UNIVERSITY OF LJUBLJANA
BIOTECHNICAL FACULTY

Katja ŠUKLJE

INFLUENCE OF VITICULTURAL PRACTICE ON DYNAMIC OF SOME SECONDARY METABOLITES IN GRAPE OF GRAPEVINE VARIETY 'SAUVIGNON BLANC' (*Vitis vinifera* L.)

DOCTORAL DISSERTATION

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DOCTORAL DISSERTATION

VPLIV VINOGRADNIŠKIH UKREPOV NA DINAMIKO IZBRANIH SEKUNDARNIH METABOLITOV GROZDJA ŽLAHTNE VINSKE TRTE (*Vitis vinifera* L.) 'SAUVIGNON BLANC'

DOKTORSKA DISERTACIJA

Ljubljana, 2014

This doctoral dissertation arises from a degree in the postgraduate study programme, of Interdisciplinary Doctoral Programme in Biosciences. The experiments were conducted at the Agricultural Institute of Slovenia, the Department of Viticulture and Oenology, University of Stellenbosch (Republic of South Africa), and in the Chair of fruit growing, viticulture and vegetables of the Biotechnical Faculty, University of Ljubljana.

The theme and title of the doctoral dissertation on the Interdisciplinary Doctoral Programme in Biosciences were adopted on the basis of the Statute of the University of Ljubljana, following the decision of the Senate of Biotechnical Faculty and Senate of University of Ljubljana on 12. 10. 2011. Assoc. Prof. Dr. Denis RUSJAN was appointed as a supervisor and Prof. Dr. Alain DELOIRE as a co-supervisor.

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AB The aim of this doctoral dissertation was to investigate the effect of modified bunch microclimate and changed leaf area-to-yield ratio on chosen grape metabolites during the growth and ripening of 'Sauvignon blanc' (*Vitis vinifera* L.) grapes. In addition, the effect of these treatments on wine chemical composition and sensory perception was studied. Bunch microclimate was modified by the removal of leaves and secondary (lateral) shoots and by reducing UV light with the installation of UV light-reducing sheets in the bunch zone. The leaf area-to-yield ratio was modified with shoot hedging and bunch thinning. The removal of leaves and secondary shoots resulted in lower 3-isobutyl-2-methoxypyrazine (IBMP) concentrations and had no effect on reduced glutathione (GSH) concentrations in the grape berry. In addition, berries of larger diameter (15.5 mm vs. 13.5 mm) and similar total soluble solids (TSS) resulted in higher IBMP concentrations. At lower leaf area-to-yield ratio a delay in TSS accumulation and GSH synthesis in the grape berry were observed. Wine chemical composition and sensory perception were significantly altered by modified bunch microclimate and changed leaf area-to-yield ratio. UV light reduction had an impact on the corresponding wines by decreasing the concentrations of 3-sulfanyl-hexan-1-ol (3SH) and 3-sulfanyhexyl acetate and some esters and had no influence on wine IBMP concentrations. The highest leaf area-to-yield ratio resulted in significantly higher 3SH and 4-methyl-4-sulfanylpentan-2-one concentrations, as well as wine with flavours reminiscent of passion fruit, mango and black currant. Wines produced from treatments without the removal of leaves and lateral shoots were associated with green descriptors (green pepper, asparagus), whereas wines from leaf and secondary shoot removal treatments were associated with fruity aromas. Such an integrated approach can assist viticulturists and winemakers to implement appropriate viticultural practices for preferred wine styles.

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AI Namen disertacije je bil raziskati vpliv spremenjene mikroklime v okolici grozdov in razmerja med listno površino in obremenitvijo vinske trte med dozorevanjem grozdja sorte (*Vitis vinifera* L.) 'Sauvignon blanc' na izbrane metabolite grozdja. Mikroklimo v okolici grozdov smo spremenili z razlistanjem ter z namestitvijo transparentih plošč, ki zmanjšajo dostop UV svetlobe. S krajšanjem mladik in redčenjem grozdov smo spremenili razmerje med listno površino in obremenitvijo trte. V dveh poskusih smo preučevali tudi vpliv razlistanja in spremenjenega razmerja med listno površino in maso grozdja na kemijsko sestavo in senzorično zaznavo vina. Razlistanje vinske trte je vplivalo na manjše vsebnosti 3-izobutil-2-metoksipirazinov (IBMP) v grozdju ob trgatvi in ni imelo vpliva na vsebnost reduciranega glutationa (GSH) v grozdju. Večje IBMP vsebnosti so bile v jagodah večjega premera (15.5 mm v primerjavi s 13.5 mm) s podobno vsebnostjo skupne suhe snovi (TSS). Razlistanje vinske trte in spremenjeno razmerje med listno površino in maso grozdja vinske trte sta imeli statistično značilen vpliv na kemijsko sestavo in senzorično zaznavo vina. Zmanjšanje dostopa UV svetlobe je vplivalo na manjšo vsebnost 3-sulfanilheksan-1-ola (3SH) in 3-sulfanilheksil acetata in nekaterih estrov, ni pa imelo vpliva na vsebnost IBMP v vinu. Največje razmerje med listno površino in maso grozdja je vplivalo na značilno večjo vsebnost 3SH in 4-metil-4-sulfanilpentan-2-ona in na večjo senzorično zaznavo po pasijonki in mangu. Vinom iz obravnavanj brez razlistanja so bili pri senzorični analizi pripisani deskriptorji po zeleni papriki, kuhanem grahu in fižolu, vinom iz grozdja z razlistanih trt pa so bile pripisane bolj sadne arome, kot sta aromi po pasijonki in mangu. Takšen celovit pristop je lahko v pomoč vinarjem in vinogradnikom pri odločitvah o ustreznih vinogradniških ukrepih in praksah ter pri pridelavi želenega stila vina.

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Article 2: Šuklje K., Antalick G., Coetzee Z., Schmidtke L., Baša Česnik H., Brandt J., Du Toit W.J., Lisjak K., Deloire A. 2014. Effects of leaf removal and ultraviolet radiation in the vineyard on the composition and sensory perception of Sauvignon Blanc (*Vitis vinifera* L.) wine. Australian Journal of Grape and Wine Research (accepted in publication)

Article 3: Šuklje K., Baša Česnik H., Janeš L., Kmecl V., Vanzo A., Deloire A., Sivilotti P. and Lisjak K. 2013. The effect of leaf area to yield ratio on secondary metabolites in grapes and wines of *Vitis vinifera* L. cv. Sauvignon blanc. Journal International des Science de La Vigne et Du Vin, 47: 83-97

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ABBREVIATIONS AND SYMBOLS

UV	ultra-violet solar radiation
MPs	methoxypyrazines
IBMP	3-isobutyl-2-methoxypyrazine
IPMP	3-isopropyl-2-methoxypyrazine
GSH	reduced glutathione
HCAs	hydroxycinnamates
TSS	total soluble solids
TA	titrable acidity
3SH	3-sulfanyl-hexan-1-ol
3SHA	3-sulfanylhexyl acetate
4MSP	4-methyl-4-sulfanylpentan-2-one
PUFAs	polyunsaturated fatty acids
NTU	nephleometric turbidity unit
PAR	photosynthetically active radiation

1 INTRODUCTION

'Sauvignon blanc' (*Vitis vinifera* L.) is the world's second most planted white grapevine cultivar, and the third most planted white grapevine variety in Slovenia (Mavrič Štrukelj et al., 2012). It is a grapevine variety that is indigenous to either the Loire Valley or the Bordeaux area in France. The origin of the name is from the French words 'sauvage' (wild) and 'blanc' (white) (Galet, 1990; Larousse, 2011).

'Sauvignon blanc' is known by its small to medium-sized bunches and vigorous growth, which can alter the aromatic quality of the wines (Sweet, 2010). The cultivar started to spread around the world when producers recognised its aromatic potential, which varies on the basis of different geographical sources (Lund et al., 2009). Wines produced from this variety are commonly described as dry and crisp, and the flavour can vary from green to tropical (Allen et al., 1991; Lund et al., 2009; Benkwitz et al., 2012). Historically, the most renowned Sauvignon blanc wines originated from Sancerre (Loire Valley, France), a region famous for producing a dry, crisp, mineral style of Sauvignon blanc wines. Nowadays, 26,062 ha is planted under 'Sauvignon blanc' grapevines in France, presenting 3 % of the total area under grapevines (France AgriMer, 2013).

In the last two decades, Sauvignon blanc wines have become the flagship of New world wine countries. For example, New Zealand's wine industry, with annual wine exports amounting to 1,200 million NZ$, increased the area under vineyard from 5,980 ha in 1991 to 34,269 ha in 2012, 20,000 of which are planted with 'Sauvignon blanc' (New Zealand annual wine report for 2012, 2013).

In South Africa, 'Sauvignon blanc' is the third most planted white cultivar. South African wine exports increased from 21.6 % of total wine production in 1999 to 49.1 % in 2009, with 86.9 % of the total yearly Sauvignon blanc wine production being exported (South African wine industry statistics, 2010).

In contrast to New Zealand and South Africa, the area under vineyard in Slovenia decreased from 17,147 ha in 1996 to 16,372 ha in 2011, with an annual export amount in 2011 of 9.4 million EUR (Mavrič Štrukelj et al., 2012; Vinska družba Slovenije, 2013). However, the 'Sauvignon blanc' in Slovenia in 2012 was planted on 6.7 % of the total area under grapevines, from which 2,245.134 L of Sauvignon blanc wines have been produced (Mavrič Štrukelj et al., 2012; RPGV, 2012). A decrease in the renewal of Slovenian vineyards under the reproductive limit has been observed in the last decade. Moreover, Slovenian vineyards traditionally have been grown on slopes, enabling them to produce high-quality grapes and wines, but the cost of such production is high (Mavrič Štrukelj et al., 2012).

Internationally, the wine industry has become increasingly competitive, as many New world wine-producing regions are advancing as a result of low-cost labour and the

production of "typical" wine styles, driven by well-studied consumer preferences. The global wine market has become more competitive and the consumers' choices regarding wine style, quality and price have become greater than ever (Swiegers et al., 2006). The question that has to be addressed is what can be done in the vineyard and in the wine cellar to achieve excellence and to produce the most appreciated wine styles.

According to our knowledge there are very few studies establishing a link between vineyard management, grape and wine composition and sensory perception. However, it is well known that the quality of wine is affected mainly by the grape composition as produced in the vineyard (Jackson and Lombard, 1993). Viticultural practices performed in the vineyard could therefore be an effective tool for viticulturists and winemakers to target specific sensory characteristics to achieve a predicted wine style by altering the fruit composition and therefore quality (Ristic et al., 2007; Kozina et al., 2008; Lohitnavy et al., 2010; Gregan et al., 2012; Scheiner et al., 2012).

The distinctive varietal aromas of Sauvignon blanc wines are reported to arise from several aromatically highly potent compounds, such as methoxypyrazines (MPs) and varietal thiols (Allen et al., 1991; Tominaga et al., 1996; Dubourdieu et al., 2006; Roland et al., 2011). Other aromatic compounds, such as esters, C-13 norisoprenoids and terpenes, can also make a considerable contribution to the overall aromatic expression of Sauvignon blanc wines (Benkwitz et al., 2012). Distinct flavour characteristics of Sauvignon blanc wines can be expressed, depending on the climatic conditions during ripening and the viticultural practices applied in the vineyard (Lund et al., 2009; Gregan et al., 2012). However, the contribution of the winemaking process in the cellar must not be overlooked.

MPs are nitrogen-containing compounds contributing to green aroma descriptors such as green pepper, cooked beans, peas, asparagus, earthy, vegetative and herbaceous, and possess a low detection threshold of around 2 ng/L in water and white wine (Buttery et al., 1969; Seifert et al., 1970; Parliament and Epstein, 1973; Maga, 1989; Allen et al., 1991; Kotseridis et al., 1998). The most important two MPs found in grapes and wines are 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) (Lacey et al., 1991).

Unlike MPs, thiols are present in grape berries in a non-volatile form. During fermentation, 3-sulfanyl-hexan-1-ol (3SH) and 4-methyl-4-sulfanylpentan-2-one (4MSP) are partly released from cysteine and the glutathionyl-bound precursors, whereas 3-sulfanylhexyl acetate (3SHA) is produced by the acetylation of 3SH by the yeast (Swiegers et al., 2005; Dubordieu et al., 2006). 4MSP is reminiscent of box tree and black currant, whereas 3SH and 3SHA are reminiscent of fruitier aromas such as guava, grapefruit, mango, passion fruit and gooseberry (Tominaga et al., 1996; Swiegers et al., 2009; Coetzee and Du Toit, 2012).

Extensive research has been conducted on the composition of Sauvignon blanc must and wine, but the complex interaction among vineyard management, grape berry and wine composition need further research (Chapman et al., 2004; Swiegers et al., 2005; Patel et al., 2010; Roland et al., 2010). Therefore the aim of this dissertation was to study the effect of some viticultural practices on grape berry and wine chemical composition and wine sensory perception.

Leaf removal, shoot hedging and bunch thinning are classical viticultural practices that are frequently used to improve grape quality (Arnold and Bledsoe, 1990; Chapman et al., 2004). Leaf removal at the bunch zone increases light intensity, which in turn aids in decreasing the green, vegetative aromas derived from MPs (Ryona et al., 2008; Scheiner et al., 2010; Gregan et al., 2012) and accelerates malic acid degradation (Kliewer and Smart, 1989; Friedel et al., 2013). This practice can be performed between the beginning of flowering and véraison (Smart, 1985; Poni et al., 2008; Sternad Lemut et al., 2011). A slight decrease (of around 1 °Brix) of the total soluble solids concentration (TSS) in the grape berries was observed when the basal leaves of 'Gewürtztraminer' variety were removed a month after anthesis (Reynolds et al., 1996). Vilanova et al. (2012) showed that manual and mechanical leaf removal of 'Tempranillo', performed before flowering, increased the concentrations of TSS for 2 °Brix compared to the control treatment, whereas the effect of leaf removal at fruit set on TSS concentration was not as pronounced (Vilanova et al., 2012). This is in agreement with Kozina et al. (2008) and Friedel et al. (2013), who found that leaf removal performed at véraison had no significant effect on TSS concentrations. Leaf removal before flowering or at berry set had no effect on TA concentration or pH values (Vilanova et al., 2012). Further more, it has been observed that leaf removal performed at the phenological stage berry size expension and véraison had no effect on the concentrations of non-flavonoids in 'White riesling' grapes (Friedel et al., 2013). It has been reported that mechanical leaf removal resulted in a significantly higher volatile acidity in wines compared to manual leaf removal performed at the same time, i.e., 468 µg/L compared to 212 µg/L respectively. Interestingly, wines from leaf removal treatments performed before flowering could be separated by means of sensory analyses from wines where leaf removal was performed at berry set and from the control treatment (Vilanova et al., 2012). Furthermore Kozina et al. (2008) reported that the removal of eight basal leaves at véraison significantly improved the overall sensory quality of Sauvignon blanc wines, whereas such effect was not observed in Riesling wines. In addition, a threefold increase in the concentrations of free volatile terpenes was observed in Sauvignon blanc wines (74 µg/L compared to 253 µg/L, respectively), and the concentration of potentially volatile terpenes increased. In contrast, the reduction of free and potentially volatile terpenes in Riesling wines was observed when the vines were subjected to removal of leaves (Kozina et al., 2008).

Shoot hedging was reported to increase bunch weight by 30 g, berry weight for 0.1 g and TSS concentrations by 1 °Brix compared to the control in 'Cabernet sauvignon' grapes. Furthermore, a 10 % increase in total flavonoid and 3 % increase in anthocyanin

concentration have been observed (Pisciotta et al., 2007). Kliewer and Bledsoe (1987) found that shoot topping to 15 nodes in 'Cabernet sauvignon' variety significantly decreased the TSS accumulation by around 1 °Brix and the acid concentration at the first sampling date, and significantly increased the pH value by 0.02 during ripening. In addition, Peterson and Smart (1975) reported decreased TSS accumulation and skin pigmentation when shoots were hedged to the 6^{th} node, and an increase in TSS concentration and yield when shoots were hedged to the 10^{th} node from the cordon, clearly showing the complexity of the relationship between leaf area and fruit composition. Reynolds et al. (1996) found that for Gewürtztraminer wines the concentration of potentially volatile terpenes can be increased slightly by vine shoot hedging to the height of 14 remaining leaves on a shoot.

Bunch thinning can be mechanised or performed manually, and is usually carried out after fruit set or around véraison. It has been reported by Kennedy et al. (2009) that bunch thinning of 'Merlot' at pea berry size and véraison had no positive effect on berry anthocyanins, which were significantly lower when the bunches were thinned at berry pea size, i.e, 1.18 mg/g in control treatment compared to 0.97 mg/g. Furthermore, polyphenol levels decreased significantly, from 1.90 mg/g in the control compared to 1.66 and 1.77 mg/g in the bunch thinning treatments, respectively. In addition, bunch thinning at both time points decreased the sensory quality of the resultant Merlot wines (Kennedy et al., 2009). In another study it was shown that bunch thinning of 'Riesling' performed at véraison significantly increased the bunch weight and increased the concentration of TSS in the berries at harvest – 220 g/L compared to 205 g/L in the control treatment (Klopčič, 2009). Bunch thinning also increased the vegetative mouthfeel perception in Cabernet sauvignon wines (Chapman et al., 2004).

The reasons for the choice of 'Sauvignon blanc' as cultivar for this research project were:
- it is a highly planted worldwide variety for the production of white wine, with an increasing market and an increase in the range of different wine styles demanded by consumers (Swiegers et al., 2006; King, 2010),
- it is a cultivar with predominant wine aromas derived from the grape berry, therefore the produced wine style is largely dependent on the viticultural practices performed in the vineyard,
- studying the fruit's responses to abiotic factors (light quality and quantity and temperature) by applying different viticultural practices, should contribute to a better understanding of the impact of environmental conditions on grape berry growth and composition, the resultant wine composition and its sensory attributes.

This study was conducted in South Africa (Overberg region in the Western Cape Province) and in Slovenia (Vipavska dolina winegrowing region) over two consecutive years. The aim was to determine the influences of common viticultural practices, such as the removal of leaves and secondary shoots in the bunch zone at the peppercorn-size berry phenological stage (E-L 29) (Eichorn and Lorenz, 1977), bunch thinning and shoot hedging on the grape

berry growth and metabolites and wine composition of 'Sauvignon blanc' (*Vitis vinifera* L.) variety. Light quality and quantity and temperature in the bunch zone was modified with the removal of leaves and secondary shoots, whereas vine balance (leaf area to yield ratio) was modified by shoot headging. A lot of research has been conducted already on the effect of modified light and air temperature at the bunch zone, as well as modified leaf area-to-yield ratio on grape berry composition, whereas very few studies report data of interactions between viticultural practice and wine chemical and sensory composition. In addition to the removal of leaves and secondary shoots in the first year, fruit classification according to their diameter and TSS concentration was introduced into the experiment, providing new insights. Grape berry classification according to the concentration of TSS has already been utilised in several studies, although mainly on red cultivars studying anthocyanin extractability and skin break force (Rolle et al., 2009; 2012). Rolle et al. (2012) suggest that berry densimetric classification could be used to produce wines of different quality, which could also hold importance for the production of white wine cultivars. The literature on this topic is very limited and, to our knowledge, there are no reports on the effect of berry size (diameter) and TSS concentration on some metabolites of 'Sauvignon blanc' grapes. Therefore, concentrations of some metabolites in the berries of the same diameters and different TSS concentrtations were compared, as well in the berries of the similar TSS concentration and different diameters.

In the second year, during the experiment in South Africa, UV light-reducing sheets were installed in addition to the removal of leaves and secondary shoots. Nowadays, UV light radiation is frequently mentioned in relation to climate change (Jug and Rusjan, 2012). As far as we are aware, the first report on the effect of UV light on IBMP and IPMP concentrations in *Vitis vinifera* L. varieties was done recently by Gregan et al. (2012). The literature on the effect of UV light on grape berry metabolites is limited and, to our knowledge, our work in this thesis presents the first results on the effect of UV light (or deficiency of UV) on the concentrations of wine aromatic compounds and the related wine sensory perception.

The third part of the thesis was conducted in the Vipavska dolina winegrowing region and the aim was to investigate the effect of the leaf area-to-yield ratio on primary and secondary metabolites in grapes and wines of *Vitis vinifera* L. 'Sauvignon blanc'. The leaf area-to-yield ratio was modified by shoot hedging and bunch thinning. Studies that focus on the interaction of leaf area and yield have been conducted mainly on red cultivars and studied the effect of bunch thinning on the concentration of anthocyanins, polyphenols and certain parameters associated with technological maturity (TSS, TA and pH or ratio Brix/TA) (Peterson and Smart, 1975; Diago et al., 2010). Literature on the effect of bunch thinning and shoot hedging on wine composition and sensory properties is also limited, especially regarding white grapevine varieties (Lohitnavy et al., 2010).

Volatile compounds to be quantified in the grape berries and wines were chosen by their importance to the expression of “typical” Sauvignon blanc wine aroma, usually described

as tropical and/or green (Lacey et al., 1991; Tominaga et al., 1996; Swiegers et al., 2005; Dubourdieu et al., 2006; Benkwitz et al., 2012). The non-volatile compounds, such as reduced glutathione (GSH) and hydroxycinnamates (HCAs), are known to be of significant importance for preserving aromatic compounds in Sauvignon blanc wines (Du Toit et al., 2007; Janeš et al., 2010; Herbst-Johnstone et al., 2011; Kritzinger et al., 2012). It was shown recently that the nitrogen and amino acid composition of Sauvignon blanc must significantly affects the composition of the wine - the release of volatile thiols during fermentation is controlled by nitrogen catabolic repression (Thibon et al., 2008). Furthermore, must nitrogen status, amino acids and lipid composition affect the ester concentration in wines (Swiegers et al., 2005). It has been shown that higher GSH concentrations in Sauvignon blanc must before fermentation can result in a higher thiol concentration in the finished wines (Roland et al., 2010), whereas the contrary has been observed in a study by Patel et al. (2010). Detailed researches have been done on the role of yeast in the release of thiols and esters during fermentation (Swiegers et al., 2006; Miller et al., 2007; King et al., 2010; Jenko et al., 2012), and it has been shown that higher temperatures at fermentation favoured higher volatile thiol and ester concentrations in Sauvignon blanc wines (Masneuf-Pomaréde et al., 2006). In contrast to thiols and esters, MPs concentrations in finished wines are more grape dependent and are decreasing with difficulty in the cellar with standard wine making processes. The final MPs concentration in wines can be halfed by must settling to the turbidity of 200 NTU before fermentation, as reported by Roujou de Boubée (2001), as well as with the addition of bentonite to the must (Kotseridis et al., 2008). Recently, it has been reported that MPs concentrations in the must can be reduced by silicon cleaning without affecting the fermentation aromas of wines (Ryona et al., 2012). Therefore, based on previous reports on 'Sauvignon blanc' grapes, musts and wines, concentrations of IBMP and IPMP, GSH and HCAs and basic parameters of maturity were measured in the grapes, whereas the concentration of thiols, esters and MPs were quantified in the wines.

An overview of the present literature and the results of previous studies led us to the following hypotheses:

- the removal of leaves and secondary shoots influences grape berry composition;
- wine chemical composition and sensory perception can be influenced by different light exposures of the bunches and by deficency UV light;
- a modified leaf area-to-yield ratio results in changes in the grape berry metabolite concentrations, and consequently results in the changing of wine composition and therefore affect the sensory perception of the related wine ; and
- canopy manipulation could be a useful method to modify Sauvignon blanc wine style, changing fruit zone microclimate. However, opening canopy should be reasoned according to the climate:cool-temperate versus warm-hot.

The viticultural practices used in this study to modify the bunch microclimate are commonly used in various wine growing regions. The obtained results could be applicable to the industry, depending on climate and row orientation, and to certain extend irrigation.

The appropriate recommendations on the leaf and secondary shoot removal in the fruit zone should be given cautiously to avoid berry sunburn damage or berry shrivelling, or to avoid important loss of acidity and aroma of the grapes and in the related wines. Therefore the obtained results might be benefitial for the viticulturists and winemakers and will upgrade the knowledge of an appropriate canopy management as a tool to already modify Sauvignon blanc wine style in the vineyard to a certain extent.

2 SCIENTIFIC WORKS

2.1 CLASSIFICATION OF GRAPE BERRIES ACCORDING TO DIAMETER AND TOTAL SOLUBLE SOLIDS TO STUDY THE EFFECT OF LIGHT AND TEMPERATURE ON METHOXYPYRAZINES, GLUTATHIONE AND HYDROXYCINNAMATES EVOLUTION DURING RIPENING OF 'SAUVIGNON BLANC' (*Vitis vinifera* L.)

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Razvrstitev grozdnih jagod po premeru in vsebnosti skupne suhe snovi pri preučevanju vpliva svetlobe in temperature zraka na vsebnost metoksipirazinov, glutationa in hidroksicimetnih kislin med dozorevanjem grozdja sorte (*Vitis vinifera* L.) 'Sauvignon blanc'

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Grozdne jagode so bile razvrščene glede na premer in glede na vsebnost skupne suhe snovi (TSS) z namenom preučitve vpliva svetlobe in temperature zraka na vsebnost metoksipirazinov (MPs), reduciranega glutationa (GSH) in hidroksicimetnih kislin (HCAs) med dozorevanjem grozdov žlahtne vinske trte sorte 'Sauvignon blanc'. Izpostavljenost grozdov svetlobi je bila modificirana z odstranitvijo listov in stranskih mladik na višini 40 cm nad kordonom na strani listne stene vinske trte, ki jo sonce obseva zjutraj v fenofazi jagod velikosti poprovega zrna (E-L 29) (Eichorn in Lorenz, 1977). Za razvrstitev grozdnih jagod glede na premer smo uporabili plastične ploščice z velikostjo luknjic od 10,5 do 16,5 mm. Jagode istega velikostnega razreda so bile glede na vsebnost TSS, izmerjeno s flotacijo v vodnih raztopinah z različno vsebnostjo sladkorja, še dodatno razvrščene v različne razrede. Porazdelitev grozdnih jagod v različne velikostne razrede je ustrezala razporeditvi Gaussove krivulje, kar dokazuje homogeno razdelitev grozdnih jagod glede na velikost v tri najbolj zastopane razrede, kar navajajo tudi Deloire in sod. (2004) ter Rolle in sod. (2012). Vsebnost GSH se je povečevala z naraščanjem vsebnosti TSS v grozdni jagodi, med katerima smo dokazali dobro korelacijo ($R^2=0,888$). Vsebnost HCAs se je med dozorevanjem zmanjševala, ob prvem vzorčenju so bile vsebnosti HCAs med 170 in 280 mg/L, medtem ko so bile ob trgatvi med 114 in 137 mg/L. V primerjavi s kontrolo (brez razlistanja) je bila vsebnost 3-izobutil-2-metoksipirazina (IBMP) v grozdnih jagodah razlistanega obravnavanja dva tedna pred trgatvijo pod mejo zaznave (0,6 ng/L). Razlistanje ni imelo značilnega vpliva na vsebnost GSH in HCAs v grozdni jagodi ob trgatvi. Premer jagod je vplival na vsebnosti IBMP v grozdni jagodi, ni pa vplival na vsebnost GSH in HCAs ob trgatvi. Grozdne jagode podobne vsebnosti TSS in večje velikosti so imele večjo vsebnost IBMP, tako so jagode podobne vsebnosti TSS, velikosti 13,5 mm in 15,5 mm, vsebovale 5,2 ng/L in 12,6 ng/L IBMP. Z raziskavo smo pokazali obstoječo heterogenost v velikosti jagod in v vsebnosti TSS znotraj vinograda.

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Classification of Grape Berries According to Diameter and Total Soluble Solids To Study the Effect of Light and Temperature on Methoxypyrazine, Glutathione, and Hydroxycinnamate Evolution during Ripening of Sauvignon blanc (*Vitis vinifera* L.)

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Supporting Information

ABSTRACT: Grape berries were classified according to diameter and total soluble solids (TSS) to study the effect of light and temperature on methoxypyrazines (MPs), glutathione (GSH), and hydroxycinnamates (HCAs) during the ripening of Sauvignon blanc. The light exposure of the fruiting zone was modified within leaf and lateral removal at the phenological stage berry of peppercorn size and no removal (control). In comparison to the control, the concentration of 3-isobutyl-2-methoxypyrazine (IBMP) was below the limit of detection in leaf removal 2 weeks before harvest. Leaf removal had no significant influence on GSH and HCAs in the grape juice at harvest. Berry diameter significantly influenced the concentration of IBMP in the grape juice and did not influence the concentration of GSH and HCAs. At harvest, the concentrations of IBMP in grape juices of similar TSS in the control were 12.6 and 5.2 ng/L in 15.5 and 13.5 mm berry diameter classes, respectively. Furthermore, the study showed that berries of the same diameter were not at the same physiological ripening level (not the same TSS).

KEYWORDS: *Sauvignon blanc, grape berry diameter, total soluble solids, light, temperature, methoxypyrazines, glutathione, hydroxycinnamates*

INTRODUCTION

Grape berry growth and maturity are characterized by asynchrony between the berries within a bunch and between bunches within the vine. Therefore, fruit classification methods are implemented to minimize berry heterogeneity and provide possible trends in the metabolism of major berry compounds.[1−4] Fruit classification according to the diameter and total soluble solids (TTS) has already been utilized in several studies, although mainly in relation to red cultivars. It has been used to enhance the understanding of grapevine fruit growth and the associated biochemical composition.[4−6]

The green aroma descriptors of Sauvignon blanc wines originate from 3-alkyl-2-methoxypyrazines (MPs), whereas volatile thiols are responsible for the tropical characteristics of the wines.[7−9] The most important MPs found in grapes and wines are 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP). IBMP contributes to the green pepper and asparagus aromas, whereas IPMP imparts earthier aromas.[10−13] The sensory detection threshold for IBMP was found to be very low, around 2 ng/L in water, 8 ng/L in Sauvignon blanc wines, and 15 ng/L in red Bordeaux wines.[10,13,14] High IBMP concentrations in grapes may have a negative impact on the quality of the wine aroma.[13]

Abiotic factors such as light and temperature at the bunch level, vine water status, and various viticulture practices can influence the concentration of MPs in the berry and wine.[15−17] It has been shown that grapes and wines from cooler climatic regions contain higher concentrations of IBMP than grapes produced in warm regions.[18] In addition, pre-véraison bunch exposure to sunlight can reduce IBMP concentrations in grapes at harvest. However, bunch exposure after véraison is reported to have little effect.[16,17]

Glutathione (GSH) and hydroxycinnamates (HCAs) are important antioxidants that preserve freshness in white wines.[19] GSH is a tripeptide composed of glutamic acid, cysteine, and glycine, which exists in a reduced or oxidized form. Its concentration ranges from 14 to 102 mg/L in grapes and up to 35 mg/L in wines.[20,21]

In grape berries, GSH synthesis starts with sugar accumulation in the berry, whereas HCAs are synthesized as early as berry formation begins. Adams and Liyanage have shown that there is a close correlation between GSH and TSS concentration until the berries reach 16 °Brix and that GSH concentration increases on a per berry basis.[22] During the oxidation of white must, the caftaric acid *O*-quinone, included in the browning of white wines, can be reduced by GSH (if present), resulting in the production of colorless 2-*S*-glutathionyl caftaric acid, also called grape reaction product (GRP).[23] Furthermore, GSH is required for the synthesis of glutathione-3-mercaptohexan-1-ol, one of the precursors of the prominent varietal thiol 3-mercaptohexan-1-ol (3MH). This

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compound imparts passion fruit aroma and plays a central role in the aromatic typicity of Sauvignon blanc wines.[24,25] GSH preserves the aromatic potential of white wines, especially varietal thiols and esters, and participates in the reversible redox reaction of the thiol group.[26,27]

HCAs found in grapes are *cis*- and *trans*-forms of caftaric, coutaric, and fertaric acids, which are tartaric esters of hydroxycinnamic acids: caffeic, *p*-coumaric, and ferulic acids, respectively. They are the major class of nonflavonoid phenolics in white wines. The free forms of HCAs appear in wine due to the hydrolytic activity of yeasts and/or grape enzymes or due to acid hydrolysis in the wine.[28] The concentration of HCAs in wines ranges from 80 to 166 mg/L.[28] Cultivars with high HCAs but low GSH concentrations have increased browning potential when exposed to oxygen.[29,30]

Internationally, including South Africa, Sauvignon blanc is an important cultivar. Although numerous studies have been conducted on this cultivar,[13,24,31−33] there are still pending questions regarding the physiology of ripening and the metabolism of aromatic precursors and their preservatives.

The aim of this work was to study the influence of bunch microclimate (light and temperature) on the evolution of MPs, GSH, and HCAs in Sauvignon blanc grapes during ripening. To understand the differences in grape berry quality within the vineyard, grape berries were classified according to their diameter and thereafter according to their TSS concentration. Such classifications provided a novel approach for studying the dynamics of MPs, GSH, and HCAs during the ripening of *Vitis vinifera* L. Sauvignon blanc grape berries.

MATERIALS AND METHODS

Experimental Vineyard. A commercial vineyard located in the Overberg region of the western coastal area, South Africa (E 19° 1′ 68″, S 34° 9′ 52.76″), was used in this study. The experiment was performed on Sauvignon blanc vines (*V. vinifera* L.), clone 316, grafted onto rootstock 101.14. The row orientation was northwest−southeast (2.5 m × 1.8 m) and the training system was vertical shoot positioning, pruned within a double cordon with two buds per spur. Canopy management was done by hedging the vines at a height of 1.4 m and lateral shoot cutting to maintain the width of the canopy at 40 cm. Irrigation was managed to avoid water constraints and was monitored by using a pressure chamber and measuring the stem water potential.[34,35]

The light exposure of the fruiting zone was modified within leaf and lateral removal (leaf removal) and no removal (control). The experimental design consisted of four rows with four replications of leaf removal and four replications of control per row. Each replicate consisted of four contiguous vines. Replicates were randomized in a block layout. In the leaf removal treatment leaves and laterals were completely removed from the bunch zone at a height of 40 cm from the cordon on the morning (eastern) side of the canopy, which resulted in 100% exposed bunches from the eastern side. Leaf and lateral removal was performed on December 17, 2010, at berry peppercorn size (E-L 29).[36] Bunches in the leaf removal treatment were 100% shaded from the afternoon (western) side of the canopy. The control consisted of 100% shaded bunches from both sides of the canopy, which was possible to realize due to the thickness of the canopy at the bunch zone.

Sampling Protocol. For the control, one bunch per vine was sampled randomly from the inside of the canopy. For the leaf removal treatment, only fully exposed bunches were sampled. For each sampling date, 40 bunches were collected per treatment. Three developmental stages were analyzed, at véraison on January 25 (E-L 35), 4 weeks after véraison on February 21 (E-L 37), and at harvest on March 1. Véraison was determined at the time when 50% of the berries were soft.

Bunch samples were kept in a cooling box and transported to the laboratory. To prevent oxidation, all berries from sampled bunches were carefully cut at the torus with a pair of scissors. The total number of berries in a sample of 40 bunches was counted, and it ranged between 2033 and 2935. Berries were classified according to their diameter using special Perspex plates. Each classification plate contained holes of different diameters from 10.5 to 16.5 mm, increasing at 1 mm intervals. Berry classification started with the classification of the largest diameter and continued to the smallest to obtain different berry size classes. Berries in each diameter class were counted, and the distribution percentage was established.

For the second classification, according to TSS concentration, two of the most representative diameter classes with at least 2 mm difference were used. Berry TSS concentration was estimated by flotation in sucrose solutions of different concentrations (from 80 to 260 g/L $C_{12}H_{22}O_{11}$).[37] The difference in density of two consecutive sucrose solutions was 10 g/L. Berries were classified, depending on the sampling date, in five to eight TSS classes. Berries of the same diameter were floated in the sucrose solution, starting with the least dense. The floated berries were considered to have the same TSS concentration as the solution. These berries were separated from the others, rinsed with water, dried, and counted. The sunken berries were collected and placed into the following, denser solution. The same procedure was repeated for all sucrose solutions. For each of the most representative berry diameter class, two classes of berries were selected according to TSS classification, with a TSS concentration difference of at least 2 °Brix. Grape berries belonging to each TSS class were counted, and their distribution percentage was established. All of the berries were inspected visually before analyses to exclude oxidation and thereby influence GSH and HCAs concentrations. These sorting methods, which were also used by other authors,[1,2,4,37,38] strongly reduce the biological heterogeneity between berry classes, which leads to replicates not being obtained.

Photosynthetic Active Radiation, Vine Water Status, and Temperature Measurements. Photosynthetic active radiation (PAR) (μmol m^{-2} s^{-1}) was measured with the Accupar PAR/LAI ceptometer, model LP-80 (Decagon Devices Inc., Pullman, WA, USA). The light sensor rod was placed parallel to the cordon to mimic the light interception of the bunches. The vine water status (Ψ_{SWP}) was measured using the stem water potential method.[34,35] Light intensity measurements were performed at 10 a.m., whereas stem water potential was measured between 12 and 2 p.m., parallel with the sampling dates. The temperature was monitored continuously inside the canopy and at the bunch-berry levels using Gemini data loggers TGP-4500 and TGP-4520, respectively (Chichester, UK).

The total soluble solids and L-malic acid concentration were determined according to standard methods.[39] A subsample of 200 berries was taken per class. Berries were weighed, transferred into a plastic bag, and crushed by hand, and the juice was collected for analyses. TSS was measured using a digital refractometer (Atago PAL-1, Tokyo, Japan) with temperature correction. The L-malic content was determined spectrophotometrically (Agilent 8453, Palo Alto, CA, USA) using enzymatic kits (Megazyme, Ireland).

Determination of Methoxypyrazines. *Preparation of Standards and Solvents.* IBMP (Sigma-Aldrich, St. Louis, MO, USA) with a purity of 99%, 2-isobutyl-3-methoxy-d_3-pyrazine ([$^2H_3$]-IBMP) (C/D/N/Isotopes, Quebec, Canada) with a purity of 99%, and IPMP (Sigma-Aldrich) with a purity of 99% were used for the preparation of standards in solvent. Stock solutions of IBMP (250 mg/L), [$^2H_3$]-IBMP (500 mg/L), and IPMP (280 mg/L) were prepared in methanol (Sigma-Aldrich). Intermediate solutions (IBMP = 2.5 mg/L, [$^2H_3$]-IBMP = 5.0 mg/L, and IPMP =2.8 mg/L) and working solutions (IBMP = 2.5 μg/L, [$^2H_3$]-IBMP = 5.0 μg/L, and IPMP = 2.8 μg/L) were prepared in methanol as well.

Preparation of Sugar Solution. Five hundred milliliters of water purified by a Milli-Q system (Bedford, MA, USA) was placed in a 1000 mL volumetric flask. Ninety grams of fructose (Sigma-Aldrich), 90 g of glucose (Sigma-Aldrich), and 1 g of tartaric acid (Merck, Darmstadt, Germany) were added and dissolved. The volumetric flask was made

Table 1. Standard Deviation and Measurement Uncertainty of the Method for Determining Methoxypyrazines (ng/L)

	dearomatized must		Sauvignon blanc from Slovenia			Sauvignon blanc from New Zealand		
	IBMP	IPMP	IBMP	IBMP	IPMP	IBMP	IBMP	IPMP
spiking level	9.8	10.0		25.0	25.0		25.0	25.0
means of the levels	10.9	10.3	1.9	20.3	21.0	6.9	23.3	20.2
standard deviation of repeatability (s_r)	0.6	0.4	0.1	0.5	0.5	0.3	0.6	0.7
relative standard deviation of repeatability (RSD_r) (%)	5.6	3.5	6.6	2.5	2.4	4.3	2.7	3.5
standard deviation of reproducibility (s_R)	1.5	0.7	0.5	1.3	1.1	1.0	1.6	1.7
relative standard deviation of reproducibility (RSD_R) (%)	14.1	6.9	25.9	6.5	5.4	15.0	7.0	8.6
uncertainty of repeatability (U_r)	1.4	0.8	0.3	1.2	1.1	0.7	1.4	1.6
relative uncertainty of repeatability (%)	14.2	8.3	15.0	4.7	4.6	9.6	5.8	6.5
uncertainty of reproducibility (U_R)	3.5	1.6	1.1	3.0	2.6	2.3	3.7	3.9
relative uncertainty of reproducibility (%)	35.5	16.2	58.7	11.9	10.3	33.9	14.7	15.6

up to volume with purified water, and the pH was adjusted to 3.2 with NaOH.

Dearomatization of Grape Juice. Forty-five milliliters of Sauvignon blanc juice was placed in the 50 mL tube and centrifuged for 5 minutes at 5000 min^{-1}. The liquid was then transferred to a 5 L flask; 3 L of previously centrifuged Sauvignon blanc juice was evaporated under reduced pressure to approximately 90% of the initial volume. The evaporated liquid was replaced by purified water. Afterward, the juice was transferred to a beaker and heated until it reached 80 °C to evaporate or decompose the MPs still present in the juice.

Preparation of Alcoholic Solution. Five hundred milliliters of purified water, 120 mL of absolute ethanol (Sigma-Aldrich), and 1 g of tartaric acid were added to a 1000 mL volumetric flask. The volumetric flask was then made up to volume with purified water, and the pH was adjusted to 3.2 with NaOH.

Preparation of Calibration Standards. Calibration standards were prepared in a sugar solution, an alcoholic solution, and a dearomatized must using working solutions of IBMP, [$^2H_3$]-IBMP, and IPMP. Some sugar solution, alcoholic solution, or dearomatized must was transferred to a 25 mL volumetric flask, [$^2H_3$]-IBMP, IBMP, and IPMP were added, and then the flask was made up to the volume to reach the final concentration of 25 ng/L of [2H3]-IBMP and IBMP and 28 ng/L of IPMP. NaCl was placed into a 20 mL SPME vial along with a stir bar, followed by 1.6 mL of the prepared solution, 6.4 mL of purified water, and 2 mL of 4 M NaOH. The vial was closed and placed onto a magnetic stir plate to dissolve the NaCl.

Preparation of Sample. The grape juice sample was prepared by hand-crushing undamaged berries in a plastic bag for 2 min. Some strained grape juice was transferred to a 25 mL volumetric flask, 125 μL of [$^2H_3$]-IBMP (internal standard) was added with a concentration of 5 μg/L, to reach the final concentration 25 ng/L of [$^2H_3$]-IBMP, and the flask was made up to the volume with grape juice. NaCl was placed into a 20 mL SPME vial along with a stir bar, followed by 1.6 mL of the prepared sample, 6.4 mL of purified water, and 2 mL of 4 M NaOH. The vial was closed and placed onto a magnetic stir plate to dissolve the NaCl.

Apparatus and Determination Procedure. The samples were analyzed using a gas chromatograph (Agilent Technologies 7890A, Shanghai, China) equipped with a Gerstel MPS2 multipurpose sampler (Gerstel, Mülheim an der Ruhr, Germany) and two successively connected columns, an HP 1 MS (Agilent Technologies, 30 m, 0.32 mm i.d., 0.25 μm film thickness) and an HP INNOWAX (Agilent Technologies, 30 m, 0.32 mm i.d., 0.25 μm film thickness), with a constant flow of helium at 1.5 mL/min. The vial was incubated for 5 min at 40 °C. The extraction on fiber DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) was performed for 40 min at 40 °C with constant stirring at 250 min^{-1}. The injector was held at 250 °C for 3 min for the analytes to desorb from the fiber.

The GC oven was programmed as follows: 60 °C for 10 min, from 60 to 100 °C at 7 °C/min, held at 100 °C for 10 min, from 100 to 170 °C at 7 °C/min, from 170 to 230 °C at 40 °C/min, held at 230 °C for 20 min, from 230 to 60 °C at 40 °C/min, and held at 60 °C for 3 min.

For the determination of analytes, a mass spectrometer (Agilent Technologies 5975C, upgraded with a triple-axis detector, Palo Alto, CA, USA) was used. The temperature of the ion source was 230 °C, the auxiliary temperature was 250 °C, and the quadrupole temperature was 150 °C. For qualitative determination, retention time and mass spectrum in selective ion monitoring mode (SIM) were used.

The mass channel was m/z 137 and 152 for IPMP, m/z 124 and 151 for IBMP, and m/z 127 and 154 for [$^2H_3$]-IBMP. Ions 137, 124, and 127 were the target ions used for quantification, whereas 152, 151, and 154 were used as qualifier ions. Calibration was performed with calibration standards in sugar solution for must and in alcoholic solution for wine. Linearity was verified by using spiked samples of dearomatized must and alcoholic solutions for wine (four repetitions for one concentration level, nine concentration levels for the calibration curve). Linearity and range were determined by multiple linear regressions, using the F test.

Calibration curves were derived using increasing amounts of IBMP (1−196 ng/L) and IPMP (1−200 ng/L) spiked in a dearomatized must, a sugar solution, and an alcohol solution. Good linearity was obtained for both analytes: IBMP (R^2 for dearomatized must was 0.9996; for sugar solution, 0.9991; and for alcohol solution, 0.9986) and IPMP (R^2 for dearomatized must was 0.9992; for sugar solution, 0.9981; and for alcohol solution, 0.9985).

The limit of detection (LD) and the limit of quantification (LQ) were calculated from the calibration curve. For IBMP, the LD of the dearomatized must was 0.6 ng/L, and for the alcohol solution it was 0.4 ng/L. The LQ for IBMP was 2.0 ng/L for the dearomatized must and 1.2 ng/L for the alcohol solution. For IPMP the LD of the dearomatized must was 0.6 ng/L, and for the alcohol solution it was 0.5 ng/L. The LQ for IPMP was 2.1 ng/L for the dearomatized must and 1.6 ng/L for the alcohol solution.

For the determination of precision,[40] that is, repeatability and reproducibility, a spiked sample of dearomatized must and two unspiked and two spiked samples of wine (Sauvignon blanc from Slovenia and Sauvignon blanc from New Zealand) were analyzed. Within a period of 10 days, two parallel samples of must and three of wine were analyzed each day. The standard deviation of repeatability (r) of the level and the standard deviation of reproducibility (R) of the level were both calculated. The results are given in Table 1.

The uncertainty of repeatability and uncertainty of reproducibility were calculated by multiplying the standard deviation of repeatability and standard deviation of reproducibility by Student's t factor for 9 degrees of freedom and a 95% confidence level ($t_{95,9}$ = 2.262). The results are presented in Table 1.

Trueness was verified by checking the recoveries. Recoveries were calculated from concentrations of samples used for the precision and uncertainty evaluation. The average of the recoveries was calculated. The results are given in Table 2.

Determination of Glutathione. Intact grape berries (200 berries) were carefully cut at the torus with scissors, transferred to a bag, purged with nitrogen for 5 min to reduce oxidation, and crushed manually. After crushing, the grape juice was immediately placed in

Table 2. Recoveries of the Method for the Determination of Methoxypyrazines

	dearomatized must		Sauvignon blanc from Slovenia		Sauvignon blanc from New Zealand	
	IBMP	IPMP	IBMP	IPMP	IBMP	IPMP
spiking level (ng/L)	9.8	10.0	25.0	25.0	25.0	25.0
recovery (%)	111.3	103.2	81.2	84.2	93.3	80.7
RSD (%)	13.8	6.8	6.3	5.3	6.8	8.3

methanol (1:10), with *N*-acetyl-L-cysteine as the internal standard, filtered through 0.45 μm Minisart RC 25 filters Sartorious (Goettingen, Germany), diluted 1:1 with a 5 mM sodium acetate buffer containing 0.1 mM EDTA, and immediately analyzed as previously described.[21] The concentration of GSH was determined by an Agilent Technologies 1200 HPLC with fluorescence detection with online precolumn derivatization, controlled by Agilent Chemstation Rev. B.03.01 from Agilent Technologies (Palo Alto, CA, USA) as previously described.[21] Briefly, separation was performed at 25 °C using a Synergi Fusion-RP 80A column (4 μm, 150 mm × 2.0 mm i.d.) from Phenomenex (Torrance, CA, USA).

The mobile phase consisted of (A) 50 mM sodium acetate buffer, pH 5.7, and (B) methanol, and the injection volume was 9 μL. The wavelength for the excitation was 340 and 450 nm for the emission. A nine-point calibration curve for standard GSH was linear over the injected range (0.2−60 mg/L) with a correlation coefficient of 0.9984.

Determination of Hydroxycinnamates. Undamaged fresh berries were cooled to 5 °C and crushed in the inert atmosphere, flushed with nitrogen for 5 min. After hand pressing in an inert atmosphere, the juice was collected, and 1000 ppm SO_2 was added to inhibit enzymatic activity. Grape juice was filtered through a 0.45 μm Millipore PVDF filter (Bedford, MA, USA) into a HPLC vial and directly injected. An Agilent Technologies 1100 HPLC with DAD connected to an Agilent NDS ChemStation was used for the detection and quantification of HCAs in grape juice as described previously.[28] The method was developed for monitoring *cis*- and *trans*-caftaric acid, coutaric acid, and fertaric acid, respectively, together with caffeic, *p*-coumaric, and ferulic acid and also a glutathione derivative of caftaric acid (GRP). Briefly, separation was performed on a 250 × 2.1 mm, 5 mm, ODS Hypersil C18 column connected to a 20 × 2.1 mm, 5 mm, ODS Hypersil guard column (Thermo Scientific). The mobile phase consisted of (A) 0.5% formic acid in water and (B) 2% formic acid in methanol, and gradient was carried out as described, [28] only here the injection volume was 10 μL. Compounds were identified by their UV−vis spectra and retention times. The quantification of compounds was based on peak areas at λ = 320 nm, and the respective concentrations in samples were expressed as *trans*-caftaric acid equivalents. A calibration curve was prepared by injecting a standard of *trans*-caftaric acid in the range from 1.05 to 500 mg/L. It was linear over the injected range with a correlation coefficient of 0.9999. The LD of *trans*-caftaric acid was 0.05 mg/L, whereas the LQ was 0.17 mg/L. To assess the repeatability of the method, 121 mg/L of standard *trans*-caftaric acid solution and a sample of grape juice were sequentially injected (both N = 10), and the relative standard deviations of repeatability RSD_r were 0.19 and 3.7%, respectively.

Data Analysis and Statistical Methods. Physiological and morphological heterogeneity in the sample was largely diminished by berry classification according to diameter and a second classification according to TSS concentration. The measured sample was homogeneous, containing berries of the same diameter and same maturation level in terms of TSS concentration. Regressions and correlations were performed with Origin 6.1 (OriginLab Corp., Northampton, MA, USA). The linearity and range of the method were determined by linear regression, using the F test. Student's t test was used for the calculation of standard deviation of the reproducibility and repeatability of the method, using Statgraphics Centurion XVI (StatPoint Technologies, Warrenton, VA, USA).

RESULTS AND DISCUSSION

Photosynthetic Active Radiation, Vine Water Status, and Temperature Measurements. The vine water measurements showed that there were no water constraints during the growing season, including during the ripening period. The stem water potential values were invariably between −0.4 and −0.45 MPa.[35,41] The mean PAR for the leaf removal was around 150 μmol m^{-2} s^{-1}, and for the control it was around 50 μmol m^{-2} s^{-1}. Therefore, the PAR at the bunch level in the leaf removal was 3 times higher than in the control, with a mean ambient PAR of 1000 μmol m^{-2} s^{-1}. The evolution of daily mean temperature during the ripening period (from January to March 2011) showed the highest temperature for bunches in the leaf removal from 10 a.m. to 2 p.m. On a daily basis, from mid-day onward the cooler wind from the Atlantic Ocean decreased the temperature of the bunches in leaf removal through the sea breeze effect (Figure 1).[42] For both treatments the mean

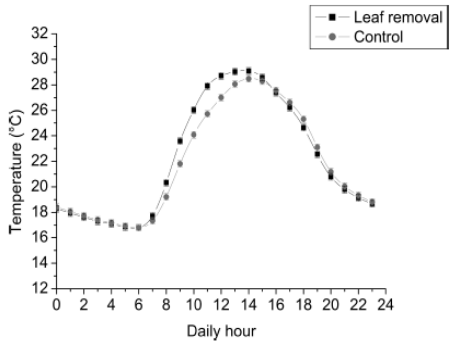

Figure 1. Mean hour temperature (°C) over the ripening period from January to March, $p < 0.05$.

temperature never exceeded 30 °C, which is the upper limit of the optimal physiological response threshold.[43] From 2 p.m. until 7 a.m. the following day, the bunch temperature of the leaf removal was slightly lower than that of the control. The coolest temperatures for both treatments were seen at 6 a.m., around sunrise, whereas the highest temperature was observed at 1 p.m.

Berry Classification. The distribution percentages of grape berries in different diameter classes during maturation are presented in Figure 2. The figure shows that the grape berries

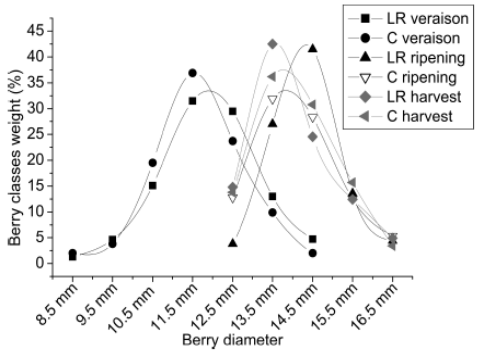

Figure 2. Distribution of Sauvignon blanc grape berries (%) in different diameter classes for all sampling dates, for leaf removal (LR) and control (C).

were distributed evenly along a Gaussian bell-shape curve for all three sampling dates, which confirmed the homogeneous distribution of the berries across the three major berry classes. These three major classes of berry diameter represented 80–85% of the berry population. This indication of low berry heterogeneity suggests that the vines did not experience any abiotic or biotic constraints.

Total Soluble Solids. The average TSS concentrations at harvest for the leaf removal and control treatments were 23.2 and 22.3 °Brix, respectively. A strong positive correlation was found between berry diameter and TSS accumulation on a per berry basis (R^2 = 0.96), irrespective of sun exposure and berry temperature (Figure 3). TSS accumulation per berry, in parallel

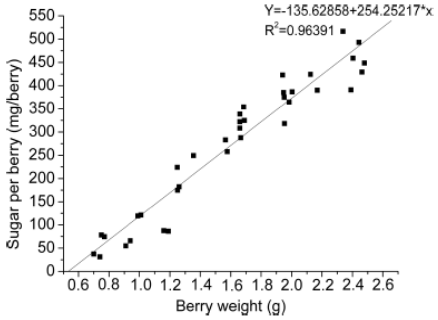

Figure 3. Relationship between sugar per berry (mg/berry) and average berry fresh mass (g) for each maturity class. The regression coefficient is calculated for all maturity classes. Regression coefficient R^2 = 0.96, p < 0.05.

with an increase in berry diameter, was continuous during the ripening period, which is related to the absence of vine water or other abiotic constraints.[41,44,45] Previous works have shown that there is a positive relationship between berry dry mass accumulation and °Brix increase up to a value of around 24 °Brix, varying on the basis of the cultivar and the production region.[1] In our study, a close correlation between berry diameter and TSS concentration was observed until 20 °Brix (data not shown). Berries of the same diameter had different TSS concentrations, which was in concurrence with previous results.[35] This is consistent with the functional link between berry sugar accumulation, fruit transpiration, and berry water accumulation, in the context of the position of a specific berry in a bunch and the related evaporative demand.[3,46] Berry sugar accumulation could also be controlled at the fruit level by the functioning of sucrose and hexose transporters.[47]

Methoxypyrazines. The concentrations of IBMP and IPMP were analyzed in grape juice from berries classified according to diameter and TSS concentration. In the leaf removal, the IPMP concentration was already below the LD at véraison, whereas it ranged from 4.1 to 2.3 ng/L in the control (data not shown). For the second and final sampling date, IPMP was not detected in either treatment (LD = 0.6 ng/L). IBMP was found in all of the berry diameter classes at véraison, irrespective of the grape light exposure, with concentrations ranging from 4.0 to 72.4 ng/L (data not shown). For the second sampling date (4 weeks after véraison) in all of the berry diameter classes, IBMP levels were under the LD in the berries from the leaf removal treatment. IBMP concentrations in grape juice from both treatments during ripening in the two most representative diameter classes are shown in Figure 4. In

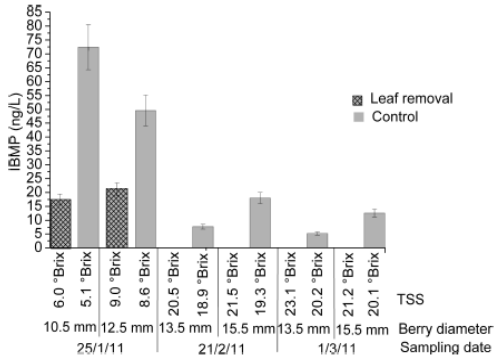

Figure 4. Effect of berry diameter (mm) and light exposure on 3-isobutyl-2-methoxypyrazine (IBMP) concentration (ng/L) in grape juice for the sampling dates January 25 (véraison), February 21 (4 weeks after véraison), and March 1 (harvest). Where no bars are shown, IBMP concentrations were below the LD. Error bars represent tolerance values for IBMP ± 2RSD$_r$ (%). At each sampling date two of the most representative diameter classes with 2 mm difference are shown.

agreement with other studies, our results indicate that bunch light exposure has a significant impact on IBMP concentrations in berries.[10,15,16] Berry diameter significantly influenced the concentration of IBMP in grape juice. At harvest, the concentrations of IBMP in grape juice of similar TSS in the control were 12.6 and 5.2 ng/L in 15.5 and 13.5 mm berries, respectively (Figure 4). Interestingly, at véraison the highest concentration of IBMP (72.4 ng/L) was found in the grape juice from berries of smaller diameter (10.5 mm) (Figure 4). However, at véraison TSS concentrations for berries of 10.5 and 12.5 mm diameter were not the same, that is, 5.1 °Brix compared to 8.6 °Brix. The IBMP concentration in grape juice of the control was significantly influenced by TSS concentration (Figure 5). At harvest the IBMP concentration in grape juice was below the limit of detection in berries with higher TSS concentration, whereas IBMP was still present in berries with lower TSS concentration, at the same berry diameter (Figure

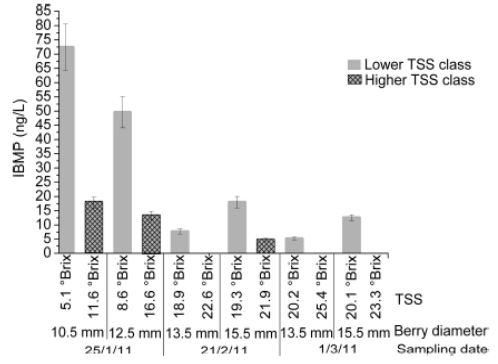

Figure 5. Effect of total soluble solids (TSS) (°Brix) on 3-isobutyl-2-methoxypyrazine (IBMP) concentration (ng/L) in grape juice for the control during the course of the ripening: January 25 (véraison), February 21 (4 weeks after véraison), and March 1 (harvest). Where no bars are shown, IBMP concentrations were below the LD. Error bars represent tolerance values for IBMP ± 2RSD$_r$ (%). At each sampling date two of the most representative diameter classes with 2 mm difference are shown.

5). This indicates that the °Brix level in unison with berry diameter strongly influences the IBMP concentration in grape juice. In our study the IBMP concentration was below LD (0.6 ng/L) when berries reached 20.2 °Brix, irrespective of the treatment.

Methoxypyrazines and L-Malic Acid. A good correlation (R^2 = 0.74) was observed between the breakdown of IBMP and L-malic acid during ripening (data not shown), which is in agreement with results found in Cabernet Sauvignon and Merlot.[10] The IBMP and L-malic acid concentrations in our study were determined in grape juice, which can explain the lower correlation compared to that of Roujou de Boubée et al., who determined IBMP concentration in whole berries.[10] These results clearly demonstrate that IBMP is quickly extracted from the skins into the grape juice.[48] The evolution and response to light and temperature exposure of IBMP and L-malic acid are distinct.[15] The IBMP concentration in grapes is related to light and temperature,[11] whereas the concentration of L-malic acid is more related to temperature.[49] The correlation between the concentrations of L-malic acid and IBMP needs further investigation; therefore, at this stage one compound could not be used to predict the degradation of the other.

Glutathione. Leaf removal had no significant effect on GSH concentration during ripening; as well, no significant effect of berry diameter was found (Supporting Information, Figure S1). A clear increase in GSH concentration in parallel with an increase in TSS was observed at véraison, whereas the concentration of GSH did not differ significantly between the different berry TSS classes at harvest for most representative diameters (Supporting Information, Figure S2). Studies of gene expression at the beginning of grape maturation showed that glutathione-*S*-transferase exhibits the same expression profile as the enzymes responsible for anthocyanin accumulation, which are strongly related to sugar accumulation.[50] This might explain the sudden increase in GSH concentration after véraison. A strong positive correlation (R^2 = 0.89) was observed between GSH and °Brix from 5.1 to 25.4 °Brix (Figure 6), whereas other

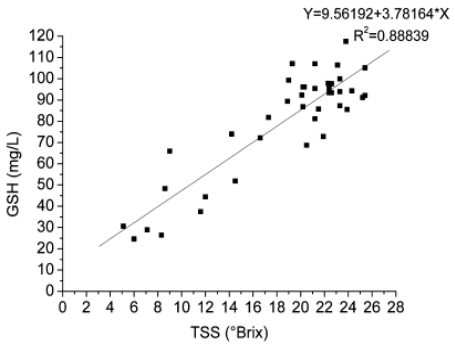

Figure 6. Relationship between glutathione (GSH) concentration in grape juice (mg/L) and total soluble solids (TSS) concentration (°Brix). The regression coefficient is calculated for all maturity classes. Regression coefficient R^2 = 0.89, p < 0.05.

studies observed a correlation between the concentration of GSH and TSS, up to 16 °Brix.[22] It should be noted that there was a strong correlation (R^2 = 0.95) between GSH content and TSS on a per berry basis (Figure 7), which shows that the increase in GSH on a per berry basis follows that of TSS.

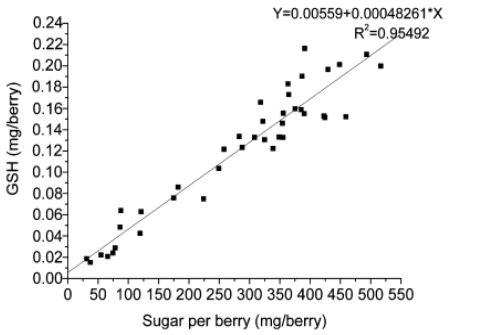

Figure 7. Relationship between glutathione (GSH) (mg/berry) and sugar per berry (mg/berry). The regression coefficient is calculated for all maturity classes. Regression coefficient R^2 = 0.95, p < 0.05.

Hydroxycinnamates. The HCAs concentration decreased with increasing °Brix, as seen in Figure 8. A decrease in HCAs

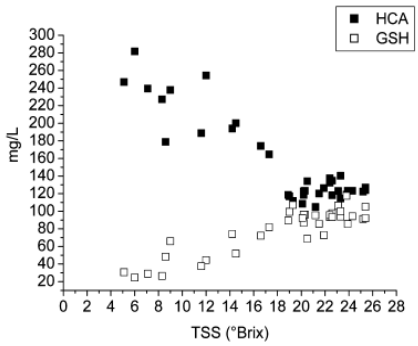

Figure 8. Relationship between concentration of total soluble solids (TSS) (°Brix), hydroxycinnamates (HCAs) (mg/L of caftaric acid), and glutathione (GSH) (mg/L) in grape juice, respectively.

concentration in grape juice occurred mainly due to the decrease in the concentrations of caftaric and coutaric acid (data not shown). A significant decrease in HCAs concentration occurred between véraison and the second sampling date (4 weeks after véraison) in both leaf removal and control, whereas the changes were not significant later (Supporting Information, Figure S3). This is in accordance with Singleton et al., who showed that HCAs concentration did not change significantly with grape maturation.[51] With the exception of sampling at véraison, later leaf removal had no significant effect on HCAs concentration in grape juice; as well, no significant effect of berry diameter was found in both treatments (Supporting Information, Figure S3). It could be that leaf removal in our study was not applied early enough to influence the concentration of HCAs at the harvest. The obtained results are in accordance with a study conducted on Pinot noir, in which it was demonstrated that leaf removal at berry set was very effective in enhancing the concentrations of caftaric and coutaric acids throughout the maturation, whereas leaf removal at véraison had no significant effect.[52] Furthermore, with the exception of sampling at véraison, there was as well no significant effect of TSS on the HCAs concentration in grape berry juice in both treatments (Supporting Information, Figure S4). The HCAs concentration decreased with TSS accumulation, whereas GSH concentration increased with increasing

TSS (Figure 8). The ratio between HCAs and GSH concentration varied from 2.0 to 11.4 at véraison and from 1.1 to 1.4 at harvest. A higher ratio at véraison could occur due to the different timing of HCAs and GSH syntheses.

On the basis of these results, it appears that classifications upon berry diameter and TSS allowed the study of the asynchronous nature of grapevine fruit maturation. These two types of successive classification provided a novel approach to study the dynamics of secondary metabolites in *V. vinifera* L. Sauvignon blanc grape berries during ripening. The classification reduced berry heterogeneity and showed relevant trends in the evolution of MPs, GSH, and HCAs. Berries of the same diameter were classified further to numerous TSS classes. It was shown in the study that berries having the same diameter can have different TSS concentrations, meaning that these berries are not at the same level of physiological ripening, which could have an impact on secondary metabolite concentrations. MPs concentrations were the most influenced by berry heterogeneity. Both diameter and TSS concentration significantly influenced MPs during ripening and did not significantly influence GSH and HCAs concentrations.

It seems that at a certain TSS concentration, berry metabolism shifts toward an aging process. Berry sugar loading seemed to be erratic from around 20 °Brix onward, meaning that there is no longer a relationship between berry volume and TSS concentration, although Garcia de Cortazar-Atauri et al. showed that a relationship exists up to 24 °Brix.[1] The most significant effect of leaf removal in this experiment was observed in the concentration of MPs, confirming that bunch light exposure drastically decreases the concentration of MPs. Leaf removal had no effect on GSH and HCAs concentrations. Separating the effects of sunlight and temperature on grape berry composition is complex and difficult, as many of the biochemical pathways are affected by light and temperature. The concentration of MPs is a relevant indicator of bunch light exposure, due to a photochemical degradation reaction that could be affected secondarily by the increase in temperature related to sun exposure. The concentrations of HCAs were negatively correlated with an increase in the TSS concentration, whereas GSH was positively correlated. A correlation was observed between GSH synthesis and TSS accumulation in the berry as well as between the degradation of HCAs concentration and an increase in °Brix. After the berries reached a certain maturation level, there were no significant changes in GSH and HCAs concentrations. When the grapes reached 20.2 °Brix, MP concentration had already decreased below the LD. Concentrations of GSH and HCAs were in the same range as already found in the South African and Slovenian grape juices and wines, which could have a potential positive effect on the sensory characteristics of Sauvignon blanc wines.[20,28] Further investigations of the effect of berry diameter and berry sugar content on the aromatic expression of Sauvignon blanc grapes and the resulting wines are needed.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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2.2 EFFECTS OF LEAF REMOVAL AND ULTRAVIOLET RADIATION IN THE VINEYARD ON THE COMPOSITION AND SENSORY PERCEPTION OF SAUVIGNON BLANC (*Vitis vinifera* L.) WINE

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Vpliv razlistanja in zmanjšanja UV svetlobe v območju grozdja v vinogradu na kemijsko sestavo in senzorične lastnosti vina sauvignon blanc

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Namen tega dela je bil preučiti vpliv intenzitete UV svetlobe v okolici grozdov na kemijsko sestavo in senzorične lastnosti vina sauvignon blanc. Z namenom spreminjanja svetlobe smo opravili razlistanje vinske trte v območju grozdov. Povprečno fotosintetsko aktivno žarčenje pri nerazlistanem obravnavanju v okolici grozdov je bilo do 60 µmol $m^{-2}s^{-1}$, medtem ko je bilo pri obravnavanjih z razlistanjem do 850 µmol $m^{-2}s^{-1}$, odvisno od ure in oblačnosti. Svetlobni spekter v območju grozdov smo spremenili z namestitvijo plošč, ki zaustavijo UV svetlobo. Zmanjšanje intenzitete UV svetlobe v območju grozdov je vplivalo na statistično manjše vsebnosti 3-sulfanil heksan-1-ola (3SH) in 3-sulfanilheksil acetata (3SHA) v vinih. Vsebnost 3SH v vinu je bila pri obravnavanju z razlistanjem na strani listne stene vinske trte, ki jo sonce obseva zjutraj (severo-vzhod) in z zmanjšanjem intenzitete UV svetlobe (LR-UV) 344 ng/L, medtem ko je bila pri obravnavanju z razlistanjem na taisti strani stene vinske trte in brez zmanjšanja UV svetlobe (M-LR) 447 ng/L. Najmanjša vsebnost 3SH, tj. 303 ng/L je bila izmerjena v kontrolnem (C) obravnavanju. Manjše vsebnosti estrov višjih alkoholov in etilnih estrov maščobnih kislin so bile v vinih iz trt z zmanjšanjem intenzitete UV svetlobe. Vsebnost estrov višjih alkoholov je bila za 17 % manjša pri obravnavanju LR-UV v primerjavi z obravnavanjem M-LR, medtem ko je bila vsebnost etilnih estrov maščobnih kislin za 18 % manjša pri obravnavanju LR-UV v primerjavi z M-LR obravnavanjem. Prav tako pa je zmanjšanje UV svetlobe in razlistanje vinske trte vplivalo na večje vsebnosti etilnih estrov razvejanih maščobnih kislin, v primerjavi s C obravnavanjem. Samo razlistanje trte brez zmanjšanja intenzitete UV svetlobe je imelo statistično značilen vpliv na večje vsebnosti 3SH, 3SHA, etilnih estrov maščobnih kislin, etilnih estrov razvejanih maščobnih kislin ter na manjše vsebnosti IBMP v vinu, medtem ko zmanjšanje UV svetlobe ni imelo statistično značilnega vpliva na vsebnosti IBMP v vinu. Vinom, pridelanih iz grozdov z nerazlistanih trt, so pripisali arome po zeleni papriki, kuhanem grahu in fižolu, medtem ko so vinom iz grozdov z razlistanih trt pripisali bolj sadne arome po pasijonki, mangu in banani. Spremenjena intenziteta svetlobe ter zmanjšanje intenzitete UV svetlobe imata lahko velik vpliv na kemijsko sestavo in senzorične lastnosti vina, s čimer določita stil vina. Preučevanje vpliva svetlobe prispeva k boljšemu razumevanju vpliva abiotskih faktorjev na kakovost in kemijsko sestavo vina ter posledične senzorične lastnosti vina. Takšen celovit pristop je lahko v pomoč vinarjem in vinogradnikom pri odločitvah o ustreznih vinogradniških praksah ter procesih pridelave vina za doseganje želenega stila vina.

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EFFECTS OF LEAF REMOVAL AND ULTRAVIOLET RADIATION IN THE VINEYARD ON THE COMPOSITION AND SENSORY PERCEPTION OF SAUVIGNON BLANC (Vitis vinifera L.) WINE

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Keywords:	esters , light , methoxypyrazines, Sauvignon Blanc , thiols, sensory analaysis

Abstract

Background and aims: The influence of fruit microclimate (light quantity, light quality and temperature) on the composition and sensory profile of South African Sauvignon Blanc wine was studied.
Materials and results: To manipulate light quantity in the bunch zone, leaf and lateral shoot removal was performed (M-LR), whereas light quality was altered by installing UV radiation reducing sheets (LR-UV). Wines were analysed for chemical attributes pertaining to aromatic composition and assessed by a trained sensory panel. Variations in chemical and sensory attributes were found to be influenced by defoliation and UV radiation reduction. Control treatment (no defoliation) was associated with attributes such as green pepper, asparagus and grassy, whereas wines from leaf and laterals shoot removal treatments were associated with tropical fruit descriptors. Moreover, this study showed for the first time that UV radiation reduction significantly decreased concentrations of varietal thiols, linalool and some yeast derived compounds, such as esters and fatty acids, in corresponding wines. Conversely, defoliation increased the levels of thiols and linalool.
Conclusions: Modified bunch microclimate can have a significant impact on wine composition and sensory properties, and therefore aid in determining wine style.
Significance: Studying the effect of environmental factors (light and temperature) in the vineyard on wine composition and sensory perception can assist winemakers and viticulturists in deciding on appropriate viticultural practices (such as canopy manipulation) and winemaking processes for preferred 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 highly potent classes of compounds, thiols and methoxypyrazines. Volatile thiols, are present in the grape berry in their non-volatile form, 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 notes, such as guava, grapefruit, mango, passion fruit and gooseberry are the main sensory characteristics of 3SH and 3SHA, whereas 4MSP is described as having box tree and passion fruit-like aromas (Tominaga et al. 1996, Swiegers et al. 2009, Coetzee and Du Toit 2012, Coetzee et al. 2013). These compounds are easily olfactorily detected, as they have low perception thresholds of 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 odours of wines (Allen et al. 1991, Pickering et al. 2007). The odour perception thresholds for IBMP and IPMP in water and in white wine are very low, in the range of 0.32-1 ng/L for IPMP and around 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 impact Sauvignon Blanc wine aroma (Benkwitz et al. 2012). At higher levels, esters are known to contribute strongly to the fruity aroma of young white wines (Ribereau-Gayon et al. 2000, Benkwitz et al. 2012). They also impact wine aroma, even at levels considerably below their perception thresholds, through complex synergistic effects (Pineau et al. 2009, Lytra et al. 2012).

Grapevine phenology and physiology, which have an impact on yield and fruit composition, are largely influenced by the climate on a macro- (regional), meso- (vineyard or site) and micro-scale (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, UV radiation has frequently been mentioned in relation to ongoing climate change (Shultz 2000, Jug and Rusjan 2012). Solar light quality, in particular UV radiation, has been shown to have a significant effect on the flavonol and stilbene composition of Cabernet Sauvignon and Riesling grapes, as well as on 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^2d and fluorescence rate of 8.25 $\mu W/cm^2$ increases the

concentrations of terpenes in grapevine leaves (Gil et al. 2012), but has no effect on IBMP concentrations in grapes (Gregan et al. 2012). However, an increase in solar radiation through bunch exposure drastically reduced the concentration of IBMP and IPMP in Cabernet Franc and Cabernet Sauvignon grape berries, when performed before veraison (Ryona et al. 2008, Scheiner et al. 2010, Koch et al. 2012).

The above studies were performed to better understand the effect of the main abiotic factors, such as temperature, light and vine water status, on vine physiology, fruit growth and fruit composition. However, very little research has focused on the effect of abiotic factors on wine composition and sensory perception. This study was undertaken to ascertain the influence of some major biochemical compounds on sensory perception of Sauvignon Blanc wine made from grapes grown under different light quality and quantity regimes in a monitored vineyard situation. To our knowledge, this study reports for the first time the effect of UV radiation reduction at the fruit zone level, on Sauvignon Blanc sensory and chemical composition.

Materials and methods

Vineyard. The experiment was performed 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). The Sauvignon Blanc vines (clone 316 grafted on 101.14) were planted in 2004 in a northeast-to-southwest row orientation, and with a 2.5 m (between row) by 1.8 m (in row) plant spacing. The 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 shoot removal was performed 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 treatment, consisting of shaded bunches within unaltered VSP canopy (C); a sun-exposed bunches treatment (M-LR), removing all leaves and lateral shoots in the bunch zone on the morning/north-eastern side of the canopy at the a height of 30-40 cm above the cordon; and a third treatment (LR-UV) utilising clear, UHI (impact modified), extruded, acrylic sheets (Perspex® 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). For the LR-UV treatment, the sheets were installed on the morning/north-eastern 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, and a replicate consisted of four consecutive vines. On each side of the experimental plot were at least 12 buffer rows, and there were six buffer vines at the beginning of the experimental row. Canopy management, including suckering and

shoot positioning, was performed rigorously in order to optimise light interception in the bunch zone.

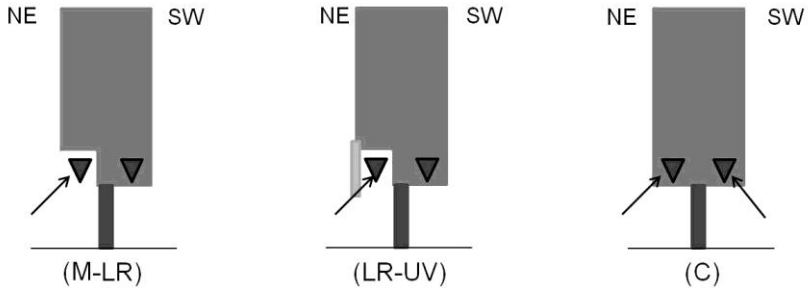

Figure 1: Schematic indication of the treatments in the experiment. The arrows indicate bunches harvested from each treatment. Exposed bunches by removing leaves and lateral shoots in the bunch zone on the morning side of the canopy (M-LR), exposed bunches on the morning side with UV radiation reducing sheets (LR-UV) and control (C).

Abiotic variables. Stem water potential measurements (Choné et al. 2001) were performed on the 6 February 2012, 3 days after veraison was determined, utilising a pressure chamber (Sholander et al. 1965), to assess the vine water status. Photosynthetic active radiation (PAR) was monitored within the canopy at the bunch zone using LI-190 quantum sensors (Li-cor Instruments, Lincoln, NE, USA) attached to a TinyTag® TGPR-1001 millivolt input data logger. UV radiation at the fruit zone was measured with a UV sensor of Davis instruments (Hayward, California, USA) attached to a Datataker DT82E series with data loggers (Thermo Fisher Scientific Pty Ltd., Victoria, Australia). 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 (north-eastern) side of the canopy. For the C treatment, 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. Microclimatic bunch temperatures were monitored at 15-minute intervals by TinyTag® dual channel external loggers, TGP-4520 (Gemini Data Loggers Ltd., Chichester, United Kingdom), 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 mean hourly value for a period of monitoring.

Winemaking practice. Grapes were harvested when juice TSS reached between 23 to 24 ° Brix and titratable acidity (TA) was around 6.5 g/L. Grapes from all three treatments in the experiment were harvested manually on 13 March 2012, 113 days after anthesis by the two authors themselves to avoid variability in harvesting regime. Only fully sun-exposed bunches from the exposed side of the canopy (north-east) were harvested in the M-LR and the LR-UV treatments (Figure 1). All the bunches from the C treatment were harvested, as they were in a permanently shaded situation and considered homogeneous in the terms of

light and temperature (Figure 1). The temperatures measured inside the bunch on the north-eastern side of the canopy and in the bunch positioned on the south-western side of the canopy in C treatment differed just for 0.5 °C for the period of monitoring (n=85 days). The treatments in the experiment were all harvested on the same day in the time frame of three hours. Grapes from the eight replicates per treatment were pooled together and stored overnight at + 4 °C prior to crushing. Forty mg/kg of sulphur dioxide 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 hours 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 hours and an enzyme was added at 2 g/hL to facilitate sedimentation (Rapidase Vino Super, DSM Food Specialists B.V., Netherlands). The clear must was divided into three volumes and fermentations, 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 total soluble solids (TSS), TA, and pH value, while additional samples were taken for GSH and grape reaction product (GRP) analysis. The must was inoculated with 30 g/hL VIN 13 yeast (Anchor, South Africa), with the addition of 30 g/hL of a yeast starter nutrient (Dynastart, Laffort, 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, South Africa) was added to avoid stuck fermentation. All fermenters proceeded to a residual sugar level 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 were measured. The TSS was measured using 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). GSH concentrations in the must before fermentation were determined by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) and on-line 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. N-acetyl-L-cysteine was added as the internal standard (8 mg/L), filtered through 0.45 µm Sartorius Minisart RC 25 filters (Goettingen, Germany), diluted 1:1 with a 5 mM sodium acetate buffer containing 0.1 mM EDTA, and analyzed as previously described (Janeš et al. 2010). For GRP analysis 5 mL of each juice sample was taken from fermenters and immediately placed in 1 000 mg/L of SO_2 in order to inhibit enzymatic activity. The sample was filtered through a 0.45 µm PVDF filter (Millipore Bedford, MA, USA) into a HPLC vial. The concentration was determined by HPLC, as

described by Vanzo et al. (2007), and expressed as aliquots of *trans*-caftaric acid. Analyses of the IBMP in the wines were performed using the headspace solid-phase microextraction method (HS-SPME), and quantification was performed by gas chromatography-mass spectrometry (GCMS). An internal standard of final concentration 25 ng/L deuterated IBMP (CDN Isotopes, Pointe-Claire, 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 deionized water and 2 mL of 4M NaOH were added. The sample was stirred until the NaCl was completely dissolved, and then analysed by GCMS (Parr et al. 2007, Šuklje et al. 2012). Quantification of 3SH and its acetate 3SHA in wines was carried out according to the method of Tominaga (Tominaga et al. 1998b, Tominaga and Dubourdieu 2006), with slight modifications and using an isotopically labelled 3SH ([$^2H_2$]-3SH) and 3SHA ([$^2H_2$]-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, Pointe-Claire, Canada). The final solution used to spike the samples was composed of [$^2H_3$]-ethyl butyrate at 40 mg/L, [$^2H_{11}$]-ethyl hexanoate at 20 mg/L, [$^2H_{15}$]-ethyl octanoate at 20 mg/L, [$^2H_{23}$]-ethyl dodecanoate at 4 mg/L, and [$^2H_5$]-ethyl cinnamate at 12 mg/L. Twenty µL of internal standard mix solution 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, Wilmington, 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 [$^2H_3$]-ethyl butyrate, 110 for [$^2H_{11}$]-ethyl hexanoate, 142 for [$^2H_{15}$]-ethyl octanoate, and 206 for [$^2H_{23}$]-ethyl dodecanoate and [$^2H_5$]-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, but with an MS-Scan mode performed simultaneously to the MS-SIM 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: [$^2H_3$]-ethyl butyrate for isobutanol and isoamyl alcohol, [$^2H_{11}$]-ethyl hexanoate for all the C6 compounds and linalool, [$^2H_{15}$]-ethyl octanoate for phenylethanol and hexanoic acid, and [$^2H_{23}$]-ethyl dodecanoate for decanoic acid.

Wine sensory analysis. Descriptive sensory analysis was performed using 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 wine industry or experienced as sensory assessors. Sensory training consisted of five one-hour 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 two-week period of intensity scaling. The aroma and mouth-feel standards used for sensorial training and wine assessment are described in Table S1 (Supporting information). 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 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 ANOVA and the means were separated using Stats-Fisher's LSD 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. PanelCheck version 1.4.0 (Nofima, Os, Norway) was used to evaluate panel performance according to the workflow proposed by Tomic et al. (2010). Tucker1 was applied to the sensory data to evaluate assessor agreement, and p*MSE graphs were assessed to evaluate assessor repeatability and discrimination ability. Sensory data were analysed using multifactoral ANOVA using Statistica version 10 (StatSoft, Tulsa, OK, USA). 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 re-scaling, 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 percentage of variation explained by this consensus compared to 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 was conducted in Metlab (Version R2012a, The Mathworks, Natick, MA). Principal component analyses (PCA) was conducted on the consensus average sensory scores using PLS Toolbox

(Eigenvector Research Inc., Wenatchee, WA, Version 5.0). Chemical data sets were related to the GPA consensus sensory matrix by Common Component and Specific Weight analyses using the SAISIR toolbox (SAISIR, 2010) on the centred and mean standardised matrices. For the purposes of clarity, multiblock analysis of datasets herein are organised and each data set was assigned a number as seen in Table 1.

Table 1: Attribute identification for data blocks.

Attribute Number	GPA Sensory Data	Chemical quantitative	Chemical Semi-quantitative
1	Overall Tropical	3-sulfanyhexyl acetate	Linalool
2	Overall Green	3-sulfanylhexan-1-ol	Phenylethanol
3	Passion Fruit	3-isobutyl-2-methoxypyrazine	Ethyl cinnamate
4	Guava	Ethyl propionate	Ethyl hydrxycinnamate
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	

Results

Abiotic variables. The experimental vineyard block was well characterised by monitoring stem water potential, light and temperature (micro, meso and macro level). Stem water potential measurements were performed at veraison, and the mean value for the C treatment 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 treatments. This was further confirmed by visual vine inspection and berry fresh mass evolution during maturation (data not shown). The PAR values in the bunch zone were significantly higher for treatments with leaf and lateral shoot removal, compared to 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^2s, 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^2s. 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 10.8 MEDs was measured in the M-LR treatment (n = 9 days), whereas lower UV radiation was measured in the C treatment 2.5 MEDs (n = 4 days) and the lowest in LR-UV treatment 1.2 MEDs (n= 6 days). Similarly as with PAR, the UV radiation measurements were not taken at the same time for all three treatments. The LR-UV treatment showed the highest mean bunch temperature readings for the period of monitoring (n = 85 days), viz. 21.4 ± 6.39 °C, whereas the C treatment (n = 85 days) showed the lowest mean bunch temperature readings 20.5 ± 5.25 °C. More precise observations can be made, when analysing the mean hourly temperature evolution (Figure 2). The elevation in bunch temperatures in the M-LR and LR-UV treatments above the C was observed in morning hours, whereas the difference in the temperatures between treatments in the afternoon was less prominent.

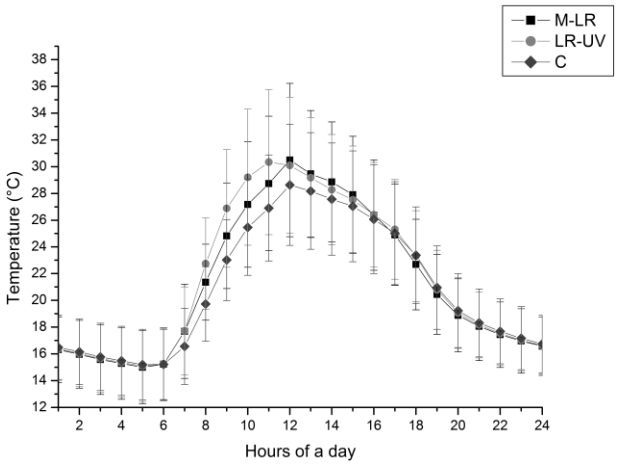

Figure 2: The mean hourly bunch temperatures from the 19 December 2011 to 13 March 2012.

Chemical analyses. Grapevine defoliation and UV radiation reduction did not influence must TA, whereas the lowest TSS were measured in C treatment (Table 2). In the current study, the GSH concentrations in must before fermentation ranged from 30.9 ± 2.11 in LR-UV to 49.2 ± 6.88 mg/L in M-LR treatments and were significantly different (Table 2). The GRP values were expressed as *trans*-caftaric acid equivalent and were the lowest in the M-LR treatment, i.e. 10.9 ± 1.08 mg/L, and the 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). Concentrations of 3SH and 3SHA were lower in the LR-UV treatment compared to the M-LR treatment, and the lowest 3SH concentration was measured in C treatment (Table 2). The observed IBMP concentrations in the wine samples were generally low. The highest IBMP concentration in the wine measured was 3.4 ± 0.31 ng/L for the C treatment, which differed significantly

from the concentrations measured in the wines of the M-LR and LR-UV treatments (Table 2). The UV radiation reduction had no significant effect on the IBMP concentrations 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 dodecanoate, which were not influenced by any of the treatments. In comparison, the M-LR treatment led to the highest concentrations of ethyl butyrate, hexanoate and octanoate in the wines. (Table 2). The wines from the LR-UV treatment recorded the lowest concentrations of 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 UV radiation reduction, increased levels of ethyl esters of branched acids compared to the C treatment. Conversely, the levels 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 compared to the M-LR treatment (Table 2). Significantly higher levels of isobutanol were measured in LR-UV treatment, whereas isoamyl alcohol and phenylethanol levels were elevated, but not significantly compared to M-LR treatment. The C treatment exhibited the lowest higher alcohol levels in the wines (Table 2). On the other hand, significantly lower levels of medium chain fatty acids were observed in the LR-UV treatment compared to M-LR and C treatments (Table 2). The highest levels of linalool were found in the M-LR treatment, whereas the lowest semi-quantitative values were observed in the C treatment. UV radiation reduction significantly reduced the linalool level in LR-UV treatment compared to the M-LR treatment (Table 2).

Table 2: Average concentrations of compounds measured in juices before fermentation and in finished wines.

Compounds	M-LR	LR-UV	C
Must before fermentation			
Total soluble solids (°Brix)	23.8 ± 0.06^{b}	24.7 ± 0.06^{a}	23.3 ± 0.01^{c}
Titratable acidity (g/L)	6.5 ± 0.05^{a}	6.3 ± 0.64^{a}	6.7 ± 0.01^{a}
pH	3.29 ± 0.03^{b}	3.41 ± 0.03^{a}	3.37 ± 0.01^{b}
Glutathione (mg/L)	49.2 ± 6.88^{a}	30.9 ± 2.11^{c}	36.3 ± 1.67^{b}
Grape reaction product (mg/L)	10.9 ± 1.08^{c}	17.6 ± 0.72^{a}	14.0 ± 2.38^{b}
Wine			
Varietal thiols (ng/L)			
3-sulfanylhexan-1-ol	447.0 ± 26.0^{a}	344.4 ± 11.2^{b}	303.7 ± 7.2^{c}
3-sulfanyhexyl acetate	186.8 ± 3.2^{a}	111.0 ± 3.2^{b}	111.1 ± 5.2^{b}
Methoxypyrazines (ng/L)			
3-isobutyl-2-methoxypyrazine	2.6 ± 0.1^{b}	2.4 ± 0.3^{b}	3.4 ± 0.3^{a}
Ethyl esters of fatty acids (µg/L)			

Ethyl butyrate	616 ± 11.9[a]	554 ± 18.6[c]	586 ± 10.4[b]
Ethyl hexanoate	1171 ± 70[a]	924 ± 73[b]	1016 ± 60[b]
Ethyl octanoate	2074 ± 206[a]	1563 ± 230[b]	1950 ± 156[a]
Ethyl decanoate	576 ± 139[a]	560 ± 75[a]	555 ± 111[a]
Ethyl dodecanoate	134 ± 32[a]	166 ± 37[a]	136 ± 23[a]
Total	4571 ± 323[a]	3767 ± 321[b]	4244 ± 110[ab]
Higher alcohol acetates(µg/L)			
Isobutyl acetate	83.7 ± 2.4[a]	81.7 ± 4.0[a]	86.4 ± 2.2[a]
Isoamyl acetate	5888 ± 513[a]	5016 ± 440[b]	5794 ± 448[a]
Hexyl acetate	238 ± 29[a]	152 ± 25[b]	225 ± 23[a]
2-phenylethyl acetate	318 ± 69[a]	166 ± 37[b]	297 ± 57[a]
Propyl acetate	186 ± 5.8[ab]	178 ± 9.7[b]	199 ± 4.5[a]
Total	6713 ± 543[a]	5593 ± 477[b]	6601 ± 488[a]
Ethyl esters of branched acids (µg/L)			
Ethyl isobutyrate	19.8 ± 1.1[a]	21.1 ± 1.3[a]	16.5 ± 0.6[b]
Ethyl 2-methylbutyrate	2.23 ± 0.05[b]	2.46 ± 0.15[a]	1.76 ± 0.10[c]
Ethyl isovalerate	4.40 ± 0.31[a]	4.70 ± 0.49[a]	3.47 ± 0.35[b]
Ethylphenyl acetate	0.41 ± 0.05[a]	0.47 ± 0.04[a]	0.31 ± 0.06[b]
Total	26.8 ± 1.3[a]	28.8 ± 1.8[a]	22.0 ± 0.8[b]
Ethyl propionate (µg/L)	83.0 ± 8.9[ab]	92.0 ± 12.5[a]	76.2 ± 5.1[b]
C6 compounds and their esters			
Ethyl cis-3-hexenoate[¥]	0.45 ± 0.05[a]	0.34 ± 0.04[b]	0.32 ± 0.04[b]
Ethyl trans-2-hexenoate (µg/L)	0.65 ± 0.08[a]	0.43 ± 0.04[b]	0.46 ± 0.04[b]
Cis-3-hexenyl acetate[¥]	0.23 ± 0.03[a]	0.17 ± 0.02[b]	0.18b ± 0.02[b]
Trans-2-hexenyl acetate[¥]	0.11 ± 0.01[a]	0.07 ± 0.01[b]	0.13 ± 0.02[a]
Hexanol[¥]	0.42 ± 0.07[a]	0.34 ± 0.04[b]	0.35 ± 0.04[b]
Ethyl esters of hydroxycinnamic acids			
Ethyl cinnamate[¥]	0.0001 ± 0.00001[a]	0.0001±0.00001[a]	0.0002 ± 0.00001[a]
Ethyl hydroxycinnamate[¥]	0.0024 ± 0.003[a]	0.0031± 0.0007[a]	0.0026 ± 0.00004[a]
Higher alcohols			
Isobutanol[¥]	3.46 ± 0.46[b]	4.31 ± 0.59[a]	2.76 ± 0.49[c]
Isoamyl alcohol[¥]	67.9 ± 10.9[a]	77.8 ± 12.0[a]	61.8 ± 8.4[a]
Phenylethanol[¥]	0.51 ± 0.10[ab]	0.58 ± 0.07[a]	0.45 ± 0.04[b]
Medium chain fatty acids			
Hexanoic acid[¥]	0.52 ± 0.06[a]	0.41 ± 0.02[a]	0.49 ± 0.09[a]

Octanoic acid¥	1.71 ± 0.15[a]	1.20 ± 0.14[b]	1.55 ± 0.22[a]
Decanoic acid¥	16.0 ± 3.4[a]	10.3 ± 1.0[b]	16.1 ± 3.6[a]
Terpenes			
Linalool¥	0.039 ± 0.007[a]	0.026 ± 0.003[b]	0.016 ± 0.002[c]

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

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 were significantly different for attributes overall tropical, overall green, passion fruit, grape fruit, banana lolly, floral and asparagus (Table 3).

Table 3: Significant sources of variation in the ANOVA model of the raw sensory data.

	Panelist	Treatment	Panelist*treatment
Overall tropical	**0.000**	**0.000**	**0.002**
Overall green	**0.000**	**0.000**	**0.023**
Passion fruit	**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

Therefore, it is obvious that the sensory attributes terms were not used consistently by panellists, and calculating a panel average as an arithmetical mean would be 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 Figure S1 (Supporting information). 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 to 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), Figure S1 (Supporting information). ANOVA was run on the consensus average scores, and post hoc results on sensory attributes are presented in Table S2 (Supporting information). The two-dimensional PCA projection applied to the consensus average scores of sensory attributes explains 76.1% of the variation, with the first component (PC1) explaining 56.6% of the variation and the second 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 UV radiation reduction. The defoliated treatments (LR-UV and M-LR) were associated with increased perception of fruity/tropical fruits attributes such as overall topical, passion fruit, grapefruit and pineapple (Figure 3). Furthermore the C treatment 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).

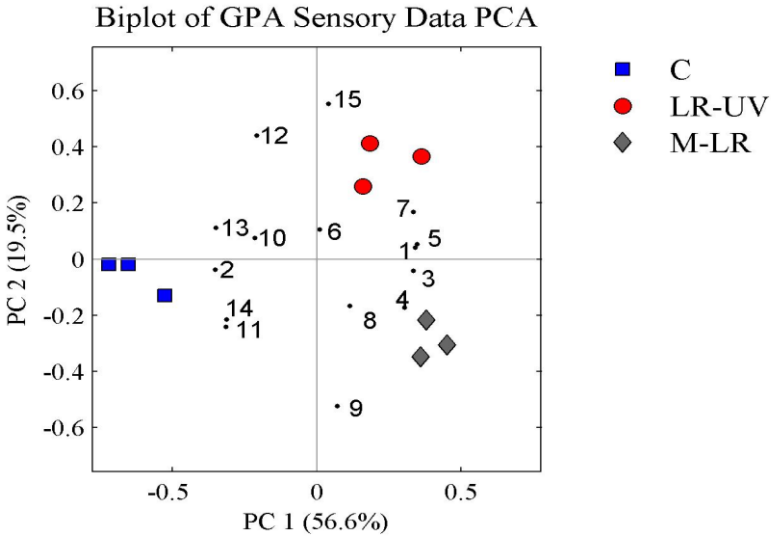

Figure 3: Principal component analyses (PCA) score plot for treatments and consensus average sensory scores calculated using Generalised Procrustes Rotation Algorithm (GPA).
Exposed bunches by removing leaves and lateral shoots in the bunch zone on the morning side of the canopy (M-LR), exposed bunches on the morning side with UV radiation reducing sheets (LR-UV) and control (C).

Correlation of sensory and chemical data sets. To assess the commonality between the GPA sensory matrix and the chemical data, Common Component Specific Weight analyses was conducted on the mean and standardised matrices. Common Component Specific Weight analyses 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 below (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, i.e. sensory attributes, quantitative chemical data and semi-quantitative chemical data has been assigned a number as presented in Materials and Methods (Table 1).

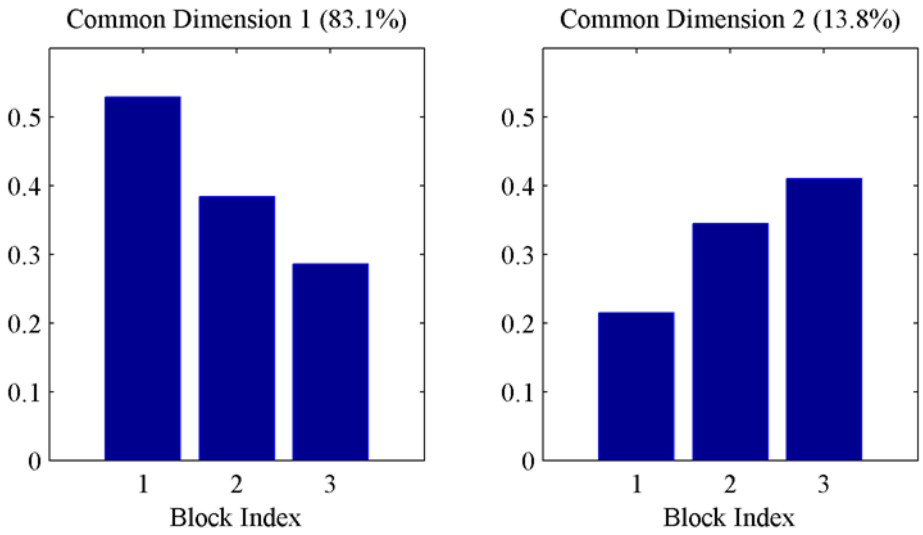

Figure 4: Salience of three data sets calculated by Common Component and Specific Weight analyses for the first two common components. Block index is indentified in Table 1.

Dividing the data into three blocks was necessary due to the orders of magnitude between sensory, quantitative and semi-quantitative data. Scores for extracted CD1 separate the C treatment from the two other treatments receiving leaf removal (M-LR and UV-LR), irrespective of UV radiation reduction, by the sensory attributes such as overall green, green pepper, grassy and cooked beans/peas, and which are all highly negatively loaded (Figure 5A, 5B). Thus, the chemical data strongly associated with the C treatment were isobutyl acetate, propyl acetate and IBMP, with the latter being known to contribute to green aromas of wines (Figure 5C, 5D). 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, 5B). 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, 5D). 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).

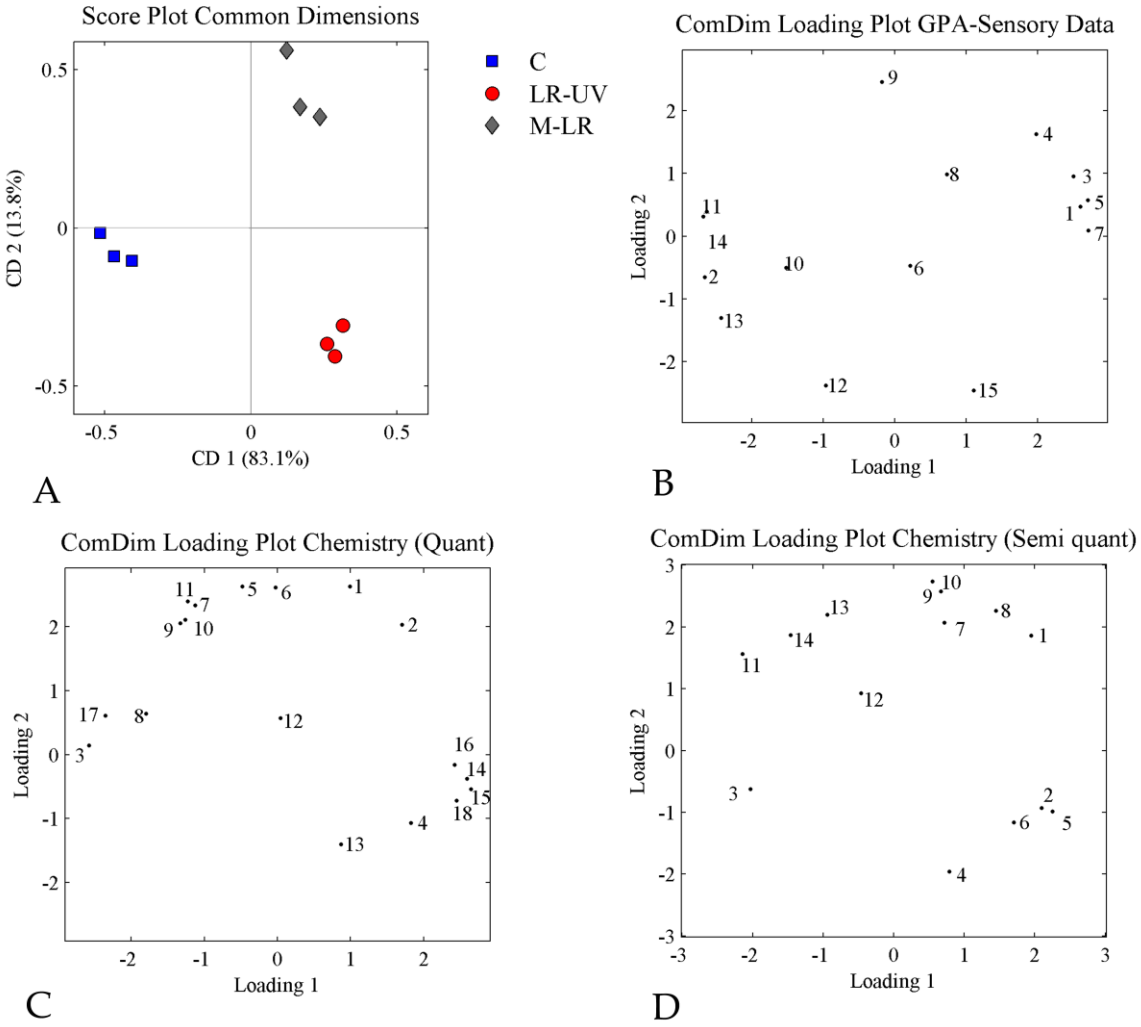

Figure 5: Common Component and Specific Weight analyses for treatments (A), a consensus average sensory scores calculated by Generalised Procrustes Rotation Algorithm (B), quantitative chemical data (C) and semi-quantitative chemical data (D). Physicochemical and sensory attributes are given in Table 1.

Discussion

The experiment was designed so that wines from three different bunch exposure treatments could be compared. One in which the fruit microclimate was not modified throughout the growth and ripening phases (C), the another where bunch exposure to sunlight was increased due to the leaf and lateral shoot removal; and a third where UV radiation was reduced. A good correlation was observed between defoliated treatments (M-LR and LR-UV) and fruity aromas, whereas the C treatment (no defoliation) was associated with acidity, green pepper and overall green attributes. Selective harvesting occurred for exposed treatments (M-LR and LR-UV) to determine the effect of light on wine composition as the defoliation was performed only on one side of the canopy to reduce the possibility of sunburns. For the C, all bunches were harvested as bunches of this treatment were in permanently shaded situation. Manual and highly controlled bunch harvesting was

adopted to avoid interference of different harvesting regime to compare wines made from sun exposed and shaded grapes. A small, but significant difference in the concentration of IBMP could probably not explain the strong separation between the treatments (leaf removal and no leaf removal). However, it has been noted, 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 with concentrations as low as 2 ng/L, and Van Wyngard (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 treatment was related to "greener attributes" regardless the small differences in the IBMP concentrations, due to the lower perception of fruity aromas (Figure 5), as wines from this treatment exhibited significantly lower concentrations of 3SH, some esters (ethyl isobutyrate, ethyl 2-methylbutyrate, ethyl isovalerate, ethylphenyl acetate, ethyl propionate) and lower levels of linalool. It is likely that higher concentrations of 3SH and ethyl esters of branched acids, the latter being known to contribute in synergistic effect to the fruity aromas of wines (Lytra et al. 2012), in M-LR and LR-UV treatments enhanced fruity notes compared to the C treatment. It has been shown that the omission of esters from the medium results in a significant intensity decrease of descriptors associated with thiols (cat pee, passion fruit, 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 differences 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 higher levels of linalool and some esters of fatty acids, responsible for floral and delicate fruity notes of white wines (Ribereau-Gayon et al. 2000). Wines from the LR-UV treatment were strongly associated with the perception of bitterness and mapped well with ethyl hydroxyicinnamate. It was shown by Fischer and Noble (1994) that bitterness in white wine was associated with increased levels of catechin and ethanol, and to increased levels of phenolics in combination with lower wine alcohol content (Gawel et al. 2013). However, the molecular base for bitterness in white wines is still widely unknown (Sokolowsky and Fischer, 2012).

This study demonstrated that wine chemical composition and sensory perception can be modified significantly, resulting from the alteration of the fruit microclimate by modifying light quantity (leaf removal) and light quality (UV radiation reduction). However, in this study the temperature effect cannot be completely excluded, as it is known that temperature of bunches is increasing with increased light penetration (Spayd et al. 2002). However, during the afternoon hours it was possible to partly separate the temperature

increase from the increased solar radiation, due to only one side of canopy defoliation, 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 concentrations of IBMP in final wines (Ryona et al. 2008, Šuklje et al. 2012). Conversely, no significant effect of UV radiation reduction on IBMP concentrations in the wines from this study was observed, 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 different treatments in the vineyard. For the first time it was observed that a reduction of UV radiation decreased the concentrations of 3SH and 3SHA in corresponding wines, whereas the lowest 3SH concentration was found in the control treatment. 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. Potentially higher concentrations 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 concentrations in the M-LR treatment could contribute to higher 3SH and 3SHA production in these wines. However, this was not the case 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. However, vineyard treatments 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 of higher alcohol acetates and ethyl esters of fatty acid concentrations in wines was observed when UV radiation reduction was performed in the vineyard, compared to the M-LR treatment. Several hypotheses for wine esters profile variation 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*), due to higher concentrations of PUFAs (Fujii et al. 1997, Fujiwara et al. 1998, Sumby et al. 2010). The observed lower levels 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, support this hypothesis. In addition, higher levels of PUFAs represent 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 to

M-LR. Moreover, the levels 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, increased levels 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 UV radiation reduction on Sauvignon Blanc wine chemical composition and sensory perception. The study demonstrated that, in a particular vineyard situation (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 sufficient wine volumes to undergo sensory analysis. The justification for this was the aim to compare wine made from bunches sourced from indisputable 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, temperatures 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. Comparing hot-warm versus temperate-cool climates could lead to different results. This study helped to understand 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 Material for:

Effects of leaf removal and ultraviolet radiation in the vineyard on the composition and sensory perception of Sauvignon Blanc (*Vitis vinifera* L.) wine

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Supporting information Table S1

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

Attribute	Reference standard
Passion fruit	5 mL passion fruit juice and pulp
Guava	10 x 10 mm cube of fresh guava
Grapefruit	5 mL of ruby grapefruit juice and a small piece of fruit
Pineapple	10 mL syrup from tinned pineapple
Banana lolly	3 drops of pure isoamyl acetate
Gooseberry	2 fresh gooseberries, quartered
Overall tropical	no standard (overall tropical aroma intensity)
Floral	5 mL rose water
Grassy	5 shredded 15 mm blades of grass
Green pepper	10 x 10 mm cube of fresh green pepper
Asparagus	5 mL brine from tinned asparagus
Cooked beans/peas	5 mL brine from tinned beans, 5 mL brine from tinned peas
Overall green	no standard (overall green aroma intensity)
Acidity	no standard
Bitterness	no standard

Supporting information Figure S1

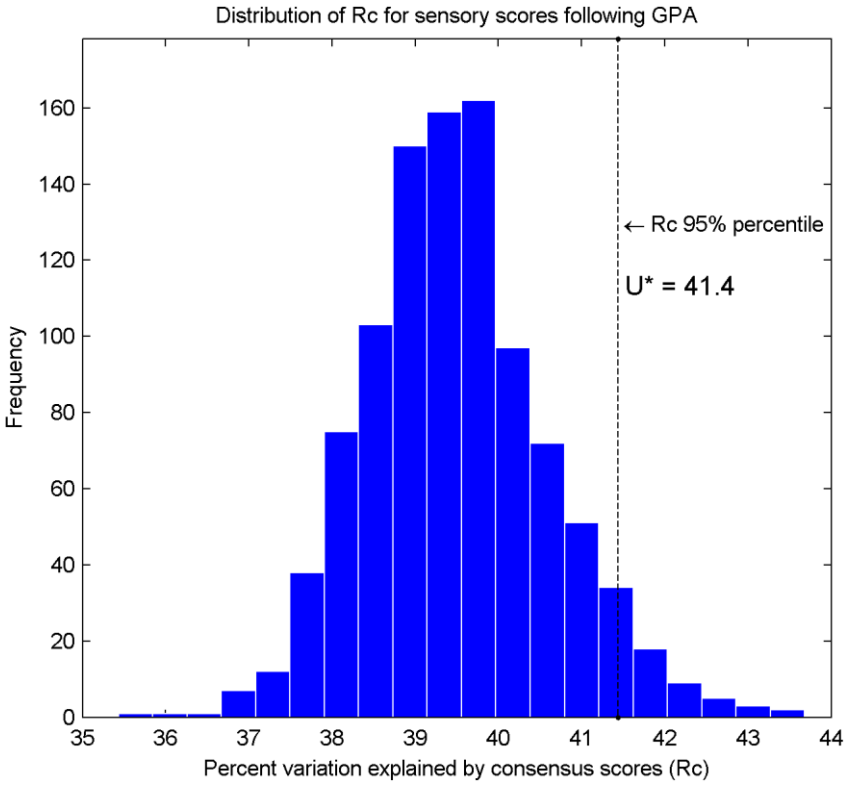

Figure S1: Generalised Procrustes Algorithm analysis (GPA) of sensory scores performed on the mean centred scores matrix for each panellist to produce a consensus mean.

Supporting information Table S2

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.

Attribute	M-LR	LR-UV	C
Overall tropical	62.4±3.9^{a}	62.2 ±4.6^{a}	43.4 ± 3.9^{b}
Overall green	27.9±3.2^{b}	31.01±8.2^{b}	58.9 ±2.2^{a}
Passion fruit	48.6±1.9^{a}	40.7±7.9^{a}	18.7±3.3^{b}
Guava	40.3±2.5^{a}	33.1±3.7^{b}	25.9±3.6^{c}
Grapefruit	39.1±4.5^{a}	37.8± 5.2^{a}	10.6±2.1^{b}
Gooseberry	26.6±1.9^{a}	28.5±2.0^{a}	26.9±5.7^{a}
Pineapple	31.8±1.9^{a}	35.1±4.0^{a}	10.4±4.6^{b}
Banana lolly	18.1±6.3^{a}	15.6±1.6^{a}	14.7±3.9^{a}
Floral	13.2±5.9^{a}	-2.54±5.7^{b}	3.8±1.5^{ab}
Grassy	18.5±6.3^{a}	21.8±1.9^{a}	28.3±12.2^{a}
Green pepper	23.6±2.9^{b}	14.5±7.6^{b}	43.9±5.6^{a}
Asparagus	15.6±1.5^{b}	30.1±5.7^{a}	30.2±2.9^{a}
Cooked beans/peas	25.7±1.1^{c}	33.8±4.2^{b}	48.2±3.3^{a}
Acidity	58.0±4.3^{b}	53.1±4.1^{b}	74.6±2.7^{a}
Bitterness	12.1±3.1^{c}	38.8±2.9^{a}	18.9±0.6^{b}

ANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leq 0.05$ (Fischer's LSD).Exposed bunches by removing leaves and lateral shoots in the bunch zone on the morning side of the canopy (M-LR), exposed bunches on the morning side with UV light-reducing sheets (LR-UV) and control (C) receiving no leaf and lateral shoot removal.

2.3 THE EFFECT OF LEAF AREA TO YIELD RATIO ON SECONDARY METABOLITES IN GRAPES AND WINES OF *Vitis vinifera* L. CV. 'SAUVIGNON BLANC'

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Vpliv razmerja med listno površino in maso grozdja vinske trte (*Vitis vinifera* L.) 'Sauvignon blanc' na izbrane sekundarne metabolite grozdja in vina
Journal International des Sciences de la Vigne et du Vin, 2013, 47: 83-97.

V poskusu smo preučevali vpliv zmanjšanja listne površine, doseženega s krajšanjem mladik z 1,6 m na dolžino 0,9 m, v kombinaciji z redčenjem grozdov, na vsebnost izbranih metabolitov grozdja med dozorevanjem, kemijsko sestavo in senzorično kakovost vina sauvignon blanc. V poskusu, zasnovanem v Vipavski dolini, smo na naključno izbranih delih vinograda in v štirih ponovitvah izvedli naslednje ampelotehnične ukrepe: krajšanje mladik in redčenje grozdov (SH/BT); krajšanje mladik brez redčenja grozdov (SH/NBT); brez krajšanja mladik z redčenjem grozdov (FC/BT) ter brez krajšanja mladik in brez redčenja grozdov (FC/NBT). Krajšanje mladik je vplivalo na značilno poznejše kopičenje skupne suhe snovi (TSS) v začetku zorenja grozdja pri obravnavanjih SH/BT in SH/NBT. Statistično značilno počasnejše kopičenje TSS je bilo pri obravnavanju SH/NBT med dozorevanjem grozdov vse do trgatve, ko se med obravnavanji v vsebnosti TSS niso več pokazale statistično značilne razlike. Prikrajševanje mladik in redčenje grozdov je vplivalo tudi na vsebnost reduciranega glutationa (GSH) v grozdnih jagodah med dozorevanjem. Vsebnost GSH je bila statistično značilno večja pri obravnavanju FC/BT z največjim razmerjem med listno površino in maso grozdja (1,85 m^2/kg) že pri drugem vzorčenju. Pri obravnavanju z najmanjšim razmerjem med listno površino in maso grozdja (0,63 m^2/kg) se je vsebnost GSH večala vse do trgatve. Ob trgatvi med obravnavanji ni bilo statistično značilnih razlik v vsebnosti hidroksicimetnih kislin, GSH, titrabilnih kislin in v vrednosti pH grozdnih jagod, vsebnost metoksipirazinov pa je bila pod mejo zaznave. Vsebnost luteina v grozdnih jagodah je bila statistično značilno večja pri obravnavanjih brez redčenja grozdov, medtem ko se v vsebnosti β-karotena in neoksantina ob trgatvi niso pokazale značilne razlike med obravnavanji. V vinu, pridelanem iz grozdov s trt z največjim razmerjem med listno površino in maso grozdja (FC/BT), je bila izmerjena statistično značilna večja vsebnost 3-sulfanil heksan-1-ola in 4-metil-4-sulfanilpentan-2-ona, medtem ko je bila vsebnost 3-sulfanilheksil acetata prav tako nekoliko večja, vendar ni bila značilna. Pri senzorični analizi je bilo za splošno kakovost najbolje ocenjeno vino obravnavanja FC/BT in intenzivnejšo aromo po pasijonki, mangu, črnem ribezu ter mačjem urinu. Vino obravnavanja FC/NBT je prejelo najboljšo oceno za svežo aromo po tropskem sadju (citrusi, guava in grenivka) in drugo najboljšo oceno za skupno kakovost. Razmerje med listno površino in maso grozdja vinske trte je vplivalo na hitrost zorenja jagod, sestavo metabolitov grozdja in vina ter senzorične lastnosti vina sauvignon blanc. Raziskava je pokazala, da je splošna senzorična kakovost vina najboljša pri največjem razmerju med listno površino in maso grozdja vinske trte.

THE EFFECT OF LEAF AREA TO YIELD RATIO ON SECONDARY METABOLITES IN GRAPES AND WINES OF *VITIS VINIFERA* L. CV. SAUVIGNON BLANC

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Abstract

Aim: To investigate the effect of reducing leaf area by shoot hedging in combination with bunch thinning on metabolite concentration and sensorial quality of Sauvignon blanc grapes and wines.

Methods and results: Four vine treatments were conducted: shoot hedging/bunch thinning (SH/BT), shoot hedging/no bunch thinning (SH/NBT), full canopy/bunch thinning (FC/BT) and full canopy/no bunch thinning (FC/NBT). Shoot hedging delayed total soluble solids accumulation at the beginning of the grape maturation in SH/BT and SH/NBT treatments. At harvest there were no significant differences in the concentration of hydroxycinnamoyl tartaric acids, glutathione, total soluble solids, titratable acidity and pH value in grape juice between all treatments and methoxypyrazines were below the limit of detection. Lutein concentration in grape berry was higher in treatments without bunch thinning, while there was no significant difference in the concentration of β-carotene and neoxanthin. The highest leaf area to yield ratio (FC/BT) resulted in higher concentration of glutathione in must and higher concentration of thiols in Sauvignon blanc wines. Upon sensory evaluation, the FC/BT wine was best scored for overall quality and heavier tropical aroma, whereas the FC/NBT wine was best scored for fresh tropical aroma and second best for overall quality.

Conclusion: Leaf area to yield ratio impacted berry ripening kinetics, grape and wine metabolite composition, and sensorial properties of Sauvignon blanc wine.

Significance and impact of the study: The study showed that the highest leaf area to yield ratio resulted in the best overall sensorial quality of wine.

Key words: Sauvignon blanc, leaf area to yield ratio, volatile thiols, glutathione, hydroxycinnamoyl tartaric acids, methoxypyrazines, carotenoids, wine style

Résumé

Objectif: Étudier l'influence de la réduction de la surface foliaire et de l'éclaircissage des grappes sur certains métabolites primaires et secondaires des baies de raisins et des vins de Sauvignon blanc.

Méthodes et résultats: Le rognage des rameaux primaires et l'éclaircissage des grappes ont été réalisés : SH/BT (rognage et éclaircissage) ; SH/NBT (rognage et 2 grappes par rameau). La modalité témoin (FC/NBT) n'a pas été traitée alors que les vignes traitées par FC/BT ont été seulement éclaircies. Le rognage a retardé l'accumulation des sucres en début de maturation pour les modalités SH/BT et SH/NBT. Aucune différence significative n'a été observée dans les moûts, parmi toutes les modalités, au moment de la vendange pour les teneurs en tartrate d'acides hydroxycinnamiques, glutathion, sucres solubles, acidité totale et pH. Les niveaux en méthoxypyrazines ont été inférieurs à la limite de détection analytique. La teneur en lutéine des baies de raisin s'est avérée plus importante dans les modalités sans éclaircissage alors qu'aucune différence n'a été mesurée pour la β-carotène et la néoxanthine. Les plus fortes teneurs en glutathion dans les moûts et en thiols dans les vins de Sauvignon blanc correspondant ont été observées avec les rapports surface foliaire exposée/charge en raisin les plus élevés (FC/BT). L'évaluation sensorielle de ces vins a permis de mettre en valeur la modalité FC/BT jugée par les dégustateurs comme ayant la meilleure qualité aromatique globale, avec des notes « tropical prononcé » intenses. Le traitement FC/NBT a été perçu comme la deuxième meilleure modalité pour la qualité aromatique globale, avec des notes « tropical frais » intenses.

Conclusion: Le rapport surface foliaire exposée/charge en raisin influence la dynamique de maturation ainsi que le profil métabolique et sensoriel des baies de raisin et des vins de Sauvignon blanc.

Signification et impact de l'étude: Les rapports surface foliaire exposée/charge en raisin élevés favorisent l'expression variétale des vins de Sauvignon blanc.

Mots clés: Sauvignon blanc, rapport surface foliaire exposée/charge en raisin, thiols volatils, glutathion, tartrate d'acides hydroxycinnamiques, méthoxypyrazines, caroténoïdes, style de vin

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INTRODUCTION

The quality of wine is affected by the composition of the grapes produced in the vineyard (JACKSON and LOMBARD, 1993). Much attention has been focused recently on the effect of bunch and canopy microclimate on the physiological, morphological and biochemical parameters of grapes and wines (BRAVDO *et al.*, 1985; BLEDSOE *et al.*, 1988; REYNOLDS *et al.*, 1996; ARNOLD and BLEDSOE, 1990; KLIEWER and DOKOOZLIAN, 2005; MYERS *et al.*, 2008). The ability of the vines to ripen the crop adequately is determined mostly by their total leaf area and the percentage of total leaf surface exposed to sunlight when other factors are not restricting growth (KLIEWER and DOKOOZLIAN, 2005). Several authors have reported that minimum leaf area for adequate grape ripening per gram of fruit is between 7 and 14 cm^2/g, varying between cultivars and trellis systems (KLIEWER and OUGH, 1970; SMITHYMAN *et al.*, 1997; KLIEWER and DOKOOZLIAN, 2005). Shoot hedging and bunch thinning are two of the many green practices performed in the vineyard to modify leaf area to yield ratio that can affect berry composition, and consequently wine quality and style.

Shoot hedging is a technique used to reduce vine vigor. It increases the light exposure of bunches and leaves and stimulates the growth of laterals when performed before flowering (JACKSON, 2008). It delays sugar accumulation, decreases berry weight and reduces berry coloration, depending on bunch microclimate (WEAVER *et al.*, 1957). The concentration of free volatile terpenes and potentially volatile terpenes can be increased by shoot hedging (REYNOLDS *et al.*, 1996). Green practices performed in the vineyard can indirectly influence the aromatic profile of the wines and grapes by modifying the bunch microclimate (DELOIRE, 2012), which in turn may affect the synthesis of aromatic compounds and its precursors, which are dependent on berry metabolism (BELL and HENSCHKE, 2005; KOCH *et al.*, 2010).

Bunch thinning reduces yield and can advance the harvest time. Some studies report that increased crop load can induce higher concentration of malic and tartaric acid and delay fruit maturation (WEAVER *et al.*, 1957; BRAVDO *et al.*, 1985). In contrast, others report that berry composition is mainly affected by the light and temperature at the bunch to berry level (SPAYD *et al.*, 2002; DELOIRE, 2012). KLIEWER and WEAVER (1971) reported that a good linear correlation exists between leaf area per fruit weight and the grape berry weight, the concentration of total soluble solids (TSS), and the concentration of proline in the grape juice. However, the main climatic drivers of berry composition are light and temperature (CONRADIE *et al.*, 2002; DEBOLT *et al.*, 2008).

There is a lack of information on the influence of shoot hedging and bunch thinning on the levels of volatile thiols and their preservative glutathione (GSH), hydroxycinnamoyl tartaric acids (HCA), methoxypyrazines (MPs) and carotenoids in Sauvignon blanc grapes and wines.

MPs are grape-delivered aroma compounds commonly found in grapes and wines of Sauvignon blanc, Cabernet-Sauvignon, Merlot and Semillon (ALLEN *et al.*, 1991; ROUJOU DE BOUBÉE *et al.*, 2000; CHAPMAN *et al.*, 2004; HUNTER *et al.*, 2004; SALA *et al.*, 2004; FALCAO *et al.*, 2007). The sensory detection threshold of 3-isobutyl-2-methoxypyrazine (IBMP) is very low, around 2 ng/L in water and around 15 ng/L in red Bordeaux wines (ROUJOU DE BOUBÉE *et al.*, 2000). MPs contribute to green pepper, green pea, herbaceous and asparagus-like aromas (MURRAY and WHITFIELD, 1975; MAGA, 1992). However, excessive IBMP concentrations can lead to unpleasant vegetative aromas, dominating the fruity sensory attributes of the wine (MARAIS and SWART, 1999; FALCAO *et al.*, 2007).

GSH and HCA are important preservatives of freshness in white wines. HCA are synthesized as the berry formation occurs. Pre-flowering leaf removal influences HCA concentration at harvest (STERNAD LEMUT *et al.*, 2011), whereas later treatment has no significant effect on HCA concentration at harvest (STERNAD LEMUT *et al.*, 2011; ŠUKLJE *et al.*, 2012). GSH synthesis in grape berry starts with berry sugar accumulation (ADAMS and LIYANAGE, 1993; ŠUKLJE *et al.*, 2012). The concentration of GSH in grapes may range from 14 to 102 mg/L, and levels of up to 35 mg/L were found in wines (DU TOIT *et al.*, 2007; JANEŠ *et al.*, 2010). GSH is one of the precursors in the synthesis of the volatile thiol 3-sulfanylhexan-1-ol (3SH), which is an aromatic compound responsible for the passion fruit aroma in Sauvignon blanc wines (PEYROT DES GACHONS *et al.*, 2002; THIBON *et al.*, 2009; ROLAND *et al.*, 2010; COETZEE and DU TOIT, 2012). GSH preserves the aromatic potential, especially volatile thiols and esters, in white wines (DUBOURDIEU *et al.*, 2001).

Volatile thiols are an important group of aromatic compounds that contribute to the aromatic profile of Sauvignon blanc wine. Unlike MPs, thiols do not exist in grapes or grape juice but are released during alcoholic fermentation from their non-volatile cysteine- and glutathione-conjugated precursors (PEYROT DES GACHONS *et al.*, 2002; SWIEGERS *et al.*, 2009). 3SH, its acetate 3-sulfanylhexyl acetate (3SHA), and 4-methyl-4-sulfanylpentan-2-one (4MSP) contribute to fruity aromas like grapefruit, passion fruit, black currant and box tree (DARRIET *et al.*, 1995; TOMINAGA *et al.*, 1998). Volatile thiols are easily detected, with a low olfactory threshold of 0.8 ng/L for 4MSP, 60 ng/L for 3SH and 4.2 ng/L for 3SHA in a model wine solution, respectively (DUBOURDIEU *et al.*, 2006). At high concentrations, they can impart strong, sweaty aromas reminiscent of cat urine (SWIEGERS *et al.*, 2009).

Carotenoid degradation in grapes is associated with the formation of C_{13}-norisoprenoids, which are compounds contributing to the aromatic profile of wines. Carotenoids are present in berry skins and berry pulp in concentrations at harvest ranging between 0.8 and 2.5 mg/kg (RAZUNGLES *et al.*, 1988; RAZUNGLES *et al.*, 1996). Some studies have investigated the effect of light and climate on the carotenoid concentration in grape berry (MARAIS *et al.*, 1991; OLIVEIRA *et al.*, 2004), but not much is known about the effect of leaf area to yield ratio on their concentration.

More research is needed not only on the effect of leaf area to yield ratio on basic physiological and chemical parameters of grapes and wines, but also on the concentrations of the varietal grape and wine aroma compounds of Sauvignon blanc and on the effect of abiotic factors on berry composition. The aim of this study was to investigate the effect of reducing leaf area by shoot hedging in combination with bunch thinning on GSH, MPs, carotenoid, HCA and volatile thiol concentration in Sauvignon blanc grapes and wines.

MATERIALS AND METHODS

1. Experimental design

The experiment was carried out in 2011 in a commercial *Vitis vinifera* L. cv. Sauvignon blanc (clone ISV-FV5) vineyard in Vipavska dolina (Vipava Valley), Slovenia. The vineyard was planted in 2002 on deep loamy Eutric gleyic Fluvisol, with the nutrient status defined as non-limiting for growth. The training system is a vertical shoot positioning, and the vines were pruned as single Guyot with nine buds per cane. The experiment was randomly designed across three rows, with four replicates of each treatment consisting of five continuous vines.

2. Shoot hedging and bunch thinning

Four treatments were introduced into the trial: shoot hedging/no bunch thinning (SH/NBT), full canopy/no bunch thinning (FC/NBT), shoot hedging/bunch thinning (SH/BT) and full canopy/bunch thinning (FC/BT).

The reduction of shoot length (primary shoot hedging) and bunch thinning were performed on 14 July 2011 (two weeks before *véraison*) at the phenological stage corresponding to 'beginning of berry touch' (E-L 33, EICHHORN and LORENZ, 1977). The shoot length and a canopy width of 30 cm were managed throughout the season. Canopy height in the SH/BT and SH/NBT treatments was 0.9 m, resulting in a reduction of 44 % of the exposed leaf area compared to the FC/BT and FC/NBT treatments. The second top bunch per shoot was removed for the bunch thinning treatments.

3. Yield and yield components

The grapes were harvested on 30 August 2011, and the harvest date was determined by the TSS level and titratable acidity (TA). The number of bunches and yield per vine were recorded to determine the bunch weight (total yield per vine/number of

Table 1 - Treatments introduced into the trial.

Treatment code	Hedging	Bunch thinning	Canopy height (m)
Shoot hedging/No bunch thinning (SH/NBT)	X		0.9
Full canopy/No bunch thinning (FC/NBT)			1.6
Shoot hedging/Bunch thinning (SH/BT)	X	X	0.9
Full canopy/Bunch thinning (FC/BT)		X	1.6

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bunches per vine). One hundred randomly sampled berries from each replicate were collected to determine the mean berry weight. The canopy external leaf area perimeter (CELAP) was calculated according to the method of DELOIRE (2012). The ratio between exposed leaf area (m^2) and yield per vine (kg) was calculated.

The temperature was recorded every 15 minutes using TinyTag® Plus 2 data loggers (Gemini Data Loggers, Chichester, United Kingdom). The canopy temperature was recorded using the TGP-4500 model and the bunch temperature was recorded with two flying lead thermistor probes (PB-5009-OM6) connected to a TGP-4520 model.

The normalized difference vegetation index (NDVI) was computed as initially described by ROUSE *et al.* (1973); it is calculated as a normalized ratio of solar radiance reflectance between the red band (670 nm, maximum chlorophyll absorbance) and near-infrared band (800 nm). This calculation is linked to the density of green vegetation. The index was derived through ARVAgreen ground sensors (ARVAtec S.R.L., Milan, Italy) mounted on four-wheel motorbikes.

4. Grape samples

Random bunches were weekly sampled from *véraison* (4.8.2011) to harvest (30.8.2011) and transported to the laboratory in cooling boxes. Berries were carefully cut and 200 berries were crushed by hand under an inert nitrogen atmosphere to prevent oxidation. The grape juice samples were used for further analyses.

The TSS concentration, pH value, TA and malic acid concentration were determined according to standard methods (EUROPEAN COMMISSION REGULATION (EEC) No. 2676/90, 1990). Malic acid was determined spectrophotometrically using a commercial enzymatic kit (Megazyme, Ireland).

The GSH concentration in grape juice was determined by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) and on-line pre-column derivatization. After crushing the berries under an inert nitrogen atmosphere, the grape juice was immediately placed in methanol and N-acetyl-L-cysteine as the internal standard was added, filtered through 0.45 µm Sartorius Minisart RC 25 filters (Goettingen, Germany), diluted 1:1 with a 5 mM sodium acetate buffer containing 0.1 mM EDTA, and immediately analyzed as previously described (JANEŠ *et al.,* 2010).

The HCA concentration in grape juice was determined on an Agilent Technologies 1100 HPLC with diode array detector (DAD) (Palo Alto, CA, USA) as described (VANZO *et al.*, 2007). After crushing the berries under an inert nitrogen atmosphere, the grape juice was collected and mixed with 1000 ppm SO_2 to inhibit enzymatic activity, filtered through a 0.45 µm Millipore PVDF filter (Bedford, MA, USA), and directly injected into the HPLC system. The method was developed for monitoring *cis*- and *trans*-caftaric acid, -coutaric acid, and -fertaric acid, respectively, together with caffeic, *p*-coumaric, ferulic, and 2-S-glutathionyl caftaric acid (GRP).

The MPs concentration in grape juice was determined by solid phase micro-extraction gas chromatography with mass spectrometric detection (SPME-GC-MS) as described by ŠUKLJE *et al.* (2012). An internal standard of deuterated IBMP (C/D/N Isotopes, Quebec, Canada) was added to the grape juice. Then 1.6 mL were transferred in a 20-mL headspace vial and 3 g of NaCl, 6.4 mL of deionized water and 2 mL of 4M NaOH were added. The sample was stirred until the NaCl was dissolved and placed on GC-MS for analyzes.

Free α-amino nitrogen (FAN) was determined spectrophotometrically as described by CORRADIN (1997) and NICOLINI *et al.* (1997). Using this method, yeast assimilable nitrogen (NH_4^+ and α-amino acid nitrogen) were determined.

Carotenoids were determined in whole grape berries according to the standard method (EUROPEAN COMMITTEE FOR STANDARDIZATION EN 12823-2, 2000). Frozen grape berries were saponified with an ethanolic potassium hydroxide solution. Carotenoids were extracted with dichloromethane and, after evaporation, the residue was dissolved in methanol. Quantifications for single carotenoids (b-carotene, lutein and neoxanthin) were performed using HPLC connected to photometric detector (UV-Vis).

5. Must and wine analyses

Winemaking practices: Approximately 5 kg of grapes were harvested for each treatment and wines were produced in triplicate using classical white wine vinification methods. Briefly, the grapes were cooled down to +4 °C and after 24 h were destemmed manually and 50 mg/L of SO_2 were added. After 3 h of cold maceration, the grapes were pressed by hand and pectolytic enzyme (Lafazym CL, Laffort, France) was added into the

juice. After 24 h of sedimentation at +4 °C, the juice was racked into 0.8-L fermenters and inoculated with 30 g/hL yeast strain VL3 (Laffort, France). The fermentation temperature was kept constant at 15 °C and 30 g/hL of ammonium salts with thiamin nutrient (Thiazote, Laffort, France) were added at one third of fermentation. After fermented to dryness, 50 mg/L of SO_2 was added and then the wine was racked and stored at +4 °C in 500-mL bottles.

The GSH concentration was monitored before yeast inoculation and four months after bottling, together with the analyses of the volatile thiols.

Volatile thiols were determined in the wines four months after bottling using a modified previously published method of TOMINAGA *et al.* (1998). Briefly, three internal standards were added in 50 mL of wine: 4-methoxy-2-methyl-2-sulfanylbutane (4MSB), [$^2H_2$]-3-sulfanylhexyl acetate (d3SHA) and [$^2H_2$]-3-sulfanylhexan-1-ol (d3SH) (University of Auckland, New Zealand). After the extraction procedure on Dowex columns as used by TOMINAGA and DUBOURDIEU (2006), the collected organic phases were evaporated under reduced pressure (250 mbar) to approximately 0.5 mL and transferred into 1.5-mL dark vials. The Soxhlet flask was rinsed with 0.5 mL of dichloromethane and then placed in an ultrasonic bath for 1 min. The samples were collected together in a 1.5-mL dark vial and concentrated under reduced pressure (100 mbar) to approximately 30 µL. Identification and quantification was performed with a gas chromatograph (Agilent Technologies 7890A) equipped with the MPS 2 automatic sampler (Gerstel, Mülheim an der Ruhr, Germany) and coupled with mass spectrometric detector (Agilent Technologies 5975C upgraded with Triple Axis detector). The thiols were separated on a HP-INNOWax column from Agilent J&W Scientific (60 m × 0.25 mm; 0.25 µm) using He carrier gas at a flow rate of 0.6 mL/min. The injector temperature was set to 240 °C; the initial oven temperature was set to 50 °C (held for 5 min) and ramped at a rate of 3 °C/min to 115 °C, then to 150 °C at 40 °C/min (held for 3 min), to 205 °C at 3 °C/min, and finally to 250 °C at 10 °C/min (held for 19.625 min) before dropping to 50 °C at 40 °C/min (held for 3 min). The ion source temperature was 230 °C, the auxiliary temperature was 250 °C and the quadrupole temperature was 150 °C. For qualitative determination, retention time and mass spectrum in Selective Ion Monitoring mode (SIM) were used. The ions m/z 116, 118, 132, 134, 134 and 136 were used as quantifiers for 3SHA, d3SHA, 4MSP, 4MSB, 3SH and d3SH, respectively. The ions m/z 101, 103, 75, 75, 100 and 102 were used as qualifiers for 3SHA, d3SHA, 4MSP, 4MSB, 3SH and d3SH, respectively. One-point calibration was performed using calibration standard in alcoholic solution with final concentration of 65 ng/L of 4MSP, 650 ng/L of 3SHA and 1202 ng/L of 3SH.

Sensory evaluation of wines: Odor comparison profile descriptive analyses were used for the sensory evaluation of the wines. The descriptors assigned were as follows: fresh tropical aromas (citrus, guava and grapefruit), heavier tropical aromas (passion fruit, mango, black currant and cat urine), fermentation aromas (pear and apple), green aromas (green pepper and asparagus) and overall quality. Eight panelists (two women and six men, ranging in age from 27 to 62), representing wine experts employed at the Agricultural Institute of Slovenia and wine producers, were asked to evaluate the wines on a five-point scale, with 5 representing the highest score and 1 the lowest.

Data analyses: Differences between the treatments were tested for significance by applying the analysis of variance (ANOVA). Statistical analyses were run using Statgraphics® Centurion XVI (StatPoint Technologies, Warrenton, VA, USA). The means were separated using Fisher's LSD test (different letters account for significant differences at $p \leq 0.05$).

RESULTS AND DISCUSSION

1. Yield and yield components

The number of bunches per vine was significantly reduced in the treatments subjected to bunch thinning. As reported in other experiments (REYNOLDS and WARDLE, 1989; HOWELL, 2001), yield reduction was directly related to the number of bunches removed, although there was a compensation effect on bunch weight. Similar results were also observed by DAMI *et al.* (2006) in Chambourcin (a French-American hybrid) and by EDSON *et al.* (1995) in Seyval grapevines. A significantly lower berry weight (as determined by weighing 100 berries) was observed in the FC/BT treatment, whereas it did not differ significantly within other treatments. No effect of shoot hedging on bunch weight could be observed in this experiment.

The evaluation of leaf area to yield ratio was done by estimation, using CELAP and yield per vine. All treatments resulted in high leaf area to yield ratio that ranged from 0.63 to 1.85 m^2/kg, as seen in

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Table 2. Total leaf surface underestimates leaf area, since there is always more than one leaf layer, thus the values of the index have to be considered more than optimal if the range of 0.8 to 1.2 m² leaf area per kg of fruit proposed by KLIEWER and DOKOOZLIAN (2005) is required to undergo optimal grape ripening. The ratio between the exposed leaf area and yield was significantly lower in the SH/NBT treatment, which was expected, as there was no significant difference between FC/NBT and SH/BT (Table 2).

The temperature in the bunch area from 10 July 2011 and harvest date did not vary significantly between the treatments with and without shoot hedging. The average daily bunch temperature in treatments without shoot hedging was 22.3°C and 22.4°C in treatments with shoot hedging. On the hottest days the maximum daily bunch temperature exceeded 37 °C in all the treatments.

No significant difference was revealed by mapping the grapevine canopy (NDVI), which confirms the vineyard homogeneity in the experiment, as seen in Table 2.

Both shoot hedging and bunch thinning had no impact on pH value, TSS and TA concentration in grape juice at harvest (Table 3). A significantly lower TSS concentration at *véraison* was found in grape juice from the treatments with shoot hedging, irrespective of bunch thinning, when compared to FC/BT and FC/NBT treatments (Table 3). From *véraison* onwards, the SH/NBT treatment resulted in significantly lower TSS concentration compared to treatments with full canopy as well as with SH/BT, but there was no significant difference in TSS concentration at harvest within the treatments. Shoot hedging delayed the decrease of TA on the

Table 2 - Effect of bunch thinning and shoot hedging on the yield and growth components of Sauvignon blanc vines at harvest.

Treatment	Berry weight (g)	Bunch weight (g)	Bunches/vine	m2 leaf area/kg fruit	CELAP (m2/vine)	NDVI total
Shoot hedging/No bunch thinning (SH/NBT)	1.93±3.30 a	97.2±9.25 a	12.3±2.52 a	0.63±0.08 c	1.87±0.06 b	0.774±0.01 a
Full canopy/No bunch thinning (FC/NBT)	1.94±2.64 a	103.2±22.96 a	13.3±2.52 a	1.07±0.08 b	3.18±0.06 a	0.748±0.04 a
Shoot hedging/Bunch thinning (SH/BT)	1.98±2.68 a	96.6±11.31 a	7.3±0.58 b	1.15±0.09 b	1.80±0.05 b	0.725±0.04 a
Full canopy/Bunch thinning (FC/BT)	1.86±4.98 b	112.1±9.19 a	7.0±1.00 b	1.85±0.12 a	3.17±0.07 a	0.736±0.03 a

CELAP = canopy external leaf area perimeter, NDVI = normalized difference vegetation index
ANOVA was used to compare data. Means followed by different letters in a column are significant at $p \leq 0.05$ (Fisher's LSD).

Table 3 - Effect of bunch thinning and shoot hedging on the yield and growth components of Sauvignon blanc vines at harvest.

Sampling dates	4 Aug 11 *véraison*	16 Aug 11	23 Aug 11	30 Aug 11 harvest
Total soluble solids (TSS) (Brix)				
Shoot hedging/No bunch thinning (SH/NBT)	11.4±0.4 b	16.3±1.43 b	17.3±1.01 b	21.7±0.50 a
Full canopy/No bunch thinning (FC/NBT)	14.1±0.35 a	18.9±0.30 a	21.1±0.64 a	22.4±0.68 a
Shoot hedging/Bunch thinning (SH/BT)	12.4±0.81 b	18.3±0.40 a	20.3±0.26 a	22.3±0.17 a
Full canopy/Bunch thinning (FC/BT)	15.1±0.61 a	19.5±0.17 a	21.2±0.15 a	22.9±0.38 a
Titratable acidity (TA) (g/l)				
Shoot hedging/No bunch thinning (SH/NBT)	24.4±0.89 a	12.7±0.42 a	9.8±0.21 a	7.6±0.45 a
Full canopy/No bunch thinning (FC/NBT)	21.1±0.53 b	11.6±0.25 b	9.6±0.51 a	7.4±0.36 a
Shoot hedging/Bunch thinning (SH/BT)	21.1±1.61 b	11.6±0.25 b	10.3±0.05 a	7.6±0.17 a
Full canopy/Bunch thinning (FC/BT)	19.2±1.97 b	10.7±0.30 c	9.6±0.41 a	7.2±0.21 a
pH				
Shoot hedging/No bunch thinning (SH/NBT)	2.64±0.04 a	2.91±0.07 b	3.05±0.06 a	3.26±0.05 a
Full canopy/No bunch thinning (FC/NBT)	2.68±0.01 a	2.96±0.04 ab	3.08±0.01 a	3.25±0.04 a
Shoot hedging/Bunch thinning (SH/BT)	2.66±0.05 a	2.97±0.02 ab	3.07±0.03 a	3.27±0.05 a
Full canopy/Bunch thinning (FC/BT)	2.72±0.04 a	3.01±0.02 a	3.11±0.02 a	3.26±0.03 a

ANOVA was used to compare data. Means followed by different letters in a column are significant at $p \leq 0.05$ (Fisher's LSD).

first and second sampling date in the SH/NBT treatment, but there was no significant difference among the treatments at harvest. In addition, shoot hedging and bunch thinning had no significant effect on pH value during the grape maturation.

Lower TSS concentration in grape juice in SH/NBT treatment during ripening, excluding harvest time, could be related to the following hypotheses : i) lower leaf area to yield ratio, which indicates that the vines were not able to compensate, at that stage, using carbohydrate reserve; ii) the remaining leaves were not able to increase their photosynthetic activity (VASCONCELOS and CASTAGNOLI, 2000); and iii) the rate of sugar accumulation per berry was different between treatments (DELOIRE, 2011). At *véraison*, shoot hedging removes young and also some photosynthetically active leaves, and the regrowth of laterals at this stage is normally very low, depending on the vigor of the vines. According to PONI and GIACHINO (2000), a strong late-season reduction in the source-to-sink relationship results in a ripening delay (sugar accumulation), even if the leaf area to yield ratio is not a limiting factor. DELOIRE (2011) observed that late treatments changing the source-to-sink relationship had no effect on berry sugar accumulation if sugar per berry had reached a plateau before the application of the treatment, mainly in situation of low to medium vigor for which irrigation is managed properly. This is in accordance with REYNOLDS and WARDLE (1989), who did not find modifications in sugar accumulation or acidity degradation with shoot hedging treatments.

The GSH concentration in grape juice ranged from 53 mg/L at the first sampling date up to 95 mg/L at harvest time (Figure 1). As described by ADAMS and LIYANAGE (1993) and ŠUKLJE *et al*. (2012), GSH synthesis in the berry and in grape juice was associated with sugar accumulation, and the concentration increased with maturation. At *véraison* (4.8.2011), no significant differences in GSH concentration in grape juice were revealed between the treatments. However, a higher concentration of GSH was found in the FC/BT treatment at the second sampling date and in the FC/NBT treatment at the third sampling date. It seems that shoot hedging delayed GSH synthesis at the first sampling dates. At harvest, neither bunch thinning nor shoot hedging had a significant effect on GSH concentration in grape juice. In the SH/NBT and SH/BT treatments, GSH accumulation continued throughout ripening, reaching significantly higher values at harvest time.

Shoot hedging and bunch thinning had no significant effect on HCA concentration in grape juice during ripening as well as at harvest (Table 4). The most abundant HCA found in Sauvignon grape juice were *trans*-caftaric acid and *trans*-coutaric acid, which ranged from 116.8±6.8 to 127.3±6.5 mg/L and from 11.7±1.6 to 13.4±0.7 mg/L (expressed as *trans*-caftaric acid equivalent) at harvest, respectively. Concentrations of *cis*-caftaric and *cis*-coutaric acid in grape juice at harvest ranged from 4.3±0.4 to 4.6±0.3 and from 3.1±0.3 to 3.4±0.2 mg/L (expressed as *trans*-caftaric acid equivalent), respectively. Caftaric acid *o*-quinone can be reduced by GSH, resulting in the production of colorless GRP (SINGELTON *et al*., 1985), which concentration was between 0.26 and 2.30 mg/L for all sampling dates and all treatments (data not shown). Free HCA (caffeic, *p*-coumaric, ferulic) were not detected as they are usually found later in wines due to yeast hydrolysis. Low GRP values indicates that the concentrations of GSH and caftaric acid were not influenced by oxidation during the sample preparation.

The IBMP concentration in the grape juice at *véraison* varied from 5.2 to 8.0 ng/L (data not shown), but the differences among treatments were not significant. After *véraison*, the concentration of IBMP dropped rapidly and by the second sampling date was already below the limit of detection (LD 0.6 ng/L), whereas the 3-isopropyl-2-

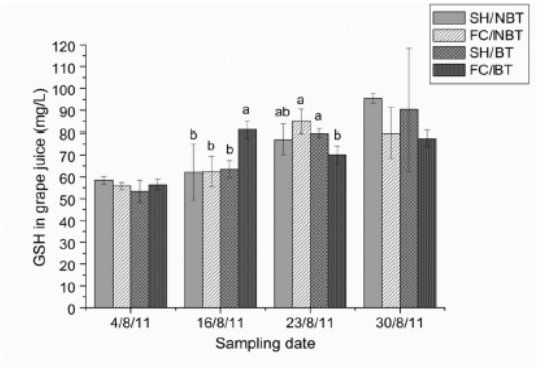

Figure 1 - The glutathione (GSH) concentration in grape juice (mg/L) during grape maturation from *véraison* to harvest in response to different canopy management treatments.
SH/NBT = Shoot hedging/No bunch thinning, FC/NBT = Full canopy/No bunch thinning, SH/BT = Shoot hedging/Bunch thinning, FC/BT = Full canopy/Bunch thinning.
Means indicated by different letters are significantly different at $p \leq 0.05$ (Fisher's LSD).

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methoxypyrazine (IPMP) concentration was already below the LD at the first sampling date. The IBMP concentration depends largely on temperature and bunch exposure to light (BELANCIC and AGOSIN, 2007; SCHEINER *et al.*, 2010). The fast decrease in IBMP concentration in our study was probably related to the high seasonal temperatures. The Huglin index was 2557 in 2011, while for the same vineyard it was 2297 in 2010, when IBMP was also detected at harvest in September. CHAPMAN *et al.* (2004) have reported that in Cabernet-Sauvignon wines the perception of green aroma decreased with increased yield, which was not observed in our study. Moreover, recent studies showed that canopy manipulation (i.e., leaf and lateral shoots removal at the bunch zone) performed in the vineyard immediately after flowering influences the IBMP concentration more drastically than the same treatment applied later in the season (RYONA *et al.*, 2008; ROBINSON *et al.*, 2011). It could be that bunch thinning and shoot hedging would have a more significant effect on the IBMP concentration when performed sooner after flowering, due to a change in the bunch microclimate (RYONA *et al.*, 2008).

Table 4 - Concentration of hydroxycinnamoyl tartaric acids (HCA) in grape juice (expressed in mg/L of *trans*-caftaric acid equivalents) during grape maturation from *véraison* to harvest in response to different canopy management treatments.

Sampling dates	4 Aug 11 *véraison*	16 Aug 11	23 Aug 11	30 Aug 11 harvest
trans-caftaric acid (mg/L expressed as *trans*-caftaric acid equivalent)				
Shoot hedging/No bunch thinning (SH/NBT)	138.8± 3.4 a	119.9±10.0 a	121.9±8.5 a	116.8±6.8 a
Full canopy/No bunch thinning (FC/NBT)	130.3±12.5 a	117.3±14.7 a	121.4±15.1 a	122.1±13.4 a
Shoot hedging/Bunch thinning (SH/BT)	135.1±18.7 a	137.0±6.3 a	125.3±17.9 a	127.3±6.5 a
Full canopy/Bunch thinning (FC/BT)	127.4±7.9 a	121.9±8.7 a	116.2±7.2 a	119.5±11.2 a
cis-caftaric acid (mg/L expressed as *trans*-caftaric acid equivalent)				
Shoot hedging/No bunch thinning (SH/NBT)	3.4±0.5 a	3.3±0.3 a	3.7±0.1 a	4.6±0.3 a
Full canopy/No bunch thinning (FC/NBT)	3.4±0.8 a	3.4±0.3 a	4.0±0.3 a	4.3±0.4 a
Shoot hedging/Bunch thinning (SH/BT)	3.1±0.1 a	3.7±0.2 a	3.9±0.5 a	4.6±0.2 a
Full canopy/Bunch thinning (FC/BT)	3.4±0.2 a	3.4±0.2 a	3.6±0.2 a	4.4±0.7 a
trans-coutaric (mg/L expressed as *trans*-caftaric acid equivalent)				
Shoot hedging/No bunch thinning (SH/NBT)	12.5±1.3 a	11.3±1.2 a	10.7±0.8 a	11.7±1.6 a
Full canopy/No bunch thinning (FC/NBT)	12.8±1.7 a	11.8±0.9 a	10.6±2.4 a	12.1±1.8 a
Shoot hedging/Bunch thinning (SH/BT)	12.3±1.9 a	12.6±1.1 a	11.4±1.4 a	13.4±0.7 a
Full canopy/Bunch thinning (FC/BT)	12.1±0.8 a	12.5±1.8 a	10.7±1.0 a	12.5±2.1 a
cis-coutaric (mg/L expressed as *trans*-caftaric acid equivalent)				
Shoot hedging/No bunch thinning (SH/NBT)	2.3±0.3 a	2.9±0.3 a	2.8±0.1 a	3.1±0.3 a
Full canopy/No bunch thinning (FC/NBT)	2.1±0.4 a	3.0±0.2 a	2.6±0.6 a	3.2±0.4 a
Shoot hedging/Bunch thinning (SH/BT)	1.9±0.2 a	3.0±0.3 a	2.8±0.2 a	3.4±0.2 a
Full canopy/Bunch thinning (FC/BT)	2.0±1.1 a	3.0±0.2 a	2.7±0.3 a	3.3±0.6 a
SUM (mg/L expressed as *trans*-caftaric acid equivalent)				
Shoot hedging/No bunch thinning (SH/NBT)	156.3±10.12 a	140.0±11.46 a	142.9±9.59 a	137.8±8.85 a
Full canopy/No bunch thinning (FC/NBT)	150.9±15.14 a	138.3±15.87 a	142.1±18.14 a	143.1±15.89 a
Shoot hedging/Bunch thinning (SH/BT)	154.5±21.10 a	159.5±7.66 a	146.9±19.81 a	150.3±7.19 a
Full canopy/Bunch thinning (FC/BT)	147.2±8.48 a	144.1±11.21 a	136.6±8.57 a	141.9±13.12 a

Sum of HCA (mg/L expressed as *trans*-caftaric acid equivalent) represents the sum of *cis*- and *trans*-caftaric acid, *cis*- and *trans*-coutaric acid, *cis*- and *trans*-fertaric acid and GRP values. ANOVA was used to compare data. Means followed by different letters in a column are significant at $p \leq 0.05$ (Fisher's LSD).

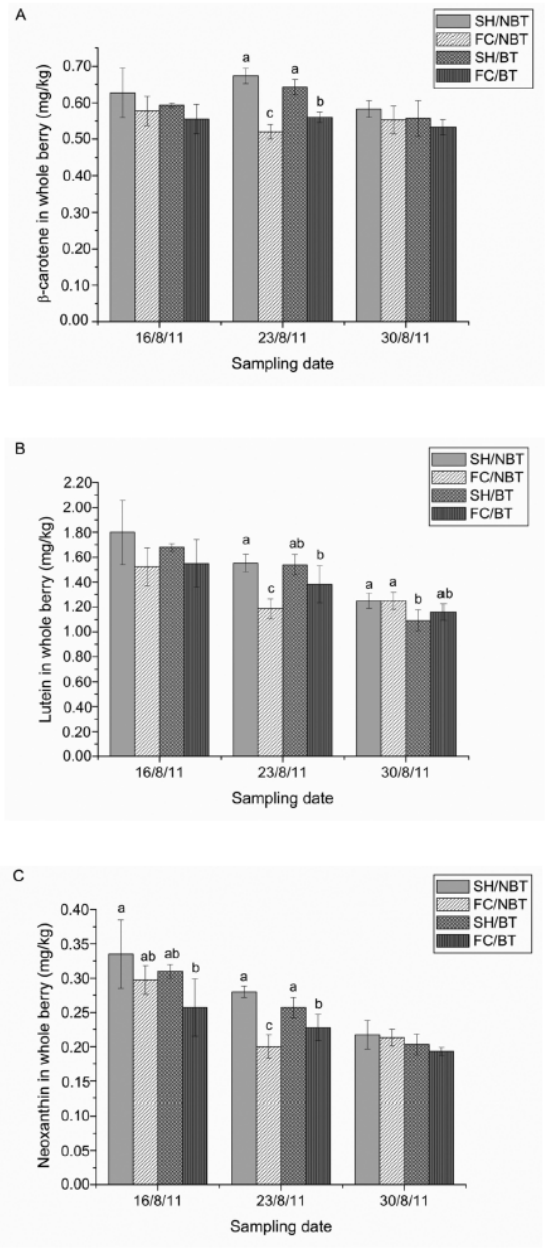

Figure 2 - Concentration of β-carotene (A), lutein (B) and neoxanthin (C) in whole berry (expressed in mg/kg) during grape maturation from the second sampling date (16.8.2011) to harvest in response to different canopy management treatments. SH/NBT = Shoot hedging/No bunch thinning, FC/NBT = Full canopy/No bunch thinning, SH/BT = Shoot hedging/Bunch thinning, FC/BT = Full canopy/Bunch thinning. Means indicated by different letters are significantly different at $p \leq 0.05$ (Fisher's LSD).

The concentrations of FAN and malic acid were determined in the grape juice at harvest. Again, no significant differences were found between treatments. The FAN concentration in must at harvest varied between 171±43 mg N/L and 164±36 mg N/L for SH/NBT and FC/NBT, respectively, and between 122±28 mg N/L and 114±15 mg N/L for SH/BT and FC/BT, respectively, and were considered as low must FAN concentrations (BISSON and BUTZKE, 2000). CHONÉ (2003) showed that high nitrogen concentration in the soil increased the concentration of thiol precursors in the berry, however, yeast strains vary in their ability to release volatile thiols from their precursors and consequently affect the concentration of 3SH and 4MSP in wine (MURAT *et al*., 2001; HOWELL *et al*., 2004). The role of must nitrogen status on thiol release is not known (BELL and HENSCHKE, 2005). The malic acid concentration of the grape juice varied from 1.70 to 1.90 g/L.

Carotenoid concentrations in whole grape berries were analyzed at the last three sampling dates. No significant difference was observed in the concentration of β-carotene and neoxanthin at harvest (Figure 2). The concentration of lutein at harvest was significantly lower in the treatments with bunch thinning. β-carotene and lutein were the predominant carotenoids in grape berry. With the exception of β-carotene at the sampling performed one week before harvest, the concentration of studied carotenoids decreased during grape maturation, as already observed in previous studies (RAZUNGLES *et al*., 1988; YOUNG *et al*., 2012). The slight increase in the β-carotene concentration in the berry one week before harvest could be due to a sampling effect. FARINA *et al*. (2010) observed that the temperature and radiation at the fruit level were positively related to carotenoid concentration. On the other hand, MARAIS *et al*. (1991) reported lower carotenoid concentration in the case of sun-exposed bunches. OLIVEIRA *et al*. (2004) reported a higher carotenoid concentration in Touriga Nacional grapes from vines with longer shoots, while in our study shoot hedging had no significant effect on the concentration of carotenoids at harvest.

Must and wine: The basic parameters of the must were analyzed before yeast inoculation (Table 5). Slight differences in TSS concentration were observed after grape maceration and pressing. The TA concentration in must decreased when compared to that in the grapes at harvest, while the pH values slightly increased.

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A reduction in the concentration of GSH was found in the musts before yeast inoculation in comparison to concentrations found in the grape juice at harvest, although this was expected due to some oxidation during pressing. The GRP values in the musts varied from 32.5 to 36.2 mg/L. The results showed that all the treatments underwent similar oxidation patterns during winemaking (Table 5). A significantly higher must GSH concentration was observed in FC/BT, even though there were no significant differences in grape GSH concentration at harvest. The must GSH concentrations did not differ significantly between the other three treatments (Table 5) and were slightly higher than the values reported by DU TOIT *et al.* (2007), who found levels of up to 35 mg/L.

The GSH concentration in wines was also determined four months after bottling, together with the determination of volatile thiols and the sensory evaluation of the wines. An important reduction in GSH concentration was observed in all treatments when compared to the must. Four months after fermentation, the GSH concentration had decreased by 77 to 82 % on average. Similar

Table 5- Concentration of total soluble solids (TSS), titratable acidity (TA), pH, 2-S-glutathionyl caftaric acid (GRP) and glutathione (GSH) in must before yeast inoculation for all treatments and of glutathione (GSH) in wine four months after bottling, before sensory evaluation.

	Must (before yeast inoculation)					Wine (4 months after bottling)
Treatments	TSS (Brix)	TA (g/L)	pH	GRP (mg/L)	GSH (mg/L)	GSH (mg/L)
Shoot hedging/No bunch thinning (SH/NBT)	20.7±0.06 c	6.3±0.06 a	3.39±0.00 a	35.8±0.11 b	42.1±4.97 b	8.7±1.42 ab
Full canopy/No bunch thinning (FC/NBT)	21.4±0.06 b	6.0±0.05 b	3.37±0.01 b	32.5±0.16 d	38.1±1.76 b	7.4±1.02 b
Shoot hedging/Bunch thinning (SH/BT)	21.4±0.06 b	6.3±0.00 a	3.41±0.02 a	36.2±0.22 a	41.6±0.90 b	7.4±2.01 b
Full canopy/Bunch thinning (FC/BT)	22.3±0.06 a	6.1±0.06 b	3.39±0.00 a	35.1±0.05 c	50.6±2.39 a	11.7±2.17 a

ANOVA was used to compare data. Means followed by different letters in a column are significant at $p \leq 0.05$ (Fisher's LSD).

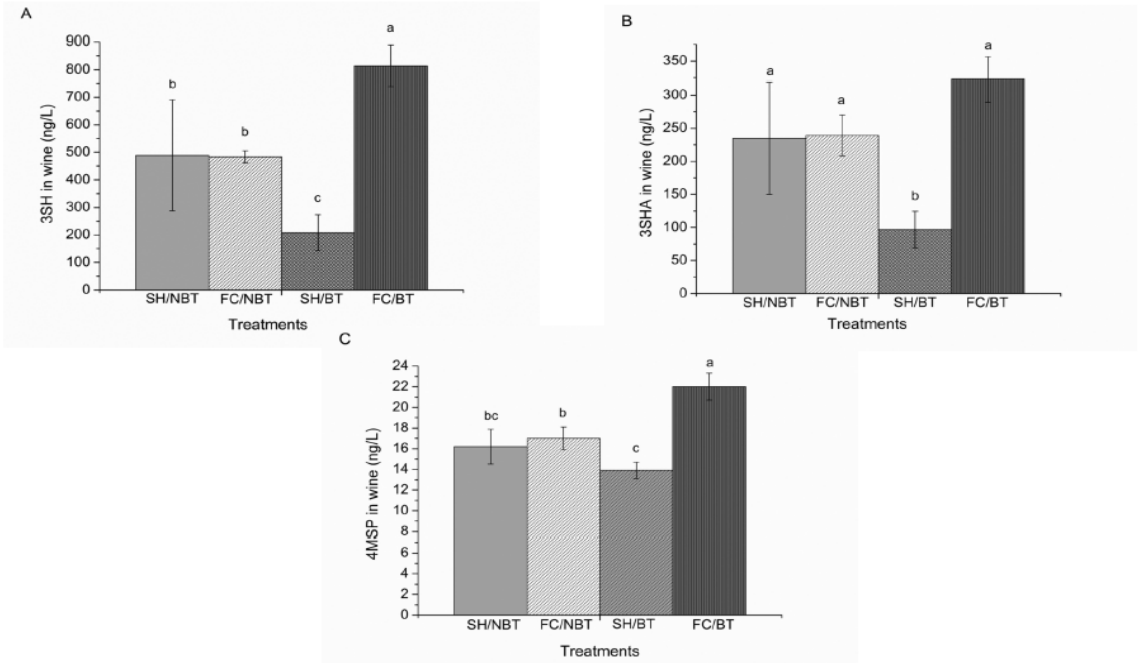

Figure 3 - Concentration of 3-sulfanylhexan-1-ol (3SH, A), 3-sulfanylhexyl acetate (3SHA, B) and 4-sulfanyl-4-methylpentan-2-ol (4MSP, C) (ng/L) in wine four months after fermentation. Means indicated by different letters are significantly different at $p \leq 0.05$ (Fisher's LSD).

results were also reported by HERBST-JOHNSTONE *et al*. (2011), who proposed a 49 to 77 % decline in GSH concentration in Sauvignon blanc wines three months after bottling.

The concentration of 3SH and 4MSP was significantly higher in FC/BT compared to the other treatments, as seen in Figure 3. The concentration of 3SH, 3SHA and 4MSP did not differ significantly between the SH/NBT and FC/NBT treatments, whereas in SH/BT treatment 3SH and 3SHA concentrations were significantly lower compared to other treatments (Figure 3). This could be due to the reduction of both the leaf area providing amino acids and the strength of the sink (bunches). PEYROT DES GACHONS *et al.* (2005) reported that nitrogen fertilization was profitable to acquire higher concentrations of thiols, which in parallel could stimulate vegetative growth.

The tasting panel confirmed the heavier tropical aroma of Sauvignon blanc in the FC/BT wines, as revealed by the higher concentration of 4MSP and 3SH found in this treatment (Figure 4), whereas the perception of the heavier tropical aroma was the lowest in the wines from SH/BT treatment.

The GRP values in must before fermentation ranged from 32.5 to 36.2 mg/L between the treatments. Beside the significant difference in GRP concentration between the treatments, the FC/BT treatment resulted in statistically higher GSH concentration in must, which could be associated to the higher production of 3SH in wine (Table 5). The concentration of the thiol precursors 3-*S*-glutathionylhexan-1-ol (G3SH), 3-*S*-cysteinylhexan-1-ol (Cys3SH) and 4-*S*-glutathionyl-4-methylpentan-2-one (G4MSP) increases significantly with grape maturation, while 4-*S*-cysteinyl-4-methylpentan-2-one (Cys4MSP) is more affected by the origin of the grapes than by the maturation stage (ROLAND *et al*., 2010). The same authors confirmed that the addition of GSH to Sauvignon blanc must resulted in a higher concentration of G3SH and consequently in a 25 to 41 % higher concentration of 3SH and 3SHA in the resulting wines, whereas in another study from Patel *et al.* (2010) higher GSH concentrations in must did not lead to higher 3SH and 3SHA production. It is likely that the significantly higher GSH concentration in the grape must positively influenced the concentration of 3SH in the FC/BT wines, while the 3SHA released from 3SH is more yeast related (SWIEGERS *et al.*, 2006). The perception of green pepper and asparagus-like nuances was quite high, even though no MPs were present in the wine. This perception of greenness may have originated from other compounds, such as hexanal and other C6 compounds (TANDON *et al*., 2000), which were not analyzed in our study. The overall quality of the wines that were produced was found to be significantly higher in the wine from the FC/BT treatment, whereas the wines from the treatments with hedging were rated as the least desirable (Figure 4).

CONCLUSIONS

Shoot hedging and bunch thinning could directly or indirectly influence the concentration of the primary and secondary metabolites of grapes by influencing their biosynthesis and/or accumulation per berry, thereby influencing the sensory properties and styles of the wines produced. Shoot hedging seems to have an effect on the kinetics of berry ripening and seems to delay TSS accumulation, which in our situation confirmed the non-compensation of the remaining leaves or the wood carbohydrate reserve. Berry maturation was slower in treatment with lower leaf area per yield. To influence the IBMP concentration of berry, shoot hedging and bunch thinning should be performed earlier in the season. The obtained results showed that higher exposed leaf area per yield significantly affects the grape juice GSH concentration, perception of tropical nuances and overall quality of the produced Sauvignon blanc wines, as confirmed by the chemical analyses and wine sensory evaluation. The sensory evaluation panel observed that overall quality of the wines was significantly higher in FC/BT treatment

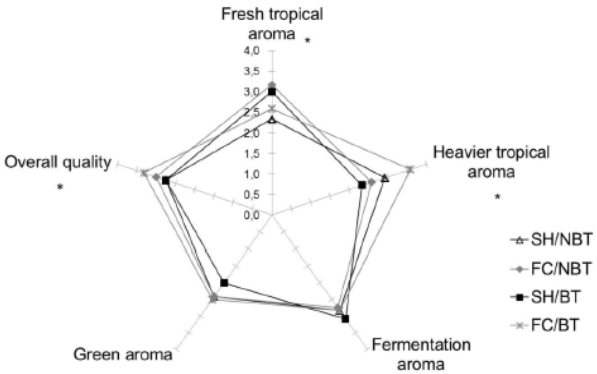

Figure 4- Sensory evaluation of the wines. Means indicated by different letters are significantly different at $p \leq 0.05$ (Fisher's LSD).

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compared to other treatments. Furthermore, this treatment had the highest leaf area to yield ratio, which can indicate that different leaf area to fruit ratio thresholds for some secondary compounds should have been proposed as suggested 7-14 cm^2/g by Kliewer and Ough (1970).

This study clearly showed the complexity of the relationship between leaf and fruit at the vine level (source-sink dynamics). Therefore, canopy manipulation in the vineyard should be reasoned carefully, according to the site (macro and meso climates: hot/warm versus temperate/cool), the row orientation, the bunch microclimate, the vine's vigor, and the desired yield per vine and wine styles (CARBONNEAU *et al.,* 2007; DELOIRE, 2012).

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3 DISCUSSION AND CONCLUSION

3.1 DISCUSSION

The overall aim of this study was to investigate the effect of some frequently used viticultural practices on the concentration of primary and secondary metabolites in 'Sauvignon blanc' (*Vitis vinifera* L.) grape berries during ripening and in the associated wines, and their effects on the sensory properties of these wines.

The main objectives were fulfilled by studying the grape berry metabolites through:
- modification of the bunch microclimate by the removal of leaves and secondary (lateral) shoots in the bunch zone, and
- by the alteration of the leaf area-to-yield ratio through shoot hedging and bunch thinning.

In addition, the classification of grape berries first according to their diameter and thereafter according to total soluble solids (TSS) was introduced in the first year to study the effect on concentrations of methoxypyrazines (MPs), reduced glutathione (GSH) and hydroxycinnamates (HCAs) in grape berry. In the second season, the effect of UV light on grape berry metabolites was investigated through the installation of UV light-reducing sheets.

Finally, the effect of the removal of leaves and secondary shoots and modification of UV light exposure of the bunches on wine chemical composition and wine sensory perception was investigated. Furthermore, the modification of the leaf area-to-yield ratio by shoot hedging and bunch thinning on the resulting wine chemical composition and sensory perception was investigated.

Briefly, in the first year of the experimentation two treatments were introduced into the trial: a control treatment, consisting of shaded bunches by not altering the natural canopy (C) and exposing bunches by removing all leaves and secondary shoots in the bunch zone on the morning side (M-LR) of the canopy at the height 40 cm above the cordon. In the second year, additional treatment (LR-UV) was introduced: i.e. removal of all leaves and secondary shoots in the bunch zone on the morning/north-eastern side of the canopy with reduction of UV light. UV light in the bunch zone was reduced with the installation of clear UHI (impact modified) extruded acrylic sheets (Perspex® South Africa), which eliminate 99 % of total UV radiation with only a 12% visible light reduction. The sheets were installed on the morning/north-eastern side of the canopy covering the bunch zone after all leaves and secondary shoots were removed.

In the experiment with the modified leaf area to yield ratio four treatments were introduced into the trial: shoot hedging/no bunch thinning (SH/NBT), full canopy/no bunch thinning (FC/NBT), shoot hedging/bunch thinning (SH/BT) and full canopy/bunch thinning (FC/BT). Canopy height in the SH/BT and SH/NBT treatments was 0.9 m, resulting in a

reduction of 44% of the exposed leaf area compared to the FC/BT and FC/NBT treatments. The second top bunch per shoot was removed for the bunch thinning treatments.

The results will be discussed in three separate paragraphs, divided according to the effect of viticultural practices on the composition of the grape berry, on wine composition and on wine sensory evaluation.

3.1.1 Grape berry composition

Heterogeneity of the grape berry composition inside the same vineyard was shown by berry classification according to diameter and TSS concentration. Grape berries were first classified according to their diameter using special Perspex plates. Each plate contained holes of different diameter, from 10.5 to 16.5 mm, increasing at 1 mm intervals. Grape berries were evenly distributed along a Gaussian bell-shaped curve according to their diameter for all three sampling dates, which confirmed the homogenous distribution of the berries across the three major berry classes, which is in agreement with Deloire et al. (2004) and Rolle et al. (2012). Berries of the same diameter were further classified according to their TSS concentration by flotation in sucrose solutions of different concentrations, which suggested that berries of the same diameter were not at the same ripening stage. Berries of the same volume could have different TSS concentration and therefore different ripening level if we do consider that the dynamic of berry sugar accumulation per fruit is a relevant physiological indicator of fruit ripening (Deloire, 2011, 2013). In our study, a decrease of 44 % of the exposed leaf area resulted in a modified leaf area-to-yield ratio, which was 0.63 m^2/kg in the treatment with shoot hedging and no bunch thinning, and significantly lower compared to the leaf area-to-yield ratio of 1.85 m^2/kg in the treatment without shoot hedging but with bunch thinning. A reduction of exposed leaf area for 44 % without bunch thinning resulted in significantly slower accumulation of TSS in the grape berry at the first samplings, probably due to the lower leaf area-to-yield ratio (0.63 m^2/kg), which could indicate that the vines were not able to compensate, at that stage, by using carbohydrate reserves, or that the remaining leaves did not increase their photosynthetic activity. No significant difference was observed at harvest in the concentration of TSS. In our study, a modified leaf area-to-yield ratio did not influence the titratable acidity (TA) concentrations or the pH value in the grapes at harvest.

It has been shown previously that a combination of leaf and secondary shoot removal causes lower 3-isobutyl-2-methoxypyrazine (IBMP) concentrations in the grape berry when performed early enough in the season (Roujou de Boubée, 2001; Ryona et al., 2008; Scheiner et al., 2010), whereas leaf removal performed after veraison had no influence on IBMP concentration. This is in agreement with our results, where removal of leaves and secondary shoots led to significantly lower IBMP and 3-isopropyl-2-methoxypyrazine (IPMP) concentrations in the grape berry compared to the control. The IBMP concentrations in the grape berries were already below the limit of detection (0.6 ng/L) at the treatment of leaf and secondary shoot removal two weeks before harvest. In the control

treatment, IBMP at harvest was present in the grape berries at concentrations between 5.2 and 12.6 ng/L. Furthermore, the berries of larger diameter and similar TSS concentration contained higher concentrations of IBMP. It has been shown by Ojeda et al. (1999) that the main determinants of the genetic variation for berry size occurs before veraison, that is during a stage of intense cell division associated with cell extension in the grapevine fruit. More intense cell division before veraison might be related to higher IBMP concentrations in grape berries of larger diameter, since IBMP is synthesised already before veraison. In addition, Dunevly et al. (2013) shown that IBMP is synthetised in the berry flesh, which suggest that bigger berry size, contribute to higher IBMP concentrations as observed in our study. At harvest, the concentration of IBMP in the grape berries of similar TSS in the control was 12.6 ng/L and 5.2 ng/L at the berry diameter classes of 15.5 mm and 13.5 mm, respectively. This is in accordance with Sala et al. (2005) who showed higher IBMP concentrations in bunches from irrigated vines. Berries of higher TSS concentrations (11.6 °Brix) resulted in significantly lower IBMP concentrations (17.8 ng/L) compared to berries of lower TSS concentrations, viz. 5.1 °Brix and IBMP concentration of 72.4 ng/L, which is in agreement with previous studies where a decrease in IBMP concentrations in grape berries during grape maturation is reported (Roujou de Boubée, 2001; Ryona et al., 2008; Koch et al., 2012). A change of a leaf area-to-yield ratio, conducted in the Vipavska dolina winegrowing district, did not affect IBMP concentrations which were below the limit of detection in grape berries (0.6 ng/L) at the second sampling (a week after veraison). That suggested that the MPs are not the aromatic compounds significantly influencing Slovenian Sauvignon blanc wines, as recently reported by Lisjak et al. (2011). Low IBMP concentrations measured in 'Sauvignon blanc' grapes and wines originating from the Vipavska dolina winegrowing district are most likely related to high seasonal air temperatures during grape maturation. It has been already reported that grapes and wines originating from cooler climatic regions contain higher concentrations of IBMP (Lacey et al., 1991).

The removal of leaves and secondary shoots had no significant effect on the GSH concentrations in the grape berry. The GSH concentration in the grape berry increased together with TSS accumulation, as well as above the value of 16 °Brix previously reported by Adams and Liyanage (1993) as a limit for GSH increase in berry. It has been shown that glutathione-S-transferase exhibits the same expression profile as the enzymes responsible for anthocyanin accumulation, which are strongly related to sugar accumulation (Terrier et al., 2005). In the recent study of Kobayashi et al. (2011), it was shown that GSH concentrations in the grapevine leaves were influenced by UV-C radiation, while extreme air temperatures (0 °C or 40 °C) or water stress had no effect on GSH concentrations. In our study, a modified leaf area-to-yield ratio significantly influenced GSH concentrations in grape berries. The highest leaf area to yield resulted in a faster GSH accumulation, and peaked in a significantly higher GSH concentration in the grape berry at the second sampling. The lowest leaf area-to-yield (0.63 m^2/kg) resulted in slower GSH accumulation, which reached a maximum concentration at harvest. Different rates of GSH accumulation in the grape berry could be related to the different speed of TSS

accumulation in the berry, as previously suggested by Adams and Liyanage (1993). At harvest, neither bunch thinning nor shoot hedging had a significant effect on GSH concentration in the grape berry.

In our study, removal of leaves and secondary shoots in the phenophase of pepper-corn size berries (E-L 29) (Eichorn and Lorenz, 1977) had no significant effect on the HCAs concentration in grape berries at harvest. Similar outcome was observed by Friedel et al. (2013), where leaves removal performed at phenological stage of berry expansion and at veraison did not affect the concentrations of non-flavonoids in 'Riesling' grapes. As reported by Sternad Lemut et al. (2011), leaf removal at berry set was an efficient method to modify the HCAs concentration in 'Pinot noir' grapes, whereas later leaf removal did not influence the HCAs concentration in the grape berry. It could be that leaf removal in our study was not performed early enough (E-L 29) to alter the final concentration of HCAs in the grape berries, since they are synthesised as early as berry formation occurs (Singleton et al., 1978). Grape berry diameter and TSS concentration in the berry had no influence on the HCAs concentration in the grape berries at harvest. The concentration of HCAs decreased during ripening from 170 to 280 mg/L at veraison to concentrations between 114 to 137 mg/L at harvest. Moreover, a modified leaf area-to-yield ratio also had no significant effect on the concentration of HCAs in the grape berry. As already stated, it could be that shoot hedging and bunch thinning were applied too late (two weeks before veraison) to modify the HCAs concentration in the grape berry, as it has been shown, that HCAs are synthesised in the grape berry in first two weeks after flowering (Singleton et al., 1978).

3.1.2 Wine composition

Grapes from the treatments applied in the vineyard were vinified in two seasons out of three. In the second year of the experiment conducted in South Africa, grapes from the eight replicates per treatment were pooled together and vinified. The concentrations of IBMP found in wines were between 2.4 and 3.4 ng/L. Wines from treatments without leaf and secondary shoot removal resulted in higher IBMP concentrations of 3.4 ng/L, above the sensory detection threshold of 2 ng/L (Allen et al., 1991). In a recent study, Gregan et al. (2012) showed the effect of UV light on IBMP concentration for the first time. Our results obtained in this study, where a 99 % reduction in UV light had no significant effect on the IBMP concentrations in the resulting Sauvignon blanc wines, are supported by Gregan et al., (2012) observations. As reported previously by Ryona et al. (2009), the concentration of IBMP in the grape berry was well correlated ($R^2=0.97$) with the IBMP found in wines. In the experiment with the modified leaf area-to-yield ratio, IBMP concentrations in the wines were not monitored due to the levels in the grape berries being below the limit of detection at the second sampling (a week after veraison). A rapid decrease in IBMP in the 'Sauvignon blanc' grapes from the Vipavska dolina winegrowing district suggested that MPs are not of significant importance for the aromatic profile of Slovenian Sauvignon blanc wines, as observed by Lisjak et al. (2011).

To our knowledge, our study reports for the first time the effect of UV light intensity on wine chemical composition and sensory perception. In this study, the concentrations of 3-sulfanyl-hexan-1-ol (3SH) and consequently, 3-sulfanylhexyl acetate (3SHA) seemed to be decreased by reduced UV light intensity, which is in agreement with the results of a preliminary study (personal data). The concentration of 3SH in the treatment with reduced UV light intensity and removal of leaves and secondary shoots (LR-UV) was 344 ng/L compared to the 447 ng/L at the treatment with removal of leaves and secondary shoots and ambient UV light radiation (M-LR). The control treatment (no defoliation and ambient UV light radiation) resulted in the lowest 3SH concentrations in wines, viz. 302 ng/L. 3SHA is formed by esterification from 3SH and, as expected, lower 3SHA concentrations were found, as observed with 3SH in treatment with UV light reduction. The significant decrease in thiols concentration in the UV light-reducing treatment could originate from variations in the degradation of grape polyunsaturated fatty acids (PUFAs) under UV solar radiation. The degradation of PUFAs is associated with up-regulated lipoxygenase activity and the production of C6 compounds, notably *trans*-2-hexenal, which are known to be stress distributers in the plant (Kalua and Boss, 2009, Kobayashi et al., 2011). The hypothesis proposed by Kobayashi et al. (2011) relating to the effect of UV light on the formation of thiol precursors through the degradation of PUFAs was strengthened in this study, where lower levels of C6 compounds and hexyl acetate were found in wines from UV light-reducing treatment. Significantly lower levels of C6 compounds observed in this study at the treatment with reduced UV light intensity in combination with removal of leaves and secondary shoots might be responsible for the lower synthesis of 3SH precursors in the grapes and, consequently, lower 3SH and 3SHA concentrations in the wines. Furthermore, our results are in accordance with the results of Kobayashi et al. (2011), who reported higher concentrations of cysteine- and glutathione-3-sulfanyl hexan-1-ol precursors in grape berries and grapevine leaves exposed to UV-C irradiation. In addition, significantly higher 3SH concentrations in wines (447 ng/L) in M-LR treatment can be related to higher GSH concentrations in the must compared to other treatments. It is known that the glutathionylated precursor of 3SH can also be synthesised in the must through the reaction between *trans*-2-hexenal, derived from lipid degradation, and GSH (Roland et al., 2010). However, Roland et al. (2010) and Patel et al. (2010) reported that higher GSH concentrations in the must before fermentation did not necessarily lead to higher thiol concentrations in the resulting wines; the thiol precursors are stable in must oxidation (Allen et al., 2011). In addition to the findings of the aforementioned studies, the absence of UV-B could increase the concentrations of nitrogen compounds in the grapes (ammonium, amino acids) as a result of an increased uptake of nitrogen, which consequently could lead to a lower thiol precursor intake by the yeast during alcoholic fermentation (Döhler, 1992; Döhler et al., 1995; Subileau et al., 2008). This hypothesis will have to be confirmed in future work by measuring variations in ammonium and amino acids in the must, in addition to volatile thiols.

In the experiment with shoot hedging and bunch thinning, significantly higher concentrations of 3SH and 4-methyl-4-sulfanylpentan-2-one (4MSP) were quantified in the

wines from the FC/BT treatment with the highest leaf area-to-yield ratio (1.85 m^2/kg). The 3SH concentration in wines from FC/BT was 814 ng/L, which was significantly higher than that observed in the treatment without shoot hedging and without bunch thinning (FC/NBT), and in treatments with shoot hedging without bunch thinning (SH/NBT) and in combination with bunch thinning (SH/BT), where they reached 484 ng/L, 489 ng/L and 208 ng/L, respectively. Furthermore, a significantly higher 4MSP concentration, viz. 22 ng/L, was observed in the FC/BT treatment compared to the 16 ng/L, 17 ng/L and 14 ng/L observed in the SH/NBT, FC/NBT and SH/BT treatments, respectively. The results of our study further imply that different leaf area-to-fruit ratio should have been proposed for some secondary compounds, such as the 7 to 14 cm^2/g (leaf area/berry fresh mass ratio) suggested by Kliewer and Ough (1970).

In our study with the removal of leaves and secondary shoots, some esters, higher alcohols, medium chain fatty acids, C6 compounds and linalool were quantified in the wines. UV light intensity reduction led to a decrease in the concentration of higher alcohol acetates and ethyl esters of fatty acids. The concentration of ethyl esters of fatty acids was 18 % lower in LR-UV treatment compared to the M-LR treatment. Due to the unfavourable conditions during fermentation, yeast overcomes the stress by increasing the fluidity of the membrane by increasing the unsaturation of cell membrane lipids and shortening their chain length (Beltran et al., 2008). The chain length can be shortened through a higher production of medium chain fatty acids (C6 to C14) (Beltran et al., 2008), which are more toxic to the yeast. However, the synthesis of medium chain fatty acids is quickly favoured during fermentation in order to improve the fluidity of the membrane at low temperature (Torija et al., 2003; Beltran et al., 2008) due to the lack of PUFAs and oxygen. In our study, it was proposed that UV light reduction might lead to higher levels of PUFAs in the must, which represents a better source for yeast than medium chain fatty acids to improve membrane fluidity. The consequence would be a decrease in medium chain fatty acids (semi-quantitative data) and ethyl esters of fatty acids levels in the wines, as observed for LR-UV compared to M-LR.

Wines from the LR-UV treatment displayed significantly lower concentrations of higher alcohol acetates, viz. 17 % lower compared to the M-LR treatment. Although the availability of higher alcohols has an impact on higher alcohol acetates final level in the wine, the limiting factor of higher alcohol acetates production is the gene expression of yeast enzymes involved in their biosynthesis (ATF1, ATF2) (Sumby et al., 2010). PUFAs are known to strongly repress ATF1 (Fujii et al., 1997; Fujiwara et al., 1998). In our study, lower levels of C6 compounds (semi-quantitative data) and hexyl acetate in the LR-UV wines might indicate that PUFAs degradation was lower in the treatments with UV light reduction, as already suggested by Kobayashi et al. (2011). Therefore, the grapes and must from the LR-UV treatment probably had higher concentration of PUFAs, leading to a stronger repression of ATF1 and consequently lower concentration of higher alcohol acetates.

Conversely, higher concentrations of ethyl esters of branched acids and ethyl propionate were found in the treatments with removal of leaves and secondary shoots (M-LR and UV-LR) compared to control. The concentration of ethyl esters of branched acids in the wines is directly dependent on the availability of their corresponding acids (Swiegers et al., 2005). Therefore, higher alcohols (semi-quantitative data), deriving from the same pathway (Swiegers et al., 2005), were also found at higher levels in this study in the treatments with leaf removal (M-LR and UV-LR) compared to the control. The trend of increased concentrations of ethyl esters of branched acids and ethyl propionate was observed as well in this study in the LR-UV compared to the M-LR treatment; however it was not always significant.

The semi-quantitative data for linalool found in the wines were significantly influenced by the light exposure of the grapes in the vineyard. Significantly higher levels of linalool were found in the wines from the treatment with the removal of leaves and secondary shoots in the M-LR treatment, whereas significantly lower values were observed at the control. UV light intensity reduction significantly decreased the linalool levels in the LR-UV treatment compared to those in the M-LR treatment.

As observed in our study, UV light intensity reduction significantly altered the chemical composition of the wine.

3.1.3 Wine sensory evaluation

A panel of 10 wine experts evaluated wines that were produced and a partial component analyses (PCA) was applied to the consensus mean centred sensory scores conducted with Generalised Procrustes Rotation Algorithm, explaining 56.6 % of the variation in PC1 and 19.5 % of variation in PC2. A clear separation was observed among treatments with and without the removal of leaves and secondary shoots. Wines from grapes produced on vines without removal of leaves and secondary shoots were associated with descriptors such as overall green, green pepper, cooked peas/beans and grassy, and a mouth feel descriptor of acidity. Wines from grapes subjected to the removal of leaves and secondary shoots were described as fruity, associated with descriptors such as pineapple, passion fruit, grapefruit, guava and overall tropical, and descriptors such as floral and banana lolly. As far as we are aware, this is the first report on the effect of UV light reduction on the sensory perception of wine. Wines from grapes subjected to UV light reduction were associated with a significantly higher mouth feel perception of bitterness. Further research on this topic would be needed to provide adequate explanations.

In order to find a possible correlation between the wine chemical and sensory data, the results were subjected to further analyses by means of Common Component Specific Weight Analyses. It is evident that common dimension 1 (CD1) explains 83 % of variation in the data set, whereas CD2 explains 14 % of data variance. Scores for extracted CD1

separate the C treatment from the two other treatments receiving leaf removal (M-LR and UV-LR), irrespective of UV radiation reduction. A positive correlation was found between the C treatment and sensory attributes such as overall green, green pepper, grassy and cooked beans/peas. The chemical data strongly associated with the C treatment were isobutyl acetate, propyl acetate and IBMP, with the latter being known to contribute to green aromas of wines. Loading scores indicate that M-LR was associated with sensory attributes such as floral, banana lolly and guava. 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. The LR-UV treatment was strongly related to the perception of bitterness.

Significantly higher concentrations of 3SH and 4MSP were found in the treatment with the highest leaf area-to-yield ratio (1.85 m^2/kg). The concentration of 3SH found in the wine from the treatment with the highest leaf area-to-yield ratio was 814 ng/L, whereas the 4MSP concentration was 22 ng/L. Wines from the treatment with the highest leaf area-to-yield ratio were scored the highest on the perception of heavier tropical aromas reminiscence of passion fruit and mango, but also on aroma linked to black currant odour were observed, both usually associated with 3SH and 4MSP (Darriet et al., 1995; Tominaga et al., 1996; Coetzee and Du Toit, 2012). The overall quality of the wines that were produced was found to be significantly higher in the wine from vines with the highest leaf area-to-yield ratio (1.85 m^2/kg), whereas wines from the treatments with hedging regardless of bunch thinning were rated as the least desirable (0.63 and 1.15 m^2/kg).

3.2 CONCLUSIONS

This section highlights the key conclusions that can be drawn from this research:

- with the introduction of a non-destructive berry classification method according to their diameter and concentration of total soluble solids (TSS), it was possible to study the grape berry heterogeneity occurring in the vineyard. Berries of the same diameter were not at the same ripening stage (°Brix);

- the removal of leaves and secondary (lateral) shoots at the phenological stage of peppercorn-size berries (E-L29) can be an efficient tool to decrease the concentrations of 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) in grapes and wines. However, UV light reduction had no significant effect on IBMP concentrations in wines. Furthermore, berries of a larger diameter and at similar TSS concentration resulted in significantly higher IBMP concentrations;

- it has been shown that the increase in light in the bunch zone as a result of the removal of leaves and secondary shoots did not significantly influence reduced glutathione (GSH) and hydroxycinnamates (HCAs) concentrations in the grape berry. It could be that removal of leaves and secondary shoots in this study, performed at the phenological stage of peppercorn-size berries (E-L 29), was not performed early enough to influence the HCAs concentrations in grape berries at harvest;

- reducing leaf area-to-yield ratio as a result of shoot hedging had an impact on the lower GSH concentrations for the first sampling dates, whereas it did not influence the HCAs concentrations in grape berries at harvest;

- reduction of UV light intensity caused significantly lower concentrations of 3-sulfanyl-hexan-1-ol (3SH) and 3-sulfanylhexyl acetate (3SHA) in resulting wines compared to the treatments without UV light reduction. The highest leaf area-to-yield ratio resulted in significantly higher concentrations of 3SH and 4-methyl-4-sulfanylpentan-2-one (4MSP) in the resulting Sauvignon blanc wines;

- UV light reduction in the vineyard resulted in an altered ester profile of the Sauvignon blanc wines. Lower concentrations of ethyl esters of fatty acids and higher alcohol acetates were observed in wines from the UV light-reduced treatments. In contrast, UV light reduction and leaf removal gave rise to significantly higher concentrations of ethyl esters of branched acids, compared to control. In terms of the different leaf and secondary shoots removal treatments, the manipulation of light quantity showed less significant modifications than UV light reduction on the ester composition of the Sauvignon blanc wines;

- UV light reduction significantly decreased levels of some medium chain fatty acids and C6 compounds and their esters (hexanol, ethyl *cis*-3-hexenoate, ethyl *trans*-2-hexenoate, *cis*-3-hexenyl and *trans*-2-hexenyl acetate) in combination with leaf and secondary shoots removal;

- significantly higher levels (semi-quantitative data) of linalool were observed in the Sauvignon blanc wines in the treatment receiving leaf and secondary shoots removal, i.e. without UV light reduction (M-LR), indicating the significance of light quantity on linalool levels in grapes and wines. UV light reduction showed a significant decrease in linalool levels, whereas the control treatment with unaltered canopy resulted in the lowest linalool levels;

- the sensory perception of Sauvignon blanc wine was significantly altered by removal of leaves and secondary shoots and UV light reduction. Wines from the grapes from the treatments with removal of leaves and secondary shoots were associated with fruitier aromas, whereas control, receiving no leaf and secondary shoot removal was associated with green descriptors. Treatment with UV light reduction was perceived as significantly more bitter compared to the treatments with no UV light reduction;

- modified leaf area-to-yield ratio significantly altered the sensory perception of Sauvignon blanc wines. The treatment with the highest leaf area-to-yield ratio resulted in a significantly higher perception of heavier tropical aromas, such as passion fruit and mango and aromas reminiscent of black currant. Furthermore, this treatment was evaluated by the panellists as being of the highest overall quality, whereas wines from the treatments receiving shoot hedging were evaluated as being of the lowest quality;

- this study reports for the first time the effect of UV light reduction on wine chemical composition and sensory perception. Furthermore, our study proposes a hypothetical influence of UV light reduction on lipoxygenase and terpene pathway and its effect on the compounds quantified in this study;

- canopy management can be an effective method (when climate and row orientation permit) to modify and target preferred Sauvignon blanc wine styles as early as in the vineyard. However, canopy manipulation in the vineyard should be reasoned carefully according to the site (hot/warm versus temperate/cool), the row orientation, the bunch microclimate, the vine’s vigour, irrigation and the desired yield per vine and wine style. The decision about the side of the canopy to remove leaves from has to be taken carefully according to row orientation and site location to prevent possible sunburns.

4 SUMMARY (POVZETEK)

4.1 SUMMARY

The overall aim of this study was to investigate the effect of modified bunch microclimate through leaf and secondary shoot removal and UV light reduction in South Africa and of a changed leaf area-to-yield ratio by shoot hedging and bunch thinning in Slovenia, on grape berry and wine composition and the related sensory evaluation of the wine.

The classification of the berries according to their diameter first and total soluble solids (TSS) concentration for berry's classes of same diameter, showed the heterogeneity in berry composition occurring in the vineyard. Grape berries were evenly distributed along a Gaussian bell-shaped curve according to their diameter at all three sampling dates, which confirms the homogenous distribution of the berries across the three major berry classes.

Removal of leaves and secondary shoots at a height of 40 cm above the cordon at the phenological stage of peppercorn-size berries has been shown to be a successful canopy management method to significantly decrease the concentration of 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) in grapes and wines. The IBMP concentrations in the treatment with removal of leaves and secondary shoots were below the limit of detection (0.6 ng/L) at the second sampling, whereas they were still measured in the control. Berries of the same diameter were not at the same ripening stage and not the same IBMP concentration because berry of the same volume could have different TSS concentration and therefore different ripening level if we do consider that the dynamic of berry sugar accumulation per fruit is a relevant physiological indicator of fruit ripening (Deloire, 2011 and 2013). Berries of a larger diameter at similar TSS concentration resulted in higher IBMP concentrations, showing the heterogeneity occouring in te vineyard in the terms of berry size, TSS concentration and concentrations of some secondary metabolites.

The removal of leaves and secondary shoots had no effect on the reduced glutathione (GSH) and hydroxycinnamates (HCAs) concentrations in the grape berries at harvest. Good correlation between the GSH and TSS concentration in grape berries was observed ($R^2=0.888$). Reduction of 44 % exposed leaf area by shoot hedging and without bunch thinning caused lower GSH concentrations in grape berries during the ripening, whereas no significant differences among the treatments were observed at harvest. Neither shoot hedging nor bunch thinning influenced HCAs concentrations in grape berries at harvest.

The chemical composition of the wines was significantly altered by the removal of leaves and secondary shoots at the phenological stage of peppercorn-size berries and installation of UV light reducing sheets. The control treatment resulted in a higher IBMP concentration compared to the treatments with removal of leaves and secondary shoots. The reduction of UV light resulted in a significantly lower concentration of 3-sulfanyl-hexan-1-ol (3SH) and

3-sulfanylhexyl acetate (3SHA) in the wine. Concentrations of ethyl esters of fatty acids and higher alcohol acetates were reduced in the wines from the treatments with UV light reduction, whereas defoliation caused higher concentrations of ethyl esters of branched acids. To our knowledge, this is the first report on the effect of UV light reduction in the bunch zone on thiols and esters concentrations in wines.

Shoot hedging and bunch thinning significantly altered thiols concentrations in resulting wines. The treatment with the highest leaf area-to-yield ratio (1.85 m^2/kg) resulted in significantly higher concentration of 3SH and 4-methyl-4-sulfanylpentan-2-one (4MSP) in the wines.

Finally, the sensory perception of the wine was altered significantly by the different viticultural practices performed in the study. Wines from treatments with removal of leaves and secondary shoots were associated with the attributes as overall fruity, passion fruit, grape fruit, pineapple and guava, whereas the control treatment was described as overall green, green pepper and cooked beans. A positive correlation between the chemical analyses and the sensory perception was shown by Common Components Specific Weight analyses. Of note is that wines from the UV light-reducing treatments were linked with a mouth feel perception of bitterness.

The highest leaf area-to-yield ratio (1.85 m^2/kg) resulted in the highest perception of heavier tropical aromas reminiscent of passion fruit and mango in wines, but also odours of black currant and cat urine were found, which was supported by the presence of the highest concentrations of 3SH and 4MSP. Wines from this treatment were also judged the highest for overall quality, whereas wines from treatments where shoot hedging was applied were evaluated as the least desirable.

'Sauvignon blanc' grape and wine composition and sensory perception can therefore be altered significantly by leaf and secondary shoot removal and a modified leaf area-to-yield ratio by shoot hedging and bunch thinning. Treatments receiving removal of leaves and secondary shoots were assosiated with fruitier aromas, whereas no leaf removal treatment resulted in elevated perception of greenes. The highest leaf area to fruit ratio resulted in the best overall quality of wines. Therefore, in this study we demonstrated that Sauvignon blanc wine composition and sensory perception can already be altered in the vineyard. It should be kept in mind, that leaf removal should be reasoned carefuly, depending on the climate, row orientation and positioning of heat waves in order to prevent possible grape sunburn and berry shrivelling, as loss of acidity and aroma. Furthermore, shoot hedging and bunch thinning should be performed carefully in regard to the expected yield/vine and hectare, wine quality and vine balance.

4.2 POVZETEK

Sorta 'Sauvignon blanc' izvira iz Francije. Originalno ime sorte je sestavljenka francoskih besed ‘sauvage’ (divji) in ‘blanc’ (bel) (Galet, 1990; Larousse, 2011) in dandanes se jo v Franciji goji na 26.062 ha. Sorta 'Sauvignon blanc' je tretja najbolj zastopana bela sorta žlahtne vinske trte v Sloveniji (Mavrič Štrukelj in sod., 2012) in druga najbolj zastopana na svetu. Z globalizacijo in s seznanjenjem potrošnikov z mednarodnimi vini je postala konkurenca na vinskem tržišču izjemna. Veliko držav pridelovalk vina Novega sveta, kot sta na primer Nova Zelandija in Republika Južna Afrika, je s cenejšo delovno silo, novejšo tehnologijo in z znanjem prodrlo na mnoga evropska tržišča, kjer ponujajo visoko kakovostna vina po enaki ali nižji ceni in konkurirajo že obstoječi domači ponudbi. Nova Zelandija je z obstoječih 5.980 ha vinogradov v letu 1991 povečala površino vinogradov na 34.269 ha v letu 2012, od katerih je 20.000 ha zasajenih s sorto 'Sauvignon blanc' (New Zealand annual wine report for 2012, 2013). Od leta 1999 se je letni izvoz vin iz Republike Južne Afrike z 21,6 % letne pridelave povečal na 49,1 % v letu 2009. V Republiki Južni Afriki je sorta 'Sauvignon blanc' tretja najbolj zastopana bela vinska sorta, 86,9 % letne pridelave vina sauvignon blanc pa izvozijo na mednarodne trge (South African wine industry statistics, 2010).

V nasprotju z Republiko Južno Afriko in Novo Zelandijo so se površine vinogradov v Sloveniji zmanjšale s 17.147 ha v letu 1996 na 16.372 ha v letu 2011, letni slovenski izvoz vina pa znaša le 9,4 milijona EUR (Mavrič Štrukelj in sod., 2012; Vinska družba Slovenije, 2013). Kar 71 % slovenskih vinogradov je zasajenih na pobočjih, kar najverjetneje omogoča pridelavo kakovostnejšega grozdja, prav tako pa vpliva na višje stroške obdelave vinogradov. Zaskrbljujoč pa je tudi podatek, da se je obnova vinogradov v Sloveniji zmanjšala že pod mejo reprodukcije (Mavrič Štrukelj in sod., 2012).

V zadnjem desetletju vino sauvignon blanc zavzema vedno večji tržni delež, saj ugaja velikemu številu potrošnikov, in sicer zaradi raznolikosti stilov vina. Vino Sauvignon blanc je običajno opisano z aromami po tropskem sadju, pasijonki, mangu, guavi, ananasu oziroma z zelenimi aromami, kot je aroma po zeleni papriki, špargljih, kuhanem grahu in fižolu (Allen in sod., 1991; Tominaga in sod., 1996; Benkwitz in sod., 2012; Coetzee in du Toit, 2012). Veliko raziskav je bilo že narejenih na sorti 'Sauvignon blanc' in kot je znano, je kakovost vina odvisna od kakovosti grozdja (Jackson in Lombard, 1993), pa tudi, da je stil vina sauvignon blanc odvisen od območja pridelave grozdja (Lacey in sod., 1991; Lund in sod., 2009), od ampelotehničnih ukrepov (Masneuf-Pomaréde in sod., 2006, Kozina in sod., 2008; Gregan in sod., 2012) in od predelave grozdja in pridelave vina (Swiegers in sod., 2005; Patel in sod., 2010). Vse te študije pa so se osredotočile le na dotične segmente, dele pridelave in predelave grozdja in pridelave vina sauvignon blanc, medtem ko je manjkal celovit pristop, ki bi preučeval vpliv različnih ampelotehničnih ukrepov na kakovost grozdja, kemijsko sestavo ter tudi na senzorične lastnosti vina.

Vinu sauvignon blanc dajejo »tipično« aromo metoksipirazini (MPs) in tioli, na vsebnost katerih je mogoče vplivati z ustreznimi ampelotehničnimi ukrepi že v vinogradu.
Namen te raziskave je bil ovrednoti učinek modificirane mikroklime grozdov in odrejenega razmerja med listno površino ter maso grozdja na trti na kemijsko sestavo grozdja in vina ter na senzorične lastnosti slednjega.

Mikroklimo grozdja smo spremenili z razlistanjem vinske trte v območju grozdov. V dveh zaporednih letih smo v Republiki Južni Afriki preučevali vpliv razlistanja vinske trte sorte 'Sauvignon blanc' na višini 40 cm nad kordonom v fenofazi jagod debeline poprovega zrna (E-L 29) na izbrane primarne in sekundarne metabolite grozdja in vina. Razlistanje je bilo v prvem letu opravljeno samo na strani listne stene vinske trte, ki jo sonce obseva zjutraj (severo-vzhodna). V drugem letu poskusa smo poleg spremembe intenzitete svetlobe v območju grozdov z razlistanjem zmanjšali tudi intenziteto UV svetlobe z namestitvijo transparentnih plošč, ki zaustavijo 99 % UV svetlobe, in sicer z zmanjšanjem intenzitete vidne svetlobe za 12 %. Plošče za zmanjšanje intenzitete UV svetlobe so bile nameščene v območju grozdov pri obravnavanju z razlistanjem na strani listne stene vinske trte, ki jo sonce obseva zjutraj (severo-vzhodna) sočasno z razlisatanjem vinske trte v fenofazi jagod debeline poprovega zrna (E-L 29). V obeh letih smo v poskusu imeli tudi kontrolno obravnavanje, pri katerem trt nismo razlistali, in smo jim v letu 2011 izmerili povprečno gostoto toka fotosintetsko aktivnega žarčenja (PAR) 50 µmol $m^{-2}s^{-1}$ ter 60 µmol $m^{-2}s^{-1}$ v letu 2012. Pri obravnavanjih z razlistanjem smo izmerili značilno večjo PAR, in sicer do 850 µmol $m^{-2}s^{-1}$, kar pa je bilo odvisno od obravnavanja, ure dneva in oblačnosti. Grozdne jagode trt, razlistanih na strani listne stene vinske trte, ki jo sonce obseva zjutraj, in kontrolnih trt smo glede na premer (mm) razvrstili v različne velikostne razrede z uporabo plastičnih ploščic z luknjicami različnih premerov. Nato so bile jagode enakega premera razvrščene še dodatno, in sicer glede na vsebnost skupne suhe snovi (TSS), ki smo jo vrednotili s flotacijo jagod v vodnih raztopinah z različno vsebnostjo sladkorja. Jagodam, razvrščenim glede na premer in vsebnost TSS, smo izmerili vsebnosti MPs, reduciranega glutationa (GSH) ter hidroksicimetnih kislin (HCAs), prav tako pa smo jim izmerili vsebnost jabolčne kisline. V vinih, pridelanih iz poskusa z razlistanjem in namestitvijo plošč, ki zmanjšajo intenziteto UV svetlobe, smo izmerili vsebnosti 3-izobutil-2-metoksipirazina (IBMP), 3-sulfanilheksil acetata (3SHA), 3-sulfanil heksan-1-ola (3SH), nekaterih estrov in semi-kvantitativno izmerili vsebnosti nekaterih višjih alkoholov, srednjeverižnih maščobnih kislin, C6 spojin ter linaloola. Vina iz poskusa so bila tudi senzorično ocenjena.

V poskusnem vinogradu v Vipavski dolini smo v fenofazi E-L 33 na trtah priredili razmerje med listno površino in maso grozdja, in sicer s krajšanjem mladik in z redčenjem grozdov. Pri obravnavanju z zmanjšano listno površino smo mladike z višine 1,6 m prikrajšali na 0,9 m, kar se je odrazilo v zmanjšanju listne površine za 44 %. Sočasno s krajšanjem mladik smo izvedli tudi redčenje grozdov, in sicer tako, da smo odstranili na vsaki mladiki vsak drugi (zgornji) grozd in tako je prišlo do spremembe razmerja med listno površino in maso grozdja na trti. V poskusu smo imeli štiri obravnavanja, in sicer

obravnavanje s krajšanjem mladik in brez redčenja grozdov (SH/NBT), obravnavanje s krajšanjem mladik in z redčenjem grozdov (SH/BT), obravnavanje brez krajšanja mladik in brez redčenja grozdov (FC/NBT) ter obravnavanje brez krajšanja mladik in z redčenjem grozdov (FC/BT). Razmerje med listno površino in maso grozdja je bilo pri SH/BT 0,63 m^2/kg, 1,15 m^2/kg pri obravnavanju SH/BT in 1,07 ter 1,85 m^2/kg pri obravnavanjih FC/NBT ter FC/BT. Med dozorevanjem grozdja smo merili vsebnosti osnovnih parametrov zrelosti (TSS, titrabilnih kislin (TA) in pH), kot tudi vsebnosti MPs, GSH in HCAs, β-karotena, luteina in neoksantina, ob trgatvi pa še vsebnost fermentabilnega dušika (FAN) ter jabolčne kisline. Ob trgatvi smo grozdje po obravnavanjih tudi potrgali in ga vinificirali v treh ponovitvah. V vinu smo izmerili vsebnosti GSH, 3SH, 3SHA in 4-metil-4-sulfanilpentan-2-ona (4MSP) ter ga tudi senzorično ocenili.

Kratek povzetek rezultatov je v nadaljevanju predstavljen v treh različnih odstavkih, zasnovanih glede na vpliv razlistanja in zmanjšanja intenzitete UV svetlobe v okolici grozdov in spremenjenega razmerja med listno površino in maso grozdja vinske trte na izbrane metabolite grozdja, na kemijsko sestavo in senzorične lastnosti vina sauvignon blanc.

- **Sestava grozdne jagode**

Porazdelitev grozdnih jagod v različne velikostne razrede je ustrezala razporeditvi Gaussove krivulje, kar dokazuje homogeno razdelitev grozdnih jagod glede na velikost v tri najbolj zastopane razrede, kar navajajo tudi Deloire in sod. (2004) ter Rolle in sod. (2012). Sprememba razmerja med listno površino in maso grozdja vinske trte s krajšanjem mladik in redčenjem grozdov je na začetku zorenja grozdov pri obravnavanjih SH/BT in SH/NBT vplivala na statistično značilno kasnejše kopičenje TSS. Statistično značilno počasnejše kopičenje TSS je bilo opazno pri obravnavanju SH/NBT med dozorevanjem grozdov vse do trgatve, ko se med obravnavanji v vsebnosti TSS niso več pokazale statistično značilne razlike. Spremenjeno razmerje med listno površino in maso grozdja vinske trte ni vplivalo na vsebnost TA in pH v grozdnih jagodah ob trgatvi.

Razlistanje v območju grozdov v fenofazi jagod debeline poprovega zrna (E-L 29) se je izkazalo kot uspešen ampelotehnični ukrep za zmanjšanje vsebnosti IBMP v grozdju ter v vinu. Vsebnosti IBMP pri razlistanem obravnavanju so bile pod mejo zaznave (0,6 ng/L) že dva tedna pred trgatvijo, medtem ko je bila vsebnost IBMP pri kontrolnem (nerazlistanem) obravnavanju ob trgatvi med 5,2 ng/L in 12,6 ng/L. Grozdne jagode enake vsebnosti TSS, ampak večje velikosti, so imele večjo vsebnost IBMP. V jagodah podobne vsebnosti TSS in velikosti 13,5 mm smo izmerili 5,2 ng/L ter pri jagodah velikosti 15,5 mm pa 12,6 ng/L IBMP. Prav tako so jagode z večjo vsebnostjo TSS v primerjavi s tistimi z manjšo vsebnostjo, imele manjšo vsebnost IBMP. Ob istem vzorčenju smo v jagodah z vsebnostjo TSS 11,6 °Brix izmerili 17,8 ng/L IBMP, medtem ko pri tistih z vsebnostjo TSS 5,1 °Brix pa 72, 4 ng/L IBMP. V poskusu zasnovanem v Vipavski dolini s prikrajševanjem mladik in redčenjem grozdov je bila vsebnost IBMP v grozdju pod mejo

zaznave (0,6 ng/L) že pri drugem vzorčenju (1 teden po fenofazi začetka mehčanja jagod), kar potrjuje, da IBMP ni spojina, ki bi vplivala na aromatiko vina sauvignon blanc, pridelanega v Vipavski dolini (Lisjak in sod., 2011). Grozdje iz hladnejših pridelovalnih območij vsebuje večje vsebnosti IBMP kot tisto, pridelano v toplejših območjih (Lacey in sod., 1991).

Razlistanje v okolici grozdov v fenofazi jagod velikosti poprovega zrna (E-L 29), ni vplivalo na vsebnosti GSH v grozdnih jagodah ob trgatvi. Vsebnosti GSH so se večale z večanjem vsebnosti TSS v grozdni jagodi. Dobili smo tudi dobro soodvisnost med vsebnostjo TSS in vsebnostjo GSH ($R^2=0,888$). Vpliv na vsebnost GSH v grozdnih jagodah med dozorevanjem sta imela tudi krajšanje mladik in redčenje grozdov. Vsebnost GSH je bila statistično značilno večja pri obravnavanju FC/BT z največjim razmerjem med listno površino in maso grozdja (1,85 m^2/kg) že pri drugem vzorčenju, medtem ko se je pri obravnavanju z najmanjšim razmerjem (0,63 m^2/kg) vsebnost GSH večala vse do trgatve.

Razlistanje vinske trte ni imelo značilnega vpliva na vsebnosti HCAs ob trgatvi. Med dozorevanjem se je vsebnost HCAs zmanjšala, ob prvem vzorčenju so bile vsebnosti HCAs med 170 mg/L in 280 mg/L, medtem ko so bile ob trgatvi med 114 mg/L in 137 mg/L. Spremenjeno razmerje med listno površino in maso grozdja vinske trte ni imelo vpliva na vsebnost HCAs, β-karotena in neoksantina, medtem ko so bile manjše vsebnosti luteina v grozdju ob trgatvi pri obravnavanjih z redčenjem grozdov.

- **Sestava vina**

V drugem letu poskusa v Republiki Južni Afriki je bilo grozdje treh obravnavanj vinificirano v treh ponovitvah. Prav tako smo ob trgatvi grozdje iz poskusa s krajšanjem mladik in redčenjem grozdja potrgali in ga vinificirali v treh ponovitvah. Razlistanje v območju grozdov in spremenjeno razmerje med listno površino in maso grozdja vinske trte je imelo statistično značilen vpliv na kemijske lastnosti vina.

Večje vsebnosti IBMP so bile v vinu, pridelanem iz grozdja nerazlistanih trt (3,4 ng/L) v primerjavi z razlistanimi, kjer so bile vsebnosti IBMP manjše, in sicer 2,4 ng/L v grozdju s trt razlistanih na jutranji strani. Namestitev transparentnih plošč za zmanjšanje intenzitete UV svetlobe ni imela statistično značilnega vpliva na vsebnost IBMP v vinih.V poskusu s krajšanjem mladik in redčenjem grozdov vsebnost IBMP v vinih ni bila izmerjena, saj je bila vsebnost IBMP pod mejo zaznave (0,6 ng/L) že dva tedna pred trgatvijo.

Zmanjšanje intenzitete UV svetlobe je vplivalo na značilno manjše vsebnosti 3SH in 3SHA v vinu. Vsebnost 3SH je bila pri obravnavanju z razlistanjem in z zmanjšano intenziteto UV svetlobo (LR-UV) 344 ng/L, medtem ko je bila pri obravnavanju z razlistanjem in brez zmanjšanja intenzitete UV svetlobe (M-LR) 447 ng/L. Prav tako je bila vsebnost 3SH statistično značilno manjša pri kontrolnem obravnavanju (C), in sicer 303 ng/L. Vsebnost 3SHA je bila v vinu obravnavanja LR-UV 111 ng/L, v obravnavanju M-LR pa 186 ng/L,

medtem ko je bila pri C 111 ng/L. Manjše vsebnosti tiolov v vinih pri obravnavanju z zmanjšano UV svetlobo so lahko posledica manjše razgradnje polinenasičenih maščobnih kislin (PUFAs), kar vpliva na zmanjšanje sinteze *trans*-2-heksenala. Posledično je lahko tudi sinteza tiolnih prekurzorjev manjša, saj je manj dostopnega *trans*-2-heksenala, na katerega bi se lahko vezal GSH (Kobayashi in sod., 2011). Samo razlistanje trte brez zmanjšanja intenzitete UV svetlobe je statistično značilnega vplivalo na večje vsebnosti 3SH in 3SHA v vinu, v primerjavi z C. Največje razmerje med listno površino in maso grozdja je vplivalo na statistično značilno večje vsebnosti 3SH in 4MSP v vinih. Vsebnosti 3SH so bile v obravnavanju FC/BT 814 ng/L in statistično značilno večje v primerjavi s 484 ng/L, 489 ng/L in 208 ng/L v obravnavanjih FC/NBT, SH/NBT ter SH/BT. Prav tako so bile značilno večje vsebnosti 4MSP v obravnavanju FC/BT, in sicer 22 ng/L, medtem ko so bile pri obravnavanjih FC/NBT, SH/BT ter SH/NBT vsebnosti 4MSP med 14 ter 17 ng/L.

V vinih obravnavanj z zmanjšano intenziteto UV svetlobe so bile izmerjene manjše vsebnosti etilnih estrov maščobnih kislin in estrov višjih alkoholov. Vsebnost etilnih estrov maščobnih kislin je bila za 18 % manjša pri obravnavanju LR-UV v primerjavi z M-LR, medtem ko je bila vsebnost estrov višjih alkoholov za 17 % manjša pri obravnavanju LR-UV pri primerjavi z obravnavanjem M-LR. Prav tako pa je razlistanje vinske trte vplivalo na večje vsebnosti etilnih estrov razvejanih maščobnih kislin, v primerjavi s C obravnavanjem. Manjše vsebnosti srednjeverižnih maščobnih kislin in večje vsebnosti višjih alkoholov (semi-kvantitativni rezultati) so bile izmerjene v obravnavanju z zmanjšano intenziteto UV svetlobe v kombinaciji z razlistanjem, kar je potrdilo našo hipotezo. Na podlagi dobljenih rezultatov smo sklepali, da zmanjšanje intenzitete UV svetlobe vpliva na manjšo razgradnjo PUFAs, kar vpliva na večje vsebnosti PUFAs v grozdju in manjše vsebnosti spojin C6. Kot je znano, večje vsebnosti PUFAs negativno vplivajo na ekspresijo gena ATF1, odgovornega za sintezo estrov višjih alkoholov (Fujii in sod., 1997, Fujiwara in sod., 1998), medtem ko dostopnost substrata (višjih alkoholov) za sintezo estrov višjih alkoholov ni običajno omejujoč faktor. To hipotezo potrjujejo manjše vsebnosti spojin C6 (semi-kvantitativni rezultati) v obravnavanju z zmanjšano intenziteto UV svetlobe, kar nakazuje na večje vsebnosti PUFAs v moštu med fermentacijo in manjše vsebnosti estrov višjih alkoholov v vinu taistega obravnavanja. Prav tako smo postavili hipotezo, da so na statistično značilne manjše vsebnosti etilnih estrov maščobnih kislin v vinih z zmanjšano UV svetlobo vplivale večje vsebnosti PUFAs (manjša razgradnja ob pomanjkanju abiotskega stresa). Znano je, da etilni estri maščobnih kislin nastanejo iz srednjeverižnih maščobnih kislin, da razstrupljajo kvasovko (Beltran in sod., 2008). V tej študiji so bile statistično značilno manjše vsebnosti srednjeverižnih maščobnih kislin v obravnavanjih z zmanjšano intenziteto UV svetlobe. Zato smo sklepali, da so kvasovke zaradi večjih vsebnosti PUFAs v moštu z redukcijo intenzitete UV svetlobe sintetizirale manj srednjeverižnih maščobnih kislin, ker so za celico bolj toksične in ker je poraba PUFAs za povečanje fluidnosti celične membrane preferenčna pot, kar se je odrazilo v manjših vsebnostih etilnih estrov maščobnih kislin. Vsebnosti etilnih estrov razvejanih maščobnih kislin v vinih so neposredno odvisne od vsebnosti ustreznih aminokislin

(Swiegers in sod., 2005). Večje vsebnosti višjih alkoholov so bile v tej študiji izmerjene v obravnavanjih z zmanjšano intenziteto UV svetlobe, kar še potrjuje postavljeno hipotezo, saj se višji alkoholi sintetizirajo iz ustreznih aminokislin.

Razlistanje vinske trte je imelo statistično značilen vpliv na vsebnosti linaloola v vinih (semi-kvantitativni rezultati). Največja vsebnost linaloola je bila v vinih obravnavanja z razlistanjem, medtem ko so bile najmanjše vsebnosti izmerjene v obravnavanjih brez razlistanja. Redukcija UV svetlobe je prav tako vplivala na manjše vsebnosti linaloola v vinih v primerjavi z razlistanim obravnavanjem.

- **Senzorična ocena**

Razlistanje in spremenjeno razmerje med listno površino in maso grozdja vinske trte je pomembno vplivalo na senzorično zaznavo vina. Vinom iz obravnavanj z razlistanjem so pripisali senzorične deskriptorje, kot so arome po pasijonki, grenivki, ananasu, guavi ter sadna aroma, medtem ko so vinu nerazlistanega obravnavanja pripisali senzorične zaznave po zeleni papriki, kuhanem fižolu in grahu. Vina iz obravnavanj z zmanjšano intenziteto UV svetlobe so bila ocenjena s statistično značilno večjo zaznavo grenkobe. Z uporabo multivariantnih statističnih analiz smo pokazali možno povezavo med kemijsko sestavo in senzorično kakovostjo vina.

Vino iz obravnavanja z največjim razmerjem med listno površino in maso grozdja vinske trte FC/BT (1,85 m^2/kg) je bilo statistično značilno najbolje ocenjeno z aromo po pasijonki, mangu, mačjem urinu ter črnem ribezu. Pri obravnavanju FC/BT so bile izmerjene tudi največje vsebnosti 3SH in 4MSP, ki dajejo vinu prej naštete arome (Tominaga in sod., 1996; Dubordieu in sod., 2006; Roland in sod., 2011). Vino iz grozdja, pridelanega po FC/BT, je bilo tudi za splošno kakovost ocenjeno kot najboljše, medtem ko so bila vina s trt, kjer smo krajšali mladike (SH/NBT in SH/BT), ocenjena kot manj kakovostna.

Kolikor nam je znano, je to prvo poročanje o vplivu zmanjšane intenzitete UV svetlobe v območju grozdov na kemijsko sestavo in senzorično zaznavo vina. V tej študiji smo predlagali tudi možen vpliv zmanjšane intenzitete UV svetlobe na dve metabolne poti, in sicer na sintezo terpenov ter na lipoksigenazno pot.

Razlistanje in spremenjeno razmerje med listno površino ter maso grozdja vinske trte lahko statistično značilno vpliva na kemijsko sestavo grozdja in vina ter senzorično zaznavo vina sauvignon blanc. Izvajanje razlistanja vinske trte mora biti dobro premišljen ampelotehnični ukrep glede na smer neba vrst v vinogradu ter glede na podnebje (makro- in mezoklimatske razmere), da se izognemo morebitnim sončnim ožigom jagod na grozdu. Prav tako mora biti ukrep krajšanja mladik in redčenja grozdov izveden premišljeno, in sicer z upoštevanjem bujnosti vinske trte in želene količine pridelka.

Ta raziskava je pokazala, da je mogoče na kemijsko sestavo grozdja in vina ter na senzorično zaznavo vina in s tem posledično stil vina vplivati že v vinogradu z ustreznimi ampelotehničnimi opravili.

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ANNEX A

09-Jan-2014

Dear Miss Suklje

It is a pleasure to accept your manuscript entitled "EFFECTS OF LEAF REMOVAL AND ULTRAVIOLET RADIATION IN THE VINEYARD ON THE COMPOSITION AND SENSORY PERCEPTION OF SAUVIGNON BLANC (Vitis vinifera L.) WINE" in its current form for publication in the Australian Journal of Grape and Wine Research.

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On behalf of the Editors of the Australian Journal of Grape and Wine Research, I thank you for your fine contribution and we look forward to your continued contributions to the Journal.

Sincerely

Dr Terry Lee
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Author:	Katja Šuklje, Klemen Lisjak, Helena Baša Česnik, Lucija Janeš, Wessel Du Toit, Zelmari Coetzee, Andreja Vanzo, and Alain Deloire
Publication:	Journal of Agricultural and Food Chemistry
Publisher:	American Chemical Society
Date:	Sep 1, 2012

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