.25 µm film thickness Agilent DB-WAX capillary column by liquid injection. The injector was held at 220 °C and the transfer line was held at 250 °C. A fast injection of 1 µL of the sample was performed in pulsed splitless mode with a pulse pressure of 40.0 psi. The carrier gas was helium (BOC Limited, North Ryde, NSW, Australia), and the flow rate was 2.0 mL/min. The initial oven temperature was 80 °C, held for 1 min, increased to 140 °C at 12 °C/min, held at 140 °C for 0.5 min, followed by an increase to 220 °C at 3.2 °C/min, and held for 5 min. Mass spectra were recorded in the selective ion monitoring (SIM) mode. The ions monitored in SIM runs were as follows: m/z 121, 93 and 136 for linalool; 142, 124 and 108 for d_7-linalool; 136, 121 and 139 for α-terpineol; 93, 124 and 142 for d_7-α-terpineol;136, 108 and 68 for d_8-naphthalene, 142, 157 and 172 for TDN; 121, 175 and 190 β-damascenone; 194 and 179 for d_4-β-damascenone; 163, 121, and 145 for actinidol; 148, 138 and 123 for Riesling acetal; and 192, 177 and 93 for vitispirane. The calibration curve for TDN was generated over a range of 2.5−200 µg/L for a free aroma analysis and 5−400 µg/L for total aroma analysis (post-hydrolysis). Identification of C_{13}-norisoprenoids with missing standards was based on published retention indices and mass spectral data (Daniel *et al*., 2009; Eggers *et al*., 2006; Gök, 2015; Janusz *et al*., 2003; Strauss *et al*., 1986) were reported in TDN equivalences. Compounds present as isomers (actinidol and vitispirane) were quantitated as one and referred to as one. Data acquisition and analyses were performed using the MassHunter Workstation software version B.09.00 (Agilent Technologies, Australia).

6. Statistical analysis

Throughout the study, analysis of variance (ANOVA) was carried out using Minitab 18 (Minitab Inc., Sydney, NSW) using a significance of $P < 0.05$, unless otherwise stated, and differences were determined using Tukey post-hoc test. Basic graphing of data was performed using Microsoft Excel 2016 (version 2101). For sensory analysis, Fisher's Least Significant Difference (LSD) value was calculated at a 95 % confidence level for the treatment effect of the sensory attributes.

RESULTS

1. Experimental field conditions

Prior to the application of the SC treatments in vineyards, the modulation of the spectral light qualities by each SC-coloured fabric was established (Figure 1). While all SCs reduced the total light quantity, the green SC specifically blocked transmittance in the near-red spectral region (Figure 1B), while the red SC preferentially blocked the green spectral region (Figure 1C), providing photoselectivity. The black SC showed no photoselectivity and yielded a near-identical spectral profile compared to the ambient light reading (Figures 1D and 1A, respectively), providing a shaded control for additional comparison with the photoselective shading provided by red and green SC.

To quantify residual radiation within the SC treatments applied to the vineyard, in situ light measurements were taken throughout the vintage 2019 growing season using

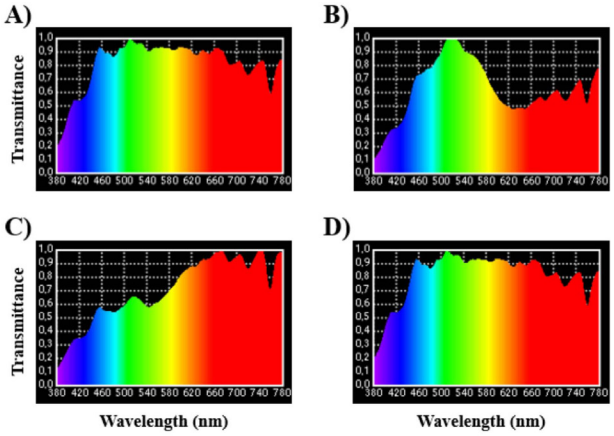


FIGURE 1. Transmittance spectral profiles (380 – 780 nm) for treatments used in this study measured under controlled conditions, with transmittance maximum in each spectrum adjusted to 1. A) full ambient light control; B) measured under green shade cloth; C) measured under red shade cloth; D) measured under black shade cloth.

TABLE 1. Daily average solar radiation was measured above the canopy (single PARbar sensor), for control and under shade cloth (green, red and black) treatments (in duplicate) at bunch zone height.*

	Above canopy	Control	Green	Red	Black
Solar exposure W/m²	350	220[a]	62[b]	81[b]	61[b]

[a-b] Different letters indicate significant differences (significance level 95 %) between treatments.

* Measurements taken between 05.01. and 13.03.2019.

light sensors that had been placed at the height of the bunch zone without leaf coverage to remove the heterogeneity of canopy light interception (Table 1). The average daily exposure above the canopy height was 350 W/m² and 220 W/m² at bunch zone height, while the SC treatments reduced the intensity of solar radiation to between 61 and 81 W/m² or 28 % to 37 % of ambient light.

To differentiate between light and temperature effects, detailed temperature data were collected across both vintages within the canopy, with temperature loggers housed inside covers to avoid temperature spikes from direct sunlight. Overall, average air temperature measurements over the period of SC application showed no significant differences (Supplementary Data, Table S3). However, in the 2019 growing season, the maximal daily temperature under all SC treatments was higher than the control by 1 °C, on average.

2. Juice—basic composition

The effects of coloured photoselective SC treatments on juice composition were assessed through one-way ANOVA of each analyte within each vintage and by two-way ANOVA of treatment and vintage effects across both vintages (Table 2). As harvest date and maturity varied slightly between vintages, the use of one-way ANOVA to explore intra-vintage treatment effects, in addition to the two-way ANOVA, was deemed necessary. Within each vintage, one-way ANOVA showed no significant differences ($P = 0.05$) for either yield or total soluble solids (TSS), although via two-way ANOVA yield comparison between vintages showed significantly lower yields in 2019 than in 2018 ($P < 0.001$, $F = 48$). For TSS, there was a significant ($P = 0.035$) but small ($F = 4$) treatment effect when compared across both vintages.

With respect to acidity, there were significant treatment and vintage effects for both titratable acidity (TA) and pH, while malic acid content was found to be highly variable within and across treatments and vintages. Juice pH from shaded fruit was generally increased in both vintages, ranging from pH 3.17 to 3.33 in 2018 and pH 3.56 to 3.59 in 2019, compared to control juice pH of 3.19 and 3.35 in 2018 and 2019, respectively. In the 2019 grapes, TA was significantly reduced in the green and black SC treatments, but this trend was not evident in the 2018 grapes. Nitrogen as yeast assimilable nitrogen (YAN) showed no consistent response to different light conditions but was significantly higher in 2019 fruit for all nitrogen measurements.

3. Post-hydrolysis 'total' aroma compounds in the juice

As with many C_{13}-norisoprenoids, free TDN is largely absent from grapes and juice, but the potential formation of TDN and other related C_{13}-norisoprenoids in wine (i.e., β-damascenone, vitispirane, actinidol, Riesling acetal) can be assessed following acid-catalysed hydrolysis of precursors in juice samples. This analysis incorporates the volatiles released from bound forms (often called 'bound aroma') and those already present in a free form, with the resulting sum of both concentrations referred to here as 'total'. During this work, the method employed for acid-catalysed hydrolysis in the 2018 vintage was optimised prior to use in the 2019 vintage (Grebneva *et al*., 2019). As such, only the results from the analysis of juice samples from the 2019 vintage are shown below, and the data resulting from the hydrolysis of vintage 2018 juice can be found in Supplementary Data (Table S4). Notably, both vintages followed a similar trend across treatments, yet the optimised method used in 2019 resulted in significantly lower values.

Photoselective shading significantly altered the profile of total C_{13}-norisoprenoids post-hydrolysis in Riesling 2019 juice samples, except for actinidol (Table 3). Total TDN concentrations, together with other C_{13}-norisoprenoids, vitispirane and Riesling acetal, were significantly reduced in shaded juice samples. Concentrations for total TDN in juice samples from shaded treatments were between 79 and 96 µg/L compared to the control juice at 150 µg/L, with the black SC treatment yielding the lowest total TDN levels among all shade treatments. Total β-damascenone concentrations followed an opposite trend to total TDN and were lower in the control samples from grapes grown under fully exposed conditions.

4. General wine composition

At the time of bottling, all wines were analysed for general wine compositional parameters (Table 4). Wines across both vintages possessed ethanol levels between 10.3 and 11.9 % (v/v), with no significant differences observed between wine made from exposed (control) grapes and SC treatments within each vintage. For the 2018 wines, the alcohol trended similarly to the grape sugar, with the control slightly higher than the SC treatments, and the control wines also showed a significantly higher residual sugar concentration (glucose + fructose). In the 2019 vintage, the control wines and wines made from grapes under red SC were slightly higher in alcohol than the wines made from grapes grown under green

TABLE 2. Basic juice composition of 2018 and 2019 Riesling grapes from control and light modulation using coloured shade cloth treatments (green, red or black), showing the outcome of one-way treatment ANOVA within each vintage and two-way treatment and vintage ANOVA across both vintages studied. Data expressed as mean of vineyard replicates (n = 3) and standard deviation.

	Treatment								ANOVA					
Analyte	2018 vintage				2019 vintage				Vintage (V)		Treatment (T)		V * T	
	Control	Green	Red	Black	Control	Green	Red	Black	F	p	F	p	F	p
Alpha Amino Nitrogen (mg/L)	142.7 ± 7.1^{b}	180.0 ± 7.9^{a}	134.0 ± 6.2^{b}	174.7 ± 8.7^{a}	318.0 ± 18.2	341.7 ± 9.9	427.0 ± 54.7	326.7 ± 66.2	229	< 0.001	3	0.087	7	0.005
Ammonia (mg/L)	122.3 ± 11.5^{a,b}	135.7 ± 6.4^{a}	109.0 ± 17.1^{a,b}	90.7 ± 11.2^{b}	165.7 ± 5.1	176.7 ± 3.1	211.0 ± 27.6	175.3 ± 27.0	104	< 0.001	3	0.043	5	0.010
Yeast assimilable nitrogen (mg/L)	243.0 ± 2.7^{b}	291.7 ± 13.3^{a}	223.3 ± 20.2^{b}	249.3 ± 17.5^{b}	454.3 ± 21.4	487.0 ± 8.2	600.3 ± 77.5	470.7 ± 88.3	199	< 0.001	3	0.089	6	0.008
Total soluble solids (°Baumé)	11.2 ± 0.4	10.4 ± 0.6	10.2 ± 0.3	10.2 ± 0.3	10.4 ± 0.4	9.9 ± 0.2	10.6 ± 0.5	10.0 ± 0.3	3	0.119	4	0.035	3	0.095
Malic acid (g/L)	3.0 ± 0.3^{a,b}	3.3 ± 0.3^{a}	2.7 ± 0.6^{a,b}	2.2 ± 0.3^{b}	2.5 ± 0.1C	2.8 ± 0.1^{B}	3.1 ± 0.1^{A}	3.1 ± 0.2^{A}	0	0.555	3	0.058	10	0.001
pH	3.19 ± 0.02^{b}	3.33 ± 0.02^{a}	3.17 ± 0.02^{b}	3.30 ± 0.03^{a}	3.35 ± 0.01^{B}	3.56 ± 0.10^{A}	3.56 ± 0.07^{A}	3.59 ± 0.06^{A}	164	< 0.001	15	< 0.001	6	0.009
Titratable acid pH 8.2 (g/L)	7.1 ± 0.4	6.8 ± 0.1	7.3 ± 0.1	6.9 ± 0.2	6.1 ± 0.1^{A}	5.4 ± 0.2B	5.9 ± 0.2^{A}	5.5 ± 0.1^{B}	284	< 0.001	12	< 0.001	2	0.156
Total weight per vine (kg)	4.2 ± 0.5	4.1 ± 0.3	4.3 ± 0.9	3.6 ± 0.6	2.4 ± 0.2	2.8 ± 0.5	2.7 ± 0.7	1.8 ± 0.8	48	< 0.001	3	0.099	0	0.855

$^{a\text{-}c,\ A\text{-}C}$ Different letters within a row and vintage indicate significant treatment differences (significance level 95 %) between treatments for each analyte, with lowercase letters used for 2018 one-way ANOVA outputs and uppercase letters used for 2019 one-way ANOVA outputs.

TABLE 3. Mean total (sum of free and bound) compound concentrations by coloured shade cloth treatments, including green, red and black colours after acid hydrolysis in the 2019 Riesling juice after pressing, as analysed by GCMS (data as mean ± SD µg/L, n = 3 vineyard replicates).

Analyte	Control	Green	Red	Black
β-Damascenone	49.5± 1.1[b]	62.2± 3.2[a]	61.7± 6.4[a]	60.7± 3.9[a]
Vitispirane*	40.3 ± 3.6[a]	26.1 ± 2.0[b]	28.8 ± 0.3[b]	26.1 ± 0.5[b]
Riesling acetal*	28.3 ± 2.2[a]	17.9 ± 2.8[b]	18.6 ± 2.8[b]	16.1 ± 2.0[b]
Actinidol*	48.8 ± 3.1	52.7 ± 5.5	47.6 ± 11.1	43.6 ± 3.9
TDN	143.7 ± 20.0[a]	95.7 ± 12.0[b]	92.1 ± 9.8[b]	79.8 ± 3.4[b]

[a-b] Different letters indicate significant differences (significance level 95 %) between treatments for each compound. *Quantified as TDN equivalences.

or black SC, although not significantly. Overall, there was no consistent impact of SC treatments on wine alcohol content in either vintage. However, a significant vintage effect could be observed in the two-way ANOVA due to the 2018 wines possessing higher concentrations than the 2019 wines.

The pH of the wines showed variation resulting from treatment ($P < 0.001$, $F = 61$) and from vintage ($P < 0.001$, $F = 67$), as pH was intentionally not adjusted in the winery to maintain any treatment effects through to the wines. The pH across the treatments of the 2018 wines matched the trend observed in the juice analysis, where the juice from control and red SC grapes were significantly lower than the juice and wine from grapes grown under black or green SC. The same was also observed in the 2019 wines, albeit with higher pH values in the 2019 green and black SC wines than in the corresponding wines from 2018. With respect to the acidity, titratable acidity (TA) and malic acid showed variable responses, which may be due to the difference in harvest timing across both vintages. In 2018 where all grapes were harvested on the same day, the resulting malic acid concentrations for the wines made from grapes grown under green or black SC were significantly higher than the control wines.

5. Wine aroma composition

Both the 2018 and 2019 wines were analysed for a selection of free aroma compounds, with analysis time points aligning with sensory evaluation. In addition to TDN, the main target related to Riesling aroma and sun exposure, C_{13}-norisoprenoids were also represented by β-damascenone, and the monoterpenes represented by linalool and α-terpineol, which are important contributors to the aroma of young Riesling (Black *et al*., 2015; Marais *et al*., 1992a). The timeline of this study allowed for the evaluation of the 2018 and 2019 wines after one year in bottle, and the second year of storage and analysis for the 2018 wines was conducted alongside the 2019 one-year timepoint (Table 5).

After one year in bottle, a significant effect was observed for linalool and α-terpineol concentrations in both vintages studied. While this decrease in linalool from the control to the SC treated wines was significant only in 2018 wines, the differences in α-terpineol between SC treatments were significant in both vintages. The wines made from grapes grown under red SC showed the lowest linalool and highest α-terpineol concentrations compared to the other SC-treated wines. Additionally, for the 2018 wines that underwent an additional year of storage, linalool levels decreased while α-terpineol was maintained at around 40 µg/L for the control and 15 µg/L for wines made from shaded grapes.

For the norisoprenoids, free β-damascenone concentrations in wine were statistically unaffected by the SC treatments applied. Free TDN was below the limit of quantification (LOQ, 5 µg/L) across all one-year-old 2018 Rieslings; however, it was detectable in two-years-old wines and showed significant differences ($P < 0.05$) related to SC treatments. The highest concentration of free TDN was observed in control wines with 16.5 µg/L followed by 8.6 µg/L in wines made from grapes grown under red SC. While TDN was absent in one-year-old 2018 wines, it was present in 2019 wines 12-months post-bottling, showing concentrations of about 10 µg/L in control wines and 6 µg/L in wines made from grapes grown under red SC. To gauge the pH impact of the SC treatments on TDN accumulation, a small aliquot of the 2018 wines were pH adjusted to 3.0 prior to bottling, albeit with the vineyard replicates combined into a pooled wine sample. After 18 months of storage at 15 °C, bottle duplicates were analysed and gave mean TDN concentrations of 9.5 µg/L for the unshaded control wine and 5.3 µg/L or lower for all the shaded treatments (Supplementary Data, Table S6). A similar trend was also observed for α-terpineol with the control wine at 16.8 µg/L and the green, red and black SC treatments at 7.6, 7.3 and 7.2 µg/L, respectively.

6. Wine sensory analysis

Descriptive analysis was conducted to quantitatively describe the sensory differences of the 2018 and 2019 Riesling wines from the SC trial compared to the control wines either one or two years post-bottling. The results of the descriptive sensory analysis are shown in Figure 2; for the mean attribute values, see Supplementary Data (Tables S7–S9).

From the ANOVA of the 2018 wines, one-year post-bottling, fourteen attributes were found to be significantly different among the treatments at $P < 0.05$: 'yellow colour intensity', 'stone fruit aroma', 'honey aroma', 'flint aroma', 'kerosene aroma', 'pungency', 'acidity', 'astringency', 'viscosity', 'hotness' (alcohol burn), 'tropical fruit flavour', 'citrus

TABLE 4. General wine composition of 2018 and 2019 Riesling wine from control and light modulation using coloured shade cloth treatments (green, red or black), showing the outcome of one-way treatment ANOVA within each vintage and two-way treatment and vintage ANOVA across both vintages studied. Data expressed as mean of vineyard replicates (n = 3) and standard deviation.

	Treatment								ANOVA					
Analyte	2018 vintage				2019 vintage				Vintage (V)		Treatment (T)		V * T	
	Control	Green	Red	Black	Control	Green	Red	Black	F	p	F	p	F	p
Alcohol % v/v	11.9 ± 0.1	11.2 ± 0.8	10.9 ± 0.3	10.9 ± 0.5	10.9 ± 0.5	10.3 ± 0.2	10.9 ± 0.7	10.3 ± 0.5	11	0.004	3	0.059	1	0.318
Glucose + Fructose (g/L)	1.8 ± 0.3^{a}	0.5 ± 0.2^{b}	0.8 ± 0.2^{b}	0.3 ± 0.1^{b}	0.3 ± 0.2	0.3 ± 0.0	0.2 ± 0.2	0.4 ± 0.3	42	< 0.001	13	< 0.001	18	< 0.001
Titratable acid at pH 8.2 (g/L)	6.9 ± 0.4	6.9 ± 0.2	8.1 ± 0.8	7.6 ± 0.6	7.7 ± 0.1^{A}	5.8 ± 1.0^{B}	8.9 ± 0.2^{A}	5.7 ± 0.7^{B}	2	0.183	17	< 0.001	9	0.001
Malic acid (g/L)	2.5 ± 0.3^{b}	3.3 ± 0.3^{a}	3.0 ± 0.1^{a,b}	3.3 ± 0.2^{a}	2.0 ± 0.1^{B}	2.2 ± 0.3 ^{A,B}	2.6 ± 0.1 ^{A}	2.1 ± 0.2 ^{A,B}	91	< 0.001	9	0.002	6	0.010
Volatile acidity as acetic acid (g/L)	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	9	0.010	2	0.105	1	0.654
pH	3.12 ± 0.04^{b}	3.25 ± 0.04^{a}	3.07 ± 0.02^{b}	3.23 ± 0.02^{a}	3.11 ± 0.02^{B}	3.64 ± 0.12^{A}	3.10 ± 0.05^{B}	3.66 ± 0.10^{A}	67	< 0.001	61	< 0.001	21	0.009

$^{a-b, A-B}$ Different letters within a row and vintage indicate significant treatment differences (significance level 95 %) between treatments for each analyte, with lowercase letters used for 2018 one-way ANOVA outputs and uppercase letters used for 2019 one-way ANOVA outputs.

TABLE 5. Free volatile composition of 2018 and 2019 Riesling wine from control and coloured shade cloth treatments, with green, red and black colours (in µg/L, n = 3 vineyard replicates).

Analyte	2018 vintage				2019 vintage			
one-year-old	Control	Green	Red	Black	Control	Green	Red	Black
Linalool	22.2 ± 4.2^{a}	10.4 ± 2.9^{b,c}	6.6 ± 1.5^{c}	9.0 ± 1.2^{b,c}	23.2 ± 1.2^{A}	18.6 ± 2.5^{A}	10.9 ± 1.4^{B}	19.2 ± 1.7^{A}
α-Terpineol	37.2 ± 5.9^{a}	12.8 ± 2.2^{b}	14.5 ± 1.3^{b}	13.1 ± 1.0^{b}	41.0 ± 2.2^{A}	17.5 ± 1.9^{B}	20.4 ± 2.0^{B}	14.8 ± 1.2^{B}
β-Damascenone	13.1 ± 4.1	10.0 ± 1.3	13.7 ± 3.2	16.5 ± 4.8	19.9 ± 1.9	23.8 ± 4.1	13.2 ± 3.0	24.2 ± 2.8
TDN	n.d.	n.d.	n.d.	n.d.	9.6 ± 1.1^{A}	n.d.	5.7 ± 0.5^{B}	n.d.
two-years-old	Control	Green	Red	Black				
Linalool	6.0 ± 2.8^{a}	6.0 ± 0.2^{a}	n.d.	n.d.				
α-Terpineol	39.3 ± 7.4^{a}	18.6 ± 0.3^{b}	13.2 ± 1.1^{b}	15.6 ± 0.7^{b}				
β-Damascenone	8.4 ± 7.0^{a}	n.d.	n.d.	5.1 ± 0.7^{a}				
TDN	16.5 ± 0.3^{a}	n.d.	8.6 ± 1.1^{b}	n.d.				

$^{a-b}$, $^{A-B}$ Different letters within a row and vintage indicate significant treatment differences (significance level 95 %) between treatments for each analyte, with lowercase letters used for 2018 one-way ANOVA outputs and uppercase letters used for 2019 one-way ANOVA outputs n.d., below the limit of quantitation for the analyte.

flavour', 'stonefruit flavour' and 'grassy flavour'. Almost all attributes, except for 'acidity', 'citrus flavour' and 'grassy flavour', were rated higher in control wines compared to shaded trial wines. Interestingly, 'kerosene aroma', whilst rated very low, was significantly higher ($P < 0.05$) in control wines from exposed grapes compared to other wines.

Curiously, after two years of storage, the wines from the 2018 vintage showed fewer differences between treatments compared to the analysis of these wines after one year. The largest difference was noted for 'kerosene aroma' only, which was rated highest in the control (mean score of 3.9), whereas wines made from grapes that received shading treatments were all significantly lower (2.1 or lower, $P < 0.05$). Wine from grapes grown under red SC had a slightly higher 'kerosene aroma' intensity compared to wine from the black and green treatments, although not significantly (2.1 and 1.9, respectively). A similar trend was found for 'yellow colour intensity' and 'viscosity', with the control, rated significantly higher than all the SC treatments. Interestingly, 'honey aroma' was elevated in the control and red SC treatments across both the one year and two year post-bottling wines, which has been linked to aged wine aroma (Black *et al*., 2012).

In the case of the 2019 wines analysed after one year of storage, attribute intensities for 'yellow colour intensity', 'citrus aroma', 'honey aroma', 'acidity', 'astringency', 'overall fruit flavour' and 'stonefruit flavour' were rated significantly differently ($P < 0.05$) among the wines. Notably, 'yellow colour intensity' and 'honey aroma' were rated highest in 2019 wines made from grapes grown under black SC, which could be an indication that some oxidation had occurred. The mean attribute scores for 'acidity' were higher for the wines made from grapes picked earlier (control and red SC) and related to wine acidity measures.

DISCUSSION

Prior to the bunch zone shading experiments described here, initial shading experiments in a previous vintage utilised whole vine shading with only green SC. However, whole vine shading in these preliminary experiments resulted in decreased sugar accumulation and yield per vine (see Supplementary Data, Table S5) to such an extent that the control and shaded treatments were not considered directly comparable due to the known correlation between maturity, or sugar accumulation, and the concentration of TDN precursors present in the juice (Strauss *et al*., 1987). Thus, in this study, the effects of different light regimes on C_{13}-norisoprenoid composition in Riesling grapes and wine were investigated through shading only the bunch zone, which left the upper canopy unshaded to maintain its full photosynthetic capabilities, intended to produce grapes with more consistent maturity between control and shaded treatments. The timing of SC application in the vineyard (20/12/2017 for the 2018 vintage and 19/12/2018 for the 2019 vintage) was chosen to align with previous reports on bunch zone light interception and key berry development stages to manipulate carotenoid and TDN concentrations (Kwasniewski *et al*., 2010).

As expected by the shade cloth product specifications, bunch zone shading under SC reduced daily solar radiation on average by at least 60 % relative to the exposed control treatment. Additionally, the green and red SC treatments altered the spectral distribution and provided photoselective shading. Green SC application preferentially allowed for the transmission of the spectral region around 520 nm (blue and green light), comparatively reducing the near-red spectral region (centred around 620–660 nm). In contrast, red SC resulted in the opposite effect for the blue-green and red spectral regions. The spectral regions selectively modulated by either the red SC or green SC photoselective shading have

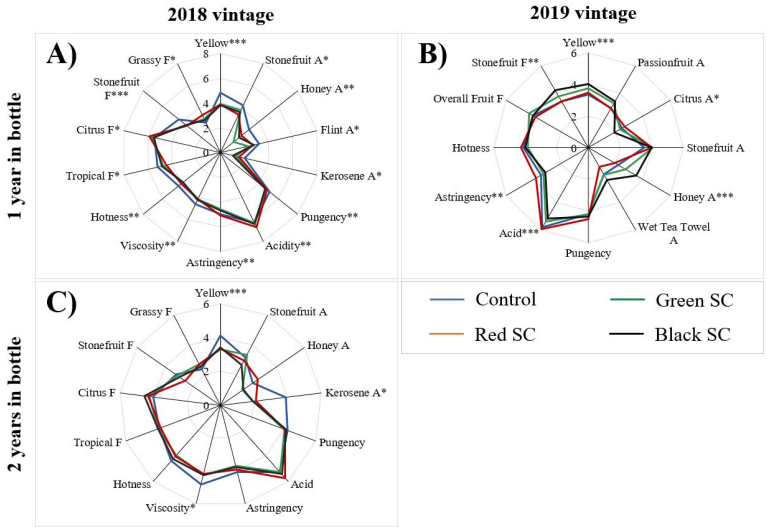


FIGURE 2. Descriptive sensory analysis results from 2018 and 2019 Riesling wines of the shading trial conducted either A and B) one year or C) two years post-bottling for selected attributes, rated on a scale from zero to ten. Significance at *P < 0.05; **P < 0.01; ***P < 0.001. SC, shade cloth; A, aroma; F, flavour.

been studied by others due to their general importance for plant photosynthesis and metabolism (Goldberg and Klein, 1977; Kotilainen *et al*., 2020) and have been shown to yield carotenoid modulation in other plants (Frede *et al*., 2019). Given the dynamic nature of carotenoid degradation and accumulation of volatile norisoprenoids and their precursors, especially under high-light conditions (Mendes-Pinto, 2009), this study focused on the impact of light modulation on the final grape composition and wine composition and sensory attributes.

Under the conditions of this shading experiment of the bunch zone, shading influenced the vines' primary metabolism, specifically grape sugar and acid accumulation. While the change of TSS between treatments in the grape berries in 2018 was not statistically significant, grapes from all shaded treatments showed a reduction by approximately 1 Baume. In an effort to standardise grape TSS across treatments and minimise any potential confounding effect of maturity on TDN accumulation (Strauss *et al*., 1987), the harvest dates were altered for the subsequent vintage. For the 2019 grapes, where harvest time was determined by sugar maturity, the resulting wines showed more consistent final alcohol content.

Increases in bunch zone light exposure from leaf removal have been noted to have differing impacts on grape composition (Zoecklein *et al*., 1992), where fruit pH effects are minimal while malic acid decreases in some situations and sugar accumulation shows variable responses. This mirrors the findings of Reynolds *et al*. (1996), where additional light exposure via leaf removal resulted in reductions of fruit TA in only 60 % of the 5 years studied (Reynolds *et al*., 1996). The differences observed in grape acidity here provide insight into the ability of bunch zone shading, photoselective or otherwise, to impact primary metabolic processes.

In the case of grape malic acid, the values observed between the two vintages were not consistent. While in 2018, fruit malic acid levels seemed not to follow any pattern, in 2019, the fruit malic levels were significantly reduced under exposed treatment (control) relative to shaded treatment. Generally, under field conditions, sun-exposed canopies produce fruit of decreased malic acid compared to shaded fruit (Crippen and Morrison, 1986; Dokoozlian and Kliewer, 1996; Friedel *et al*., 2015; Rojas-Lara and Morrison, 1989; Sweetman *et al*., 2014), so it is expected that the control grapes may have lower malic acid concentrations as observed in the 2019 vintage. This is normally attributed to higher temperatures of sun-exposed grape berries (Burbidge *et al*., 2021), and while no temperature differences were observed between the treatments here, these measurements were taken within the canopy and did not account for the exposed berries. Furthermore, for the two vintages studied, on average, the 2017-2018 growing season was colder and wetter than the 2018-2019 growing season, with growing degree days of 1918 and 2031, respectively, and growing season rainfall of 145 mm and 101 mm respectively (Supplementary Data, Table S1). However, the 2018-2019 growing season in Eden Valley and its surroundings were characterised by frost and hail events in spring, which contributed to a 50 % reduction in the grape yield in the region (Wine Australia, 2019). This trend was mirrored in the trial vineyard, where the yield per vine dropped from approximately 4 kg/vine in 2018 to below 2.5 kg/vine in 2019, which may have contributed to the significant vintage effects for grape and wine chemistry observed throughout and possibly confounded the influence of vintage-to-vintage temperature variations and the treatment effects.

When following the pH and acidity concentrations in the resulting wines, most wines, regardless of vintage, showed a slight decrease in pH, which is often attributed to the precipitation of potassium hydrogen tartrate during

winemaking (Boulton, 1980). However, the red SC wines in 2019 showed a significant decrease in pH, outside that observed in any other wines, and requires further investigation to explain this outcome. The red SC treatment in the 2018 vintage also showed the largest pH decrease during winemaking, albeit only slightly, but further highlights the anomalous pH outcomes created by red SC.

Post-hydrolysis total aroma composition from juice, as well as free volatile composition in wine obtained in the current study, were both significantly reduced upon shading. Similar results were observed in previous studies (Gerdes *et al*., 2002; Kwasniewski *et al*., 2010; Lee *et al*., 2007; Marais *et al*., 1992b). Nevertheless, the current study additionally examined the effect of light spectral composition on TDN formation, which appeared to be less affected by the quality of light than the overall reduction in sun exposure. This was evident in the reduced total TDN levels observed in grape samples from black SC treatment compared to other SC colours. Moreover, due to the significant reduction of total TDN concentrations achieved with SC application in grapes, more time is required to establish TDN formation in the resulting wines. However, while the highest free TDN concentrations were observed in wines from sun-exposed grapes, they were followed by wines made from grapes grown under red SC. This points towards a combined effect of light interception achieved in this experiment and the more acidic wine conditions observed for control and red treatment. Especially when considering that C_{13}-norisoprenoids are degradation products of carotenoids, the different light spectral distribution affected carotenoid metabolism while the acidic conditions favoured TDN liberation from its glycosylated precursors. The absence of free TDN in wines made from other shading treatments in comparison to wines from red treatment can similarly be attributed to the reduction of light interception in conjunction with the higher pH levels (Daniel *et al*., 2009) observed in wine from the green and black wine treatments and suggests the importance of pH control on TDN formation in wine.

Notably, differences observed for free TDN levels in wine between the two vintages, where one-year-old wines from 2019 showed significantly higher free TDN levels compared to TDN absence (not detected) in one-year-old 2018 wines, may be attributable to the climatic conditions in the warmer 2019 growing season (see Supplementary Data, Table S1) (Marais *et al*., 1992). Nevertheless, despite pronounced free TDN concentrations in 2019 one-year-old control and in red treatment wines, these wines were not associated with kerosene/petrol-like aromas. Especially control wines with free TDN concentrations of 9.6 µg/L that exceeded the reported detection threshold of TDN reported as 2-4 µg/L (Sacks *et al*., 2012; Tarasov *et al*., 2020; Ziegler *et al*., 2019) were not linked to kerosene-related aromas.

Conversely, control wines from the 2018 vintage after 12 months of storage were rated significantly higher for petrol/kerosene-like aroma, while no free TDN levels were detected in any of those wines. Not surprisingly, after two years of ageing under screwcap, the 2018 wines showed increased free TDN concentrations in control wines made from exposed grapes, as well as a lower 'kerosene' rating for all SC treatments compared with the control wines. However, much like for the one-year-old 2019 wines, no direct correlation was observed between the higher free TDN levels and the perception of a 'kerosene-like' aroma in the wines.

Both arguments indicate that possible masking or enhancing effects occurred that depending on the overall wine aroma composition determined the perception of kerosene aroma in the resulting wines, similarly as previously reported (Black *et al*., 2012; Robinson *et al*., 2009; Ziegler *et al*., 2019). Additionally, the study by Robinson *et al*. (2009) studied the effect of wine ethanol concentration on the partitioning of TDN into the headspace above wine, observing a reduction with increasing ethanol levels. While here, ethanol was higher in the 2018 wines after one year than in the 2019 wines and would not account for the sensory differences observed, the possibility that other wine components were affecting the partitioning of the compounds present must be considered. Of note was the high nitrogen content of the 2019 grapes, which has the potential to result in an increase in fermentation-derived aroma compounds (Bell and Henschke, 2005) and contributed the masking the impact of TDN.

With respect to the monoterpene content of the finished wines, the SC treatments showed consistently lower linalool and α-terpineol concentrations, which aligns with previous reports linking sun exposure to increases in bound monoterpenes in grapes (Hernandez-Orte *et al*., 2015). The change in the ratio between α-terpineol and linalool in wine from grapes grown under red SC compared to the other shaded treatments can be most likely attributed to the lower pH in the red SC wines (Williams *et al*., 1982).

In this study, the aim was to maintain the compositional differences produced by the shaded treatments throughout the life of the wine, including pH, to gauge the holistic treatment effect on the wines. However, in an exploratory experiment, the 2018 wines were pH adjusted prior to bottling, and the results clearly emphasised that some of the differences in TDN accumulation and monoterpene ratios observed in the shaded treatments were artefacts of differences in wine pH. The chemical analysis of the pH-adjusted wines highlighted that the reduction in sunlight quantity from the control wine to the shaded wines had more of an impact than the light quality or the photoselective shading that occurred within the shaded treatments.

Overall, vineyard light modifications conducted in this study significantly affected volatile aroma composition and development in Riesling wines. However, the degree of difference among the wines for each vintage after one year in bottle was rather less pronounced or even not present after two years in bottle for wine made in the 2018 vintage. In fact, an obvious effect amongst the longer ageing wine samples was only observed with regard to TDN formation and, in some cases, was related to the 'kerosene' perception, which indicates that the application of shade cloth can be effective

in selectively reducing these attributes over others. At the same time, chemical analysis of pH-adjusted wines showed that reductions in TDN accumulation by shading were also associated with reductions in monoterpene levels also.

CONCLUSION

The objective of this work was to evaluate photoselective bunch zone shading using shade cloths as a practical means for modulating the formation of the potent aroma compound, TDN, and assess the overall impact on Riesling wine. The application of different coloured SC during grape ripening allowed the establishment of reduced light conditions in combination with an altered spectral composition (light quality) reaching the grapes. Photoselective shadings resulted in slight delays in sugar maturity, which were less evident for grapes grown under red SC compared to grapes grown under green or black SC; it also resulted in significant changes to malic acid, titratable acidity and juice and wine pH. Specifically, decreases in pH during winemaking for the red SC treatments require further investigation to explain the large decrease, especially that observed from the 2019 vintage. Grapes and wines from these treatments over two consecutive vintages showed a consistent decrease in the amount of TDN (total or free) and monoterpenes; wine made from grapes grown under red SC wines had a lower pH which might have further altered its aroma profile. While there were some differences observed between the wines made from grapes grown under red SC compared to the black or green SC treatments with respect to volatile composition, these were diminished when pH adjustment was made. The overall effect appears to reflect reductions in overall light quantity compared to the control (fully exposed treatment) rather than changes in light quality due to photoselective shading. In future, pH adjustment of all wines is recommended to assess better the role of shading on TDN accumulation and monoterpene rearrangement independent of differences in wine pH.

In summary, the application of photoselective SC in viticulture warrants further studies, especially with respect to the potential for delaying and extending the ripening period. Moreover, further sensory analysis, together with more detailed compositional data for Riesling and other varietals, are required to establish the impact of light quantity and quality on wine chemical and sensory attributes beyond TDN.

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