Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of *Vitis vinifera* L. cv. Sauvignon Blanc wine

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Abstract

Background and Aims: This article studies the influence of the microclimate (light quantity, light quality and temperature) around fruit on the composition and sensory profile of South African Sauvignon Blanc wine.
Materials and Results: We manipulated the light quantity in the bunch zone through leaf and lateral shoot removal, and light quality was altered by installing ultraviolet (UV) radiation-reducing sheets. We analysed wines made from fruit subjected to these treatments for chemical attributes pertaining to aromatic composition and assessed by a trained sensory panel. Variation in chemical and sensory attributes was found to be influenced by defoliation and UV radiation reduction. Control (no defoliation) was associated with green pepper, asparagus and grassy attributes, whereas wines from treatments where leaf and laterals shoot were removed were associated with tropical fruit attributes. Moreover, this study showed for the first time that UV radiation reduction significantly decreased the concentration of varietal thiols, linalool and some yeast derived compounds, such as esters and fatty acids, in the corresponding wines. Conversely, defoliation increased the concentration of thiols and linalool.
Conclusions: Modification of the bunch microclimate can significantly affect wine composition and sensory properties, and therefore contribute to wine style.
Significance of the Study: Understanding the effect of environmental factors (light and temperature) in the vineyard on wine composition and sensory attributes can assist winemakers and viticulturists in implementing appropriate viticultural practices (such as canopy manipulation) to assist in obtaining desired wine styles.

Keywords: *esters, light, methoxypyrazines, Sauvignon Blanc aroma, thiols*

Introduction

The distinctive varietal aromas of Sauvignon Blanc wines are reported to arise from several classes of highly potent compounds, such as the thiols and methoxypyrazines. Volatile thiols, present in the grape berry in a non-volatile form, are bound to glutathione (GSH) or cysteine (Tominaga et al. 1998a, Peyrot des Gachons et al. 2002, Capone et al. 2010, Roland et al. 2011). During fermentation, 3-sulfanylhexan-1-ol (3SH) and 4-methyl-4-sulfanyl pentan-2-one (4MSP) are released partly from non-odiferous precursors, whereas 3-sulfanylhexyl acetate (3SHA) is produced through the acetylation of 3SH by yeast metabolism (Darriet et al. 1995, Tominaga et al. 1998a). Fruity aromas, such as guava, grapefruit, mango, passionfruit and gooseberry, are the main sensory characteristics of 3SH and 3SHA, whereas 4MSP is described as having box tree and passionfruit-like aromas (Tominaga et al. 1996, Swiegers et al. 2009, Coetzee and Du Toit 2012, Coetzee et al. 2013). These compounds are easily detected olfactorily, as they have a low perception threshold, being 0.8 ng/L for 4MSP, 4.2 ng/L for 3SHA and 60 ng/L for 3SH in model wine solutions (Dubourdieu et al. 2006).

Conversely, methoxypyrazines, such as 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP), are responsible for green pepper, asparagus, grassy and vegetative aromas of wines (Allen et al. 1991, Pickering et al. 2007). The perception thresholds for IBMP and IPMP in water and in white wine are low, in the range of 0.32–1 ng/L for IPMP and about 2 ng/L for IBMP (Buttery et al. 1969, Allen et al. 1991, Kotseridis et al. 1998, Pickering et al. 2007). Recently, it has also been shown that yeast-derived metabolites such as esters can significantly affect Sauvignon Blanc wine aroma (Benkwitz et al. 2012). At higher concentration, esters are known to contribute strongly to the fruity aroma of young white wines (Ribéreau-Gayon et al. 2000, Benkwitz et al.

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2012). Esters can also affect wine aroma at a concentration considerably below their perception threshold, through complex synergistic effects (Pineau et al. 2009, Lytra et al. 2012).

Grapevine phenology and physiology, which influence yield and fruit composition, are largely under the control of climate on a macro (regional), meso (vineyard or site) and microscale (canopy and fruit zone). Much previous research has reported the use of canopy manipulation and irrigation to change the vine microclimate (Bergqvist et al. 2001, Sala et al. 2004, Falcão et al. 2007, Ryona et al. 2008, Jreij et al. 2009, Greer et al. 2010, Scheiner et al. 2010, Gregan et al. 2012, Šuklje et al. 2012). Furthermore, increased ultraviolet (UV) radiation has been related to ongoing climate change (Schultz 2000, Jug and Rusjan 2012). Solar light quality, in particular UV radiation, can significantly affect the flavonol and stilbene composition of Cabernet Sauvignon and Riesling grapes, as well as the concentration of amino acids (Schultz et al. 1998, Keller and Torres-Martinez 2004). Furthermore, it has been reported that UV-B radiation at a dose of 4.65 kJ/($m^3 \cdot$ d) and fluorescence rate of 8.25 μW/cm^3 for 16 h per day increases the concentration of terpenes in grapevine leaves (Gil et al. 2012), but has no effect on IBMP concentration in grapes (Gregan et al. 2012). An increase in solar radiation, however, through bunch exposure prior to veraison drastically reduced the concentration of IBMP and IPMP in Cabernet Franc and Cabernet Sauvignon grape berries (Ryona et al. 2008, Scheiner et al. 2010, Koch et al. 2012).

The above studies aimed to understand better the effects of the main abiotic factors, such as temperature, light and vine water status, on vine physiology, fruit growth and fruit composition. Less research, however, has focused on studying the effect of these factors on wine composition and sensory attributes. This study aimed to ascertain the influence of some major biochemical compounds on sensory attributes of Sauvignon Blanc wine made from grapes grown under several light quality and quantity regimes in the vineyard. To our knowledge, this study reports for the first time the effect of reduced UV radiation on fruit on the sensory and chemical composition of Sauvignon Blanc wine.

Materials and methods

Vineyard

The experiment was undertaken in a commercial *Vitis vinifera* L. cv. Sauvignon Blanc vineyard located in the Overberg region of the southern coastal area, South Africa (34°9′53.10′S; 19°0′50.51′E). Sauvignon Blanc vines (clone 316 grafted on 101.14) were planted in 2004 in a northwest to southeast row orientation, with a 2.5 m (row) × 1.8 m (vine) spacing. Vines were trained on a double cordon with vertical shoot positioning (VSP) and were not irrigated during the season. To examine the influence of bunch microclimate manipulation on wine composition, leaf and lateral shoots were removed on 13 December 2011, at the phenological stage of berries at peppercorn size (E-L 29) (Eichorn and Lorenz 1977). Three treatments were established: a control (C) consisting of shaded bunches within unaltered VSP canopy; a sun-exposed bunches treatment (M-LR), removing all leaves and lateral shoots in the bunch zone on the morning/northeastern side of the canopy at the a height of 30–40 cm above the cordon; and a third treatment (LR-UV) utilising clear, extruded high impact acrylic sheets [Perspex South Africa (Pty) Ltd, Umbogintwini, South Africa] to reduce UV radiation to bunches exposed as per the second treatment (Figure 1). These sheets eliminate 99% of the total UV radiation, with visible light reduction of only 12% [Perspex South Africa (Pty) Ltd]. For the LR-UV treatment, sheets were installed on the

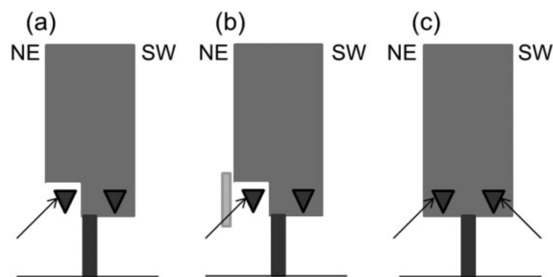

Figure 1. Schematic indication of the defoliation treatments applied to Sauvignon Blanc grapevines. The arrows indicate the bunches harvested from each treatment. The treatments were: (a) M-LR, exposed bunches by removing leaves and lateral shoots in the bunch zone on the morning side of the canopy; (b) LR-UV, exposed bunches on the morning side with UV radiation-reducing sheets; and (c) control (C). NE, northeast; SW, southwest; UV, ultraviolet.

morning/northeastern side of the canopy, covering the bunch zone after all the leaves and lateral shoots had been removed at the height 30–40 cm above the cordon. The installation of the UV radiation-reducing sheets coincided with the date of leaf and lateral shoot removal. The treatments were replicated eight times across the layout, with a replicate consisting of four consecutive vines. On each side of the experimental plot, there were at least 12 buffer rows, and there were six buffer vines at the beginning of the experimental row. The canopy, including suckering and shoot positioning, was managed evenly across treatments to optimise light intensity in the bunch zone.

Abiotic variables and plant responses

To assess vine water status, stem water potential was measured (Choné et al. 2001) on 6 February 2012, 3 days after veraison with a pressure chamber (Sholander et al. 1965). Photosynthetic active radiation (PAR) was monitored within the canopy at the bunch zone with LI-190 quantum sensors (LI-COR Inc., Lincoln, NE, USA) attached to a TinyTag TGPR-1001 millivolt input data logger (Gemini Data Loggers Ltd, Chichester, England). Ultraviolet radiation at the fruit zone was measured with a UV sensor of Davis Instruments (Hayward, CA, USA) attached to a Datataker DT82E series with data loggers (Thermo Fisher Scientific Australia Pty Ltd, Scoresby, Vic., Australia). UV radiation was measured as minimum erythemal dose, which was converted to Commission Internationale de l'Eclairage-weighted irradiation (mJ/cm^3) using a conversion factor of 21 (Swedish Radiation Safety Authority 2014).

For the exposed treatments (M-LR and UV-LR) the PAR and UV radiation sensors were positioned parallel with the cordon at the bunch zone on the defoliated (northeastern) side of the canopy. For the C, PAR and UV radiation sensors were positioned parallel with the cordon inside the canopy at the bunch zone. As only two units for measuring PAR and UV radiation were available, light sensors were positioned consecutively within two treatments for a predetermined period of time, therefore comparing two treatments per logging interval. The temperature of the bunch microclimate was monitored at 15-min intervals by TinyTag dual channel external loggers, TGP-4520 (Gemini Data Loggers Ltd), with flying lead thermistor probes positioned inside the bunch on both sides of the canopy. The bunch temperature loggers were installed on 19 December 2011, and removed at harvest, on 13 March 2012. The PAR, UV radiation and temperature data are presented as a mean hourly value for the period of monitoring.

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Winemaking

Grapes were harvested when juice total soluble solids (TSS) reached between 23 and 24°Brix and titratable acidity (TA) was about 6.5 g/L. Grapes from all three treatments in the experiment were harvested manually on 13 March 2012, 113 days after anthesis by two authors to avoid variability in the harvesting regime. Only fully sun-exposed bunches from the exposed side of the canopy (northeast) were harvested in the M-LR and the LR-UV treatments (Figure 1). All the bunches from the C were harvested, as they were permanently shaded and considered homogeneous in the terms of light and temperature (Figure 1). The air temperature measured inside the bunch on the northeastern side of the canopy and in the bunch positioned on the southwestern side of the canopy in C differed by 0.5°C for the period of monitoring (n = 85 days). The treatments in the experiment were all harvested on the same day within 3 h. Grapes from the eight replicates per treatment were pooled together and stored overnight at +4°C prior to crushing. Sulfur dioxide (SO_2) (40 mg/kg) was added during de-stemming and crushing, along with the addition of solid carbon dioxide and a flow of nitrogen gas (N_2). After cold maceration for 24 h at +4°C, the grapes were pressed under a constant flow of N_2 in combination with the addition of solid carbon dioxide to prevent oxidation of the must. The must was clarified at +4°C for 48 h and an enzyme was added at 2 g/hL to facilitate sedimentation (Rapidase Vino Super, DSM Food Specialists B.V., Delft, Netherlands). The clear must was divided into three volumes, after which it was vinified in triplicate. For each treatment, 4 L of the clear must was decanted into three 4.5-L N_2-filled fermenters. Prior to inoculation, a 50-mL sample of must was taken for analysis of TSS, TA and pH, while additional samples were taken for analysis of GSH and grape reaction product (GRP). The must was inoculated with 30 g/hL VIN 13 yeast (Anchor Yeast, Industria, South Africa) with the addition of 30 g/hL of a yeast starter nutrient (Dynastart, Laffort, Bordeaux, France). Fermentations were conducted in a temperature-controlled room at +15°C. Six days after inoculation, 50 g/hL of an additional yeast nutrient (Nutrivin, Anchor Yeast) was added to avoid a stuck fermentation. All fermenters proceeded to a residual sugar concentration of below 4 g/L. Wines were cold stabilised at −4°C for 16 days, after which, free SO_2 was adjusted to 35 mg/L and wines were bottled. Bottled wines were stored at +4°C until sensory evaluation.

Chemical analysis

In the must, a set of physiochemical parameters relating to maturity and oxidation were measured before fermentation, whereas in wines, a set of compounds relating to wine aroma was measured. The TSS was measured with a digital refractometer (Atago PAL-1, Tokyo, Japan) with temperature correction. The pH value and TA were determined through sodium hydroxide titration with a Metrohm titrator and sample changer (785 DMP Titrino with a LL-Unitrode Pt1000 F P, Metrohm AG, Herisau, Switzerland). The concentration of GSH in the must before fermentation was determined by high-performance liquid chromatography (HPLC) with fluorescence detection (HPLC-FLD) and online pre-column derivatisation, as described previously (Janeš et al. 2010). Clear grape juice after sedimentation and before fermentation was taken from fermenters and immediately placed in methanol. The internal standard, N-acetyl-L-cysteine (8 mg/L), was added to the juice, which was filtered through 0.45-μm Sartorius Minisart RC 25 filters (Sartorius, Goettingen, Germany), diluted 1:1 with a 5-mmol sodium acetate buffer containing 0.1-mmol ethylenediaminetetraacetic acid, and analysed as previously described (Janeš et al. 2010). For GRP analysis 5 mL of each juice sample was taken from the fermenters and immediately placed in 1000 mg/L of SO_2 in order to inhibit enzymatic activity. The sample was filtered through a 0.45-μm polyvinylidene difluoride filter (EMD Millipore Corporation, Billerica, MA, USA) into an HPLC vial. The concentration was determined by HPLC, as described by Vanzo et al. (2007), and expressed as aliquots of *trans*-caftaric acid. The wines were analysed for IBMP with the headspace solid-phase microextraction method (HS-SPME) and with quantification by gas chromatography-mass spectrometry (GC-MS). An internal standard of final concentration 25 ng/L deuterated IBMP (CDN Isotopes, Pointe-Claire, QC, Canada) was added to the wine. Then 1.6 mL of wine was transferred into a 20-mL headspace vial containing 3 g of NaCl, and 6.4 mL of deionised water and 2 mL of 4 mol NaOH were added. The sample was stirred until the NaCl was completely dissolved, and then analysed by GC-MS (Parr et al. 2007, Šuklje et al. 2012). 3-Sulfanylhexan-1-ol and its acetate 3SHA in wines were measured according to the method of Tominaga (Tominaga et al. 1998b, Tominaga and Dubourdieu 2006) with slight modifications and using an isotopically labelled 3SH ($[^3H_2]$-3SH) and 3SHA ($[^3H_2]$-3SHA) as internal standards (Šuklje et al. 2013). All esters, except ethyl 3-*cis*-hexenoate, *cis*-3-hexenyl and *trans*-2-hexenyl acetate were quantified as described by Antalick et al. (2010), with slight modifications. The sample volume was reduced from 10 mL to 5 mL, and alternate internal standards were added. A mix of isotopically labelled esters was prepared from commercial deuterated esters (CDN Isotopes). The final solution used to spike the samples was composed of $[^3H_3]$-ethyl butyrate at 40 mg/L, $[^3H_{11}]$-ethyl hexanoate at 20 mg/L, $[^3H_{15}]$-ethyl octanoate at 20 mg/L, $[^3H_{23}]$-ethyl dodecanoate at 4 mg/L and $[^3H_5]$-ethyl cinnamate at 12 mg/L. An internal standard mix solution (20 μL) was added to an exact volume of 10 mL of wine. An aliquot of 5 mL of this wine was placed into a 20-mL SPME vial previously filled with 1.5 g of NaCl. The samples were analysed by GC-MS in selected ion monitoring (SIM) mode as described previously by Antalick et al. (2010) using a DB-FFAP capillary column (60-m, 0.25-mm, 0.5-μm film thickness, Agilent Technologies, Little Falls, DE, USA) and a 6890 gas chromatograph coupled to a 5975C mass spectrometer (Agilent Technologies) equipped with Enhanced Chemstation version D.01.02.16 software (Agilent Technologies). Quantifying ions chosen for the internal standards were 74 for $[^3H_3]$-ethyl butyrate, 110 for $[^3H_{11}]$-ethyl hexanoate, 142 for $[^3H_{15}]$-ethyl octanoate, 206 for $[^3H_{23}]$-ethyl dodecanoate and 181 for $[^3H_5]$-ethyl cinnamate. Ethyl 3-*cis*-hexenoate, *cis*-3-hexenyl and *trans*-2-hexenyl acetates, hexanol, higher alcohols, medium chain fatty acids and linalool were measured in a semi-quantitative way (peak area ratio, compounds/internal standard) by the same method with a scan mode in mass spectrometry performed simultaneously to the SIM mode for esters. Quantifying ions chosen were 43 for isobutanol and hexenyl acetates, 55 for isoamyl alcohol, 91 for phenylethanol, 69 for ethyl *cis*-3-hexenoate, 56 for hexanol, 93 for linalool and 60 for hexanoic, octanoic and decanoic acids. The internal standards were chosen as follows: $[^3H_3]$-ethyl butyrate for isobutanol and isoamyl alcohol, $[^3H_{11}]$-ethyl hexanoate for all the C6 compounds and linalool, $[^3H_{15}]$-ethyl octanoate for phenylethanol and hexanoic acid, and $[^3H_{23}]$-ethyl dodecanoate for decanoic acid.

Wine sensory analysis

Descriptive sensory analysis was undertaken by a trained panel consisting of 10 panellists (nine women and one man), ranging in age from 22 to 45 years and who were either working in the

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Table 1. Attribute identification for data blocks.

Attribute number	GPA sensory data	Chemical attributes – quantitative	Chemical attributes – semi-quantitative
1	Overall tropical	3-Sulfanyhexyl acetate	Linalool
2	Overall green	3-Sulfanylhexan-1-ol	Phenylethanol
3	Passionfruit	3-Isobutyl-2-methoxypyrazine	Ethyl cinnamate
4	Guava	Ethyl propionate	Ethyl hydroxycinnamate
5	Grapefruit	Ethyl butyrate	Isobutanol
6	Gooseberry	Ethyl hexanoate	Isoamyl alcohol
7	Pineapple	Ethyl octanoate	Hexanol
8	Banana lolly	Isobutyl acetate	Ethyl *cis*-3-hexenoate
9	Floral	Isoamyl acetate	Ethyl *trans*-2-hexenoate
10	Grassy	2-Phenylethyl acetate	*Cis*-3-hexenyl acetate
11	Green pepper	Hexyl acetate	*Trans*-2-hexenyl acetate
12	Asparagus	Ethyl decanoate	Hexanoic acid
13	Cooked beans/peas	Ethyl dodecanoate	Octanoic acid
14	Acidity	Ethyl isobutyrate	Decanoic acid
15	Bitterness	Ethyl 2-methylbutyrate	—
16	—	Ethyl isovalerate	—
17	—	Propyl acetate	—
18	—	Ethylphenyl acetate	—

GPA, Generalised Procrustes Rotation Algorithm. —, No attribute.

wine industry or experienced as sensory assessors. Sensory training consisted of five 1-h training sessions. The panellists initially generated descriptors individually, and these were then discussed in a group to choose the predominant attributes ($n = 15$). The panel was then trained in the recognition and discrimination of the selected attributes using reference standards (Noble et al. 1987) and a 2-week period of intensity scaling. The aroma and mouth-feel standards used for sensory training and wine assessment are described in Supporting Information Table S1. Each attribute was rated for intensity on a 10-cm unstructured line scale. The line scale was anchored at 0 for 'none' and 10 for 'intense'. Wines were evaluated in triplicate and each fermentation triplicate was evaluated three times per assessor. Wines were served to tasters according to a William design Latin-square and assigned a randomised three-digit number for identification. Wines were presented in black ISO-tasting glasses to exclude colour differences, and the tastings were conducted in a well-ventilated sensory lab, at 20 ± 2°C, with separate tasting booths.

Statistical analysis

Chemical data were analysed using Statistica, Version 10 (StatSoft, Tulsa, OK, USA). The significance was checked using one-way analysis of variance (ANOVA) and the means were separated using Stats-Fisher's least significant difference test (different letters account for significant differences at $P \leq 0.05$). All quoted uncertainty is the standard deviation of the replicates of one treatment. Panel performance was evaluated with PanelCheck version 1.4.0 (Nofima, Os, Norway) according to the workflow proposed by Tomic et al. (2010). Tucker1 was applied to the sensory data to evaluate assessor agreement, and p-value* mean square error values (p*MSE) graphs were assessed to evaluate assessor repeatability and discrimination ability. Sensory data were analysed using multifactoral ANOVA using Statistica version 10 (StatSoft). The averaging of the panel scores was considered necessary as the ANOVA revealed a significant panellist effect. Simple averaging of the sensory data is inappropriate; therefore, a consensus average of sensory scores was determined on mean-centred sensory scores using a Generalised Procrustes Rotation Algorithm (GPA), followed by a permutation test as described in Schmidtke et al. (2010). The Procrustes algorithm employed in this study aims to mitigate confusion of attributes and differences in panellist use by an interactive rescaling, reflection and projection to minimise the differences between each combination of answers (ten Berge 1977). As GPA may produce a consensus for random data, it is necessary to test significance if the consensus average is obtained and permutation test was used for this purpose (Wakeling et al. 1992, Dijksterhuis and Heiser 1995). Following calculation, the consensus average as a proportion of variation explained by this consensus compared with the total variation of the initial new data was calculated. Permutations of samples within the score tables were conducted 1000 times, and comparison of the distribution of the permutated data variance with the variable for the initial data to estimate the significance of the consensus was done. The GPA and permutation test were conducted in Matlab (Version R2012a, The Mathworks, Natick, MA, USA). Principal component analysis (PCA) was conducted on the consensus average sensory scores using PLS Toolbox version 5.0 (Eigenvector Research Inc., Wenatchee, WA, USA). Chemical data sets were related to the GPA consensus sensory matrix by Common Component and Specific Weight Analyses using the SAISIR toolbox (Bertrand and Cordella 2011) on the centred and mean standardised matrices. For the purposes of clarity, multiblock analysis of data sets herein is organised, and each data set was assigned a number as seen in Table 1.

Results

Abiotic variables

The experimental vineyard block was characterised by monitoring stem water potential, light and temperature (micro,

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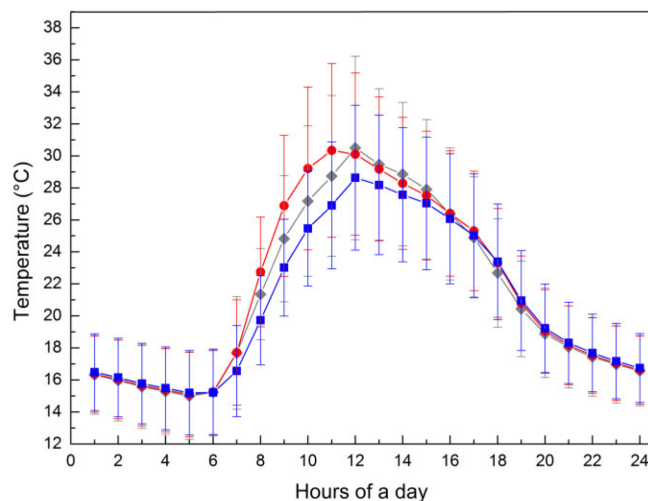

Figure 2. Effect of treatments on the mean hourly temperature of bunches from the 19 December 2011 to 13 March 2012 in Sauvignon Blanc vines. The treatments were: M-LR, exposed bunches by removing leaves and lateral shoots in the bunch zone on the morning side of the canopy (■); LR-UV, exposed bunches on the morning side with ultraviolet radiation-reducing sheets (■); and C, control (■). Error bars represent the standard deviation of the mean hourly temperature of the treatments for the period 19 December 2011 to 13 March 2012.

meso and macrolevel). Stem water potential was measured at veraison, and the mean value for the C was −715 ± 132 kPa, −761 ± 115 kPa for the M-LR treatment and −717 ± 154 kPa for the LR-UV treatment. The stem water potential measurements confirmed the homogeneity of the experimental block and showed that vines did not experience water constraint irrespective of the treatment. This was further confirmed by visual vine inspection and the evolution of berry fresh mass during maturation (data not shown). The PAR values in the bunch zone were significantly higher for treatments with leaf and lateral shoot removal, compared with the values observed in the C treatment. The mean PAR in the C treatment (n = 59 days) remained relatively stable during the entire day, reaching a mean maximum hourly value of around 60 μmol/(m^3 · s), whereas in the M-LR (n = 59 days) and UV-LR (n = 12 days) treatments measured PAR reached the mean maximum hourly value for a period of monitoring, 450 and 830 μmol/(m^3 · s). As PAR was not measured in all the treatments at the same period of monitoring the observed variations in the PAR in the exposed treatments could be mainly due to the extent of cloud cover at the time of measurement. The highest mean maximum hourly UV radiation of 226.8 mJ/cm^3 was measured in the M-LR treatment (n = 9 days), whereas lower UV radiation was measured in the C 52.5 mJ/cm^3 (n = 4 days) and the lowest in LR-UV treatment 25.2 mJ/cm^3 (n = 6 days). Similarly as with PAR, the measurement of UV radiation was not taken at the same time for all three treatments. The LR-UV treatment showed the highest reading of mean bunch temperature for the period of monitoring (n = 85 days), viz. 21.4 ± 6.39°C, whereas the C (n = 85 days) showed the lowest reading of mean bunch temperature of 20.5 ± 5.25°C. More precise observations can be made when analysing the evolution of mean hourly temperature (Figure 2). The elevation in bunch temperature in the M-LR and LR-UV treatments above that of C was observed in the morning hours, whereas the difference in the temperature between treatments in the afternoon was less prominent.

Chemical analyses

Grapevine defoliation and reduced UV radiation did not influence must TA, whereas the lowest TSS were measured in C (Table 2). In the current study, the GSH concentration in must before fermentation ranged from 30.9 ± 2.11 in LR-UV to 49.2 ± 6.88 mg/L in M-LR treatment and was significantly different (Table 2). The GRP values were expressed as *trans*-caftaric acid equivalent and were the lowest in the M-LR treatment, that is, 10.9 ± 1.08 mg/L, and highest in the LR-UV treatment, 17.6 ± 0.72 mg/L (Table 2). The highest concentration of 3SH and 3SHA was observed in the M-LR treatment, 447.0 ± 26.0 and 186.8 ± 3.2 ng/L, respectively (Table 2). The concentration of 3SH and 3SHA was lower in the LR-UV treatment compared with that of the M-LR treatment, and the lowest 3SH concentration was measured in C (Table 2). The observed concentration of IBMP in the wine samples was generally low. The highest IBMP concentration in the wine measured was 3.4 ± 0.31 ng/L for C, which differed significantly from that measured in the wines of the M-LR and LR-UV treatments (Table 2). The reduced UV radiation had no significant effect on the IBMP concentration in Sauvignon Blanc wines. In general, ethyl esters of fatty acids were produced in lesser quantities by yeast in LR-UV treatment wines, excluding ethyl decanoate and ethyl dodecanoate, which were not influenced by any of the treatments. In comparison, the M-LR treatment led to the highest concentration of ethyl butyrate, ethyl hexanoate and ethyl octanoate in the wines (Table 2). The wines from the LR-UV treatment recorded the lowest concentration of the higher alcohol acetates. A decrease in the concentration of hexyl acetate, isoamyl acetate and 2-phenylethyl acetate in the LR-UV treatment was observed (Table 2). No significant difference in the concentration of higher alcohol acetates was found within the M-LR and C treatments. Leaf and lateral shoot removal in the bunch zone, irrespective of reduced UV radiation, increased the concentration of ethyl esters of branched acids compared with that of the C. Conversely, the relative-concentration of hexanol and C6 esters, such as ethyl *cis*-3-hexenoate, ethyl *trans*-2-hexenoate, *cis*-3-hexenyl and *trans*-2-hexenyl acetate, decreased significantly in the LR-UV and C wines compared with that of the M-LR treatment (Table 2). A significantly higher relative concentration of isobutanol was measured in the LR-UV treatment, whereas the relative concentration of isoamyl alcohol and phenylethanol was elevated, but not significantly compared with that of the M-LR treatment. The C exhibited the lowest concentration of higher alcohols in the wines (Table 2). In contrast, a significantly lower relative concentration of medium chain fatty acids was observed in the LR-UV treatment compared with that of the M-LR and C treatments (Table 2). The highest relative concentration of linalool was found in the M-LR treatment, whereas the lowest relative concentration was observed in the C. Reduced UV radiation significantly reduced the relative linalool concentration in the LR-UV treatment compared with that in the M-LR treatment (Table 2).

Wine sensory evaluation

From the ANOVA results conducted on the raw sensory data, it is evident that some sensory attributes were different for panellists, and the interaction of panellists*treatments was significantly different for the attributes overall tropical, overall green, passionfruit, grapefruit, banana lolly, floral and asparagus (Table 3). Therefore, it is obvious that the sensory attributes terms were not applied consistently by panellists, and calculating a panel average as an arithmetical mean would be

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Table 2. Average concentration of compounds measured in juices before fermentation and in finished Sauvignon Blanc wines.

Compounds	M-LR	LR-UV	C
Must before fermentation			
Total soluble solids (°Brix)	23.8 ± 0.06b	24.7 ± 0.06a	23.3 ± 0.01c
Titratable acidity (g/L)	6.5 ± 0.05a	6.3 ± 0.64a	6.7 ± 0.01a
pH	3.29 ± 0.03b	3.41 ± 0.03a	3.37 ± 0.01b
Glutathione (mg/L)	49.2 ± 6.88a	30.9 ± 2.11c	36.3 ± 1.67b
Grape reaction product (mg/L)	10.9 ± 1.08c	17.6 ± 0.72a	14.0 ± 2.38b
Wine			
Varietal thiols (ng/L)			
3-Sulfanylhexan-1-ol	447.0 ± 26.0a	344.4 ± 11.2b	303.7 ± 7.2c
3-Sulfanyhexyl acetate	186.8 ± 3.2a	111.0 ± 3.2b	111.1 ± 5.2b
Methoxypyrazines (ng/L)			
3-Isobutyl-2- methoxypyrazine	2.6 ± 0.1b	2.4 ± 0.3b	3.4 ± 0.3a
Ethyl esters of fatty acids (µg/L)			
Ethyl butyrate	616 ± 11.9a	554 ± 18.6c	586 ± 10.4b
Ethyl hexanoate	1171 ± 70a	924 ± 73b	1016 ± 60b
Ethyl octanoate	2074 ± 206a	1563 ± 230b	1950 ± 156a
Ethyl decanoate	576 ± 139a	560 ± 75a	555 ± 111a
Ethyl dodecanoate	134 ± 32a	166 ± 37a	136 ± 23a
Total esters of fatty acidss	4571 ± 323a	3767 ± 321b	4244 ± 110ab
Higher alcohol acetates (µg/L)			
Isobutyl acetate	83.7 ± 2.4a	81.7 ± 4.0a	86.4 ± 2.2a
Isoamyl acetate	5888 ± 513a	5016 ± 440b	5794 ± 448a
Hexyl acetate	238 ± 29a	152 ± 25b	225 ± 23a
2-Phenylethyl acetate	318 ± 69a	166 ± 37b	297 ± 57a
Propyl acetate	186 ± 5.8ab	178 ± 9.7b	199 ± 4.5a
Total higher alcohol acetates	6713 ± 543a	5593 ± 477b	6601 ± 488a
Ethyl esters of branched acids (µg/L)			
Ethyl isobutyrate	19.8 ± 1.1a	21.1 ± 1.3a	16.5 ± 0.6b
Ethyl 2-methylbutyrate	2.23 ± 0.05b	2.46 ± 0.15a	1.76 ± 0.10c
Ethyl isovalerate	4.40 ± 0.31a	4.70 ± 0.49a	3.47 ± 0.35b
Ethylphenyl acetate	0.41 ± 0.05a	0.47 ± 0.04a	0.31 ± 0.06b
Total esters of branched acids	26.8 ± 1.3a	28.8 ± 1.8a	22.0 ± 0.8b
Ethyl propionate	83.0 ± 8.9ab	92.0 ± 12.5a	76.2 ± 5.1b
C6 compounds and their esters			
Ethyl *cis*-3-hexenoate†	0.45 ± 0.05a	0.34 ± 0.04b	0.32 ± 0.04b
Ethyl *trans*-2-hexenoate (µg/L)	0.65 ± 0.08a	0.43 ± 0.04b	0.46 ± 0.04b
Cis-3-hexenyl acetate†	0.23 ± 0.03a	0.17 ± 0.02b	0.18b ± 0.02b
Trans-2-hexenyl acetate†	0.11 ± 0.01a	0.07 ± 0.01b	0.13 ± 0.02a
Hexanol†	0.42 ± 0.07a	0.34 ± 0.04b	0.35 ± 0.04b
Ethyl esters of hydroxycinnamic acids			
Ethyl cinnamate†	0.0001 ± 0.00001a	0.0001 ± 0.00001a	0.0002 ± 0.00001a
Ethyl hydroxycinnamate†	0.0024 ± 0.003a	0.0031 ± 0.0007a	0.0026 ± 0.00004a
Higher alcohols			
Isobutanol†	3.46 ± 0.46b	4.31 ± 0.59a	2.76 ± 0.49c
Isoamyl alcohol†	67.9 ± 10.9a	77.8 ± 12.0a	61.8 ± 8.4a
Phenylethanol†	0.51 ± 0.10ab	0.58 ± 0.07a	0.45 ± 0.04b
Medium chain fatty acids			
Hexanoic acid†	0.52 ± 0.06a	0.41 ± 0.02a	0.49 ± 0.09a
Octanoic acid†	1.71 ± 0.15a	1.20 ± 0.14b	1.55 ± 0.22a
Decanoic acid†	16.0 ± 3.4a	10.3 ± 1.0b	16.1 ± 3.6a
Terpenes			
Linalool†	0.039 ± 0.007a	0.026 ± 0.003b	0.016 ± 0.002c

†Indicates relative concentration of compounds where semi-quantitative data are shown, showing a peak ratio. Analysis of variance was used to compare data. Means followed by different letters in a row are significant at $P \leq 0.05$ (Fischer's least significant difference). C, control receiving no leaf and lateral shoot removal; LR-UV, exposed bunches on the morning side with UV radiation reducing sheets; M-LR, exposed bunches by removing leaves and lateral shoots in the bunch zone on the morning side of the canopy.

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Table 3. Significant sources of variation in the analysis of variance model of the raw sensory data.

	Panellist	Treatment	Panellist*treatment
Overall tropical	**0.000**	**0.000**	**0.002**
Overall green	**0.000**	**0.000**	**0.023**
Passionfruit	**0.000**	**0.000**	**0.007**
Guava	**0.014**	**0.000**	0.458
Gooseberry	0.489	**0.000**	0.443
Grapefruit	**0.000**	**0.000**	**0.001**
Pineapple	**0.000**	**0.000**	0.074
Banana lolly	0.119	**0.000**	**0.002**
Floral	**0.000**	**0.000**	**0.000**
Grassy	**0.002**	**0.000**	0.169
Green pepper	**0.000**	**0.000**	0.340
Asparagus	**0.000**	**0.000**	**0.000**
Cooked beans/peas	**0.000**	**0.000**	0.565
Acidity	**0.000**	**0.000**	0.805
Bitterness	**0.000**	**0.000**	0.328

Bold numbers indicate significance at $P \leq 0.05$ (Fischer's least significant difference).

inappropriate for some attributes. Thus, a GPA on the mean-centred scores matrix for each panellist was used to mitigate the variability of the panellists performance by calculating a consensus average of the sensory response (Gower 1975, ten Berge 1977). The distribution of the permuted data variance is illustrated in Supporting Information Figure S1. The upper band for the 95% confidence limit of the variance distribution (U*) is chosen as the critical value in determining the significance of the consensus results (King and Arents 1991), and it is compared with the total variance of the new sensory data (R_c). In this study, the consensus variance is larger than U^*_9 and $P < 0.001$, F (1008, 112). Therefore, it can be concluded that the consensus for the GPA represents a true consensus among panellists (King and Arents 1991) (Supporting Information Figure S1). ANOVA was run on the consensus average scores, and post-hoc results on sensory attributes are presented in Supporting Information Table S2. The two-dimensional PCA projection applied to the consensus average scores of sensory attributes explains 76.1% of the variation, with the first principal component (PC1) explaining 56.6% of the variation and the second principal component (PC2) explaining 19.5% of the variation (Figure 3). Examination of the biplot shows that treatments are separated by PC1, according to increased light penetration at the bunch zone achieved through the leaf removal, regardless of the reduced UV radiation. The defoliated treatments (LR-UV and M-LR) were associated with increased perception of the attributes fruity/tropical fruits, such as overall topical, passionfruit, grapefruit and pineapple (Figure 3). Furthermore, the C was associated with the increased perception of green attributes, such as cooked beans/peas, acidity and green pepper (Figure 3). The LR-UV treatment was associated with a perception of bitterness, whereas the M-LR treatment was strongly related to the increased perception of floral, and was separated along the PC2 (Figure 3).

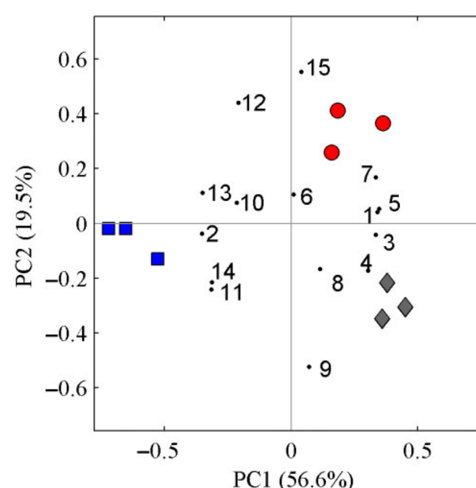

Figure 3. Principal component analysis score plot for treatments and consensus average sensory scores calculated using Generalised Procrustes Rotation Algorithm. Treatments applied to the Sauvignon Blanc vines were: M-LR, exposed bunches by removing leaves and lateral shoots in the bunch zone on the morning side of the canopy (◆); LR-UV, exposed bunches on the morning side with UV radiation reducing sheets (●); and C, control (■). UV, ultraviolet; PC1, first principal component; PC2, second principal component.

Correlation of sensory and chemical data sets

To assess the commonality between the GPA sensory matrix and the chemical data, Common Component Specific Weight Analysis was conducted on the mean and standardised matrices. Common Component Specific Weight Analysis defines the common space and block weighting for the relative importance of multiple blocks of data in the same sample set for each common dimension. The salience of each data block for each extracted common dimension is shown on Figure 4. It is evident that each data set contributed approximately the same variance for the first two common components. Loading plots for common dimensions and their respective groups are illustrated in the Figure 4. Common dimension 1 (CD1) explains 83% of data variance and CD2 explains 14% of the data variance (Figure 4). A clear grouping of the treatment replicates is evident, and a separation of treatments in CD1 and CD2 is noted (Figure 5). Each measured attribute, that is, sensory attributes, quantitative chemical data and semi-quantitative chemical data,

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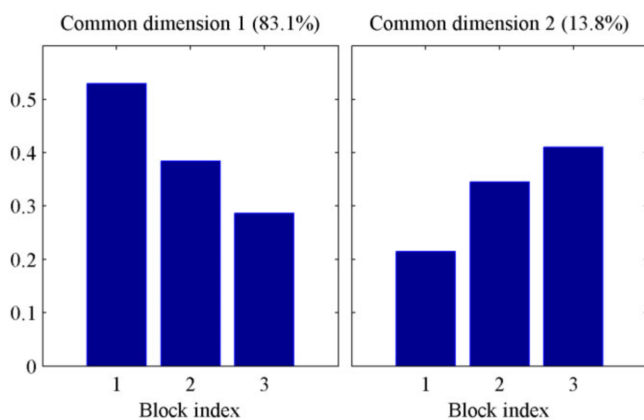

Figure 4. Relative importance and contribution (salience) of each data block used in the Common Components and Specific Weights Analysis for the first two common components of the three blocks of data. Block 1: Sensory data of wines following a Generalised Procrustes Analysis of panellist sensory data; Block 2: Chemical-quantitative analysis of volatile compounds; and Block 3: Chemical semi-quantitative analysis of volatile compounds.

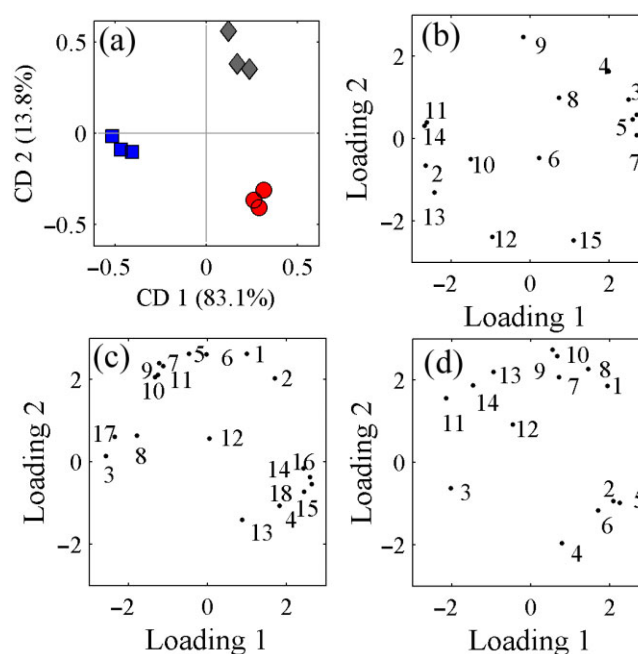

Figure 5. Common Components and Specific Weights Analysis scores and loadings plots of the three data blocks.: (a) Scores of common dimensions 1 and 2 for wine samples from viticultural treatments on Sauvignon Blanc vines: M-LR, exposed bunches by removing leaves and lateral shoots in the bunch zone on the morning side of the canopy (◆); LR-UV, exposed bunches on the morning side with UV radiation reducing sheets (●); and C, control (■); (b) loadings plot for data block 1 Sensory scores of wines following a Generalised Procrustes Analysis of panellist sensory data (numbers refer to closest sensory attribute); (c) loadings plot for data block 2 quantitative analysis of volatile compounds; (d) loadings plot for data block 3 semi-quantitative analysis of volatile compounds; The numbered sensory, quantitative chemical and non-quantitative chemical attributes are listed in Table 1. UV, ultraviolet; CD1, common dimension 1; CD2, common dimension 2.

has been assigned a number as presented in Materials and methods (Table 1). Dividing the data into three blocks was necessary because of the orders of magnitude among sensory, quantitative and semi-quantitative data. Scores for extracted CD1 separate C from the two other treatments receiving leaf removal (M-LR and UV-LR), irrespective of reduced UV radiation, by the sensory attributes, such as overall green, green pepper, grassy and cooked beans/peas (Figure 5a,b). Thus, the chemical data strongly associated with C were isobutyl acetate, propyl acetate and IBMP, with the latter being known to contribute to green aromas of wines (Figure 5c,d). On the positive side of CD1, the loading scores indicate that M-LR is high in CD2, the dimension associated with GPA sensory loadings, such as floral, banana lolly and guava (Figure 5a,b). In parallel, wines from the M-LR treatment were correlated with compounds responsible for floral and fruity aromas of wines, such as thiols (3SH, 3SHA), ethyl esters of fatty acids (ethyl butyrate, ethyl hexanoate, ethyl octanoate), higher alcohol acetates (isoamyl acetate, 2-phenylethyl acetate and hexyl acetate) and linalool (Figure 5c,d). Moreover, ethyl *trans*-2-hexenoate, *cis*-3-hexyl-acetate, isoamyl alcohol and hexanol were found in this dimension (Figure 5d). The LR-UV treatment was low in GD2 and strongly related to the perception of bitterness (Figure 5a).

Discussion

The experiment was designed so that the effect of three bunch exposure treatments on wines could be compared: one in which the fruit microclimate was not modified throughout the growth and ripening phases (C); another where bunch exposure to sunlight was increased because of the leaf and lateral shoot removal (M-LR); and a third where UV radiation was reduced (LR-UV). A strong correlation was observed between defoliation treatments (M-LR and LR-UV) and fruity aromas, whereas the C (no defoliation) was associated with acidity, green pepper and overall green attributes. Exposed treatments were selectively harvested (M-LR and LR-UV) to determine the effect of light on wine composition as only one side of the canopy was defoliated to reduce the possibility of sunburn. For the C, all bunches were harvested as bunches of this treatment were permanently shaded. Manual and highly controlled bunch harvesting was adopted to avoid interference of different harvesting regimes to compare wines made from sun-exposed and shaded grapes. A small, but significant difference in the concentration of IBMP was unlikely to explain the strong separation between the treatments (leaf removal and no leaf removal). It has been noted, however, that a wine aroma profile is rarely related to solely one compound such as IBMP (Marais and Swart 1999, Noble and Ebeler 2002). It has been reported by Allen et al. (1991) that IBMP can be detected in wines at a concentration as low as 2 ng/L, and Van Wyngaard (2013) noted that Sauvignon Blanc wines spiked with 2 ng/L of IBMP and 250 ng/L of 3SH are associated with greener rather than tropical attributes. Furthermore, greenness in Sauvignon Blanc wines was related to some enantiomers of 3SH, 3SHA and 4MSP (Roland et al. 2011). Masking effects of IBMP and the consequent suppression of fruity aromas in wines has long been known, whereas it has only recently been reported that thiols have the same ability (Benkwitz et al. 2012, Van Wyngaard 2013). Therefore, it is likely that the C was related to 'greener attributes' regardless of the small differences in the IBMP concentration, because of the lower perception of fruity aromas (Figure 5), as wines from this treatment exhibited a significantly lower concentration of 3SH, some esters (ethyl isobutyrate, ethyl 2-methylbutyrate, ethyl isovalerate, ethylphenyl acetate, ethyl propionate) and a lower relative-concentration of linalool. It is likely that a higher concentration of 3SH and ethyl esters of branched acids, the latter being known to contribute in synergistic effect to the fruity

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aromas of wines (Lytra et al. 2012), in M-LR and LR-UV treatments enhanced fruity notes compared with that of the C. It has been shown that the omission of esters from the medium results in a significant decrease in the intensity of descriptors associated with thiols (cat pee, passionfruit, stalky), as well as a decrease in apple, stone fruit and overall tropical perception (Benkwitz et al. 2012). Furthermore, other volatiles not quantified in this study, such as β-damascenone, could also contribute to a difference in wine sensory profiles (Benkwitz et al. 2012). In addition, the M-LR treatment was strongly associated with the perception of floral, which could be related to a higher relative-concentration of linalool and some esters of fatty acids, responsible for floral and delicate fruity notes of white wines (Ribéreau-Gayon et al. 2000). Wines from the LR-UV treatment were strongly associated with the perception of bitterness and mapped well with ethyl hydroxycinnamate. It was shown by Fischer and Noble (1994) that bitterness in white wine was associated with an increased concentration of catechin and ethanol and to an increased concentration of phenolic substances in combination with lower wine alcohol content (Gawel et al. 2013). The molecular base for bitterness in white wines, however, is still largely unknown (Sokolowsky and Fischer 2012).

This study demonstrated that wine chemical composition and sensory attributes can be modified significantly, resulting from the alteration of the fruit microclimate by modifying light quantity (leaf removal) and light quality (reduced UV radiation). In this study, however, the temperature effect cannot be excluded, as it is known that the temperature of bunches increases with increased light penetration (Spayd et al. 2002). During the afternoon hours, however, it was possible to partly separate the temperature increase from the increased solar radiation, because of defoliation of only one side of the canopy, and the occurrence of a cooling breeze coming from the Atlantic Ocean onto the experimental site (Bonnardot et al. 2005). In accordance with previous work, leaf and lateral shoot removal in this study decreased the concentration of IBMP in final wines (Ryona et al. 2008, Šuklje et al. 2012). Conversely, no significant effect of reduced UV radiation on IBMP concentration in the wines from this study was observed, what is in agreement with the results reported by Gregan et al. (2012) on Sauvignon Blanc grapes.

Thiols were another group of compounds that appeared to be influenced by the different treatments in the vineyard. For the first time, it was observed that a reduction of UV radiation decreased the concentration of 3SH and 3SHA in the corresponding wines, whereas the lowest 3SH concentration was found in the C. It has been shown by Kobayashi et al. (2011) that increased UV radiation favours higher production of 3SH thiol precursors in the grape berry, whereas an increase in grape bunch temperature had no effect. A potentially higher concentration of thiols in the M-LR treatment originated from higher thiol precursors formation in the grapes. Consequently, the reduction of UV radiation might decrease the formation of thiols precursors in grapes. In addition, higher GSH and lower GRP concentration in the M-LR treatment could contribute to higher 3SH and 3SHA production in these wines. This was not the case, however, when comparing the C and LR-UV treatments. Lack of consistency between GSH in must and thiol concentration in wines has been observed by Patel et al. (2010) and Roland et al. (2010). Nonetheless, the origin of thiols in wines remains unclear (Coetzee and Du Toit 2012).

In contrast to thiols and IBMP, esters are not varietal compounds and are mainly derived from yeast metabolism during alcoholic fermentation. Vineyard treatments, however, can have an indirect impact on ester biosynthesis by influencing the composition of grape amino acids, ammonium or lipids (Roufet et al. 1987, Bell and Henschke 2005, Sumby et al. 2010). In this study, a decrease in the concentration of higher alcohol acetates and ethyl esters of fatty acids in wines was observed when UV radiation was reduced in the vineyard, compared with that of the M-LR treatment. Several hypotheses for the variation in the profile of wine esters could be advanced. Reduction of UV radiation is reported to decrease the degradation of polyunsaturated fatty acids (PUFAs) in grapes as a result of a lack of abiotic stress (Kalua and Boss 2009, Kobayashi et al. 2011). This could result in the repression of genes involved in yeast and higher alcohol acetates synthesis (*ATF1, ATF2*) because of a higher concentration of PUFAs (Fujii et al. 1997, Fujiwara et al. 1998, Sumby et al. 2010). The observed lower concentration of C6 compounds and consequently hexyl acetate as shown by Dennis et al. (2012), originating from lipids degradation and measured in the LR-UV wines supports this hypothesis. In addition, a higher concentration of PUFAs represents a better source of yeast to improve the membrane fluidity than medium chain fatty acids (Torija et al. 2003, Beltran et al. 2008). The consequence could be a decrease in medium chain fatty acids and ethyl esters of fatty acid levels in the wines, as observed for LR-UV compared with that of M-LR. Moreover, the concentration of ethyl esters of branched acids in wines might be directly dependent of the availability of their corresponding acids (Sumby et al. 2010). As for higher alcohol compounds, branched acids also derive from the Erlich pathway (Swiegers et al. 2005). Therefore, the increased relative-concentration of higher alcohols and ethyl esters of branched acids measured in the wines corresponding to the LR-UV treatment could be related.

This work provides a first report on the effect of reduced UV radiation on the chemical composition and sensory perception of Sauvignon Blanc wines. The study demonstrated that in a particular vineyard location (a cool site in South Africa subjected to a sea breeze effect), light quantity and quality are important abiotic variables influencing wine chemical and sensory composition and consequently wine style. A potential drawback of this study was the harvesting of grapes from replicates that were pooled together to produce a sufficient volume of wine to undergo sensory analysis. The justification for this was the aim to compare wine made from bunches sourced from indisputably exposed and shaded treatments. Therefore, selective harvesting occurred for the defoliated treatments (bunches taken from the exposed canopy side only) whereas for the control, all bunches were harvested. The homogeneity of the experimental site was confirmed by monitoring stem water potential, temperature and light as these are the main drivers of homogeneity/heterogeneity in the vineyard in terms of canopy size and fruit microclimate (Choné et al. 2001, Deloire et al. 2004). In parallel, the vigour assessment of the canopy was made by multispectral imaging at veraison (data not shown). Further work should be done on this topic, researching the response of vine and fruit to different abiotic stresses at the genetic level. Comparison of hot-warm versus temperate-cool climates could lead to different results. This study provided some understanding of the relevance of the fruit zone microclimate linked to canopy manipulation and vine architecture, and also enhanced the depth of knowledge on the relationship between wine composition and wine sensory attributes and style.

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Supporting information

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Figure S1. Generalised Procrustes Algorithm analysis (GPA) of sensory scores performed on the mean centred scores matrix for each panellist to produce a consensus mean.

Table S1. Attributes and reference standards used for sensory descriptive analysis, prepared as described by Noble et al. (1987).

Table S2. Mean values of consensus average scores for intensity of sensory and mouthfeel attributes in Sauvignon Blanc wines made from grapes that had undergone three different canopy manipulation treatments in the vineyard.

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